Processes driving freshwater plant production and diversity in upland streams

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Abstract

Upland headwater streams are important sources of freshwater in mountainous temperate to sub-arctic latitude European countries like Scotland. Yet much less is known about the ecology of small, characteristically oligotrophic, mountain streams supporting periphyton and aquatic bryophyte dominated vegetation, and their potential bioindicator capacity of environmental water quality, than lowland rivers impacted by anthropogenic disturbance, in this context.

This scarcity of knowledge has significant implications for the success of the recently implemented Water Framework Directive (WFD: 2000/60/EC). The WFD is a major piece of environmental legislation for water policy and sustainable water management in Europe. New contributions are fundamental to environment agencies, such as the Scottish Environment Protection Agency (SEPA), tasked with the responsibility of enforcing WFD statutory requirements and developing effective biomonitoring tools for assessing water quality status in Scotland.

A major aim of the WFD is to achieve at least 'good' ecological status of inland waterbodies by 2015. Further, in doing so, to ascertain ecological benchmark communities of near-pristine (or minimally-impacted) reference conditions as indicators of high water quality status. The objective is to improve understanding of the environmental processes driving the production and diversity of freshwater plant species-assemblages in upland streams. Such information can be used for assessing perturbations threatening the ecological integrity of rivers impacted by anthropogenic disturbances (human pressure). This enables environment agencies such as SEPA, to respond appropriately by implementing corrective measures and sustainable management strategies.

This project monitored a range of near-pristine headwater streams of contrasting underlying geology in the Scottish Highlands. The approach adopted was compatible with current WFD river characterisation and biomonitoring strategies.

These were used to investigate the structural and functional response of freshwater plant communities (chiefly diatoms and other algal groups; aquatic bryophyte and vascular submerged macrophyte vegetation) to environmental drivers (e.g. flow, substrate morphology, nutrient inputs, water chemistry, underwater light availability). The work was carried out with the aim of contributing to future development of baseline monitoring tools for assessing upland stream habitat quality in Scotland.

Substantial datasets were collected from intensive surveys of three small streams in the Scottish Highlands. These revealed the existence of three ecological benchmark communities of freshwater vegetation characterising suites of environmental habitat conditions in near-pristine reference streams of ranging water chemistry, buffering capacity and substrate particle composition, as determined by the underlying geology. The vegetation types were indicative of base-poor, acid sensitive streams dominated by boulders (e.g. *Frustulia rhomboides*, *Scapania undulata*, *Hygrohypnum ochraceum*: Group III); circumneutral, weakly alkaline conditions characterised by cobbled, frequently disturbed habitats (e.g. *Gomphonema acuminatum*, *Blindia acuta*, *Schistidium agassizii*: Group II); and mineral-rich, calcareous streamwaters characterised mostly by pebbles and sand (e.g. *Fragilaria pulchella*, *Chiloscyphus polyanothos*, *Hygrohypnum luridum*, *Palustriella falcata*, *Potamogeton polygonifolius*, *Chara globularis*, *Myriophyllum alterniflorum*: Group I).

The outcome of multivariate analyses and multiple regression modelling using the datasets suggested that environmental gradients of water chemistry and (where relevant) substrate morphology were the principal drivers of the distribution and species diversity of the aquatic plant assemblages present. Whilst flow, water temperature and underwater light regime factors were generally more influential predictors of the functional attributes (e.g. standing crop) of the vegetation.

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Figure 4.37 CCA ordination of epilithic periphyton (on naturally-occurring mineral substrata), aquatic bryophyte, vascular submerged macrophyte species and environmental variables. Periphyton species codes: *Eunotia arcus* sensu (Earc), *Eunotia muscicola* var. *tridentula* (Emus), *Eunotia* cf. *incisa* (Einc), *Eunotia meisteri* (Emei), *Eunotia bilunaris* var. *linearis* (Ebil), *Eunotia exigua* (Eexi), *Eunotia bilunaris* var. *mucophila* (Ebmu), *Eunotia serra* (Eser), *Eunotia implicata* (Eimp), *Fragilaria capucina* var. *vaucheriae* (Fcva), *Fragilaria capucina* var. *gracilis* (Fcgr), *Fragilaria virescens* (Fvir), *Fragilaria arcus* (Farc), *Fragilaria pulchella* (Fpul), *Synedra ulna* (Suln),*Gomphonema* cf. *parvulum* var. *exilissimum* (Gpxs), *Gomphonema clavatum* (Gcla), *Gomphonema truncatum* (Gtru), *Gomphonema acuminatum* (Gacu), *Gomphonema olivaceum* (Goli), *Gomphonema olivaceum* var. *olivaceoides* (Gool), *Gomphonema gracile* (Ggra), *Gomphonema ventricosum*

(Gven), *Meridion circulare* var. *constrictum* (Mcco), *Meridion circulare* (Mcir), *Frustulia rhomboides* var. *rhomboides* (Frho), *Frustulia rhomboides* var. *crassinervia* (Frcs), *Frustulia vulgaris* (Fvul), *Navicula rhynchocephala* (Nrhy), *Navicula lanceolata* (Nlan), *Navicula* cf. *aquaedurae* (Naqu), *Navicula angusta* (Naan), *Navicula radiosa* (Nrad), *Navicula tripunctata* (Ntri), *Navicula* cf. *gregaria* (Ngre), *Navicula capitatoradiata* (Ncpr), *Navicula* cf. *pygmaea* agg. (Npyg), *Navicula jaernefeltii* (Njae), *Navicula minima* (Nmin), *Craticula acidoclinata* (Crac), *Nitzschia dissipata* (Ndis), *Nitzschia sinuata* (Nsin), *Nitzschia gracilis* (*Ngra*), *Nitzschia hantzschiana* (Nhan), *Nitzschia perminuta* agg. (Nper), *Nitzschia intermedia* agg. (Nint), *Nitzschia* cf. *acula* (Nacu), *Nitzschia palea* agg. (Npal), *Nitzschia sublinearis* (Nsbl), *Nitzschia angustata* (Nian), *Nitzschia* undefined sp. (Nspp), *Tabellaria flocculosa* (Tflo), *Cymbella silesiaca* (Csle), *Cymbella gracilis* (Cgra), *Cymbella cistula* (Ccis), *Cymbella cymbiformis* (Ccym), *Cymbella helvetica* (Chel), *Cymbella affinis* (Caff), *Cymbella lanceolata* (Clan), *Cymbella caespitosa* (Ccae), *Cymbella naviculiformis* (Cnav), *Cymbella microcephala* (Cmic), *Didymosphenia geminata* (Dgem), *Pinnularia subcapitata* (Psub), *Pinnularia* cf. *sudetica* (Psud), *Pinnularia* cf. *divergens* (Pdiv), *Surirella roba* (Srba), *Surirella brebissonii* (Sbre), *Tetracyclus glans* (Tgla), *Diatoma mesodon* (Dmes), *Diatoma moniliformis* (Dmon), *Diatoma tenuis* (Dite), *Denticula tenuis* (Dten), *Epithemia adnata* (Eadn), *Epithemia sorex* (Esor), *Rhopalodia gibba* (Rgib), *Nedium bisulcatum* (Nebi), *Achnanthes lanceolata* (Alan), *Achnanthidium minutissima* (Amin), *Cocconeis placentula* (Cpla), *Diploneis* cf. *elliptica* (Dell), *Diploneis marginestriata* (Dmar), *Diploneis oblongella* (Dobl), *Brachysira vitrea* (Bvit), *Brachysira procera* (Bpro), *Closterium* sp. (Clos), *Cosmarium* sp. (Cosm), *Spirogyra* sp. (Spir), *Zygnema* sp. (Zygn), *Microspora* sp. (Micr), *Mougeotia* sp. (Moug), *Ulothrix* sp. (Ulox), *Stigeoclonium* sp. (Stig), *Bulbochaete* sp. (Bulb), *Rivularia* sp. (Rivu), *Lemanea fluviatilis* (Lema), *Batrachospermum* sp. (Batr). Aquatic bryophyte species codes: *Blindia acuta* (Bacu), *Brachythecium plumosum* (Bplu), *Ctenidium molluscum* (Cmol), *Fissidens adianthoides* (Fadi), *Fontinalis*

antipyretica (Fant), *Hygrohypnum luridum* (Hlur), *Hygrohypnum ochraceum* (Hoch), *Mnium hornum* (Mhor), *Palustriella falcata* (Pfal), *Platyhypnidium riparioides* (Prip), *Racomitrium aciculare* (Raci), *Schistidium agassizii* (Saga), *Schistidium rivulare* (Sriv), *Warnstorfia exannulata* (Wexa), *Chiloscyphus polyanthus* (Cpol), *Pellia epiphylla* (Pepi), and *Scapania undulata* (Sund). Vascular submerged macrophyte species codes: *Potamogeton polygonifolius* (Ppol), *Chara globularis* var. *globularis* (Cglo), *Eleogiton fluitans* (Eflu), *Ranunculus flammula* (Rfla), and *Myriophyllum alterniflorum* (Malt). Environmental variables: Underlying geology: Granite (%GRAN), Mica Schist (%SCHI), Granodiorite (%GDIO), Diorite (%DIOR), Quartz/Psammite (%QP), Quartz/Psammite/Pelite (%QPP), Diorite/Amphibolite (%DA), Amphibolite (%AMPH), Serpentinite (%SERP), Metamorphic Limestone (%MLIM), Durness Limestone (%DURL), Moine Schist (%MOIN), Eriboll Sandstone Group (%ESG), Applecross Formation (%APCF) and An-T'sron (%ANT). Substrate morphology: substrate particle diversity (SubH), substrate particle dominance (SubDom), hydromorphological diversity (HyMoH), streambed cover of Boulders (%BO), Large Stones (%LS), Small Stones (%SS), Gravel (%GR), and Sand (%SA). Water physico-chemistry: benthic depth (D: m), light attenuation coefficient (K: m^{-1}), euphotic depth 1% (Zeu^1: m^{-1}), $Zeu:D^1$ ratio 3%, euphotic depth 3% (Zeu^3: m^{-1}), $Zeu:D^3$ ratio 1%pH, alkalinity (Alk: mg/l), conductivity (Cond: $\mu S\ cm^{-1}$), water temperature (Temp: ^oC), current velocity (Flow: $m\ s^{-1}$), %Shade and height of riparian vegetation (Hrip). Water Chemistry: Phosphate (PO_4-P), Chloride (Cl), Sulphate (SO_4), Cadmium (Cd), Chromium (Cr), Copper (Cu), Lead (Pb), Nickel (Ni), Zinc (Zn), Aluminium (Al), Vanadium (V), Arsenic (As), Sodium (Na), Potassium (Kpot), Calcium (Ca), Magnesium (Mg), Iron (Fe), and Manganese (Mn). Monte Carlo significance test: Axis 1: $P<0.005$; all canonical axes: $P<0.005$. Eigenvalues: Axis 1: 0.543; Axis 2: 0.363.....648

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Author's Declaration

I declare that the work described in this thesis has been carried out by myself, except where otherwise acknowledged. It is entirely of my own composition and has not, in whole or part, been submitted for any other degree.

Pauline Lang

April 2010

Chapter 1. Introduction

1.1 Project Outline

The introduction of new European legislation under the Water Framework Directive ("Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy": WFD; European Communities 2000) has led to renewed interest in the relationships between aquatic plants and environmental conditions, in particular the environmental drivers which control assemblage and functional attributes of river plant communities (e.g. Ali *et al*. 1999, Sabbatini *et al*. 2002, Garbey *et al*. 2004). Much less is known about upland than lowland streams in this context, and least of all about small mountain streams, supporting mainly aquatic bryophyte and periphyton-dominated vegetation. Few plants other than periphytic algae, aquatic bryophytes, and a handful of specialised vascular species, are capable of enduring the cold-stressed, and turbulent, high-disturbance conditions provided by upland stream habitats in temperate to sub-arctic latitudes, but these plants are now of considerable importance as potential bioindicators of the environmental quality of upland streams.

A major aim of this project was to determine the relative importance of flow, substrate morphology, water chemistry and other environmental habitat conditions as processes driving production and diversity for three groups of freshwater plants (periphytic algae, aquatic bryophytes and vascular macrophytes) dominating small streams across a gradient of underlying catchment geology in upland Scotland. This new knowledge can provide useful information on reference ("near-pristine") conditions for this type of habitat, needed for further development of biomonitoring procedures for upland stream water quality (Soulsby *et al*. 2002a), legally-required in Scotland from 2008, under the WFD. Such ecological information not only provides new fundamental knowledge about the ecology of upland streams, but is also urgently needed (by

environmental regulators such as the Scottish Environment Protection Agency, SEPA, which is responsible for WFD implementation in Scotland) for implementation of biomonitoring procedures in upland streams.

1.2 Project Aims & Objectives

The four main aims of the project and specific questions to be addressed were as follows:

1. To categorise and characterise the nine sampling sites of three target streams (Water of Dye, River Girnock and Knockan Burn) located in two geologically-contrasting regions of the Scottish Highlands in terms of their similarities (e.g. flow regime, nutrient status) and perhaps more crucially their differences in environmental habitat conditions (e.g. substrate morphology, water chemistry) that may influence freshwater plant community abundance, species composition and diversity.

- *Can knowledge of the environmental characteristics of upland stream habitats be used to predict the abundance and composition of plant communities expected to occur in upland streams?*

2. To determine the relative importance of environmental processes potentially driving freshwater plant production, species-assemblage and diversity in upland stream habitats, through individual assessments of periphytic algae, aquatic bryophytes and (where present) vascular submerged macrophytes and also by combining the whole plant community.

- *How is the growth of each of the three target aquatic plant groups (periphytic algae, aquatic bryophytes and vascular submerged macrophytes) affected by environmental variation?*

- *What sets of environmental habitat conditions drive the formation of these freshwater vegetation assemblages, and plant species diversity, in such streams?*

3. To characterise the stream habitat conditions associated with the communities of freshwater vegetation present; to identify potential indicator species and/or plant assemblages indicative of high environmental quality; and to use this information to determine near-pristine (reference) conditions for use in the implementation of biomonitoring protocols to assess environmental quality for upland stream habitats in Scotland.

- *Can the data be used to establish type-specific reference conditions (Annex II of WFD)?*

- *Can data derived from this project be used to develop a minimal model system to effectively predict reference (near-pristine) conditions for upland stream habitats in Scotland?*

4. To determine the value or otherwise of artificial substrate sampling procedures for assessing periphyton production and community composition, in comparison with direct sampling of naturally-occurring microhabitats in upland stream habitats.

- *Do artificial substrate samplers make effective surrogates for naturally-occurring microhabitats and periphyton colonisation?*

1.3 The Water Framework Directive: Statutory Legislation for the Protection and Biomonitoring of Freshwater Ecosystems

The Water Framework Directive (WFD: 2000/60/EC) is a major piece of environmental legislation for water policy and sustainable water management in Europe. The WFD was implemented by the European Commission in December 2000, and transposed into law in Scotland in 2003, through the Water Environment and Water Services Act (2003).

The WFD legislation brought together previously fragmented EC water directives to drive a more comprehensive approach to water policy, aimed at achieving sustainable water management and protection to improve environmental quality of waters in Europe (Jekel 2005).

Primarily the WFD aims to achieve at least 'good ecological status' (or a quality as possibly close to reference conditions), by 2015, of all EU member state water bodies, and prevent further deterioration of such ecological status (EC 2000, Jekel 2005). Central to WFD objectives is the pending requirement to establish near-pristine baseline reference or benchmark conditions (i.e. the expected ecological quality in the absence of anthropogenic influence or in minimally-impacted systems), and further to identify indicator species or ecological assemblages representing a given set of environmental habitat conditions to ascertain ecological status for classification purposes (EC 2000, Jekel 2005).

In accordance with the WFD, environment agencies responsible for upholding this piece of environmental legislation in the UK (e.g. SEPA for Scotland; Environment Agency: EA for England & Wales; Environment and Heritage Service: EHS for Northern Ireland) and other EU member states, have adopted an integrated approach to monitoring water quality by gathering information regarding the physical habitat (e.g. River Habitat Survey: RHS), chemical analyses, and biological elements using metrics for diatoms (e.g. Trophic Diatom Index: TDI; Diatom Assessment for River/Lake Ecological Status: DARES/DALES), macroinvertebrates (e.g. River Invertebrate Prediction and Classification System: RIVPACS), aquatic macrophytes (e.g. LEAFPACS), and fish (e.g. Fish-based Assessment for the Ecological status of European rivers: FAME) of inland surface waters (Jekel 2005). There are clear benefits of employing a multidisciplinary approach to monitor water quality: physical and chemical data alone can only provide snapshot indications of environmental status at the time of sampling. However, aquatic biota (e.g. periphyton, hydrophytes) interact with and respond to their surrounding environment, to provide an integrated, longer-term indication of environmental habitat conditions. This is incorporated into the

structural and functional response of the enduring biotic assemblages present at the time of sampling (Boon & Howell 1997). By using this combined information, WFD classification currently recognises five levels of ecological status: high, good, moderate, poor and bad. Classifying water bodies in this way reflects a water quality gradient from near-pristine mostly undisturbed habitats (e.g. high or good status), to those which deviate significantly from reference conditions, having been exposed to sufficient anthropogenic disturbance(s) to be considered polluted and/or modified, and are thereby classified (e.g. moderate, poor or bad status) on the basis of how severely these communities diverge from those expected to occur in comparable ecological benchmark conditions (Boon & Howell 1997, EC 2000).

Therefore water bodies must be continuously monitored to:

- measure water quality
- enable the development and implementation of sustainable management plans to attain and maintain good ecological status
- identify any perturbations in ecological status particularly those changes related to anthropogenic disturbances (human pressure) that threaten ecological integrity and respond by implementing corrective measures where necessary
- partition natural environmental variation from human impacts on the ecological structure and functioning of inland waters using long-term historical data-sets
- protect environmental status, ecosystem functioning and human health

Yet one must remain cautious when applying the term 'reference condition' to inland freshwaters. Ideally, this expression should encompass 'pristine, unmodified habitats', but in reality 'near-natural' freshwater habitats are rare entities (Moss 1988). Upland headwater streams are especially vulnerable to the consequences of human disturbance such as acidification from atmospheric deposition and climate change. Palaeolimnology is one way of overcoming the

hurdle of defining baseline reference conditions using historical records of past environments and the fossilised remains of aquatic biota accumulating in lake sediments, such as diatoms and cladocerans (e.g. Bennion *et al*. 2004, Simpson *et al*. 2005). However it can be more challenging to apply similar approaches to inland flowing waters, though some recent studies have highlighted the value of using historical records to identify stream reference communities (e.g. Baattrup-Pedersen *et al*. 2008). Therefore with respect to characterising streams and rivers, often the 'best available' sampling sites, those considered as close to near-pristine conditions as possible, are used as a benchmark for good ecological integrity, in which assemblages of aquatic biota reflect the characteristics of their surrounding physical and chemical environment.

In Scotland, the majority of inland waters are generally considered to be of high quality, with approximately 79% achieving (at least) good ecological status or better, following a recent review of Scotland's water environment (SEPA 2007). However anthropogenic pressures (e.g. nutrient enrichment, acid mine drainage, habitat modifications) have impacted many of lowland Scottish rivers (e.g. 12%), particularly those flowing through urbanised and industrialised regions. In contrast, the majority of headwater streams and rivers (e.g. 31%) in Scotland are considered to be predominantly of near-pristine reference condition, or high water quality status. Yet a substantial proportion of biomonitoring is focussed in easy-to-access lowland rivers of Scottish towns and cities affected by pollution and other forms of human disturbance. This emphasizes the requirement for improved monitoring of upland streams, unpolluted near-pristine habitats, which could potentially fulfil WFD requirements for defined baseline reference conditions in Scotland, as part of the larger European initiative to better equip environment agencies with the ability to predict ecological status from a reliable number of similar physical and chemical features across the UK (Boon & Howell 1997, EC 2000). Moreover, this strategy would help clarify the extent to which impacted stream assemblages of aquatic biota deviate in their composition and

abundance from benchmark reference communities occurring in defined river habitat typologies (EC 2000).

Additionally, current assessments of ecological status are based on the outcome of individual protocols using different aquatic biological assemblages such as diatoms (TDI in DARES/DALES), macrophytes (LEAFPACS), macroinvertebrates (RIVPACS) and fish (FAME) which are used independently of each other to classify the status of inland freshwater habitats. This could potentially lead UK and other European environment agencies to run into problems concerning the comparability of these biological methods in classifying ecological status and thereby, water quality. Consequently, there is a push for the existing 'integrated approach' to mature into a more complementary approach which will combine the 'whole biological community' (e.g. diatoms, macrophytes, macroinvertebrates and fish) together with the physical and chemical habitat components, directed towards manufacturing a more robust tool for evaluating the ecological status of inland waters in the UK (Boon & Howell 1997).

This project monitored a range of near-pristine headwater streams in the Scottish highlands and adopted an approach compatible with current WFD river characterisation and biomonitoring strategies for investigating the structural and functional response of freshwater plant communities (chiefly diatoms and other algal groups; aquatic bryophyte and vascular submerged macrophyte vegetation) to a range of environmental drivers (e.g. flow, substrate morphology, nutrient inputs, water chemistry, underwater light availability), with the aim of contributing to the development of baseline monitoring tools for assessing upland stream habitat quality in Scotland.

1.4 A Review of the Ecosystem Support Function and Ecological Importance of Freshwater Plants as Biomonitors in Streams and Rivers

In high altitude fast-flowing turbulent headwater streams and rivers, such as those examined in this study, periphyton and aquatic bryophytes are usually the dominant primary producers, and vascular macophytes are often scarce or absent. Nevertheless, all three plant groups undertake pivotal roles in ecosystem support functioning of other lotic biota (Biggs 1996), and are also fundamental to the biomonitoring of freshwaters in the UK and other EU constituent countries under the WFD (EC 2000).

1.4.1 Periphyton

Periphyton is the term given to the attached algae, specifically adhering to and forming biofilms over the surfaces of benthic habitats in streams, rivers and lakes (Weitzel 1979, Sládečková 1962, Whitton 1975, Dennis & Isom 1984, Stevenson *et al*. 1996). "Aufwuchs" is a synonymous German term used to describe the epiphytic growth of algae occurring on the surfaces of aquatic macrophyte foliage (Weitzel 1979, Hynes 1970, Sládečková 1962, Carpenter & Lodge 1986, Stevenson *et al*. 1996).

1.4.1.1 Ecosystem support function of periphyton in streams and rivers

Periphytic algae are cosmopolitan to most freshwater habitats, often embracing the foundations of autochthonous production in turbulent streams and rivers wherein the occurrence of macrophytic plant life (e.g. aquatic bryophytes) may be confined by specific substrate requirements (Weitzel 1979, Whitton 1975, Winterbourn & Ryan 1994, Stevenson *et al*. 1996). Thereby periphytic algae undertake an important ecological functioning role as a direct food source for

grazing macroinvertebrate fauna and occasionally fish, but also indirectly contribute to secondary or tertiary consumer pathways, nutrient cycling and energy budgets. Additionally, thick mats of periphyton, especially erect diatoms and filamentous growth forms provide microhabitat enveloped by a hydraulically-altered boundary layer (Jones *et al*. 2000b), capable of supporting higher densities of smaller meiofauna, macroinvertebrates and refuge for other benthic algae (e.g. diatoms) entangled in the matrix (Stevenson *et al*. 1996, Dodds & Biggs 2002, Passay 2007). Furthermore some mucilaginous prostrately-attached diatoms can play a key role during flow-scours by acting as protective microcosms for heterotrophic bacteria and other micro-organisms, by shielding them from the effects of physical disturbance (Blenkinsopp & Lock 1994), and may even facilitate post-disturbance colonisation of other periphytic algae (Lamb & Lowe 1987).

The structural and functional attributes of periphyton assemblages have been extensively tested in laboratory streams (e.g. Kevern & Ball 1965, McIntire & Phinney 1965, McIntire 1966ab, 1968, Steinman & McIntire 1987, Lamberti *et al*. 1989, Steinman *et al*. 1989 and 1991, Horner *et al*. 1990, Mulholland *et al*. 1991, Biggs & Thomsen 1995), and much knowledge has been gained from this type of work. Experimental field studies have also made substantial contributions to understanding periphyton-environment interactions (e.g. Fairchild *et al*. 1985, Lowe *et al*. 1986, Hill & Knight 1987, Mosisch *et al*. 1999, 2001, Kiffney *et al*. 2003). However, much remains to be learned about the ecology of stream periphyton communities, particularly in the context of attempts to establish suites of environmental habitat conditions characterising benchmark species assemblages in upland streams in Scotland. This new information is required imminently for WFD to assist more reliably in revealing the extent to which our rivers are impacted (i.e. deviate from near-pristine conditions), and to establish the principal environmental drivers responsible for the ecological shifts in community composition, and control measures that need to be implemented to regain good water quality status in those habitats most affected by anthropogenic disturbance.

1.4.1.2 Use of periphyton as biomonitors in streams and rivers

Amongst the periphyton, diatom communities are recognised as important indicators of water quality and are extensively used, worldwide, in biomonitoring programs to assess the trophic status of freshwater habitats, for example under the WFD in the UK and other EU member states (Bona *et al*. 2007, Fisher & Dunbar 2007, Blanco *et al*. 2008, Kelly *et al*. 2008). Elsewhere around the globe, analogous sampling protocols are being developed which incorporate diatoms as an integral group for assessing the ecological status of inland water bodies (e.g. Lavoie *et al*. 2008: Canada; Taylor *et al*. 2005 & 2007b, van Vuuren *et al*. 2008: South Africa). Diatom-based indices of community structure (e.g. TDI: Kelly & Whitton 1995, Kelly *et al*. 2001) are a valuable way to monitor general water quality and detect environmental perturbations such as acidification, heavy metal contamination, organic pollution and eutrophication of streams and rivers (Patrick 1949, Lavoie *et al*. 2004, De la Rey *et al*. 2004, Taylor *et al*. 2005, Charles *et al*. 2006, Archibald & Taylor 2007, Kelly *et al*. 2008). Diatoms are also a potentially valuable tool for assessing long-term trends in river water quality from historical data (e.g. Lang & Krokowski 2010), or hind-casting environmental change in lakes from fossil records (e.g. Battarbee *et al*. 1996, Bennion *et al*. 2004, Simpson *et al*. 2005).

Diatom communities offer several key attributes which make them excellent bioindicators for reflecting water quality:

- Universally distributed microflora (Taylor *et al*. 2005, Lavoie *et al*. 2008).

- The limited mobility of diatom communities coupled to their rapid and integrative response to environmental variation makes them especially reliable for detecting anthropogenic disturbances of freshwater habitats (Stevenson *et al*. 1996, De la Rey *et al*. 2004, Lavoie *et al*. 2004, Harding *et al*. 2005, Taylor *et al*. 2005, Bona *et al*. 2007, Lavoie *et al*. 2008).

- Exhibit specific ecological niche preferences and environmental tolerances, thus structuring species assemblages (De la Rey *et al*. 2004, Harding *et al*. 2005, Blanco *et al*. 2008).

- Representative diatom communities can usually be sampled from a relatively small unit area, and harvested from any form of available substratum (Harding *et al*. 2005).

- Permanent mounts of diatoms can be prepared for inter-calibration (quality control) purposes and future library reference (Harding *et al*. 2005).

Perhaps one downside of their merit is that to-date pre-existing biomonitoring protocols for have relied heavily on diatoms, whilst other components of periphytic algal communities have largely been ignored and typically not incorporated into WFD assessments of river water quality. Although more recently LEAFPACS acknowledged the worth of macrophytic filamentous algae in riverine surveys, ecological analysis is mostly concerned with nuisance growths (e.g. *Cladophora glomerata*) often linked to nutrient enrichment (Biggs 1995, Biggs *et al*. 1998, Foerster *et al*. 2004).

Collectively, there are several viable areas for the application of project outputs emerging from this study. Principally, the periphyton data presented herewith will contribute to further development of an already existing approach in ecological biomonitoring protocols implemented under the WFD and similar legislation worldwide by providing specific knowledge of indicator species characterising near-pristine habitat conditions. Yielding such presently scant information regarding upland streams would potentially enable SEPA to utilise these reference communities as ecological benchmarks for those occurring in disturbed rivers of similar typology in Scotland. The work undertaken will also provide an opportunity to evaluate the worth of employing entire periphytic algal communities, rather than with sole emphasis on diatoms, for ascertaining water quality status of streams and rivers in the UK.

1.4.1.3 Alternative tactics for sampling periphyton from streams and rivers: use of naturally-occurring vs. artificial substrata

Most standard biomonitoring protocols stipulate that, as far as possible, periphyton should be harvested directly from naturally-occurring substrata (e.g. cobbles) to obtain an integrated sample (Kelly *et al*. 2001). Nevertheless this sampling method is not without its drawbacks (see Table 1.1). Alternatively, many ecological studies have employed the use of artificial substrates to collect periphyton (e.g. Lowe & Gale 1980, Lane *et al*. 2003, Coe *et al*. 2006). However, there has been much debate in the literature over their use, often questioning amongst other points (see Table 1.2), whether artificial substrates make good surrogates for natural microhabitats. An overview of the inherent advantages and disadvantages for the alternative tactics used for sampling periphyton from streams and rivers is provided in Table 1.1 and Table 1.2 (e.g. Grzenda & Brehmer 1960, Sládečková 1962, Vollenweider 1969, Ertl 1971, Whitton 1975, Brown 1976, Saunders & Eaton 1976, Lowe & Gale 1980, Weber & McFarland 1981, Antoine & Benson-Evans 1985, Biggs 1988, Aloi 1990, Stevenson *et al*. 1996, Boon & Howell 1997, Lane *et al*. 2003, Taylor *et al*. 2005, Bergey & Getty 2006).

To address the controversy surrounding the alternative tactics used to sample periphyton from streams and rivers, I decided it was sensible to adopt an integrated approach which involved sampling periphyton from a variety of artificial substrates (e.g. linoleum, Astroturf, plastic aquarium plants) for comparison with complementary direct sampling of naturally-occurring solid substrata and submerged plants (refer to Chapter 3, section 3.3.1 for more detail), and thereby allow me to tackle directly the ongoing controversy over the value or otherwise of artificial substrate sampling procedures for assessing periphyton production and community composition in upland stream habitats.

Advantages of using naturally-occurring substrata	*Disadvantages of using naturally-occurring substrata*
• Periphyton communities harvested from naturally-occurring substrata provide an integrated representation of preceding environmental conditions and therefore a reliable indicator of ecological quality	• Naturally-occurring substrata are highly variable in size, texture, surface area, architecture or morphology, and perhaps microhabitat conditions. This heterogeneity introduces problems for comparing periphyton communities and their growth that has developed on naturally-occurring surfaces
• Guaranteed that substratum will be available at time of sampling	• Samples generally not reproducible as naturally-occurring substrates not standardised like artificial samplers
• Cost-effective	• Neither ecologically nor logistically viable to repeatedly remove substratum from field and transport to lab
	• Periphyton becomes established at an unknown rate and colonisation occurs during an exposure interval unknown to the sampler
	• May be difficult or a safety risk to access streams or rivers to obtain samples from representative naturally-occurring microhabitat

Table 1.1 Evaluated advantages and disadvantages of utilising naturally-occurring substrata to collect benthic periphyton

Advantages of using artificial substrata	*Disadvantages of using artificial substrata*
• Provide a homogenous, more comparable surface (together with a precise unit area) for sampling periphyton: eliminating problems encountered from inherent differences in naturally-occurring substratum surfaces	• Artificial substrata captures only the community that develops during the sampling period, and may not show much value as indicators of environmental conditions prior to sampling
• Reproducible samples easily acquired from repeat-sampling of uniform substrates	• Can be difficult to anchor in a fixed position
• Easily transported from field to lab	• Susceptible to vandalism, damage, loss or displacement
• Flexible substrates types more resistant to breakage	• Ceramic, clay or other less malleable substrates types prone to fracturing
• Periphytic colonisation occurs during an exposure interval defined by the experimenter	• Can be costly to employ and maintain this approach
• Artificial samplers can be positioned in easy-to-access regions of streams or rivers	

Table 1.2 Evaluated advantages and disadvantages of utilising artificial substrata to collect benthic periphyton

1.4.2 Aquatic Bryophytes

Bryophytes are a group of non-vascular plants, including the true mosses (Bryophyta) and liverworts (Hepatophyta) characterised by their lack of protective structures, absence of well-developed transport system and lack of a functionally-developed root system, compared to higher plants (Stream Bryophyte Group 1999). Bryophytes attach themselves to appropriate substrates using rhizoids (hair-like extensions of epidermal cells) which act as holdfasts. The plants often form carpets over the surfaces upon which they become established (Stream Bryophyte Group 1999). More specifically, aquatic bryophytes encompass that relatively small proportion of the moss and liverwort flora which is capable of exploiting habitats frequently inundated with water. Such bryophytes are considered to be either obligate aquatic species (e.g. *Fontinalis antipyretica*, *Platyhypnidium riparioides*), facultative species (e.g. *Hygrohypnum luridum*, *Palustriella falcata*), or semi-aquatic species (e.g. *Blindia acuta*, *Schistidium rivulare*), and are defined by their dependence (for growth and sporophyte production) on being fully, or partially submerged at some stage during their life cycle (Cook 1999). Although some of the bryophyte species encountered in this study are not strictly aquatic, the majority preference moist conditions, and commonly occur in wet habitats such as streams, rivers and cascading waterfalls.

Although there is a reasonably comprehensive literature on aquatic bryophyte ecology and distribution (e.g. Watson 1981, Hill *et al*. 1991, 1992a and 1994, Paton 1999, Smith 2004), much remains to be learned about the ecology and environmental factors that influence the distribution of stream bryophyte communities which, together with periphyton, usually dominate primary production in low-order streams in temperate to sub-arctic upland areas throughout Europe and the Northern hemisphere. This is especially important given the high vulnerability of such stream habitats to the likely consequences of global climate change (Hynes 1970, Vitt *et al*. 1986, Suren 1996, Stream Bryophyte Group 1999, Vanderpooten *et al*. 1999, Virtanen *et al*. 2001, Martínez-Abaigar *et al*. 2002, Arróniz-Crespo *et al*. 2004).

1.4.2.1 Ecosystem support function of aquatic bryophytes in streams and rivers

Few macrophytes other than aquatic bryophytes are capable of enduring the variable and turbulent, high-disturbance conditions provided by upland stream habitats in temperate to sub-arctic latitudes. In the absence of other macrophytes (e.g. O'Hare & Murphy 1999), bryophytes fulfil vital ecological roles such as the provision of microhabitat and shelter from extreme flow conditions for periphyton and macroinvertebrate fauna utilising the large, complex surface area of the plants (Glime & Clemons 1972, Dawson 1973, Westlake 1975, Suren 1991, Suren & Winterbourn 1992, Lancaster & Hildrew 1993, Winterbourn & Ryan 1994, Suren 1996, Englund *et al*. 1997, López *et al*. 1997, Suren & Ormerod 1998, Nikora *et al*. 1998, Stream Bryophyte Group 1999, Garcia-Alvaro *et al*. 2000, Suren *et al*. 2000, Linhart *et al*. 2002a, Heino & Korsu 2008). Besides offering refuge to other lotic organisms, particularly macroinvertebrate instars and juvenile fish, aquatic bryophytes also harbour mats of detritus and periphyton which accumulate upon their foliage, and are therefore also ideal grazing grounds for many freshwater invertebrates (Glime & Clemons 1972, Suren 1991, Suren & Winterbourn 1992, Steinman & Boston 1993, Finlay & Bowden 1994, Nikora *et al*. 1998, Suren & Ormerod 1998, Muotka & Laasonen 2002, Muotka & Syrjanen 2007, Heino & Korsu 2008). For these reasons, stands of aquatic bryophytes often support a higher abundance and diversity of other organisms, compared to nearby exposed substrata like unvegetated stoney riffles (Glime & Clemons 1972, Suren & Winterbourn 1992, Englund *et al*. 1997, Nikora *et al*. 1998, Stream Bryophyte Group 1999, Linhart *et al*. 2002ab).

1.4.2.2 Use of aquatic bryophytes as biomonitors in streams and rivers

Although they are a characteristic feature of upland streams and rivers, aquatic bryophytes have been amongst the most overlooked and under-utilised of plant groups, with regards to the biomonitoring of inland waters (Scarlett & O'Hare

2006), despite their potential applicability in classifying trophic status (Newman & Dawson 1996).

However, more recently, the usefulness of aquatic bryophytes as bioindicators of freshwater pollution, particularly as accumulators of heavy metal contaminants and affinity for other trace elements or substances, has received recognition and been the research focus of several studies (e.g. Say & Whitton 1983, Wehr & Whitton 1983, Mouvet 1985, Jackson *et al*. 1991, López & Carballeira 1993a, Claveri *et al*. 1994, López *et al*. 1994, Claveri *et al*. 1995, Claveri & Mouvet 1995, Engleman & McDiffett 1996, Roy *et al*. 1996, Siebert *et al*. 1996, Bruns *et al*. 1997, Gagnon *et al*. 1998, Mersch & Reichard 1998, Samecka-Cymerman & Kempers 1998, 1999, Mouvet & Claveri 1999, Vázquez *et al*. 1999, 2000, Carballeira *et al*. 2001, Croisetiere *et al*. 2001, 2005, Vincent *et al*. 2001, Hongve *et al*. 2002, Martins & Boaventura 2002, Nimis *et al*. 2002, Delepee *et al*. 2004, Martins *et al*. 2004, Bleuel *et al*. 2005, Figueira & Ribeiro 2005, Aronsson & Ekelund 2006, Cesa *et al*. 2006, Fernández *et al*. 2006, Samecka-Cymerman *et al*. 2002, 2005, and 2007). A number of these authors outline the benefits of utilising aquatic bryophytes in water quality biomonitoring protocols:

- Widespread distribution throughout European rivers and relative ease of sampling aquatic bryophytes in-situ (passive) and via transplantation (active) methods for biomonitoring purposes to determine ecological status.

- Sessile organisms, forming relatively stable and fixed communities that reflect environmental status through their ability to endure selection pressures, and disturbances.

- Some common aquatic bryophyte species (e.g. *Fontinalis antipyretica*, *Platyhypnidium riparioides*) known to bioaccumulate heavy metals via cation exchange mechanisms, show relative tolerance to substances accumulated and an ability to survive these contaminants. Thus such species can be used as monitors of the occurrence of metal contaminations.

- Lack well-developed vascular and root systems, meaning that aquatic bryophytes rapidly respond to environmental conditions and pollutant uptake is direct from the surrounding environment through the leaf surface and therefore reflects water quality (unlike integrated water and sediment properties depicted by rooted vascular submerged macrophytes).

- Economical to sample, generally less expensive than water chemical analyses

- For example; both *Fontinalis antipyretica* and *Platyhypnidium riparioides*, mainly because of their particularly extensive distribution, relative pollution tolerance and bioaccumulator capacity for heavy metals, have been used in such river biomonitoring studies.

A less studied aspect of aquatic bryophyte ecology is the abundance, distribution and species-composition of aquatic bryophyte communities in relation to underlying environmental gradients, particularly water quality (Muotka & Virtanen 1995, Suren & Ormerod 1998, Thiebaut *et al*. 1998, Stream Bryophyte Group 1999, Scarlett & O'Hare 2006). Studies of the habitat ecology of aquatic bryophytes are relatively neglected particularly in the upland stream habitats that form important headwaters in Europe, including Scotland and other high-latitude regions. Only a handful of studies have addressed this particular topic, to date. There is one recent comprehensive UK-based study (e.g. Scarlett & O'Hare 2006) of the occurrence of stream bryophyte assemblages in response to environmental variables, restricted in its focus to England and Wales. Pentecost (1991) and Ormerod *et al*. (1987) studied the macro-floral (periphyton and aquatic bryophyte) assemblages of upland streams in England and Wales, respectively, but neither extended their investigation to other parts of Britain. This further emphasizes the general lack of knowledge regarding stream bryophyte distribution on a nationwide basis, especially for Scotland. Moreover, the limited number of studies that have been conducted tend to examine aquatic bryophyte species-assemblages occurring in riverine ecosystems subjected to a range of environmental habitat conditions and water qualities, often including assessments of communities exposed to anthropogenic disturbances such as acidification (e.g.

Thiebaut *et al*. 1998, Stetzka & Baumann 2002), nutrient enrichment (e.g. Claveri *et al*. 1995, Suren 1996, Suren & Ormerod 1998, Vanderpooten 1999ab, Vanderpooten *et al*. 1999, Vanderpooten & Klein 1999ab, Stetzka & Baumann 2002), and flow regulation (e.g. Englund *et al*. 1997, Vanderpooten & Klein 1999a, Vanderpooten & Klein 2000). Given the lack of research it is highly unlikely that previous studies can provide a full reflection of the naturally-occurring aquatic bryophyte communities to be found in near-pristine and unmodified habitats in British upland streams. Nor is it likely that knowledge is complete about how aquatic bryophyte species-assemblages may deviate, in perturbed conditions, away from ecological benchmark reference communities. In order to make such assessments correctly, information on species-environment interactions is needed across a broad range of habitat conditions, taking into account macro- (e.g. underlying geology, climate), meso- (e.g. water chemistry) and micro-scale (e.g. flow regime, substrate morphology and stability) environmental factors potentially affecting community composition. Relevant comparable research has mostly been undertaken in other European countries such as Spain, France and Germany (e.g. García-Álvaro *et al*. 2000, Claveri *et al*. 1995, Vanderpooten *et al*. 1999, Vanderpooten & Klein 1999b), as well as elsewhere around the world including Western Canada (e.g. Vitt *et al*. 1986), Nepalese Himalayas (e.g. Suren & Ormerod 1998), and New Zealand (e.g. Suren 1996). Such studies are clearly useful in gaining knowledge of upland stream bryophyte ecology, but also emphasize the paucity of knowledge about such systems in the UK, in particular in the context of the potential value of stream bryophytes to assess water quality. Therefore the research undertaken in this study could contribute to the basic knowledge needed to establish reference conditions and communities, needed for development of aquatic bryophyte biomonitoring protocols in not only the UK, but also in temperate to sub-arctic upland stream ecosystems in other parts of the Northern Hemisphere.

Of late aquatic bryophytes have been increasingly recognised as good bioindicators of water quality. This is because, like periphyton, they endure

naturally disturbed and/or stressed conditions, characteristic of upland stream habitats, and are likely to respond rapidly, via changes in production, diversity and species-set, to "adding-in" of additional stress or disturbance as a result of human activity. Altogether, the widespread distribution, ecosystem support role and potential bioindicator capacity of these plants fulfil criteria for the suitability of aquatic bryophytes to be included in biomonitoring protocols and river management within the UK, with potential application in other European and high latitude countries.

In 2009, SEPA and their collaborative partners were in the early stages of developing the work needed, as part of WFD implementation (e.g. LAKE & RIVER LEAFPACS: WFD-UKTAG 2008ab, Willby *et al*. 2009ab), to determine a direct role for aquatic bryophytes (along with vascular macrophytes and phytobenthos) as suitable candidates in sampling protocols and biomonitoring procedures of inland waters. Given their ecological importance there is a fundamental requirement for improved understanding of the environmental constraints governing assemblage, diversity and abundance of aquatic bryophytes in stream ecosystems. Not least, the availability of improved knowledge could help identify marker species characterising suites of environmental habitat conditions, for possible future implementation in biomonitoring schemes for upland rivers, appropriate under WFD and similar legislation worldwide. The work undertaken in this study represents the first study of its kind (probably for the UK and certainly for Scotland), to provide information which could potentially be used as a prerequisite and potential feeder strategy for WFD progression *a propos* river biomonitoring protocols utilising aquatic bryophyte communities.

1.4.3 Vascular Submerged Macrophytes

Aquatic macrophytes are defined as "aquatic photosynthetic organisms, large enough to see with the naked eye…which actively grow either permanently or periodically (for at least several weeks each year) submerged below,

floating on, or growing up through the water surface" (Chambers *et al*. 2008). In LEAFPACS this concept applies broadly to filamentous growths of macroalgae, as well hydrophytes including bryophytes and other aquatic plants (Willby *et al*. 2009ab). However, in this thesis I mostly restrict the term macrophytes for referring specifically to vascular river plants: aquatic angiosperms possessing well-developed vascular transport systems (i.e. xylem and phloem) particularly submerged forms rooted in the sediment with their vegetative parts occurring mostly underwater, except perhaps for leaves floating at the water surface. Throughout this thesis, periphyton and aquatic bryophytes are considered separately from vascular macrophytes, except in sections 3.6.4 and 4.5.23, when the whole freshwater plant community was examined.

1.4.3.1 Ecosystem support function of vascular submerged macrophytes in streams and rivers

Where present vascular submerged macrophytes perform important ecosystem support functioning roles similar to those of aquatic bryophytes (refer back to section 1.4.2.1), as providers of microhabitat for other lotic organisms particularly epiphytes and macroinvertebrates (Carpenter & Lodge 1986). The morphological complexity and reduced flows within river macrophyte beds facilitate niche-diversification, especially by providing hydraulic refugia, often enhancing the abundance and influencing the community composition of macroinvertebrates (e.g. O'Hare & Murphy 1999, Ali *et al*. 2007). Additionally, the increased habitat heterogeneity offered by aquatic macrophytes also benefits fish assemblages and their diversity (e.g. Brazner & Beals 1997, Agostinho *et al*. 2003). However, stands of submersed aquatic macrophytes may be important in other ways in running waters by locally altering other physico-chemical conditions (e.g. light, temperature), providing both a direct and indirect (epiphyte grazing) food source for macroinvertebrates and fish, acting as detrital traps, and particularly in biogeochemical (e.g. oxygen, dissolved organic carbon) and nutrient (e.g. N, P) cycling as they inhabit the water interface thereby linking sediment and

atmospheric exchange (Westlake 1975, Carpenter & Lodge 1986, Clarke & Wharton 2001, Chambers *et al*. 2008).

1.4.3.2 Use of vascular submerged macrophytes as biomonitors in streams and rivers

Vascular macrophytes are a much more extensively studied group of river plants, than aquatic bryophytes. As discussed above for aquatic bryophytes, they are useful indicators or "longer-term integrators" of habitat conditions in streams and rivers, worldwide (Daniel & Haury 1996, Lancaster *et al*. 1996, Ali *et al*. 1999, Ellwood *et al*. 2008). Furthermore, aquatic macrophyte assemblages tend to be spatially-organised in relation to environmental gradients and species co-occurrence is essentially non-random (Boschilia *et al*. 2008).

Also for reasons similar to those of aquatic bryophytes (refer back to section 1.4.2.2), the propensity for heavy metal uptake has also been used to investigate potential bioindicator capacity of submerged vascular plants in polluted freshwater systems (e.g. Lewander *et al*. 1996, Samecka-Cymerman & Kempers 1996, Cardwell *et al*. 2002, Ngayila *et al*. 2007, Hassan *et al*. 2009 in press).

Together the early work of Butcher (1933), Haslam (1978, 2006), and Holmes (1983) paved the way for river classification using aquatic plants in the UK, and since then they have been the focus of many other studies directed towards the biomonitoring of running waters in Britain (e.g. Holmes *et al*. 1998, 1999ab, Dawson *et al*. 1999ab), Northern Ireland (e.g. Dodkins *et al*. 2005) and elsewhere in Europe (e.g. Daniel & Haury 1996, Haury 1996, Haury *et al*. 1996, 2006, Schneider & Melzer 2003, Szoszkiewicz *et al*. 2007, Fabris *et al*. 2009). Some of these macrophyte-based tools have specifically been developed to classify river systems by using them as indicators of trophic status, such as the Mean Trophic Rank (MTR: Dawson *et al*. 1999a, Holmes *et al*. 1999b), Trophic Index of Macrophytes: (TIM: Schneider & Melzer 2003), and Macrophyte Biological Index for Rivers

(IBMR or MBIR: Haury *et al*. 2006). Fewer studies (e.g. Meilinger *et al*. 2005, Fabris *et al*. 2009) have attempted to assess the deviation in freshwater macrophyte species-assemblages from reference vegetation as required by the WFD. Until more recently (e.g. Baattrup-Pedersen *et al*. 2006, 2008) knowledge of reference macrophyte communities characterising unimpacted streams and rivers in the UK and other EU constituent countries was particularly scarce.

The work of this study will build on that undertaken by UK environment agencies (e.g. SEPA, EA) who have recently begun developing the use of aquatic macrophytes in assessing the water quality status of inland waters for WFD classification purposes (e.g. LAKE & RIVER LEAFPACS: WFD-UKTAG 2008ab, Willby *et al*. 2009ab).

1.5 Approaches

In this study I took a comparative, intensive-survey approach, which followed variation in stream vegetation over time, in relation to a broad range of environmental habitat conditions, in order to assess the likely relevance and importance of potential environmental drivers of upland stream plant community production, diversity and species-assemblages.

Each results chapter in this thesis is structured to meet the aims of the project:-

Chapter Two: Characterises and describes the environmental habitat conditions associated with the nine sampling sites in the three target streams of the study (Aim 1). The work presented in this chapter uses an approach similar to the WFD River Habitat Survey, combined with the application of multivariate analyses for clustering together and differentiating between samples based on their inherent similarities and differences in habitat variables (e.g. nutrient status, substrate morphology, streamwater chemistry etc.).

Chapter Three: Investigates relationships between freshwater plant production and environmental habitat variables, indicating which are the most influential to the functional growth and abundance of periphyton, aquatic bryophytes, and (where present) vascular submerged macrophytes in upland streams in Scotland of near-pristine water quality (Aim 2). A minimal modelling approach was developed as a tool for predicting freshwater plant chlorophyll content in response to combinations of measured environmental variables using linear regression analysis. This chapter also provides a comparative analysis of periphyton production on various types of artificial substrata compared to their corresponding naturally-occurring microhabitat (Aim 4).

Chapter Four: Explores relationships of freshwater plant species-assemblages and diversity with underlying environmental habitat gradients, using multivariate analyses to detect the major influential factors driving the community structure of periphyton, aquatic bryophytes, and (where present) vascular submerged macrophytes in upland streams in Scotland of near-pristine water quality (Aim 2). Species lists for periphyton, aquatic bryophyte and vascular submerged macrophyte flora are provided. For prospective biomonitoring purposes, this chapter also identifies the major freshwater vegetation community-types occurring in near-pristine reference headwater streams in the Scottish Highlands of contrasting underlying geology and characterises the environmental habitat conditions driving these species-assemblages (Aim 3). A minimal modelling approach was developed as a tool for predicting freshwater plant species diversity in response to combinations of measured environmental variables using linear regression analysis. This chapter also includes a comparative analysis of periphyton community composition and diversity colonising various types of artificial substrata compared to their corresponding naturally-occurring microhabitat (Aim 4).

Chapter 5: Integrates findings of the three main results chapters, summarises their main conclusions and discusses the potential implementation of the results of this study, as well as the scope for future research.

Chapter 2. Upland Stream Environmental Habitat Characterisation

2.1 Objectives

- To characterise the three target streams, and quantify between- and within-stream differences, at a set of sampling sites within each stream, in terms of environmental habitat conditions; sub-catchment geology, streambed substrate morphology, physico-chemistry variables, nutrient status, heavy metal composition, flow regime, and extent of shade from riparian vegetation.

- To use multivariate approaches to group sampling sites based on their similarities in geomorphological features and other environmental habitat conditions in accordance with the approach followed by the River Habitat Survey.

- To determine the nature, strength and significance of any associations between these habitat conditions.

2.2 Introduction

2.2.1 The River Habitat Survey and stream characterisation

The River Habitat Survey (RHS: Raven *et al.* 1997) was developed by the Environment Agency as a tool for assessing the physical habitats of rivers and streams throughout the United Kingdom (Raven *et al*. 1998abc, Boon *et al*. 1998, Raven *et al.* 2000, Newson 2002, EA 2003). The RHS is a legislative requirement of the European Water Framework Directive (2000/60/EC) to classify lotic freshwaters based on their hydromorphological features, and to ascertain river habitat quality (Boon & Howell 1997, Fox *et al.* 1998, Raven *et al.* 1998ab, 2000, Newson 2002, Sear & Newson 2003, Balestrini *et al.* 2004, Vaughan & Ormerod 2005, Šporka *et al.* 2006). Although the RHS is considered one of the most effective and widely used approaches, similar protocols have been developed in other EU

member countries: Ökomorphologische Gewässerbewertung, Austria; SEQ Physique, France; and LAWA-Field Survey, Germany (Raven *et al.* 2002, Szoszkiewicz *et al.* 2006, Kamp *et al.* 2007, Weiß *et al.* 2008).

In the field, river habitat surveys follow a standardised field procedure set out in EA (2003) and derived from Raven *et al*. (1997) that encompasses an entire suite of hydromorphological features (e.g. substrate composition, flow type, depth, extent of riparian vegetation, etc.) and aims to describe river habitats as fully as is possible. Survey data are incorporated into a RHS database along with other catchment specifics derived from maps (e.g. geology, altitude, slope, etc.) and compared to existing habitat information. Derived from this are the Habitat Quality Assessment (HQA) and Habitat Modification Score (HMS), indicating the integrity of river habitats and extent to which they have been impinged upon by humans, respectively. One shortcoming of the RHS is that habitat quality indices do not take into account the presence of alien invasive or rare native species which could potentially affect HQA scores (Raven *et al*. 2000). However, the System for Evaluating Rivers for Conservation (SERCON: Boon *et al*. 1998) incorporates rare native and also introduced species into its approach.

To date, most RHS work in the U.K. has been confined to England and Wales, thus knowledge of upland habitats in Scotland is more limited (Raven *et al*. 1998c). The work that has been conducted suggests that nearly half of all upland streams in Scotland are in pristine condition, and c. 70% considered semi-natural: predominantly unmodified (Raven *et al*. 2000). Therefore there is an ongoing requirement to characterise and assess the remaining upland stream habitats in Scotland.

Stream habitat characterisation is fundamental to the understanding of the response of aquatic biota to their physical habitat, and can be utilised coherently with other WFD criteria (e.g. water chemistry) to predict the occurrence, diversity and abundance of these communities (Fox *et al.* 1998). RHS embraces a crucial role in determining the ecological interactions that exist between aquatic assemblages

and prevailing environmental habitat conditions (Raven *et al.* 1998b, 2000, Balestrini *et al.* 2004, Turak & Koop 2008).

In this study, a concept and sampling framework similar to that of the RHS (EA 2003) was adopted in the field. Field methods differed from those stated in the survey guidance manual in the following ways: different dimensions and fewer size categories of stoney substrate particles were used, and predominant flow-types were simplified from the nine types available to three basic surface patterns (e.g. pool, glide, riffle). The primary intention was to characterise natural variation in hydromorphological features between the target three streams and relate these habitat attributes to predominant geologies of the drainage basins. Stream characterisation is fundamental to understanding the environmental habitat conditions driving the abundance and community composition of aquatic vegetation (periphyton) characteristic of upland watercourses: especially diatoms (organisms used in WFD classification of trophic status: Kelly *et al*. 2001) as well as aquatic bryophytes and, less commonly, vascular macrophytes. Stream characterisation helps divulge the principal habitats exploited by specified groups of aquatic vegetation tending to occur together (e.g. Haslam 1978, 1987, 2006, Holmes *et al*. 1998). Such methods integrate the physical and chemical habitat together with stream ecology. This establishes ecological reference or benchmark conditions that pinpoint which groups of vegetation are expected to occur within certain stream typologies or a given set of environmental habitat conditions. For the purposes of this study, stream characterisation methods were used to determine which sites were most similar in habitat composition and therefore most likely to accommodate similar assemblages of aquatic flora.

Multivariate analyses such as Principal Components Analysis and Hierarchical Clustering were applied to the abiotic data sets gathered to determine underlying environmental gradients, which may help to explain natural variation in the occurring assemblages of aquatic vegetation present. I anticipated that such analyses would create visual representations of stream habitat characteristics and yield further information about the ecological habitat preferences of groups of

aquatic vegetation, giving the data ecological significance. Thus data derived from this study could potentially be utilised to ascertain near-pristine reference conditions in the biomonitoring of upland stream water quality in Scotland, and potentially in similar Northern high latitude temperate zones (e.g. Alaska, Canada, Scandinavia).

2.3 Study Areas and Sampling Sites

Separated by the Great Glen Fault, the Grampian Mountains and North West Highlands form two separate regions of the Scottish Highlands, a spectacular mountainous range extending from the south-east to the north-west region of Scotland.

The three target streams of this study are headwaters located in two near-pristine, geologically contrasting catchments in upland Scotland. The R. Dee catchment sourced from the Cairngorms is part of the Grampian Mountain range wherein the geology is composed of igneous granite with metamorphic rocks of the Grampian Group and Glenshirra Subgroup, belonging to the more recent Dalradian Supergroup (Allison *et al.* 1988, Trewin 2002). The R. Kirkaig catchment is an area of the North West Highlands renowned for shaping the history of Scottish geology, as it is characterised by the older metamorphic Moine Supergroup and Moine Thrust Zone (Allison *et al.* 1988, Trewin 2002).

These study areas were selected for the purpose because they represented regions of the Scottish Highlands underlain with contrasting geologies. This made it possible to examine natural variation in habitat conditions and aquatic vegetation occurring in the streams, across environmental gradients of streamwater chemistry and substrate morphology. Sampling sites were established in the upper, mid- and lower parts of each stream to account for spatial variation. Prior to the study, the floristic composition of the three streams was unknown. These streams are considered relatively pristine in the nearest sense: in terms of their

characteristically low nutrient status. However, they are not exclusive of exposure to anthropogenic pressure from atmospheric SO_2 deposition, though levels have declined across Europe in recent years (Ferrier *et al*. 2001). Further in review of this, catchment vegetation is semi-natural mixed heath due to historic forest clearances and management of upland moorland for sheep grazing and grouse shooting.

The Water of Dye (an acid, granite rock stream) and the River Girnock (a mixed acid rock and limestone stream), sub-catchments of the R. Dee in Aberdeenshire NE Scotland, support an aquatic flora consisting only of periphyton (attached algae) and bryophytes (mosses and liverworts). Knockan Burn in Sutherland NW Scotland (a calcareous base-rich stream), draining a Durness limestone sub-catchment of the R. Kirkaig, contains periphyton and bryophytes, as well as the macrophytic alga *Chara globularis* and submerged aquatic vascular macrophyte species such as *Ranuculus flammula*, *Eleogiton fluitans*, *Potamogeton polygonifolius* and *Myriophyllum alterniflorum*; reflecting its more calcareous and nutrient-richer status compared with the Aberdeenshire streams. A species of freshwater sponge (Porifera) was also found occurring at each of the three sites along Knockan Burn, reflecting the calcareous nature of this stream (Wissmar *et al*. 1997). All three streams are comparable in terms of depth, range of flow conditions, size, gradient and altitude. Suitable datasets from six sites within the first two streams were collected on repeated sampling occasions during 2004 – 2006. Similarly, further data was collected from three sites within the third stream during 2005 – 2006. Sampling overlapped between all three streams in April 2006.

The Water of Dye and River Girnock form major freshwater sub-catchments of the River Dee, NE Scotland draining the high altitude western Cairngorm mountains (c. 1300m) and flowing out towards the North Sea. The Water of Dye (Latitude 56^o 58'N; Longitude 2^o 40'W) is a peatland-dominated system, wherein acidic granite dominates the underlying geology (Dawson *et al.* 2001, Smart *et al.* 2001, Dawson *et al.* 2004, Soulsby *et al.* 2003, Rogers *et al*. 2005, Tetzlaff *et al*. 2007b). In the sub-catchment of the River Girnock (Latitude: 57^o 00'N; Longitude 3^o 10'W) peat cover

is less extensive and granite is interspersed with base-rich rocks which occupy a greater proportion of catchment geology (Soulsby *et al.* 2007, Tetzlaff *et al*. 2007a).

In the Water of Dye catchment terrestrial flora is typically a mosaic of peat bog and heather moorland vegetation: *Calluna vulgaris* (L.) Hull, *Erica tetralix* (L.), *Erica cinerea* (L.), and bracken (*Pteridium aquilinum* L.), produced and maintained by periodic burning to manage the age structure of the inhabiting population of red grouse, *Lagopus lagopus* (Dawson *et al.* 2001, 2004, Soulsby *et al.* 2002b, 2003, Rogers *et al*. 2005, Tetzlaff *et al*. 2007b). Grazing pressure from red deer (*Cervus elaphus*), hill-sheep and cattle rearing occurs in the Dee catchment; the intensity of which generally increases towards the lower part of the catchment. This lower region is used mainly for settlement and agriculture, both arable and pastoral. Riparian forestry also occupies a significant proportion of land-use in this vicinity (Smart *et al.* 2001, Soulsby *et al.* 2002b). Land use is similar in the River Girnock, although upland peat habitat is sparser. Additionally, gravelly substrates in the River Girnock are excellent spawning sites for Atlantic salmon (*Salmo salar*) and have been the focus of extensive research for many years (Malcolm *et al*. 2005); conducted by FRS (Fisheries Research Services) Freshwater Laboratory, and the University of Aberdeen.

In each river three sampling sites were located in the upper, mid- and lower basins: Brocky Burn (BB), Charr Flume (CF), and Bogendreip (BD) in the Water of Dye; and Iron Bridge (IB), Hampshire's Bridge (HB), and Littlemill (LM) in the River Girnock.

Brocky Burn [BB: NO 614 833] is a narrow stoney stream of intricate and meandering microhabitat (Figure 2.1). This sampling site lies at an altitude of c. 300m and is fed directly from the peatland dominated landscape in the upper region of the sub-catchment. Brocky Burn functions as a first order tributary of the Water of Dye, adjoining with the main stream approx 1 km from the mid basin, above the Water of Charr. The streambed at Brocky Burn is bordered with dense

growth of terrestrial vegetation dominated by bracken (*Pteridium aquilinum*), which heavily shades the stream during the summer months.

Charr Flume [CF: NO 625 835] is situated on the main river channel of the Water of Dye (Figure 2.1), here known as the Water of Charr, residing at an altitude of c. 250m. In the mid basin of the sub-catchment, geology is mostly granite, with some mica schist. Bracken is the dominant vegetative community on the left back slope at Charr Flume (during peak growth season), with rush grassland (dominated by *Juncus effusus* L.) lining the opposite side of the channel. Although some shading occurs at the bank edges, the inner channel at the Water of Charr is mostly unaffected by riparian vegetation shading due to its broad width. Many years prior to sampling, the Scottish Environment Protection Agency (SEPA) commissioned Grampian Regional Council to construct and station a large pressure transducer flume on the Water of Charr to monitor water volume and discharge (repaired in 1983), which granted us easy access to the main river. Directly upstream of the flume, the streambed remained evidently disturbed from when the flume had been constructed. Therefore this part of the river was avoided, and all sampling was undertaken further downstream of the flume where distribution of substrata and flow regime patterns were unaffected by construction activities.

Bogendreip [BD: NO 662 910] is situated in the lower valley of the Water of Dye sub-catchment (Figure 2.1), at an altitude of c. 100m. Underlying geology is similar in composition to that occurring at CF, but with less mica schist. Bogendreip is moderately afforested by riparian vegetation and abundant in tree species; Birch (*Betula* sp.), Alder (*Alnus glutinosa*) and Scots Pine (*Pinus sylvestris* L.). These trees form a corridor along the embankments of the stream, with some overhang into the waters causing a shaded edge effect. However, the wide channel limits the impact of shading, leaving the mid-channel to remain relatively open to light penetration. Furthermore there is evidence that patches of terrestrial vegetation have been cut-back to accommodate concrete embankments supporting the Bridge of Bogendreip.

Figure 2.1 Water of Dye sampling sites, from L-R: Brocky Burn, Charr Flume, and Bogendreip

Iron Bridge [IB: NO 293 909] is a small, boulder dominated stream with abundant periphyton and bryophyte vegetation, situated in the upper River Girnock sub-catchment (Figure 2.2), at an altitude of c. 360m, draining the surrounding heather moorland. The granitic massif, Lochnagar provides a spectacular backdrop to the site. Geology in this part of the upper Girnock is predominantly granite and granodiorite. Shrubby vegetation of short stature borders this stream, mainly a combination of common gorse (*Ulex europaeus* L.) and heather, with bracken a scarcity in this part of the sub-catchment.

Hampshire's Bridge [HB: NO 312 912] is a wide and shallow part of the River Girnock situated in the mid-basin of the sub-catchment (Figure 2.2), at an altitude of c. 310m. Granitic rocks are less abundant here, and more base-rich geologies are present (diorite; amphibolite; serpentinite; quartz/ psammite, QP; diorite/ amphibolite, DA; and quartz/ psammite/ pelite, QPP, and mixed calcareous limestone). The stream experiences minimal riparian shade, being bordered by short stature vegetation similar to that of upper Girnock. Most of the fishery-related research is concentrated in the main valley of the River Girnock, due to the incidence of salmon redds in these parts. Fine gravelly substrates can be easily manipulated by the swift, directed movement by the tail of female salmon in their attempt to sequester lain eggs to secure offspring survival (C. Soulsby pers. comm.).

Littlemill [LM: NO 330 961] is located in the lower region of the Girnock sub-catchment (Figure 2.2), at an altitude of c. 250m. Underling geology is similar to that occurring at HB. However, this site is heavily shaded from dense canopy closure by dominant riparian vegetation on either side of the stream, comprised mostly of tall tree species; Scots Pine, Alder, Birch, and Willow (*Salix* sp.).

Figure 2.2 River Girnock sampling sites, from L-R: Iron Bridge, Hampshire's Bridge, and Littlemill

Knockan Burn (Abhainn a'Chnochain) in Sutherland NW Scotland, is a calcareous base-rich stream draining a Durness limestone sub-catchment (Longitude: 4° 58' W, Latitude: 58° 3' N). Draining to Loch Veyatie, the Knockan Burn forms a sub-catchment of the complex R. Kirkaig catchment, which enters the sea just south of Lochinver. Knockan Burn drains a medium altitude small range of hills (the Cromalt hills rising to an altitude of c. 500m) rising from the Moine Schist (metamorphosed Torridonian sandstone), with the basin also characterised by fine-grained Durness Limestone (the Durness Group is a series of sedimentary dolimite rocks or dolostones comprised of mineral calcium magnesium carbonate). The Eriboll Group is also characteristic of the Moine Thrust Zone that underpins the catchment (Figure 2.3), and is further subdivided into the following geological formations; Applecross (Conglomerate and Basal Quartzite; pure quartz Torridonian sandstone), Eriboll Sandstone (Pipe Rock Member; quartz sandstone with vertical *Skolithos* burrows), and An-t'Sron (upper Salterella Grit; quartz sandstone and calcareous shales characterised by the shells of *Salterella maccullochi* and lower Fucoid Beds; calcareous dolimitic siltstones containing other fossilised marine fauna of the Cambro-Ordovician): as detailed in the British Geological Survey (1989) and Trewin (2002). The upper catchment is mostly covered by sphagnum-peat bog moorland, with some settlement occurring at the small village of Elphin in the lower region. The limestone supports abundant fertile grassland on the Urigill Estate, which is used mostly for hill sheep rearing, including rare breeds. Soils on the Urigill Estate are a mixture of blanket peat, peat podzols, peaty gleys and brown rendzinas (The Macaulay Institute for Soil Research, 1981). Although Knockan falls within the boundary of a Special Site of Scientific Interest (S.S.S.I.), little to nothing was hitherto known about the flora and water chemistry of this upland burn (Alex Scott, SNH West Sutherland; Ross Doughty, SEPA East Kilbride; pers comm.).

The NW Highlands contain outstanding landscape scenery arising from the complex of geologies underpinning this part of Scotland. The Moine Thrust Zone (Figure 2.3) formed between 430-400 m.y.a. has younger rocks (e.g. Durness

Limestone) overlain by much older strata (e.g. Moine Schists), that were overturned and displaced westwards during tectonic land mass collision (Young *et al.* 1994, Livingstone 2002, Trewin 2002). Mountains of rust-coloured Torridonian Sandstone punctuate the horizon. These are underlain by Lewisian Gneiss, Europe's oldest metamorphic rock (c. 3,000 m.y.a.) which formed the Isles of the Outer Hebrides. Detailed background geology on the NW Highland region is described elsewhere (Peach *et al.* 1907, Allison *et al.* 1988, Young *et al.* 1994, Trewin 2002).

Figure 2.3 Layered geological composition of the Moine Thrust Zone: Moine Schist on the top, with Durness Limestone underneath, followed by Salterella Grit, Fucoid Beds, Pipe Rock, Basal Quartzite overlying Torridonian Sandstone and Lewisian Gneiss (photographs taken by Pauline Lang at Knockan Crag Visitor Centre, Sutherland, NW Scotland).

Three sampling sites were established on the upper, mid and lower portions of Knockan Burn: Upper Knockan (UK), Mid-Knockan (MK), and Lower Knockan (LK), to reflect site locations in the target streams of the R. Dee catchment.

Upper Knockan [UK: NC 221 099] is a slender and meandering tributary stream of upland Knockan (Figure 2.4), which sources from the Cromalt hills and emerges from a fissure in the Durness limestone, at an altitude of c. 170m. Substrate particles are quite fine (e.g. gravel, sand) and the stream gradient is gentle. Growth of the great water moss, *Fontinalis antipyretica* Hedw., is extensive in this part of the stream. Furthermore several aquatic submerged vascular macrophyte species occurred here including bog pondweed: *Potamogeton polygonifolius* Pourret, and, floating clubrush: *Eleogeton fluitans* L., along with small populations of the lesser spearwort: *Ranunculus flammula* L., and dense stands of the fragile stonewort, a form of macrophytic alga: *Chara globularis* var. *globularis* Thuill. Some shading by riparian vegetation occurred due to narrow stream width, coupled to the development of moderately tall plant growth in the summer; *Equisetum* sp., *Carex* sp., *Juncus* sp., and several varieties of thistle.

Mid-Knockan [MK: NC 221 100] is formed at the mid-basin of the Knockan Burn sub-catchment (Figure 2.4), at an altitude of c. 170m. The geology is principally composed of dolomitic limestone. However, in contrast to UK, this stream flows over a steeper gradient and rapid flows are thus characteristic of this site. There are areas of solid calcium magnesium carbonate outcrops on the streambed. Some shade occurs at this site due to the steep bank side covered with sphagnum mosses, but there is little in the way of riparian vegetation and submerged vascular macrophytes are entirely absent from the site.

Lower Knockan [LK: NC 210 105] is located downstream in the Knockan sub-catchment and flows past the village of Elphin (Figure 2.4), sited at an altitude of c. 140m. Eriboll Group geology becomes a feature of the lower basin and substrate morphology is quite variable. Flow regime is similar to that experienced in the R. Dee catchment. This site is very open, and practically unshaded. The alternate

flowered milfoil, *Myriophyllum alterniflorum* (DC.), was the only species of submerged vascular macrophyte to occur in the lower basin of Knockan Burn. Terrestrial vegetation was primarily natural grassland kept short by grazing pressure from sheep in this part of the Knockan catchment.

In the 1970's Loch Urigill, among many other small lochs in the Ullapool area were surveyed floristically (Spence & Allen 1979). Some of these sites were revisited during 2003-2004 for a Glasgow University research project supervised by Dr. Kevin Murphy (Kathariou 2004). Some of the aquatic macrophytes identified as present in these adjoining lochs also occurred in Knockan Burn; *Myriophyllum alterniflorum*, *Potamogeton polygonifolius*, *Eleogiton fluitans*, *Ranunculus flammula*, *Chara* sp., and *Fontinalis antipyretica*.

In the R. Dee catchment sampling commenced on the Water of Dye in October 2004, and subsequently on the River Girnock in April 2005, and continued on both rivers until April 2006. In December 2005 sampling began on the Knockan Burn sub-catchment stream and ended in November 2006.

Figure 2.4 Knockan Burn sampling sites, from L-R: Upper Knockan, Mid Knockan, and Lower Knockan

2.4 Methods

2.4.1 Geological and Soil Reference Maps

Information from Soulsby *et al*. (2003, 2007) was used to determine geological and soil composition of the R. Dee sub-basins. Since no previous similar research had been undertaken for the Knockan Burn sub-catchment, data was obtained from relevant geological and soil-survey maps (British Geological Survey 1989; The Macaulay Institute for Soil Research 1981).

2.4.2 Field Surveys

Field survey campaigns were conducted on three separate occasions over the course of a full growing season (one year) within three 100m stretches in the upper, mid- and lower basins of each of the three sub-catchment streams: during May (MY) and August (AU) 2005 and finally in April (AP) 2006 for the Water of Dye and River Girnock; and during April (AP), September (SM) and finally in November (NV) 2006 for Knockan Burn. The primary objectives of the surveys were to characterise environmental habitat conditions of the streams and quantify natural variation in substrate morphology, physico-chemistry variables, nutrient status, heavy metal composition, flow regime, and shade from riparian vegetation in response to ranging underlying geology, and further to examine the spatial and seasonal response of these environmental parameters.

In accordance with RHS methods (Raven *et al*. 1997, EA 2003), a stratified random sampling procedure was adopted. This involved using a sub-divided 1 m^2 quadrat to determine substrate composition and calculate % frequency of substrate particles within 6 sub-samples, for each of "low", "intermediate" and "high" abundance strata of aquatic vegetation, in each of three habitat flow-types present in the river (determined by observed flow characteristics - P: pool, G: glide, R: riffle): hereafter referred to as 'hydromorphological units'. Pools were

identified as stretches of standing water with barely observable or detectable flows (EA 2003). Glides occurred where noticeable water currents flowed downstream, but without turbulent flow breaking the surface. Riffle habitats were often characterised by waterfalls, flumes, white water, and surface bubbles (EA 2003). These flow units approximately correspond to a 3-point scale; slow <0.2 ms^{-1}, moderate 0.2 - 0.4 ms^{-1}, and fast >0.4 ms^{-1} (Ali *et al.* 1999). To ensure consistency, the primary surveyor (P. Lang) categorised the hydromorphological units by assessing the predominant flow regime (pool, glide or riffle) and aquatic vegetation abundance (low, intermediate or high). Agreement was sought from the secondary surveyor (K. Murphy) before proceeding with the survey of each hydromorphological unit. Stream substrate characteristics were assessed visually as median % cover of each class of stony particle diameter (boulders: >1 m, large stones: 1 - 0.5 m, small stones: 0.5 - 0.1 m, gravel: <0.1 – 0.002 m, and sand: <0.002 m) present within a sample unit (Tominga & Ichmura 1966, Saunders & Eaton 1976, Gordon *et al*. 2004). Median % cover of each substrate particle type identified within each hydromorphological unit was categorised on a five-point scale: scarce, ≤3%; occasional, 15.5%; frequent, 38%; highly abundant, 63%; and dominant, ≥88%. For the first two surveys, the sampling regime used was n = 18 hydromorphological units per site, but this was reduced for the third (final) survey, n = 9 hydromorphological units per site. In total (across all sampling occasions), n = 45 hydromorphological units were assessed per site; 135 hydromorphological units were assessed per sub-catchment stream; and 405 hydromorphological units were assessed combining data from the three target streams.

The stratified random sampling procedure was adopted from guidelines described by Dennis & Isom (1984), so that "observations are most alike within strata and most different between strata". This technique ensured that sampling was undertaken without exhibiting preferences for dense stands of vegetation biomass by sub-sampling an equivalent number of zones considered intermediate or meagre in their extent of aquatic flora, thereby minimising observer bias in the

results (Jeffers 1998a). The stratified random sampling regime incorporates the added advantage that a considerable streambed stretch of each site could be covered during the survey period. Further, this approach to sampling is designed to standardize the samples, thus reducing the variance in plant production associated with heterogeneous microhabitat, common in streams (Dennis & Isom 1984, Jeffers 1998a). A standard quadrat-size of 1 m^2 was chosen as a compromise which could accommodate the narrow streambeds of the upland sites. Although a smaller unit area would be easier to survey (visually) under lotic, frequently disturbed conditions, quadrats of small size are susceptible to the 'edge-effect', the bias caused by the sampler's decision on whether any plant (or other survey parameter) that borders the edge is included or excluded from the quadrat (Dennis & Isom 1984).

Environmental variables measured at each hydromorphological unit (n = 18 per site) comprised snapshot flow data (current velocity ms^{-1}: using a SENSA electromagnetic portable flow meter, and averaged from three readings measured across the area of each hydromorphological unit), mean water depth [D m], and underwater light regime (measured using a twin sensor Skye PAR meter to determine values of light attenuation [K m^{-1}]: Moss 1988, euphotic depth [Z_{eu} m] (calculated as 1% for algae, and 3% for aquatic bryophytes and vascular submerged macrophytes) and ratio of Z_{eu}:D, an indication of whether light reaches the actual bed of the stream, under extant water clarity: Sabbatini *et al*. 1998). The sensors were secured to a metre stick and deployed perpendicular to the direction of incident solar radiation to avoid the effect of self-shading. Mean % shade was used to quantify the reduction in incoming light from terrestrial bankside vegetation. This was undertaken by conducting synchronized simultaneous paired light measurements at a set of randomly-located stations (e.g. n = 6) at water level, within each site, and at an unshaded, open reference station up on the bank or further away from the stream as necessary to avoid shading from trees, ground structures and overhanging vegetation. This required use of two light meters and appropriate communications (using mobile phones if necessary if the

reference site was too far from the stream to be within earshot) to permit simultaneous measurements. Percentage light reduction for incoming light reaching the stream surface was then calculated as % loss of light at water level compared to incoming light at the open adjacent reference site.

Temperature, conductivity and pH were also recorded during sampling (using a Schott Handylab pH/LF 12 meter). Alkalinity concentrations were measured as calcium carbonate ($CaCO_3$) by the Scottish Environment Protection Agency (SEPA: East Kilbride laboratory) from water samples collected at each site during each field survey. SEPA also undertook measurements of concentrations of nutrients in water samples collected from each site during each field survey campaign [ammonia-nitrogen, NH_3-N; nitrate-nitrogen, NO_3-N; phosphate, PO_4-P; chloride, Cl; and sulphate, SO_4] and heavy metals [cadmium, Cd; chromium, Cr; copper, Cu; lead, Pb; nickel, Ni; zinc, Zn; aluminium, Al; vanadium, V; arsenic, As; sodium, Na; potassium, K; calcium, Ca; magnesium, Mg; iron, Fe; and manganese, Mn] to determine water quality status directly. The number of water samples permitted due to lab time restrictions, was limited to collections of one water sample per site, per survey. In total 27 water samples were collected during the course of this project (9 sites x 3 surveys). Water samples were collected using two types of plastic containers specified and provided by SEPA East Kilbride. This was necessary to comply with SEPA standards and to facilitate separate nutrient and heavy metal analyses in the laboratory. Sampling was done by submerging each of the sample bottles in the stream and rinsing several times with streamwater before filling (container lid closed underwater to prevent atmospheric contamination). Water samples were always collected at the end of each field survey, on the same day and within reasonable time limits as close to one another as was possible. Water samples were kept cold in a refrigerator to preserve the chemical constituents and transported in a cool box from the field directly to the laboratory based at East Kilbride and analysed by SEPA staff shortly thereafter.

2.5 Data Analysis

Normality of the response data was examined visually in the form of histograms and analysed statistically using the Ryan-Joiner test. Data with a p-value >0.05 were considered normal. Most response variables were either normally distributed or those that were skewed could be normalised by natural log transformation. All statistical analyses were conducted using Minitab version 15.1.0. One-way ANOVA was performed on response variables with normal distribution, and Tukey's multiple comparison method was used to identify where significant differences occurred. Logistic values were back-transformed where necessary to display original data. Data that was not considered to be normally distributed ($p<0.05$) and could not be normalised by transformation were analysed using the Kruskal-Wallis test (non-parametric equivalent of one-way ANOVA) and the median value(s) quoted where relevant for non-normal variables.

Pearson product-moment correlation coefficients (r) were used to analyse normally distributed data to determine the nature and strength of relationships (if any) between pairs of variables. A strong negative relationship is indicated by a value close to -1, whilst +1 indicates a strong positive relationship. Values closer to 0 suggest no relationship between variables. Correlations were considered significant if $P < 0.05$.

2.5.1 Multivariate approaches to the characterisation of stream environmental habitat conditions

Principal Components Analysis (PCA: Goodall 1954) is an ordination technique used in this chapter to explore and characterise the sampling sites in terms of environmental habitat conditions (variables) to reveal predominant patterns in the data, and will be referred to in subsequent chapters to help explain the occurrence of aquatic vegetation communities. PCA is a method of reducing multivariate (or multidimensional) data onto two axes. PCA diagrams arrange data points (e.g.

sites, samples or species) based on their similarity or dissimilarity in variable composition to one another, and ordinates this information in a two-dimensional space according to their eigenvalues (proportion of the variance explained) on the two major ordination axes (Gauch 1982). Thus samples positioned closer together within the ordination plot are expected to be more similar in terms of the environmental variables measured (and are hence likely to share overlapping environmental habitat conditions), than points distributed further away in the ordination space. PCA performs eigenanalysis on the data matrix, and produces an eigenvalue for each PCA axis (or component: the number of components extracted is equal to the number of variables analysed), arranging these successively from the first and second major axes which capture optimal data ordination and possess the highest eigenvalues. The first three eigenvalues of the ordination represent the largest proportion of cumulative variance explained and are therefore considered the principal components, with each ensuing component carrying smaller proportions of variance. PCA is recognized as a useful technique for ecological application by the River Habitat Survey approach (Jeffers 1998b, Vaughan & Ormerod 2005). Non-linearity can be a problem for PCA (e.g. species data responding unimodally to environmental variables), and one potential drawback of using PCA is the horseshoe effect, which describes the curved distortion of the ordination when axes one and two are quadratically related each other resulting from non-linear distribution (Gauch 1982).

Cluster Analysis is a hierarchical classification method used to partition data into groups (clusters) which are most similar in terms of variable composition and separate these from data that is considered dissimilar, presenting this information in a dendrogram (Gauch 1982). Clustering is useful for defining the environmental variables that characterise a group of sample points that have been deemed similar by PCA. Average-linkage variable clustering was used to distinguish samples by their average dissimilarity (spatial distance) and classify sampling sites by their similarity in environmental habitat characteristics from the principal groups identified from PCA. Once the primary clusters had been

defined by the multivariate analyses, one-way ANOVA was conducted to determine the redundant variables that showed no significant variation between PCA clusters (Table 2.3). This improved the strength of subsequent ordinations once data had been reanalysed by eliminating environmental factors which did not contribute to sample characterisation. Note: colours produced from cluster variable analysis (e.g. red, green, blue) to partition data into groups was followed through on the PCA diagrams to distinguish between clusters (e.g. 1, 2, 3).

Minitab version 15.1.0. was used to perform the PCA and Cluster analyses.

Sample site-codes: Water of Dye sites: Brocky Burn (BB), Charr Flume (CF) and Bogendreip; River Girnock sites: Iron Bridge (IB), Hampshire's Bridge (HB) and Littlemill (LM); Knockan Burn sites: Upper Knockan (UK), Mid-Knockan (MK) and Lower Knockan (LK). Each site code is completed using code letters for survey date (AP: April; MY: May; AU: August; SM: September; NV: November), flow regime (P: Pool; G: Glide; R: Riffle) and year sampled (05: 2005; 06: 2006). Example: BBMYR05 = Brocky Burn May Riffle 2005. Categorising the sites in this way produced a total of 79 sample observations (technically there should have been 81 sample observations, but at UK evident riffle zones were not observable during both the September and November surveys: hence 81 - 2 sample observations = 79 in total).

As there was no significant variation in ammonia-nitrogen (<0.04 mg l^{-1}) or nitrate-nitrogen (<0.01 mg^{-1}) between sub-catchments, sites and dates sampled, these particular nutrient variables were omitted from multivariate analyses from the beginning since they would not be useful in explaining variation in the abundance or community composition of the aquatic vegetation sampled, and were consistently below the limit of detection throughout the field campaigns. Overall, phosphate concentrations remained below the limit of detection (<0.003 mg l^{-1}) and showed no significant variation between sub-catchments or sites sampled. However, streamwater phosphate concentrations were significantly elevated in each of three target streams during April 2006 (refer to Table 2.4, Table 2.5, and

Table 2.6, respectively). Phosphate showed a pulse phenomenon probably attributed to the spring flush. However, like ammonia-nitrogen and nitrate-nitrogen, PO_4-P did not vary significantly between the three clusters characterising environmental habitat conditions: subsequently described in this chapter (refer to Table 2.3 for an overview). Consequently, phosphate was omitted from PCA and cluster analyses in this chapter. However, I decided to retain PO_4-P as a potential contributory factor in other multivariate analyses conducted in subsequent chapters, reasoning that the significant seasonal variation exhibited by nutrient may exert an effect on freshwater plant biomass or community composition.

Physical diversity of the streambed habitat was assessed using Simpson's Diversity Index to measure substrate diversity (variation in size particle composition), and hydromorphological diversity (variation in substrate composition and flow pattern), with higher values indicating increased substrate diversity (concept akin to the Shannon Wiener Index: refer to Chapter 4, section 4.4). The Berger-Parker Dominance Index was used to assess substrate dominance, with values closer to 1 indicating that the substrate is predominated by one particular particle size class (e.g. boulders, large stones, small stones, gravel or sand), and values closer to 0 representing assorted substrate morphologies, with no particular size class prevailing above the other. These substrate indices were calculated using the *Species Diversity and Richness* software package version 4 (Seaby & Henderson 2006).

2.6 Results

2.6.1 Habitat characteristics of the Water of Dye, River Girnock and Knockan Burn; their sub-catchments and sites

There is significant variation in underlying geology between the three sub-catchment basins (Table 2.1). The Water of Dye is an acidic, granite-rock dominated sub-catchment with some mica schist occurring in the lower valley.

Underlying geology of the River Girnock sub-catchment comprises mixed acid rock and limestone. Knockan Burn is a calcareous base-rich stream draining a Durness limestone catchment, and other rocks characteristic of the Moine Thrust Zone.

PCA ordination (Figure 2.5) and cluster analysis (Figure 2.6) partitioned the nine sampling sites of the study into three distinct clusters, on the basis of their similarity in geological composition (refer to Table 2.2 for individual site characteristics). Brocky Burn, Charr Flume, Bogendreip (of the Water of Dye) and Iron Bridge (of the upper Girnock) were clustered together as being functionally similar habitats and were characterised by a high proportion of granite in their drainage basins (indicated by Cluster 1; red, n = 4 sites). Hampshire's Bridge and Littlemill (of the River Girnock) were clustered together as these underlying geologies were comprised of lower proportions of granite and granodiorite, with the introduction of varying extents of diorite, amphibolite, serpentinite, QP, DA, QPP, and mixed calcareous limestone (Cluster 2; green, n = 2 sites). The three sites of the Knockan Burn sub-catchment (UK, MK and LK) are underpinned by Moine Thrust Zone geology; Durness limestone, Moine schist, Eriboll sandstone, Applecross formation and An-t'Sron (Cluster 3; blue, n = 3 sites). The eigenvalues for the PCA analysis were 6.796, 3.359 and 1.887, for axes one, two and three, respectively. The cumulative proportion of variation explained was 46.5% by the first, 68.7%, by the first and second together, and 80.3% by inclusion of the third axis. This was indicative that the ordination explained the data well.

Streambed substrate morphology in the Water of Dye was characterised by a predominance of boulders, compared to the River Girnock and Knockan Burn which lacked this feature. The River Girnock was strewn with a significantly higher proportion of large stones compared to both the Water of Dye and Knockan Burn. A significant assemblage of finer particles characterised the Knockan Burn streambed, mainly comprised of small stones, gravel and sand. Although a higher abundance of small stones occurred in the River Girnock compared to the Water of Dye, generally these streams possessed lower proportions of fine particle

substrates compared to Knockan Burn. Furthermore, no significant differences in gravel cover were detected between the two R. Dee sub-catchment streams, and sandy loams were not observed during sampling in these rivers. Overall, the composition of streambed particles varied significantly: Knockan Burn had greater physical habitat diversity compared to the Water of Dye and River Girnock, which were similar and tended to be dominated by more stable substrates. Details are provided in Table 2.1.

PCA ordination (Figure 2.7) and cluster analysis (Figure 2.8) grouped the nine sampling sites based on predominant substrate morphologies in relation to underlying geology, to produce three main clusters (similar to those described in Figure 2.5 and Figure 2.6). As in the previous ordination (Figure 2.5), Brocky Burn, Charr Flume, Bogendreip and Iron Bridge are clustered together, their streambeds characterised by hard resistant geology and dominated by boulder morphology (Cluster 1; red, n = 36 sample observations). Hampshire's Bridge and Littlemill are indicated by their similar mix of geologies and streambed occupied principally by large stones (Cluster 2; green, n = 18 sample observations). Softer, sedimentary rock types and finer substrate morphologies (small stones, gravel and sand) comprised the relatively diverse streambed structure of upper, mid and lower Knockan (Cluster 3; blue, n = 25 sample observations). The PCA eigenvalues were 7.318, 4.051 and 2.253, for axes one, two and three, respectively. The cumulative proportion of variation explained axis was 36.6%, 56.8% and 68.1% respectively by the first, second and third axes, combined. This showed that a reasonable proportion of the data had been explained by the ordination.

Although there was no significant variation in streamwater depth between the three sub-catchments, significant differences in underwater light regime were detected. Water of Dye streamwaters were characterised by a significantly stronger light attenuation (K) value, compared to the River Girnock and Knockan Burn. Significantly higher values for euphotic depth (Z_{eu}) and Z_{eu}:D were associated with Knockan streamwaters, unlike the Water of Dye and River

Girnock which exhibited shallower euphotic zones and smaller Zeu:D ratios. Refer to Table 2.1 for details.

Table 2.1 shows that strong significant variation was detected for streamwater pH and conductivity between the three sub-catchment streams, with the Water of Dye having the lowest pH and conductivity values and Knockan Burn possessing the highest. Streamwater alkalinity concentrations followed a similar trend (i.e. Water of Dye < River Girnock < Knockan Burn).

Streamwater temperatures differed significantly (Table 2.1), between the River Girnock and Knockan Burn sub-catchments (with the Water of Dye differing significantly from neither of these two).

Mean current velocities did not differ significantly between the Water of Dye and River Girnock. However, Knockan Burn was characterised by significantly higher streamwater flows (attributed to MK) compared to the two R. Dee sub-catchment streams (Table 2.1).

The nine sampling sites were again grouped similarly by PCA ordination (Figure 2.9) and cluster analysis (Figure 2.10) based on variation in water physico-chemistry (minus redundant variables, Table 2.3) in relation to underlying geology. Brocky Burn, Charr Flume, Bogendreip and Iron Bridge were amalgamated to form a single cluster, characterised by hard resistant geology, with low pH and conductivities (Cluster 1; red, n = 36 sample observations). Hampshire's Bridge and Littlemill were underlain by more base-rich geologies and exhibited intermediate pH and conductivities. Furthermore the lower portion of the stream was heavily shaded at LM by riparian vegetation (Cluster 2; green, n = 18 sample observations). Upper, mid and lower Knockan streambeds were characterised by softer, sedimentary rock types with high values Z_{eu}:D, along with high pH and conductivities (Cluster 3; blue, n = 25 sample observations). The PCA eigenvalues were 7.457, 4.812 and 1.862, for axes one, two and three, respectively.

The cumulative proportion of variation explained by the first axis was 39.2%, with 64.6% and 74.4% by including the second and third axes, respectively.

Refer respectively to Table 2.1 and Table 2.2 for details of variation in streamwater chemistry between sub-catchments and sites. There were no significant differences detected for ammonia-nitrogen, nitrate-nitrogen or phosphate between the three sub-catchments. Neither did concentrations of cadmium, chromium, copper, nickel, arsenic, sodium, potassium or iron, vary significantly between these three streams. Streamwater chloride concentrations were significantly higher for Knockan Burn, than for the Water of Dye and River Girnock. Strong significant variation in sulphate concentrations occurred between the three sub-catchment streams, with the highest content of SO_4 recorded in the Water of Dye, and of least abundance in Knockan Burn streamwaters. Streamwater concentrations of lead, zinc, aluminium, vanadium, calcium, magnesium and manganese exhibited strong significant differences between the three sub-catchment streams.

Ordination and grouping of the nine sampling sites into three primary clusters was again conducted by PCA and cluster analyses (Figure 2.11 and Figure 2.12), based on variation in water chemistry (with redundant variables omitted, Table 2.3) in relation to underlying geology. Brocky Burn, Charr Flume, Bogendreip and Iron Bridge formed a single cluster, characterised by hard resistant geology (primarily granite) and elevated levels of sulphate, lead, zinc, aluminium, vanadium, iron, and manganese (Cluster 1; red, n = 12 sample observations). Hampshire's Bridge and Littlemill (Cluster 2; green, n = 6 sample observations) are underlain by mixed geological composition, having intermediate levels of streamwater sulphate and metallic minerals (as detailed in Table 2.3). Upper, mid and lower Knockan streambeds are characterised by softer, sedimentary rock types, with streamwaters high in calcium magnesium carbonates and chloride (Cluster 3; blue, n = 9 sample observations). The eigenvalues from the PCA analysis were 8.433, 6.895 and 2.384, for axes one, two and three, respectively. The

cumulative proportion of variation explained by the first and second axis was 33.7% and 61.3%, increasing to 70.8% by including the third axis.

PCA ordination (Figure 2.13) and Cluster analysis (Figure 2.14) of the entire environmental data set with redundant variables omitted (Table 2.3) supported the three principal clusters produced by preceding ordinations (Figure 2.5, Figure 2.7, Figure 2.9 and Figure 2.11). The first cluster (red, n = 36 sample observations) encompassed four sites, three from the Water of Dye and one from the upper Girnock: Brocky Burn, Charr Flume, Bogendreip and Iron Bridge. These were classified as naturally acidic, acid-sensitive streams underlain by base-poor geologies (principally granite), with sulphate and heavy metals prevalent, particularly Pb, Zn, Al, V, Fe and Mn. Stable streambed morphology dominated by resistant geology and high boulder cover, was another common feature of these four sites. The second cluster (green, n = 18 sample observations) consisted of Hampshire's Bridge and Littlemill of the River Girnock. Herein water quality was markedly influenced by the presence of base-rich geologies (amphibolite, serpentinite and metamorphic limestone), containing sulphate and heavy metal levels midway between that of the Water of Dye and Knockan Burn. Streambed structure was largely cobbled, with fewer boulders compared to the Water of Dye. Heavy shade was a prominent feature in the lower valley of the River Girnock. Upper, mid and lower Knockan were constituents of the third cluster (blue, n = 25 sample observations), representative of a well-buffered calcareous base-rich stream. The highly weatherable geologies contributed high loads of mineral cations (Ca^{2+} and Mg^{2+}), producing high streamwater pH and conductivities. Streambed morphology was characterised by mostly finer, unstable substrates. Furthermore, the strong maritime influence from chloride was most prevalent in Knockan Burn. PCA eigenvalues were 10.776, 7.638 and 2.979, for axes one, two and three, respectively. The cumulative proportion of variation explained by progressively adding in the first, second and third axis was 30.8%, 52.6%, and 61.1%.

Mean Variable	**Water of Dye**		**River Girnock**		**Knockan Burn**		**P_{ANOVA}**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
% Granite	84.9[a]	1.00	62.2[b]	1.49	0.0[c]	0.00	P<0.001***
% Granodiorite	0.0[a]	0.00	9.0[b]	0.32	0.0[a]	0.00	P<0.001***
% Diorite	0.0[a]	0.00	0.3[b]	0.04	0.0[a]	0.00	P<0.001***
% Mica Schist	11.5[a]	0.76	0.0[b]	0.00	0.0[b]	0.00	P<0.001***
% Amphibolite	0.0[a]	0.00	7.7[b]	0.47	0.0[a]	0.00	P<0.001***
% Serpentinite	0.0[a]	0.00	1.0[b]	0.07	0.0[a]	0.00	P<0.001***
% QP	0.0[a]	0.00	0.7[b]	0.04	0.0[a]	0.00	P<0.001***
% DA	0.0[a]	0.00	2.3[b]	0.15	0.0[a]	0.00	P<0.001***
% QPP	0.0[a]	0.00	9.5[b]	0.60	0.0[a]	0.00	P<0.001***
% Limestone	0.0[a]	0.00	7.4[b]	0.55	0.0[a]	0.00	P<0.001***
% Durness Limestone	0.0[a]	0.00	0.0[a]	0.00	73.3[b]	2.48	P<0.001***
% Eriboll Sandstone	0.0[a]	0.00	0.0[a]	0.00	10.0[b]	1.47	P<0.001***
% Moine Schist	0.0[a]	0.00	0.0[a]	0.00	6.7[b]	1.22	P<0.001***
% Applecross Formation	0.0[a]	0.00	0.0[a]	0.00	6.7[b]	0.82	P<0.001***
% An-t'Sron	0.0[a]	0.00	0.0[a]	0.00	3.3[b]	0.41	P<0.001***
% Boulders	40.9[a]	2.82	21.8[b]	2.17	13.9[b]	2.34	P<0.001***
% Large Stones	25.3[a]	2.19	41.5[b]	2.22	26.8[a]	2.23	P<0.001***
% Small Stones	14.4[a]	1.63	21.1[b]	1.92	30.0[c]	2.39	P<0.001***
% Gravel	14.9[a]	2.00	10.9[a]	1.53	23.9[b]	2.41	P<0.001***
% Sand	0.0[a]	0.00	0.0[a]	0.00	6.1[b]	1.42	P<0.001***
D (m)	0.14	0.17	0.12	0.16	0.13	0.19	NS
K (m^{-1})	2.99[a]	0.15	2.32[b]	0.15	2.57[b]	0.14	P<0.01**
$Z_{eu}^{1\%}$ (m)	0.25[a]	0.15	0.26[a]	0.19	0.36[b]	0.14	P<0.01**
Z_{eu}:$D^{1\%}$	1.81[a]	0.17	2.14[a]	0.22	2.84[b]	0.17	P<0.001***

$Z_{eu}^{3\%}$ (m)	0.84[a]	0.15	0.89[a]	0.19	1.24[b]	0.14	P<0.01**
Z_{eu}:$D^{3\%}$	6.18[a]	0.16	7.37[a]	0.21	9.96[b]	0.17	P<0.001***
pH	6.33[a]	0.07	6.93[b]	0.05	7.56[c]	0.03	P<0.001***
Conductivity (µS cm^{-1})	45.8[a]	0.11	51.8[b]	0.13	138.5[c]	0.12	P<0.001***
Alkalinity (mg l^{-1})	5.94[a]	5.52	20.48[b]	8.72	56.05[c]	5.63	P<0.001***
Water Temperature (^oC)	10.2[ab]	0.05	11.2[a]	0.07	9.0[b]	0.02	P<0.05*
Flow (m s^{-1})	0.218[a]	0.02	0.203[a]	0.02	0.290[b]	0.03	P<0.01**
% Shade	26.1[a]	1.42	29.9[a]	2.88	8.4[b]	0.68	P<0.001***
Height of Riparian Vegetation (m)	2.82[a]	0.29	3.52[a]	0.36	0.19[b]	0.03	P<0.001***
NH_3-N (mg l^{-1})	<0.04		<0.04		<0.04		NS
NO_3-N (mg l^{-1})	<0.01		<0.01		<0.01		NS
PO_4-P (mg l^{-1})	<0.003		<0.003		<0.003		NS
Cl (mg l^{-1})	9.00[a]	0.71	8.03[a]	0.51	12.84[b]	0.53	P<0.001***
SO_4 (mg l^{-1})	2.49[a]	0.54	1.11[b]	0.27	0.15[c]	0.06	P<0.001***
Cd (µg l^{-1})	0.03	0.02	0.02	0.02	0.02	0.01	NS
Cr (µg l^{-1})	0.21	0.25	0.22	0.41	0.13	0.05	NS
Cu (µg l^{-1})	0.32	0.05	0.25	0.05	0.16	0.08	NS
Pb (µg l^{-1})	0.53[a]	0.23	0.17[b]	0.26	0.06[c]	0.19	P<0.001***
Ni (µg l^{-1})	0.28	0.18	0.27	0.23	0.24	0.26	NS
Zn (µg l^{-1})	3.38[a]	0.17	1.84[ab]	0.15	1.00[b]	0.20	P<0.001***
Al (µg l^{-1})	129.7[a]	18.24	82.5[ab]	12.39	48.6[b]	11.63	P<0.01**
V (µg l^{-1})	0.36[a]	0.13	0.24[ab]	0.23	0.15[b]	0.17	P<0.01**
As (µg l^{-1})	0.49	0.14	0.37	0.23	0.59	0.00	NS
Na (mg l^{-1})	5.24	0.37	4.65	0.36	5.71	0.33	NS
K (mg l^{-1})	0.42	0.07	0.58	0.13	0.53	0.14	NS
Ca (mg l^{-1})	2.06[a]	0.57	3.28[a]	0.55	10.74[b]	0.56	P<0.001***

Mg (mg l^{-1})	0.82[a]	0.13	1.28[a]	0.17	6.56[b]	0.18	P<0.001***
Fe (mg l^{-1})	0.28	0.18	0.25	0.22	0.16	0.17	NS
Mn (mg l^{-1})	0.015[a]	0.001	0.013[a]	0.002	0.006[b]	0.003	P<0.01**
Substrate diversity (Simpson's D)	3.05[a]	0.12	3.11[a]	0.10	3.50[b]	0.18	P<0.01**
Substrate dominance (Berger-Parker)	0.45[a]	0.03	0.45[a]	0.02	0.39[b]	0.03	P<0.01**
Hydromorphological diversity (Simpson's D)	3.16[a]	0.12	3.22[a]	0.10	3.59[b]	0.18	P<0.01**

Table 2.1 Mean values (± 1 standard error) of normally distributed environmental habitat variables: proportion of underlying geology, substrate morphology, physico-chemistry, nutrient status, heavy metal composition, shade and height of riparian vegetation (data back-transformed where necessary) between study stream sub-catchments (n = 405). Note that for water chemical parameters (NH_3-N – Mn inclusive), n = 27. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values are quoted for non-normal variables compared using Kruskal-Wallis test.

	Water of Dye						River Girnock								Knockan Burn				
Mean Variable	**BB**		**CF**		**BD**		**IB**		**HB**		**LM**		**UK**		**MK**		**LK**		**PANOVA**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
% Granite	100.0	0.00	72.1	0.00	82.5	0.00	86.0	0.00	55.0	0.00	45.7	0.00	0.0	0.00	0.0	0.00	0.0	0.00	N/A
% Granodiorite	0.0	0.00	0.0	0.00	0.0	0.00	14.0	0.00	7.9	0.00	5.2	0.00	0.0	0.00	0.0	0.00	0.0	0.00	N/A
% Diorite	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.9	0.00	0.0	0.00	0.0	0.00	0.0	0.00	N/A
% Mica Schist	0.0	0.00	21.5	0.00	12.9	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	N/A
% Amphibolite	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	11.2	0.00	11.9	0.00	0.0	0.00	0.0	0.00	0.0	0.00	N/A
% Serpentinite	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	1.8	0.00	1.2	0.00	0.0	0.00	0.0	0.00	0.0	0.00	N/A
% QP	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	1.2	0.00	0.8	0.00	0.0	0.00	0.0	0.00	0.0	0.00	N/A
%DA	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	4.1	0.00	2.7	0.00	0.0	0.00	0.0	0.00	0.0	0.00	N/A
% QPP	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	12.1	0.00	16.3	0.00	0.0	0.00	0.0	0.00	0.0	0.00	N/A
% Limestone	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	6.6	0.00	15.5	0.00	0.0	0.00	0.0	0.00	0.0	0.00	N/A
% Durness Limestone	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	70.0	0.00	100.0	0.00	50.0	0.00	N/A
% Eriboll Sandstone	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	10.0	0.00	0.0	0.00	20.0	0.00	N/A
% Moine Schist	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	20.0	0.00	0.0	0.00	0.0	0.00	N/A
% Applecross Formation	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	20.0	0.00	N/A
% An-t'Sron	0.0	0.00	0.0	0.00	0.0	0.0	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	10.0	0.00	N/A

% Boulders	29.7[a]	4.31	31.9[a]	4.45	61.0[b]	4.50	26.7[a]	3.83	10.0[cd]	2.58	28.5[a]	4.10	3.80[d]	1.99	19.0[c]	4.25	21.1[c]	4.88	P<0.001***
% Large Stones	37.5[a]	3.59	28.8[a]	3.75	9.7[b]	2.76	30.2[a]	3.68	56.6[c]	3.15	37.6[a]	3.63	26.6[a]	3.52	34.9[a]	4.07	18.8[d]	3.64	P<0.001***
% Small Stones	15.7[a]	3.03	19.0[a]	2.82	8.6[b]	2.45	20.7[a]	3.14	20.1[a]	3.27	22.5[a]	3.61	34.3[c]	3.98	20.3[a]	3.53	35.0[c]	4.55	P<0.001***
% Gravel	7.9[a]	2.55	26.3[b]	3.97	10.4[a]	3.15	12.7[a]	3.28	9.5[a]	2.50	10.5[a]	2.06	30.7[b]	3.99	12.0[a]	3.03	29.1[b]	4.80	P<0.001***
% Sand	0.0[a]	0.00	0.0[a]	0.00	0.0[a]	0.00	0.0[a]	0.00	0.0[a]	0.00	0.0[a]	0.00	12.2[b]	3.15	5.0[a]	2.52	1.3[a]	0.91	P<0.001***
D (m)	0.09[a]	0.30	0.17[b]	0.27	0.18[b]	0.24	0.12[ab]	0.25	0.11[a]	0.28	0.13[ab]	0.30	0.13[ab]	0.38	0.11[a]	0.29	0.13[ab]	0.30	P<0.001***
K (m^{-1})	4.39[a]	0.22	2.37[b]	0.22	2.58[b]	0.26	2.50[b]	0.25	2.43[b]	0.24	2.05[b]	0.28	2.64[b]	0.22	2.35[b]	0.22	2.64[b]	0.29	P<0.001***
$Z_{eu}^{1\%}$ (m)	0.17[a]	0.22	0.35[bd]	0.22	0.26[d]	0.26	0.36[b]	0.25	0.40[b]	0.24	0.12[c]	0.28	0.33[bd]	0.22	0.37[b]	0.22	0.37[b]	0.29	P<0.001***
Z_{eu}:$D^{1\%}$	1.91[a]	0.26	2.08[ab]	0.31	1.48[a]	0.30	2.87[b]	0.25	3.66[b]	0.31	0.93[c]	0.36	2.46[b]	0.32	3.34[b]	0.27	2.81[b]	0.30	P<0.001***
$Z_{eu}^{3\%}$ (m)	0.56[a]	0.26	1.20[bd]	0.22	0.90[d]	0.25	1.25[b]	0.25	1.41[b]	0.24	0.39[c]	0.27	1.14[bd]	0.24	1.29[b]	0.22	1.31[b]	0.28	P<0.001***
Z_{eu}:$D^{3\%}$	6.42[a]	0.26	7.21[ab]	0.30	5.09[a]	0.27	10.06[b]	0.25	12.80[b]	0.31	3.11[c]	0.31	8.59[b]	0.30	11.67[b]	0.27	9.86[b]	0.30	P<0.001***
pH	5.87[a]	0.13	6.80[b]	0.07	6.29[c]	0.12	6.51[c]	0.08	7.15[d]	0.06	7.13[d]	0.06	7.31[d]	0.01	7.43[d]	0.02	7.95[e]	0.03	P<0.001***
Conductivity ($\mu S\ cm^{-1}$)	38.4[a]	0.18	48.1[b]	0.19	52.1[bc]	0.19	39.0[a]	0.22	58.6[c]	0.22	60.9[c]	0.21	116.8[d]	0.19	110.9[d]	0.18	205.2[e]	0.19	P<0.001***
Alkalinity ($mg\ l^{-1}$)	2.49[a]	9.81	10.21[a]	13.12	5.15[a]	10.27	13.58[a]	14.68	22.65[ab]	17.98	25.18[ab]	16.44	47.72[b]	11.62	40.59[b]	5.34	79.84[c]	10.63	P<0.001***
Water Temperature (^oC)	9.9[ab]	0.10	10.7[ab]	0.06	10.1[ab]	0.10	10.9[ab]	0.12	12.8[a]	0.16	9.9[ab]	0.05	8.8[b]	0.05	8.2[b]	0.03	10.1[ab]	0.02	P<0.001***
Flow ($m\ s^{-1}$)	0.228[a]	0.06	0.216[a]	0.06	0.209[a]	0.06	0.224[a]	0.07	0.198[a]	0.07	0.188[a]	0.06	0.168[a]	0.08	0.475[b]	0.08	0.267[a]	0.08	P<0.001***

% Shade	26.6[a]	3.25	18.5[b]	1.20	33.2[c]	2.01	11.2[d]	0.01	1.90[e]	0.02	76.5[f]	0.72	13.6[bd]	1.56	10.0[d]	0.03	1.60[e]	0.08	P<0.001***
Height of Riparian Vegetation (m)	0.54[a]	0.10	0.45[ae]	0.08	7.45[b]	0.00	0.47[ae]	0.00	0.66[a]	0.00	9.44[c]	0.00	0.37[e]	0.07	0.16[e]	0.03	0.04[f]	0.01	P<0.001***
NH_3-N (mg l^{-1})	<0.04		<0.04		<0.04		<0.04		<0.04		<0.04		<0.04		<0.04		<0.04		NS
NO_3-N (mg l^{-1})	<0.01		<0.01		<0.01		<0.01		<0.01		<0.01		<0.01		<0.01		<0.01		NS
PO_4-P (mg l^{-1})	<0.003		<0.003		<0.003		<0.003		<0.003		<0.003		<0.003		<0.003		<0.003		NS
Cl (mg l^{-1})	8.38[a]	1.06	8.03[a]	1.06	10.56[ab]	1.37	7.30[a]	0.83	7.99[a]	0.90	8.78[a]	1.05	12.50[b]	1.16	12.67[b]	0.98	13.38[b]	0.94	P<0.01**
SO_4 (mg l^{-1})	3.00[a]	1.46	1.47[ab]	0.79	2.99[a]	0.10	1.03[b]	0.47	0.72[bc]	0.62	1.57[b]	0.25	0.10[c]	0.00	0.10[c]	0.00	0.27[c]	0.17	P<0.05*
Cd (µg l^{-1})	0.04	0.00	0.02	0.00	0.03	0.01	0.02	0.01	0.03	0.012	0.02	0.00	0.02	0.00	0.02	0.00	0.02	0.00	NS
Cr (µg l^{-1})	0.25	0.52	0.41	0.43	0.14	0.25	0.48	0.90	0.56	0.72	0.24	0.73	0.14	0.10	0.12	0.00	0.12	0.00	NS
Cu (µg l^{-1})	0.27	0.07	0.40	0.10	0.28	0.03	0.18	0.08	0.26	0.13	0.30	0.01	0.25	0.20	0.11	0.06	0.12	0.07	NS
Pb (µg l^{-1})	1.21[a]	0.11	0.35[b]	0.31	0.35[b]	0.17	0.38[b]	0.19	0.15[c]	0.45	0.10[c]	0.25	0.09[c]	0.56	0.05[c]	0.00	0.05[c]	0.00	P<0.001***
Ni (µg l^{-1})	0.44	0.19	0.23	0.36	0.22	0.31	0.25	0.49	0.33	0.50	0.25	0.32	0.25	0.66	0.18	0.33	0.30	0.44	NS
Zn (µg l^{-1})	4.60[a]	0.25	2.58[ab]	0.28	3.00[a]	0.37	2.41[ab]	0.30	1.69[b]	0.25	1.43[b]	0.18	1.46[b]	0.61	0.79[b]	0.00	0.79[b]	0.00	P<0.01**
Al (µg l^{-1})	140.8	21.32	109.7	37.22	138.7	42.99	93.3	10.53	74.0	29.19	80.2	27.94	67.8	34.86	44.1	9.69	34.0	3.57	NS
V (µg l^{-1})	0.49	0.25	0.36	0.07	0.27	0.21	0.30	0.57	0.24	0.50	0.19	0.16	0.18	0.50	0.13	0.16	0.13	0.15	NS
As (µg l^{-1})	0.60	0.20	0.42	0.37	0.44	0.28	0.37	0.51	0.36	0.51	0.36	0.49	0.59	0.00	0.59	0.00	0.59	0.00	NS
Na (mg l^{-1})	4.57	0.68	5.05	0.62	6.10	0.45	4.22	0.63	4.64	0.72	5.08	0.65	5.47	0.67	5.54	0.69	6.11	0.51	NS

K (mg l^{-1})	0.35	0.07	0.43	0.12	0.48	0.13	0.48	0.20	0.60	0.27	0.67	0.25	0.55	0.36	0.49	0.22	0.55	0.22	NS
Ca (mg l^{-1})	1.47[a]	1.08	2.54[a]	0.99	2.35[a]	0.91	2.49[a]	0.97	3.52[a]	1.00	4.05[a]	0.97	8.66[b]	0.97	8.21[b]	0.85	17.42[c]	0.83	P<0.001***
Mg (mg l^{-1})	0.58[a]	0.20	0.94[a]	0.20	1.01[a]	0.15	0.80[a]	0.26	1.42[a]	0.26	1.53[a]	0.26	5.45[b]	0.35	4.85[b]	0.21	10.67[c]	0.23	P<0.001***
Fe (mg l^{-1})	0.50	0.25	0.20	0.17	0.23	0.18	0.42	0.46	0.24	0.16	0.16	0.30	0.18	0.41	0.17	0.34	0.14	0.28	NS
Mn (mg l^{-1})	0.027[a]	0.00	0.013[b]	0.00	0.013[b]	0.00	0.036[a]	0.05	0.009[b]	0.00	0.007[b]	0.00	0.005[b]	0.00	0.007[b]	0.00	0.008[b]	0.00	P<0.01**
Substrate diversity (Simpson's D)	3.10[a]	0.16	3.32[a]	0.14	2.74[b]	0.29	3.25[a]	0.14	2.77[b]	0.19	3.34[a]	0.12	3.75[c]	0.25	3.12[a]	0.39	3.64[ac]	0.26	P<0.05*
Substrate dominance (Berger-Parker)	0.42[a]	0.02	0.40[a]	0.02	0.53[b]	0.06	0.42[a]	0.02	0.50[b]	0.04	0.41[a]	0.02	0.33[c]	0.02	0.47[b]	0.06	0.38[ac]	0.03	P<0.05*
Hydromorphological diversity (Simpson's D)	3.22[a]	0.17	3.43[a]	0.14	2.83[b]	0.29	3.37[a]	0.14	2.87[b]	0.19	3.45[a]	0.12	3.84[c]	0.26	3.22[a]	0.40	3.72[ac]	0.26	P<0.05*

Table 2.2 Mean values (± 1 standard error) of normally distributed environmental habitat variables: proportion of underlying geology, substrate morphology, physico-chemistry, nutrient status, heavy metal composition, shade and height of riparian vegetation (data back-transformed where necessary) between sampling sites (n = 405). Note that for water chemical parameters (NH_3-N – Mn inclusive), n = 27. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. [Note: N/A represents data with no variation and therefore one-way ANOVA could not be performed due to limited number of replicates]. Note also that median values quoted for non-normal variables compared using Kruskal-Wallis test.

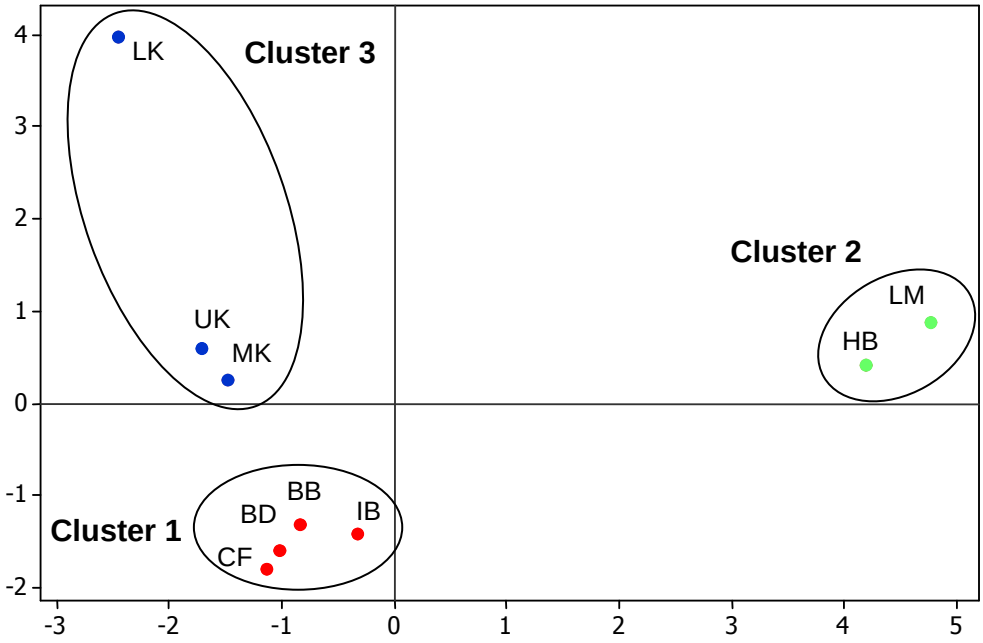


Figure 2.5 PCA ordination of 9 sampling sites by variation in underlying geology; Granite (GRAN), Mica Schist (SCHI), Granodiorite (GDIO), Diorite (DIOR), Quartz/Psammite (QP), Quartz/Psammite/Pelite (QPP), Diorite/Amphibolite (DA), Amphibolite (AMPH), Serpentinite (SERP), Metamorphic Limestone (MLIM), Durness Limestone (DURL), Moine Schist (MOIN), Eriboll Sandstone Group (ESG), Applecross Formation (APCF) and An-T'sron (ANT). Clusters indicated from cluster variables analysis dendrogram (Figure 2.6, below).

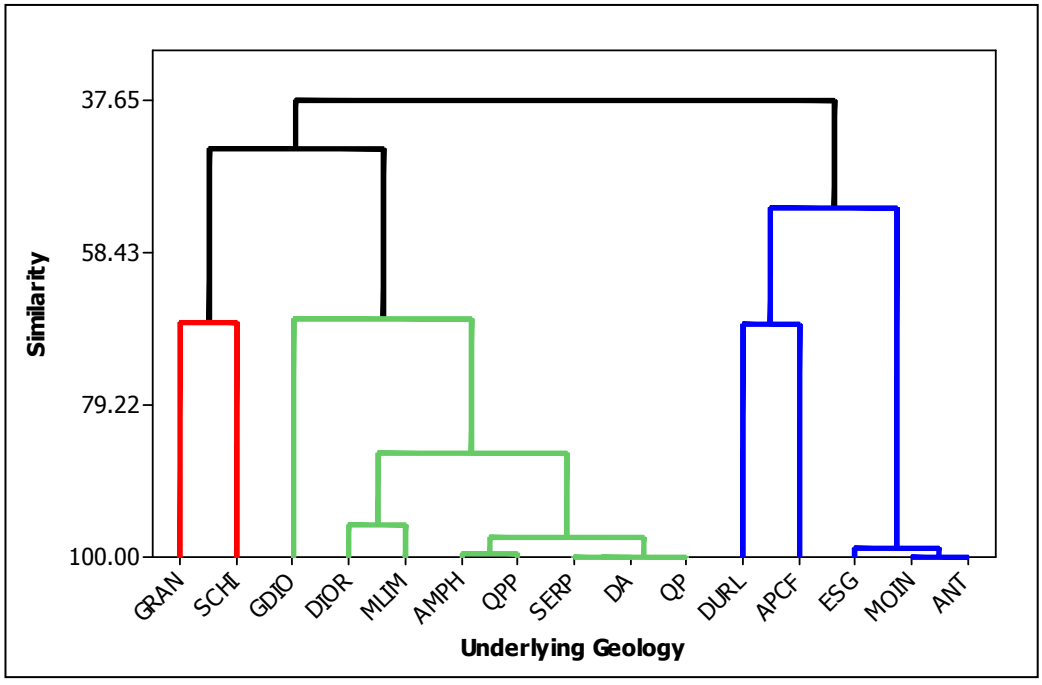


Figure 2.6 Dendrogram showing variable clustering of 9 sampling sites by variation in underlying geology; Granite (GRAN), Mica Schist (SCHI), Granodiorite (GDIO), Diorite (DIOR), Quartz/Psammite (QP), Quartz/Psammite/Pelite (QPP), Diorite/Amphibolite (DA), Amphibolite (AMPH), Serpentinite (SERP), Metamorphic Limestone (MLIM), Durness Limestone (DURL), Moine Schist (MOIN), Eriboll Sandstone Group (ESG), Applecross Formation (APCF) and An-T'Sron (ANT).

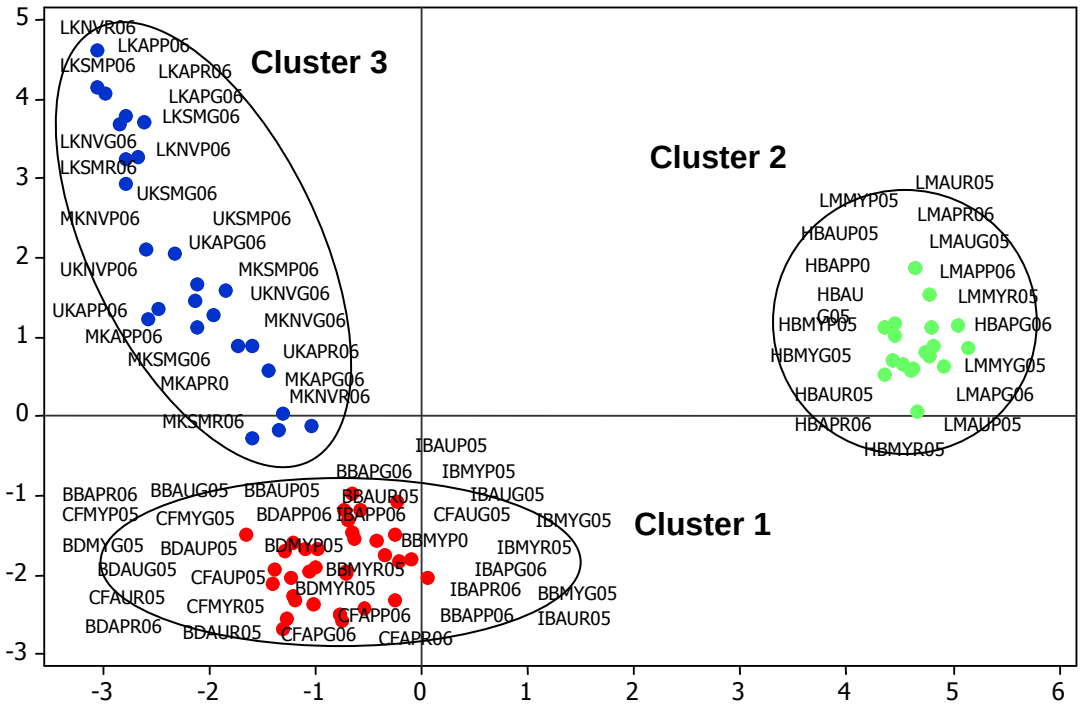


Figure 2.7 PCA ordination of 79 sample observations by variation in underlying geology and substrate morphology; streambed cover of Boulders (BO), Large Stones (LS), Small Stones (SS), Gravel (GR), and Sand (SA). Clusters indicated from cluster variables analysis dendrogram (Figure 2.8, below).

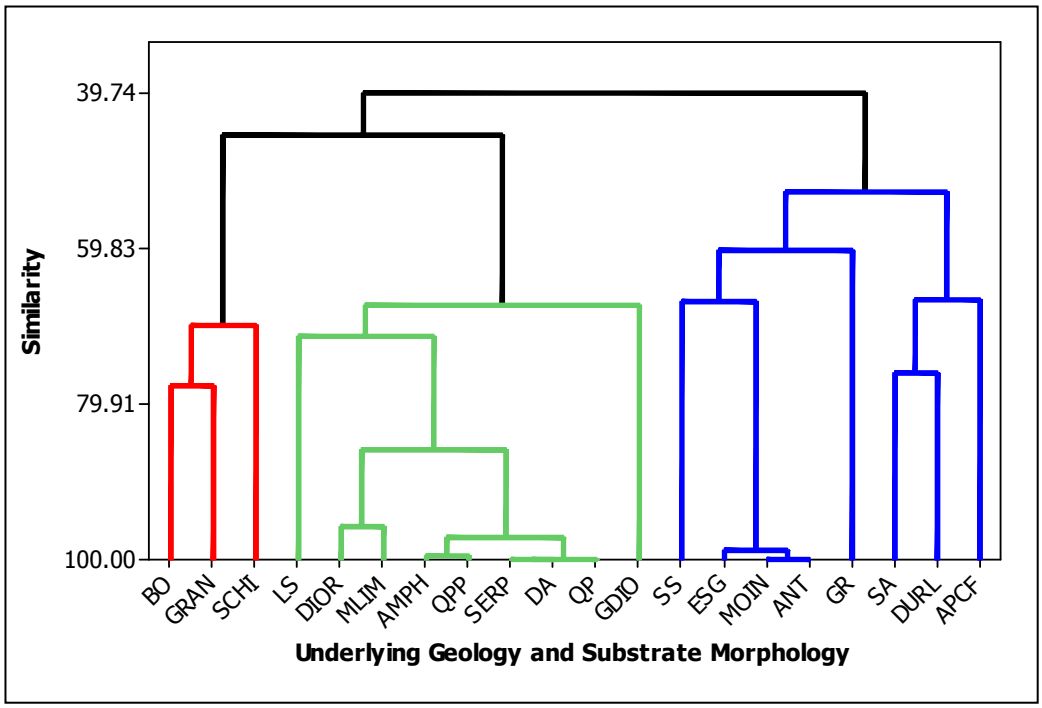


Figure 2.8 Dendrogram showing variable clustering of 79 sample observations by variation in underlying geology and substrate morphology; streambed cover of Boulders (BO), Large Stones (LS), Small Stones (SS), Gravel (GR), and Sand (SA).

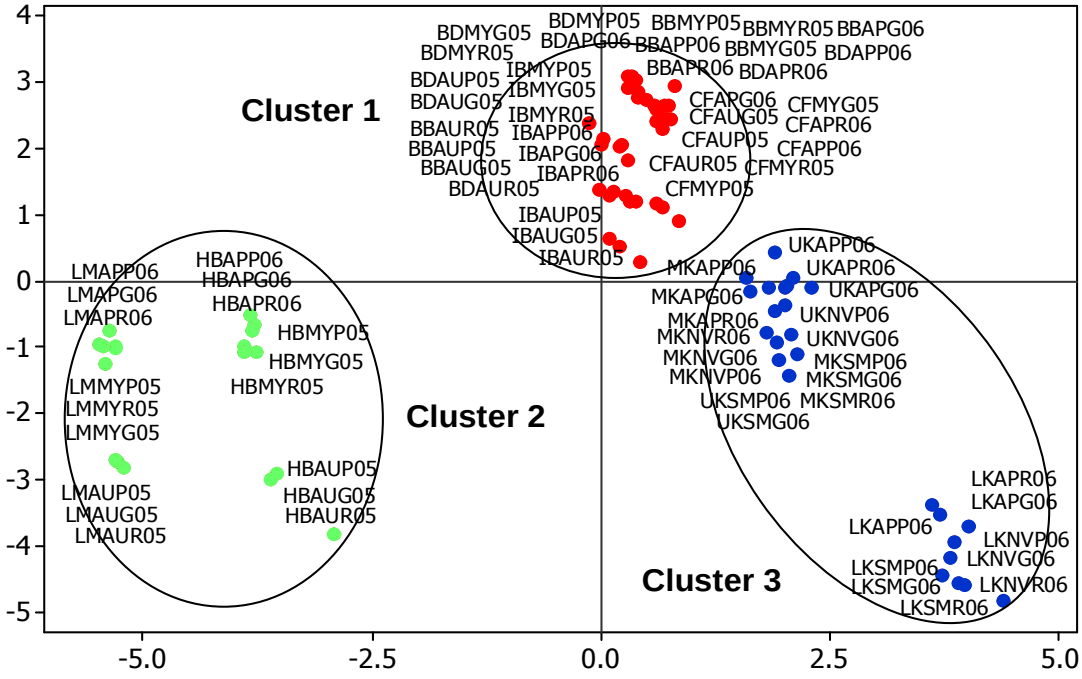


Figure 2.9 PCA ordination of 79 sample observations by variation in underlying geology and water physico-chemistry; Z_{eu}:D, %Shade (Shad), pH, and conductivity (Cond). Clusters indicated from cluster variables analysis dendrogram (Figure 2.10, below), and redundant variables omitted (Table 2.3).

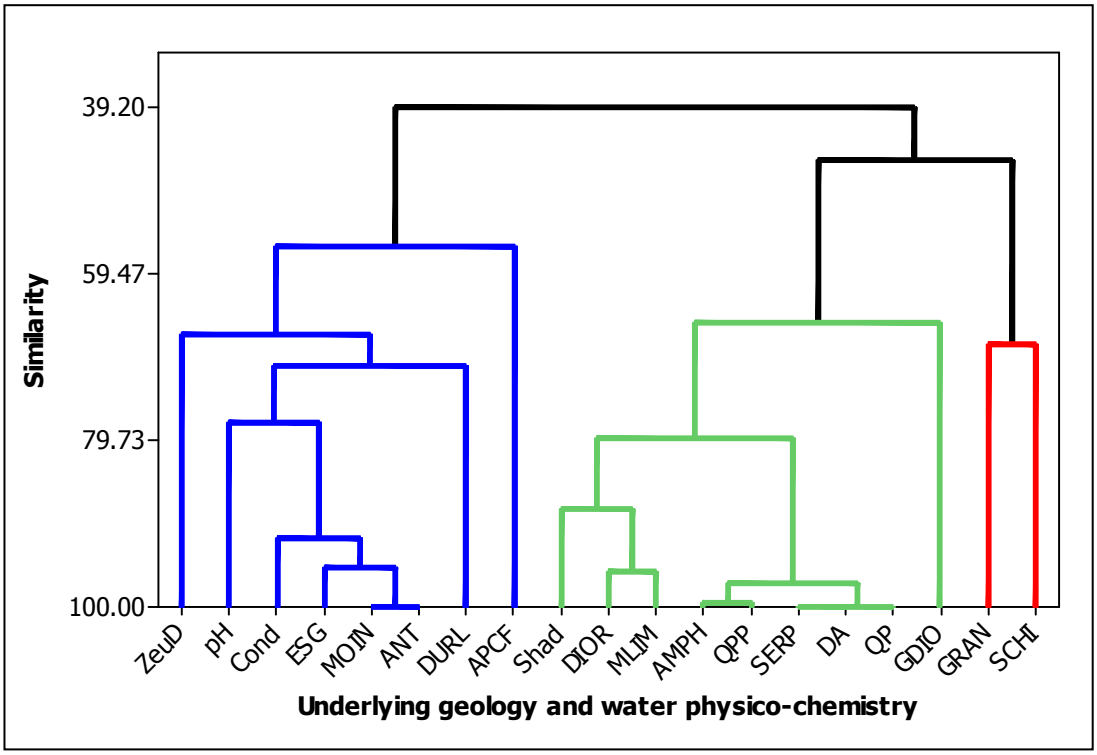


Figure 2.10 Dendrogram showing variable clustering of 79 sample observations by variation in underlying geology and water physico-chemistry; Z_{eu}:D, %Shade (Shad), pH, and conductivity (Cond), with redundant variables omitted (Table 2.3).

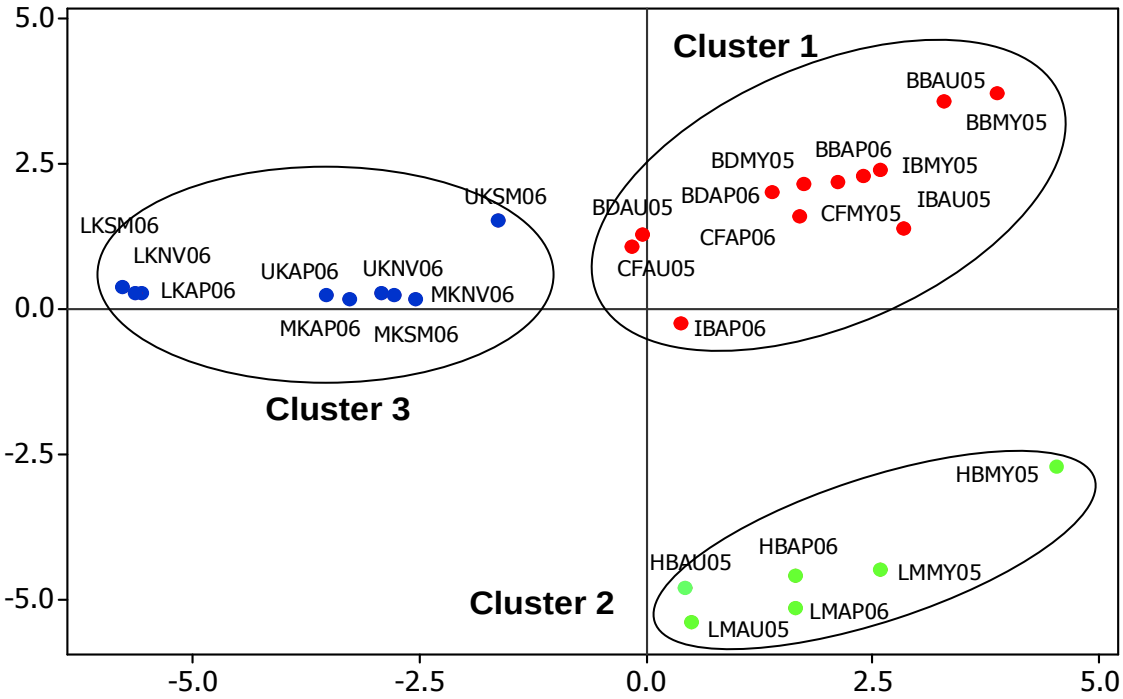


Figure 2.11 PCA ordination of 27 sample observations by underlying geology and water chemistry; Chloride (Cl), Sulphate (SO_4), Lead (Pb), Zinc (Zn), Aluminium (Al), Vanadium (V), Calcium (Ca), Magnesium (Mg), Iron (Fe), and Manganese (Mn). Clusters indicated from cluster variables analysis dendrogram (Figure 2.12, below), and redundant variables omitted (Table 2.3).

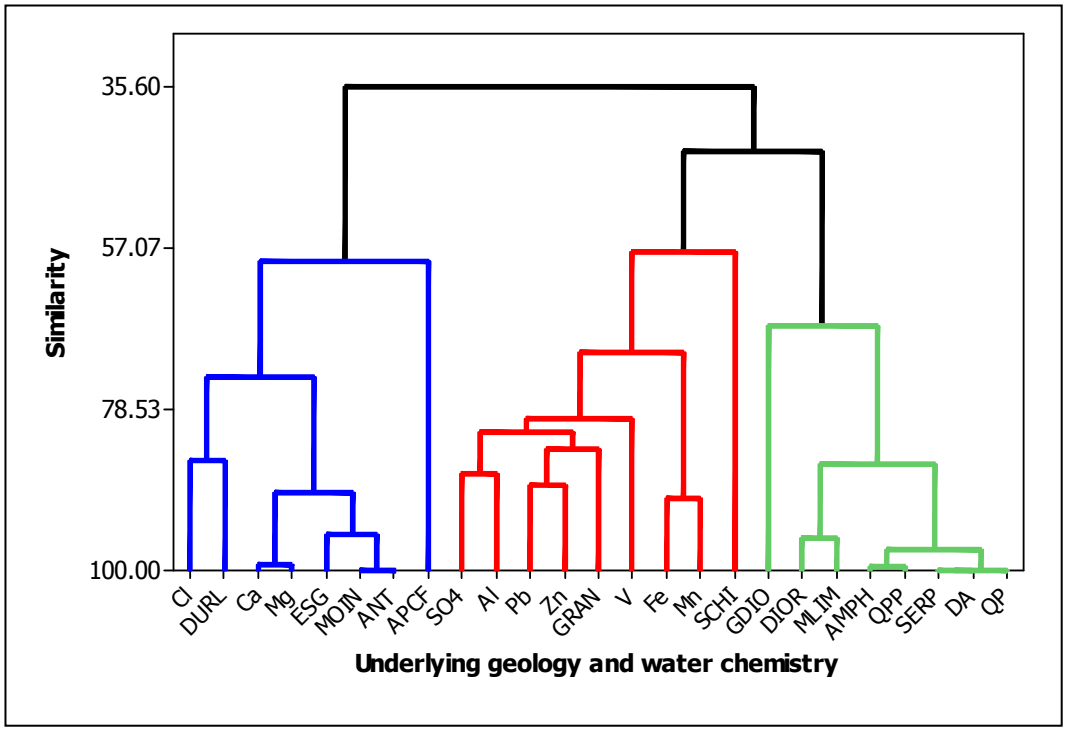


Figure 2.12 Dendrogram showing variable clustering of 79 sample observations by variation in underlying geology and water chemistry; Chloride (Cl), Sulphate (SO_4), Lead (Pb), Zinc (Zn), Aluminium (Al), Vanadium (V), Calcium (Ca), Magnesium (Mg), Iron (Fe), and Manganese (Mn), with redundant variables omitted (Table 2.3).

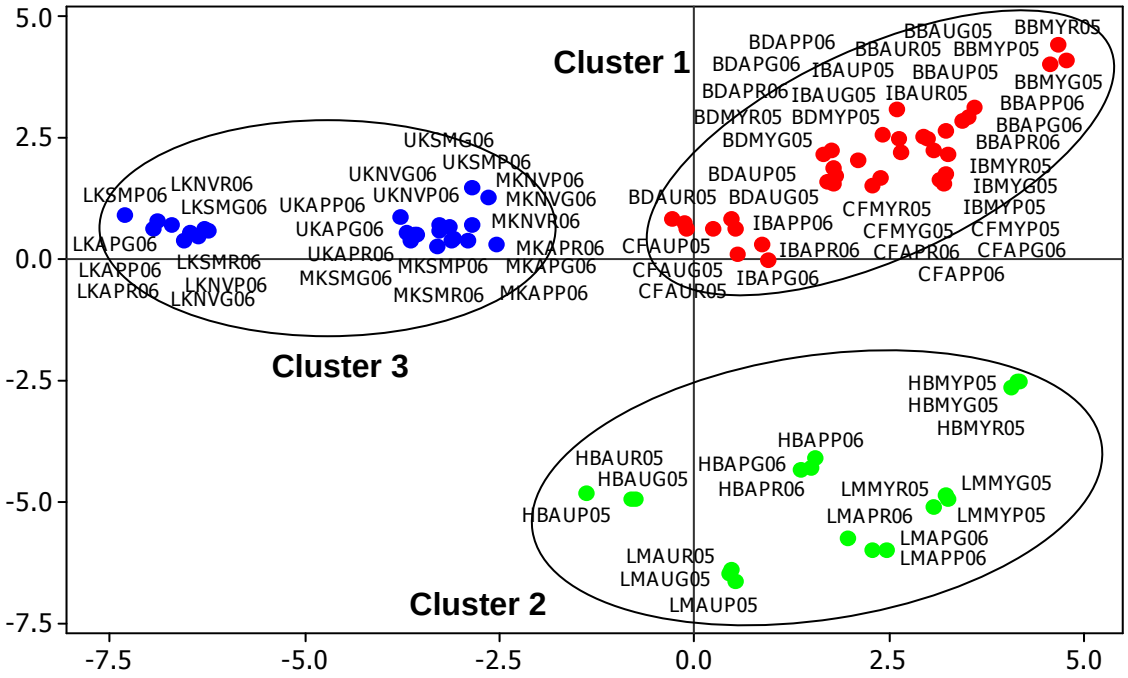


Figure 2.13 PCA ordination of 79 sample observations by underlying geology and significant environmental habitat variables (substrate morphology, water physico-chemistry and water chemistry). Clusters indicated from cluster variables analysis dendrogram (Figure 2.14, below), and redundant variables omitted (Table 2.3).

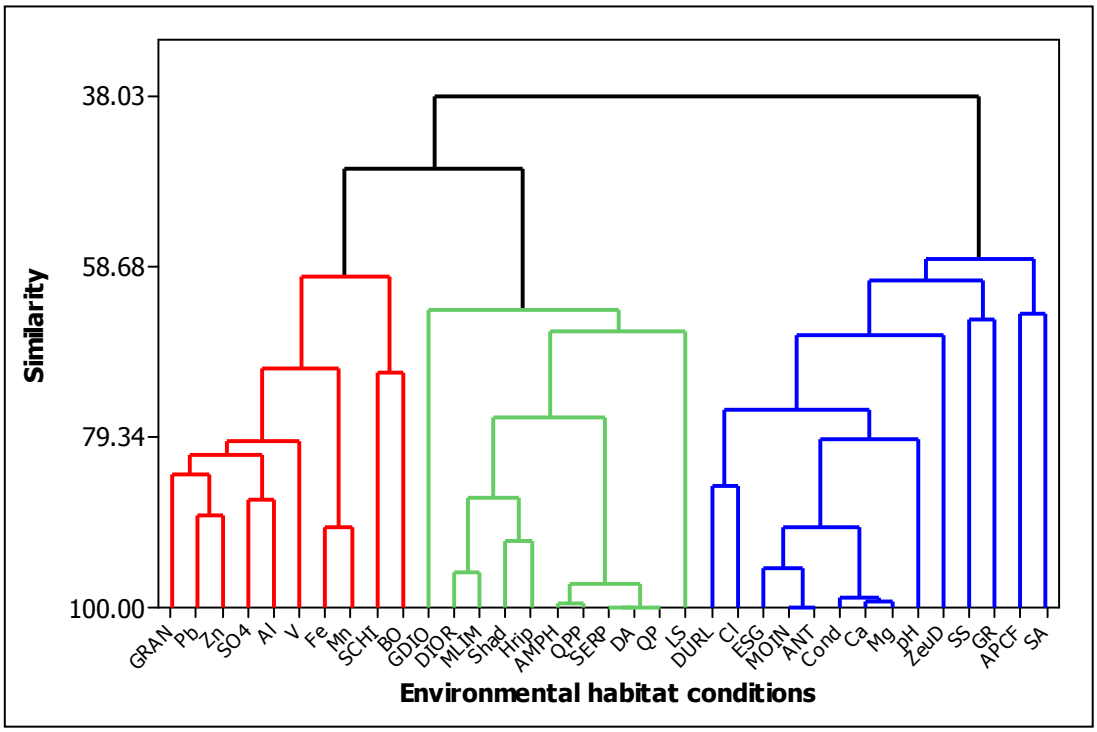


Figure 2.14 Dendrogram showing variable clustering of 79 sample observations by variation in underlying geology and significant environmental habitat conditions, with redundant variables omitted (Table 2.3).

Mean Variable	**Cluster 1 Red (n = 36)**		**Cluster 2 Green (n = 18)**		**Cluster 3 Blue (n = 25)**		**PANOVA**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
% Granite	85.2[a]	1.69	50.4[b]	1.13	0.0[c]	0.00	P<0.001***
% Granodiorite	3.5[a]	1.03	6.6[b]	0.33	0.0[c]	0.00	P<0.05*
% Diorite	0.0[a]	0.00	0.5[b]	0.11	0.0[a]	0.00	P<0.001***
% Mica Schist	8.6[a]	1.54	0.0[b]	0.00	0.0[b]	0.00	P<0.001***
% Amphibolite	0.0[a]	0.00	11.6[b]	0.10	0.0[a]	0.00	P<0.001***
% Serpentinite	0.0[a]	0.00	1.5[b]	0.07	0.0[a]	0.00	P<0.001***
% QP	0.0[a]	0.00	1.0[b]	0.06	0.0[a]	0.00	P<0.001***
%DA	0.0[a]	0.00	3.4[b]	0.18	0.0[a]	0.00	P<0.001***
% QPP	0.0[a]	0.00	14.2[b]	0.51	0.0[a]	0.00	P<0.001***
% Limestone	0.0[a]	0.00	11.1[b]	1.08	0.0[a]	0.00	P<0.001***
% Durness Limestone	0.0[a]	0.00	0.0[a]	0.00	73.3[b]	2.48	P<0.001***
% Eriboll Sandstone	0.0[a]	0.00	0.0[a]	0.00	10.0[b]	1.47	P<0.001***
% Moine Schist	0.0[a]	0.00	0.0[a]	0.00	6.7[b]	1.22	P<0.001***
% Applecross Formation	0.0[a]	0.00	0.0[a]	0.00	6.7[b]	0.82	P<0.001***
% An-t'Sron	0.0[a]	0.00	0.0[a]	0.00	3.3[b]	0.41	P<0.001***
% Boulders	42.1[a]	3.13	24.0[b]	3.55	13.9[b]	3.07	P<0.001***
% Large Stones	32.3[a]	2.63	48.7[b]	2.98	23.1[a]	3.27	P<0.001***
% Small Stones	22.0[a]	1.95	26.7[ab]	3.34	30.6[b]	3.04	P<0.05*
% Gravel	21.7[a]	2.91	16.9[a]	3.04	30.1[b]	4.33	P<0.01**
% Sand	0.0[a]	0.00	0.0[a]	0.00	6.1[b]	1.42	P<0.01**
D (m)	0.16	0.27	0.14	0.40	0.13	0.39	NS
K (m^{-1})	2.83	0.26	2.37	0.33	2.72	0.30	NS
$Z_{eu}^{1\%}$ (m)	0.27	0.23	0.22	0.44	0.36	0.33	NS

Z_{eu}:$D^{1\%}$	2.03[a]	0.35	1.85[a]	0.50	2.84[b]	0.37	P<0.001***
$Z_{eu}^{3\%}$ (m)	1.06	0.27	0.84	0.48	1.27	0.31	NS
Z_{eu}:$D^{3\%}$	6.84[a]	0.36	5.91[a]	0.56	10.02[b]	0.33	P<0.001***
pH	6.38[a]	0.12	7.13[b]	0.10	7.58[c]	0.06	P<0.001***
Conductivity (μS cm^{-1})	43.0[a]	0.05	56.8[b]	0.07	137.7[c]	0.09	P<0.001***
Alkalinity (mg l^{-1})	7.86[a]	6.49	23.92[b]	7.35	56.05[c]	5.63	P<0.001***
Water Temperature (^oC)	9.1	0.15	9.9	0.23	8.9	0.10	NS
Flow (m s^{-1})	0.241	0.04	0.209	0.04	0.289	0.06	NS
% Shade	20.9[a]	2.50	38.9[b]	9.01	7.3[c]	1.35	P<0.001***
Height of Riparian Vegetation (m)	2.19[a]	0.52	5.05[b]	1.07	0.14[c]	0.06	P<0.001***
NH_3-N (mg l^{-1})	<0.04		<0.04		<0.04		NS
NO_3-N (mg l^{-1})	<0.01		<0.01		<0.01		NS
PO_4-P (mg l^{-1})	<0.003		<0.003		<0.003		NS
Cl (mg l^{-1})	8.58[a]	0.60	8.39[a]	0.64	12.84[b]	0.53	P<0.001***
SO_4 (mg l^{-1})	2.13[a]	0.46	1.15[b]	0.36	0.15[c]	0.06	P<0.001***
Cd (μg l^{-1})	0.03	0.01	0.02	0.01	0.02	0.00	NS
Cr (μg l^{-1})	0.21	0.26	0.21	0.50	0.13	0.05	NS
Cu (μg l^{-1})	0.28	0.04	0.28	0.06	0.16	0.08	NS
Pb (μg l^{-1})	0.48[a]	0.18	0.12[b]	0.25	0.06[c]	0.19	P<0.001***
Ni (μg l^{-1})	0.27	0.17	0.29	0.27	0.24	0.26	NS
Zn (μg l^{-1})	3.03[a]	0.15	1.57[b]	0.14	1.00[c]	0.20	P<0.001***
Al (μg l^{-1})	120.6[a]	14.46	77.1[ab]	18.13	48.6[b]	11.63	P<0.05*
V (μg l^{-1})	0.35[a]	0.16	0.21[ab]	0.24	0.15[b]	0.17	P<0.05*
As (μg l^{-1})	0.45	0.16	0.37	0.32	0.59	0.00	NS
Na (mg l^{-1})	4.98	0.33	4.86	0.45	5.71	0.33	NS
K (mg l^{-1})	0.43	0.07	0.64	0.17	0.53	0.14	NS

Ca (mg l^{-1})	2.16^{a}	0.28	3.77^{b}	0.37	10.74^{c}	0.33	P<0.001***
Mg (mg l^{-1})	0.81^{a}	0.11	1.48^{b}	0.17	6.56^{c}	0.18	P<0.001***
Fe (mg l^{-1})	0.31^{a}	0.17	0.25^{ab}	0.22	0.16^{b}	0.17	P<0.05*
Mn (mg l^{-1})	0.019^{a}	0.006	0.008^{b}	0.005	0.006^{b}	0.003	P<0.01**
Substrate diversity (Simpson's D)	3.10^{a}	0.10	3.06^{a}	0.13	3.48^{b}	0.19	P<0.05*
Substrate dominance (Berger-Parker)	0.44	0.02	0.46	0.03	0.41	0.03	NS
Hydromorphological diversity (Simpson's D)	3.21^{a}	0.10	3.13^{a}	0.13	3.59^{b}	0.19	P<0.05*

Table 2.3 Mean values (± 1 standard error) of normally distributed environmental habitat conditions (data back-transformed where necessary) between the three PCA clusters identified from multivariate analyses (n = 79 sample observations), with non-significant factors representing redundant or 'non explanatory' variables. Note that for water chemical parameters (NH_3-N – Mn inclusive), n = 27. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values quoted for non-normal variables compared using Kruskal-Wallis test.

2.6.2 Seasonal variation in environmental habitat conditions of the Water of Dye, River Girnock and Knockan Burn

2.6.2.1 Water of Dye

There was no significant variation in the proportion of streambed occupied by boulders, large stones and small stones between sampling dates (Table 2.4). However, the occurrence of gravel-sized particles was significantly sparse in late spring (May) 2005 compared to the summer (August) of 2005 and spring (April) of 2006. Overall, physical habitat diversity and substrate dominance did not vary significantly between field surveys.

Strong significant differences in mean streamwater depth were recorded between sampling dates (Table 2.4). In August 2005, streamwater depth was significantly shallower than in either May 2005 or April 2006. Furthermore, streamwaters were significantly deeper in April 2006 than in May 2005.

Strong significant variation in underwater light regime factors were observed between sampling dates (Table 2.4). Light attenuation was significantly higher in May 2005 than in August 2005 and April 2006, and was significantly negatively correlated with shallower waters and higher water temperatures. The euphotic depth (Z_{eu}) was significantly greater in April 2006 than in May and August 2005. During the summer (August) 2005, Z_{eu}:D was significantly higher in comparison to that of May 2005 and April 2006.

Mean streamwater pH, conductivity and alkalinity were significantly higher in the summer (August) 2005 compared to May 2005 and April 2006. Mean streamwater pH was significantly lower in May 2005, compared to April 2006, although mean conductivity and alkalinity did not differ significantly between these sampling dates (Table 2.4).

Mean streamwater temperature was significantly warmer in the summer (August) 2005, compared to the late spring (May) of 2005 and spring (April) of 2006.

Furthermore, streamwater temperatures were significantly colder in April 2006, than in May 2005 (Table 2.4).

Mean flow was significantly higher in April 2006 in comparison to May and August 2005.

Shading of the streambed increased significantly in the summer (August) 2005, compared to May 2005 and April 2006, although the mean height of riparian vegetation did not vary significantly between the seasons (Table 2.4).

Details of seasonal variation in streamwater chemistry in the Water of Dye are also provided in Table 2.4. Streamwater phosphate concentration was significantly higher in April 2006, than in May or August 2005. There was no significant variation in the concentrations of nitrogen-containing compounds (e.g. NH_3-N, NO_3-N) between sampling dates. Seasonal variations in sulphate, cadmium, chromium, copper, sodium and potassium concentrations were also insignificant. Although occurring in lower concentrations in the summer, there was no significant variation in streamwater content of lead, nickel and manganese between sampling dates. Seasonal variation was also insignificant for vanadium, arsenic and iron. There were however, significant reductions in the concentrations of zinc and aluminium in August 2005. The reverse trend was observed for chloride, calcium and magnesium, which showed significantly higher concentrations in August 2005 compared to May 2005 and April 2006.

2.6.2.2 River Girnock

Similar to the response of substrate morphology in the Water of Dye, there were no significant differences in streambed cover of boulders, large stones and small stones in the River Girnock between sampling dates. Also, significantly higher proportions of gravelly substrata were found in the summer (August) of 2005 and

spring (April) of 2006 (Table 2.5). Generally, there was no significant difference in physical habitat diversity or substrate dominance between field surveys.

Significant variation in water depth, underwater light climate (K, Z_{eu} and Z_{eu}:D), and water-physico-chemistry (pH, conductivity, alkalinity and temperature) occurred between sampling dates (Table 2.5), following trends similar to those observed in the Water of Dye (Table 2.4).

No significant difference in mean current velocity was detected between sampling dates in the River Girnock (Table 2.5).

There was no significant change in the proportion of shade or height of riparian vegetation between seasons (Table 2.5).

Seasonal variation in streamwater chemistry in the River Girnock is detailed in Table 2.5. There was a significant rise in streamwater phosphate concentration in April 2006 compared to other dates sampled, but no significant seasonal variation in streamwater levels of ammonia-nitrogen, nitrate-nitrogen, sulphate, cadmium, chromium, copper, vanadium, arsenic, iron, and manganese were detected. Aluminium showed significant variation between sampling dates, but lead, nickel and zinc did not, despite also occurring at lower concentrations in August 2005 (compared to spring of 2005 and 2006). Conversely, sodium, potassium, calcium and magnesium concentration were significantly higher in August 2005 compared to May 2005 and April 2006.

2.6.2.3 Knockan Burn

Streambed substrate morphology did not very significantly except for the presence of fine sandy particles which were significantly more abundant in the late summer (September), compared to the spring (April) and winter (November) of 2006

(Table 2.6). On the whole, physical habitat diversity and substrate dominance did not vary significantly between field surveys.

Mean streamwater depth was significantly deeper in the spring (April 2006) than in the late summer (September) and winter (November) of 2006, which were characterised by shallower habitat conditions (Table 2.6).

In Knockan Burn, light attenuation [K] was significantly greater in the summer, coupled to a lower euphotic depth. Although strong significant differences in light attenuation (K) and euphotic zone (Z_{eu}) occurred, Z_{eu}:D was unaffected by these changes and did not show significant variation between sampling dates (Table 2.6).

Mean streamwater pH, conductivity and alkalinity were significantly higher in the late-summer (September) compared to the spring (April) and winter (November) of 2006 (Table 2.6).

Mean streamwater temperatures varied significantly between the seasons, which were coldest in April, and warmest in September 2006 (Table 2.6).

Significantly faster stream flows occurred in April, and were more subdued in both September and November 2006 (Table 2.6).

Refer to Table 2.6 for seasonal variation in streamwater chemistry in Knockan Burn. Mean streamwater concentrations of ammonia-nitrogen, nitrate-nitrogen, sulphate, cadmium, chromium, copper, lead, nickel, zinc, aluminium, vanadium and arsenic showed no significant variation between sampling dates. However, streamwater content of potassium, calcium, magnesium, iron and manganese peaked significantly in the summer of 2006, unlike concentrations of phosphate, chloride and sodium which were most pronounced in April 2006.

Mean Variable	**May 2005**		**August 2005**		**April 2006**		**PANOVA**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
% Boulders	38.7	4.41	42.3	4.69	42.4	5.95	NS
% Large Stones	24.1	3.15	24.3	3.51	29.9	5.65	NS
% Small Stones	12.5	2.46	16.9	2.77	13.5	3.48	NS
% Gravel	9.6[a]	2.73	17.2[b]	3.25	20.7[b]	5.12	P<0.01**
D (m)	0.15[a]	0.27	0.10[b]	0.24	0.23[c]	0.30	P<0.001***
K (m^{-1})	3.73[a]	0.21	2.76[b]	0.24	2.25[b]	0.30	P<0.001***
$Z_{eu}^{1\%}$ (m)	0.20[a]	0.22	0.26[ab]	0.24	0.33[b]	0.29	P<0.001***
Z_{eu}:$D^{1\%}$	1.37[a]	0.27	2.68[b]	0.25	1.43[a]	0.28	P<0.001***
$Z_{eu}^{3\%}$ (m)	0.80[a]	0.22	0.71[a]	0.26	1.31[b]	0.20	P<0.001***
Z_{eu}:$D^{3\%}$	5.55[a]	0.27	7.10[b]	0.26	5.80[a]	0.28	P<0.05*
pH	5.56[a]	0.08	7.07[b]	0.03	6.37[c]	0.11	P<0.001***
Conductivity ($\mu S\ cm^{-1}$)	37.6[a]	0.15	59.5[b]	0.16	40.4[a]	0.24	P<0.001***
Alkalinity ($mg\ l^{-1}$)	1.86[a]	0.62	83.80[b]	7.06	1.38[a]	0.45	P<0.001***
Water Temperature (^oC)	9.8[a]	0.02	15.4[b]	0.02	3.7[c]	0.03	P<0.001***
Flow ($m\ s^{-1}$)	0.217[a]	0.02	0.172[a]	0.03	0.326[b]	0.03	P<0.01**
% Shade	14.4[a]	0.78	43.6[b]	1.50	14.4[a]	1.12	P<0.001***
Height of Riparian Vegetation (m)	2.49	0.48	3.31	0.40	2.49	0.69	NS
NH_3-N ($mg\ l^{-1}$)	<0.04	0.00	<0.04	0.00	<0.04	0.00	NS
NO_3-N ($mg\ l^{-1}$)	<0.01	0.00	<0.01	0.00	<0.01	0.00	NS
PO_4-P ($mg\ l^{-1}$)	<0.003[a]		<0.003[a]		0.32[b]		P<0.001***
Cl ($mg\ l^{-1}$)	6.69[a]	0.66	10.19[b]	0.65	9.90[b]	0.70	P<0.05*
SO_4 ($mg\ l^{-1}$)	3.60	0.57	2.70	0.88	1.17	0.97	NS
Cd ($\mu g\ l^{-1}$)	0.03	0.01	0.03	0.01	0.04	0.01	NS

Cr (μg l^{-1})	0.24	0.06	0.20	0.42	0.18	0.75	NS
Cu (μg l^{-1})	0.34	0.13	0.33	0.04	0.29	0.04	NS
Pb (μg l^{-1})	0.71	0.37	0.39	0.56	0.53	0.32	NS
Ni (μg l^{-1})	0.47	0.15	0.18	0.34	0.26	0.18	NS
Zn (μg l^{-1})	3.79^{a}	0.26	1.89^{b}	0.20	4.96^{a}	0.11	P<0.05*
Al (μg l^{-1})	182.9^{a}	6.72	86.5^{b}	19.97	119.7^{ab}	9.35	P<0.05*
V (μg l^{-1})	0.38	0.06	0.43	0.32	0.29	0.05	NS
As (μg l^{-1})	0.56	0.11	0.58	0.09	0.30	0.17	NS
Na (mg l^{-1})	4.87	0.44	6.38	0.33	4.46	0.64	NS
K (mg l^{-1})	0.39	0.02	0.45	0.08	0.43	0.04	NS
Ca (mg l^{-1})	1.71^{a}	0.80	3.88^{b}	0.73	1.45^{a}	0.82	P<0.01**
Mg (mg l^{-1})	0.70^{a}	0.11	1.17^{b}	0.18	0.66^{a}	0.13	P<0.05*
Fe (mg l^{-1})	0.33	0.21	0.35	0.40	0.20	0.26	NS
Mn (mg l^{-1})	0.015	0.01	0.012	0.01	0.024	0.01	NS
Substrate diversity (Simpson's D)	3.05	0.14	3.18	0.22	2.92	0.28	NS
Substrate dominance (Berger-Parker)	0.42	0.02	0.45	0.04	0.48	0.06	NS
Hydromorphological diversity (Simpson's D)	3.17	0.13	3.29	0.22	3.02	0.28	NS

Table 2.4 Mean values (± 1 standard error) of normally distributed environmental habitat variables (data back-transformed where necessary) between sampling dates in the Water of Dye sub-catchment (n= 135). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values quoted for non-normal variables compared using Kruskal-Wallis test.

Mean Variable	**May 2005**		**August 2005**		**April 2006**		**PANOVA**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
% Boulders	18.1	3.44	22.7	3.16	27.3	5.47	NS
% Large Stones	41.3	3.58	39.1	3.42	46.5	5.08	NS
% Small Stones	17.4	2.93	21.3	3.00	28.2	4.48	NS
% Gravel	4.70^{a}	1.36	16.0^{b}	3.09	13.3^{b}	2.93	P<0.01**
D (m)	0.14^{a}	0.20	0.08^{b}	0.25	0.20^{c}	0.32	P<0.001***
K (m^{-1})	2.90^{a}	0.21	2.10^{b}	0.26	1.80^{b}	0.26	P<0.001***
$Z_{eu}^{1\%}$ (m)	0.21^{a}	0.29	0.28^{ab}	0.28	0.34^{b}	0.42	P<0.01*
Z_{eu}:$D^{1\%}$	1.51^{a}	0.33	3.46^{b}	0.30	1.62^{a}	0.46	P<0.001***
$Z_{eu}^{3\%}$ (m)	0.75^{a}	0.28	0.90^{a}	0.30	1.20^{b}	0.40	P<0.01**
Z_{eu}:$D^{3\%}$	5.51^{a}	0.31	11.01^{b}	0.32	5.91^{a}	0.47	P<0.001***
pH	6.50^{a}	0.06	7.38^{b}	0.04	6.90^{c}	0.09	P<0.001***
Conductivity (µS cm^{-1})	39.4^{a}	0.18	78.5^{b}	0.16	39.0^{a}	0.23	P<0.001***
Alkalinity (mg l^{-1})	6.73^{a}	0.53	140.48^{b}	6.13	5.28^{a}	0.68	P<0.001***
Water Temperature (^{o}C)	10.3^{a}	0.29	17.2^{b}	0.40	3.9^{c}	0.22	P<0.001***
Flow (m s^{-1})	0.217	0.02	0.167	0.03	0.253	0.03	NS
% Shade	28.5	4.31	31.8	4.93	28.5	6.16	NS
Height of Riparian Vegetation (m)	3.52	0.58	3.52	0.58	3.52	0.82	NS
NH_3-N (mg l^{-1})	<0.04	0.00	<0.04	0.00	<0.04	0.00	NS
NO_3-N (mg l^{-1})	<0.01	0.00	<0.01	0.00	<0.01	0.00	NS
PO_4-P (mg l^{-1})	$<0.003^{a}$		$<0.003^{a}$		0.52^{b}		P<0.001***
Cl (mg l^{-1})	6.18^{a}	0.29	9.07^{b}	0.44	8.76^{b}	0.55	P<0.01**
SO_4 (mg l^{-1})	1.86	0.12	1.01	0.46	0.45	0.35	NS
Cd (µg l^{-1})	0.03	0.01	0.02	0.00	0.02	0.01	NS

Cr (μg l^{-1})	0.46	0.27	0.11	0.43	0.10	0.34	NS
Cu (μg l^{-1})	0.38	0.07	0.19	0.07	0.17	0.06	NS
Pb (μg l^{-1})	0.24	0.37	0.13	0.73	0.20	0.26	NS
Ni (μg l^{-1})	0.45	0.28	0.26	0.47	0.28	0.44	NS
Zn (μg l^{-1})	1.82	0.23	1.75	0.43	1.83	0.11	NS
Al (μg l^{-1})	118.9^{a}	5.16	49.22^{b}	24.07	79.36^{ab}	3.96	P<0.05*
V (μg l^{-1})	0.33	0.26	0.24	0.33	0.15	0.13	NS
As (μg l^{-1})	0.44	0.14	0.42	0.18	0.26	0.12	NS
Na (mg l^{-1})	4.26^{a}	0.19	5.94^{b}	0.28	3.74^{a}	0.29	P<0.01**
K (mg l^{-1})	0.44^{a}	0.09	0.93^{b}	0.13	0.48^{a}	0.09	P<0.01**
Ca (mg l^{-1})	2.60^{a}	0.72	6.13^{b}	0.75	2.35^{a}	0.75	P<0.01**
Mg (mg l^{-1})	1.02^{a}	0.18	2.10^{b}	0.39	0.92^{a}	0.17	P<0.05*
Fe (mg l^{-1})	0.31	0.03	0.29	0.67	0.18	0.13	NS
Mn (mg l^{-1})	0.013	0.00	0.017	0.05	0.009	0.00	NS
Substrate diversity (Simpson's D)	2.92	0.23	3.37	0.12	3.09	0.08	NS
Substrate dominance (Berger-Parker)	0.47	0.05	0.40	0.02	0.46	0.02	NS
Hydromorphological diversity (Simpson's D)	3.03	0.24	3.49	0.13	3.18	0.08	NS

Table 2.5 Mean values (± 1 standard error) of normally distributed environmental habitat variables (data back-transformed where necessary) between sampling dates in the River Girnock sub-catchment (n = 135). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values quoted for non-normal variables compared using Kruskal-Wallis test.

Mean Variable	**April 2006**		**September 2006**		**November 2006**		**PANOVA**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
% Boulders	15.5	3.59	14.3	3.78	13.5	5.44	NS
% Large Stones	31.1	3.60	23.1	3.02	25.4	5.91	NS
% Small Stones	30.0	3.93	29.7	3.61	29.8	5.55	NS
% Gravel	20.0	3.59	24.0	3.73	31.6	6.07	NS
% Sand	4.6[a]	2.11	9.7[b]	2.77	2.10[a]	0.95	P<0.05*
D (m)	0.21[a]	0.23	0.08[b]	0.26	0.11[b]	0.46	P<0.001***
K (m^{-1})	1.76[a]	0.20	3.61[b]	0.20	2.77[c]	0.28	P<0.001***
$Z_{eu}^{1\%}$ (m)	0.52[a]	0.20	0.25[b]	0.20	0.33[c]	0.29	P<0.001***
Z_{eu}:$D^{1\%}$	2.50	0.25	3.19	0.28	2.92	0.42	NS
$Z_{eu}^{3\%}$ (m)	1.87[a]	0.20	0.85[b]	0.20	1.19[c]	0.28	P<0.001***
Z_{eu}:$D^{3\%}$	9.03	0.24	10.68	0.27	10.59	0.41	NS
pH	7.45[a]	0.03	7.70[b]	0.05	7.52[a]	0.05	P<0.001***
Conductivity (μS cm^{-1})	116.9[a]	0.17	173.4[b]	0.18	124.3[a]	0.29	P<0.001***
Alkalinity (mg l^{-1})	33.82[a]	4.37	95.71[b]	12.33	43.87[a]	9.25	P<0.001***
Water Temperature (^oC)	6.4[a]	0.04	12.5[b]	0.02	8.3[c]	0.02	P<0.001***
Flow (m s^{-1})	0.514[a]	0.04	0.141[b]	0.04	0.259[b]	0.05	P<0.001***
% Shade	5.7[a]	0.44	12.5[b]	1.44	5.7[a]	0.63	P<0.001***
Height of Riparian Vegetation (m)	0.0	0.00	0.48	0.12	0.0	0.00	P<0.001***
NH_3-N (mg l^{-1})	<0.04	0.00	<0.04	0.00	<0.04	0.00	NS
NO_3-N (mg l^{-1})	<0.01	0.00	<0.01	0.00	<0.01	0.00	NS
PO_4-P (mg l^{-1})	0.02[a]		<0.003[b]		<0.003[b]		P<0.001***
Cl (mg l^{-1})	14.73[a]	0.24	12.57[b]	0.15	11.23[b]	0.45	P<0.01**
SO_4 (mg l^{-1})	0.10	0.00	0.27	0.17	0.10	0.00	NS

Cd (μg l^{-1})	0.02	0.00	0.02	0.00	0.02	0.00	NS
Cr (μg l^{-1})	0.12	0.00	0.12	0.00	0.12	0.00	NS
Cu (μg l^{-1})	0.10	0.00	0.28	0.14	0.10	0.00	NS
Pb (μg l^{-1})	0.05	0.00	0.12	0.07	0.05	0.00	NS
Ni (μg l^{-1})	0.15	0.00	0.60	0.28	0.20	0.33	NS
Zn (μg l^{-1})	0.79	0.00	2.18	0.14	0.79	0.00	NS
Al (μg l^{-1})	28.56	1.92	79.90	2.92	37.40	4.12	NS
V (μg l^{-1})	0.11	0.00	0.25	0.30	0.11	0.10	NS
As (μg l^{-1})	0.59	0.00	0.59	0.00	0.59	0.00	NS
Na (mg l^{-1})	6.82^{a}	0.15	5.61^{b}	0.09	4.69^{b}	0.37	P<0.01**
K (mg l^{-1})	0.36^{a}	0.04	0.88^{b}	0.12	0.45^{a}	0.05	P<0.001***
Ca (mg l^{-1})	7.34^{a}	0.86	16.62^{b}	0.80	10.17^{ab}	1.00	P<0.05*
Mg (mg l^{-1})	4.73^{a}	0.23	11.29^{b}	0.22	6.50^{ab}	0.32	P<0.05*
Fe (mg l^{-1})	0.08^{a}	0.01	0.27^{b}	0.04	0.19^{b}	0.02	P<0.01**
Mn (mg l^{-1})	0.004^{a}	0.00	0.008^{b}	0.00	0.007^{b}	0.01	P<0.05*
Substrate diversity (Simpson's D)	3.51	0.29	3.84	0.29	3.08	0.38	NS
Substrate dominance (Berger-Parker)	0.40	0.05	0.35	0.03	0.43	0.06	NS
Hydromorphological diversity (Simpson's D)	3.62	0.30	3.94	0.28	3.17	0.38	NS

Table 2.6 Mean values (± 1 standard error) of normally distributed environmental habitat variables (data back-transformed where necessary) between sampling dates in the Knockan Burn sub-catchment (n = 135). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values quoted for non-normal variables compared using Kruskal-Wallis test.

2.6.3 Response of habitat characteristics in the Water of Dye, River Girnock and Knockan Burn to variation in flow regime: pool, glide and riffle zones

2.6.3.1 Water of Dye

Overall, physical habitat diversity and substrate dominance did not vary significantly between flow regimes as mostly there was no significant difference in the proportion of streambed occupied by boulders, large stones and small stones between pool, glide, and riffle habitats. However, gravel cover showed a significant response to variation in flow regime, with a significant reduction in percent cover in fast flowing riffles (Table 2.7).

Riffles were characterised as significantly shallower regions of the streambed, unlike pools and glides which were, not surprisingly, found to occur as deeper habitats (Table 2.7).

There were no significant differences in underwater light climate (K, Z_{eu} and Z_{eu}:D), and water physico-chemistry (pH, conductivity and temperature) between varying flow regimes (Table 2.7).

Current velocities were significantly faster in riffle zones, compared to glides and pools. Also, flows were significantly slower in pools than in glides (Table 2.7).

2.6.3.2 River Girnock

Although physical habitat diversity and substrate dominance showed no significant variation to flow regime, a significantly higher proportion of boulders occurred in riffle habitats. Large stone and small stone cover did not vary significantly in response to flow regime, but gravel substrates were a highly significant feature of pools, and less abundant in glide and riffle zones (Table 2.8).

Similarly to the Water of Dye, underwater light regime and water physico-chemistry parameters (except flow) did not vary significantly between flow habitat conditions (Table 2.8).

Riffles were classified as zones with significantly higher flows, and pools had significantly low current velocities. Glides occupied a moderate flow pattern, ranging between these two distinct habitats (Table 2.8).

2.6.3.3 Knockan Burn

Riffle zones were characterised by significant boulder cover, compared to glides and pools. The proportion of large stones and small stones did not vary significantly, however both gravel and sandy substrates were significantly more abundant in pools than in either glides or riffle habitats. In general, pools and riffles were lowest in terms of physical habitat diversity, and highest in terms of substrate dominance, with riffles predominated by boulders and pools characterised by finer particles (Table 2.9).

No significant variation in underwater light climate, pH, conductivity and temperature, was detected between flow patterns (Table 2.9).

As described for the Water of Dye and River Girnock, significant differences in mean current velocity were exhibited by the three basic flow patterns: pools, glides and riffle habitats (Table 2.9).

2.6.3.4 Amalgamated sub-catchment data

All substrate morphologies showed a significant response to variation in flow regime (Table 2.10). High boulder cover occurred in riffle zones. Glides had the highest proportion of large stones and contained moderate proportions of other substrate particles. Small stones were least abundant in riffle habitat, as were

gravelly substrates and sand which were most abundant in pools. Physical habitat diversity was significantly higher in glides than in riffles, but pools did not vary significantly from either. There was no significant variation in substrate dominance, although clearly particle size distribution varied significantly in response to flow regime. In general, these data agree with the normally-found response of current velocities to varying proportions of substrate morphologies (Table 2.11 - Table 2.15, inclusive).

There was no significant variation in water physico-chemical parameters between flow regimes. Riffles were shallower habitats than glides and pools. Current velocities were highest in riffles, moderate in glides and slowest in pool habitats.

Variable	Pool		Glide		Riffle		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
% Boulders	35.8[a]	5.27	34.9[a]	4.81	51.9[b]	4.48	P<0.001***
% Large Stones	20.4	3.74	32.7	3.93	22.9	3.52	NS
% Small Stones	14.5	2.86	19.2	3.24	9.6	2.15	NS
% Gravel	20.6[a]	4.26	14.9[ab]	3.38	9.1[b]	2.38	P<0.05*
D (m)	0.15[a]	0.26	0.15[a]	0.29	0.11[b]	0.31	P<0.05*
K (m^{-1})	2.78	0.24	2.84	0.25	3.40	0.26	NS
$Z_{eu}^{1\%}$ (m)	0.27	0.24	0.26	0.26	0.23	0.27	NS
Z_{eu}:$D^{1\%}$	1.74	0.32	1.78	0.27	1.90	0.30	NS
$Z_{eu}^{3\%}$ (m)	0.91	0.26	0.89	0.27	0.74	0.27	NS
Z_{eu}:$D^{3\%}$	5.95	0.30	6.07	0.26	6.52	0.29	NS
pH	6.33	0.34	6.33	0.34	6.32	0.34	NS
Conductivity (μS cm^{-1})	45.9	0.19	45.8	0.19	45.7	0.19	NS
Water Temperature (^oC)	10.1	0.08	10.3	0.09	10.3	0.09	NS
Flow (m s^{-1})	0.009[a]	0.01	0.066[b]	0.02	0.164[c]	0.02	P<0.001***
Substrate diversity (Simpson's D)	3.15	0.17	3.30	0.17	2.71	0.26	NS
Substrate dominance (Berger-Parker)	0.42	0.02	0.41	0.03	0.52	0.06	NS
Hydromorphological diversity (Simpson's D)	3.20	0.17	3.40	0.17	2.87	0.27	NS

Table 2.7 Mean values (± 1 standard error) of normally distributed environmental habitat variables (data back-transformed where necessary) between flow regime (pool, glide, riffle habitats) in the Water of Dye sub-catchment (n = 135). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Variable	Pool		Glide		Riffle		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
% Boulders	13.3[a]	2.80	17.1[a]	3.12	34.2[b]	4.36	P<0.001***
% Large Stones	42.7	3.83	45.6	3.95	36.1	3.70	NS
% Small Stones	26.3	3.29	20.9	3.19	16.5	3.39	NS
% Gravel	19.4[a]	3.55	9.6[b]	2.02	4.3[b]	1.68	P<0.001***
D (m)	0.14[a]	0.26	0.13[a]	0.28	0.10[b]	0.27	P<0.05*
K (m^{-1})	2.25	0.28	2.27	0.23	2.43	0.26	NS
$Z_{eu}^{1\%}$ (m)	0.26	0.32	0.26	0.32	0.25	0.34	NS
Z_{eu}:$D^{1\%}$	1.90	0.35	2.02	0.34	2.50	0.44	NS
$Z_{eu}^{3\%}$ (m)	0.90	0.31	0.91	0.32	0.85	0.34	NS
Z_{eu}:$D^{3\%}$	6.54	0.35	6.98	0.33	8.70	0.43	NS
pH	7.04	0.09	6.93	0.08	6.84	0.08	NS
Conductivity (μS cm^{-1})	53.1	0.23	50.9	0.22	51.5	0.22	NS
Water Temperature (^oC)	11.2	0.12	11.1	0.11	11.2	0.11	NS
Flow (m s^{-1})	0.069[a]	0.02	0.205[b]	0.02	0.389[c]	0.02	P<.0001***
Substrate diversity (Simpson's D)	3.09	0.16	3.10	0.20	3.17	0.15	NS
Substrate dominance (Berger-Parker)	0.45	0.03	0.46	0.04	0.42	0.03	NS
Hydromorphological diversity (Simpson's D)	3.15	0.16	3.22	0.20	3.33	0.15	NS

Table 2.8 Mean values (± 1 standard error) of normally distributed environmental habitat variables (data back-transformed where necessary) between flow regime (pool, glide, riffle habitats) in the River Girnock sub-catchment (n = 135). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Variable	Pool		Glide		Riffle		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
% Boulders	7.3^{a}	3.12	14.9^{ab}	3.56	25.4^{b}	5.80	P<0.05*
% Large Stones	18.9	2.96	31.7	3.53	28.8	5.24	NS
% Small Stones	32.8	4.00	29.7	3.52	25.5	5.54	NS
% Gravel	38.6^{a}	4.45	18.6^{b}	3.06	12.3^{b}	4.47	P<0.001***
% Sand	14.2^{a}	3.45	3.1^{b}	1.52	0.10^{b}	0.10	P<0.001***
D (m)	0.12	0.36	0.14	0.26	0.11	0.38	NS
K (m^{-1})	2.79	0.23	2.40	0.22	2.60	0.30	NS
$Z_{eu}^{1\%}$ (m)	0.32	0.24	0.38	0.22	0.36	0.29	NS
Z_{eu}:$D^{1\%}$	2.90	0.33	2.64	0.25	3.22	0.33	NS
$Z_{eu}^{3\%}$ (m)	1.09	0.25	1.36	0.22	1.26	0.30	NS
Z_{eu}:$D^{3\%}$	9.78	0.32	9.47	0.24	11.43	0.33	NS
pH	7.54	0.20	7.57	0.18	7.59	0.26	NS
Conductivity (μS cm^{-1})	148.1	0.20	134.2	0.19	133.6	0.26	NS
Water Temperature (^{o}C)	9.5	0.07	8.7	0.06	8.4	0.09	NS
Flow (m s^{-1})	0.001^{a}	0.01	0.208^{b}	0.04	0.589^{c}	0.05	P<0.001***
Substrate diversity (Simpson's D)	3.50^{ab}	0.38	3.95^{a}	0.19	2.85^{b}	0.27	P<0.05*
Substrate dominance (Berger-Parker)	0.43^{a}	0.06	0.34b	0.02	0.47^{a}	0.05	P<0.01**
Hydromorphological diversity (Simpson's D)	3.55^{ab}	0.38	4.07^{a}	0.19	2.99^{b}	0.27	P<0.05*

Table 2.9 Mean values (± 1 standard error) of normally distributed environmental habitat variables (data back-transformed where necessary) between flow regime (pool, glide, riffle habitats) in the Knockan Burn sub-catchment (n = 135). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Variable	Pool		Glide		Riffle		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
% Boulders	19.6[a]	2.58	22.2[a]	2.32	37.1[b]	2.84	P<0.001***
% Large Stones	27.0[a]	2.23	36.2[b]	2.23	29.4[a]	5.24	P<0.001***
% Small Stones	24.5[a]	2.07	24.0[a]	1.99	16.1[b]	2.10	P<0.001***
% Gravel	26.3[a]	2.48	14.8[b]	1.72	8.0[c]	1.55	P<0.001***
% Sand	4.8[a]	1.30	1.2[b]	0.62	0.03[c]	0.03	P<0.001***
D (m)	0.14[a]	0.17	0.14[a]	0.16	0.11[b]	0.18	P<0.01**
K (m^{-1})	2.60	0.15	2.48	0.14	2.80	0.16	NS
$Z_{eu}^{1\%}$ (m)	0.28	0.16	0.30	0.15	0.26	0.18	NS
Z_{eu}:$D^{1\%}$	2.13	0.20	2.18	0.17	2.40	0.22	NS
$Z_{eu}^{3\%}$ (m)	0.96	0.16	1.06	0.16	0.89	0.18	NS
Z_{eu}:$D^{3\%}$	7.26	0.19	7.57	0.16	8.34	0.22	NS
pH	6.97	0.07	7.01	0.06	6.82	0.08	NS
Conductivity ($\mu S\ cm^{-1}$)	71.5	0.16	72.8	0.15	62.0	0.16	NS
Water Temperature (^oC)	10.6	0.06	9.7	0.06	10.1	0.07	NS
Flow ($m\ s^{-1}$)	0.062[a]	0.01	0.289[b]	0.02	0.465[c]	0.03	P<0.001***
Substrate diversity (Simpson's D)	3.25[ab]	0.15	3.45[a]	0.13	2.92[b]	0.13	P<0.05*
Substrate dominance (Berger-Parker)	0.43	0.03	0.40	0.02	0.47	0.03	NS
Hydromorphological diversity (Simpson's D)	3.30[ab]	0.15	3.56[a]	0.13	3.07[b]	0.14	P<0.05*

Table 2.10 Mean values (± 1 standard error) of normally distributed environmental habitat variables (data back-transformed where necessary) between flow regime (pool, glide, riffle habitats) for amalgamated sub-catchment data (the Water of Dye, River Girnock, and Knockan Burn, n = 405). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

2.6.4 Response of habitat characteristics in the Water of Dye, River Girnock and Knockan Burn to variation in substrate morphology

2.6.4.1 Amalgamated sub-catchment data

Streambed habitats lacking boulders were significantly higher in light availability (Z_{eu}:D), pH and conductivity, compared to habitats wherein boulders were present (Table 2.11 and Appendix 2a). Current velocities increased significantly in response to increased boulder cover, and were lowest where boulders were absent (Table 2.11). Variation in the proportion of large stones did not appear to have a significant effect on stream habitat conditions, including flow (Table 2.12). An increase in the proportion of finer substrate particles (such as small stones, gravel and sand) were associated with a significant increase in Z_{eu}:D (small stones only), pH and conductivity, and a significant reduction in mean current velocities (Table 2.13, Table 2.14 and Table 2.15, respectively). Overall, physical habitat diversity exhibited a significant humpback response to increasing proportions of substrate particles, with substrate dominance showing the inverse relationship. At the extreme proportions of streambed cover (e.g. 0% and 88%), physical habitat diversity was lowest and substrate dominance highest. At intermediate proportions of substrate particles (e.g. 15.5% - 38%), most commonly associated with moderate flows or glides, physical habitat diversity was highest with no single particle size-class predominating above the others in terms of streambed substrate composition. This again reflects the significant hydromorphological interactions occurring in upland stream habitats and supports previous findings in this chapter that low energy habitats are predominated by finer particles and high flow zones are defined by larger substrate morphologies, with both inherently low in terms of physical habitat diversity. On the other hand, moderate velocity conditions tend to possess a mixed composition of substrate particles and greater physical habitat diversity. Details are provided in Table 2.11 - Table 2.15, inclusive.

Variable	0% BO		3% BO		15.5% BO		38% BO		63% BO		88% BO		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
D (m)	0.12	0.06	0.14	0.11	0.13	0.09	0.14	0.09	0.14	0.06	0.13	0.09	NS
K (m^{-1})	2.65	0.14	2.86	0.23	2.47	0.21	2.34	0.25	2.54	0.23	2.86	0.29	NS
$Z_{eu}^{1\%}$ (m)	0.31	0.15	0.26	0.28	0.25	0.24	0.29	0.28	0.28	0.22	0.25	0.31	NS
Z_{eu}:$D^{1\%}$	2.82^{a}	0.19	1.91^{b}	0.31	2.04^{b}	0.28	2.07^{b}	0.34	2.00^{b}	0.24	1.78^{b}	0.36	P<0.001***
$Z_{eu}^{3\%}$ (m)	1.12	0.16	0.91	0.29	0.84	0.25	1.04	0.29	0.96	0.23	0.81	0.30	NS
Z_{eu}:$D^{3\%}$	9.81^{a}	0.18	6.70^{b}	0.29	6.83^{b}	0.27	7.33^{b}	0.34	6.87^{b}	0.23	6.10^{b}	0.35	P<0.001***
pH	7.27^{a}	0.05	6.68^{b}	0.10	6.85^{b}	0.10	6.84^{b}	0.12	6.71^{b}	0.11	6.80^{b}	0.14	P<0.001***
Conductivity ($\mu S\ cm^{-1}$)	95.9^{a}	0.05	52.3^{b}	0.06	59.8^{b}	0.06	56.6^{b}	0.08	59.9^{b}	0.07	65.0^{b}	0.10	P<0.001***
Water Temperature (^{o}C)	10.3	0.06	9.7	0.11	10.8	0.09	9.8	0.13	9.6	0.09	10.1	0.12	NS
Flow (ms^{-1})	0.190^{a}	0.02	0.241^{ab}	0.03	0.210^{ab}	0.03	0.288^{b}	0.03	0.323^{b}	0.03	0.268^{b}	0.04	P<0.01**
Substrate diversity (Simpson's D)	2.04^{a}	0.06	1.97^{a}	0.09	2.35^{b}	0.10	2.59^{b}	0.09	1.62^{c}	0.05	1.18^{d}	0.05	P<0.001***
Substrate dominance (Berger-Parker)	0.65^{a}	0.02	0.68^{a}	0.03	0.60^{a}	0.02	0.49^{b}	0.02	0.77^{c}	0.02	0.93^{d}	0.02	P<0.001***
Hydromorphological diversity (Simpson's D)	2.11^{a}	0.06	2.05^{a}	0.09	2.45^{b}	0.10	2.71^{b}	0.09	1.71^{c}	0.05	1.23^{d}	0.05	P<0.001***

Table 2.11 Mean values (± 1 standard error) of normally distributed environmental habitat variables (data back-transformed where necessary): response to variation in the abundance (median % cover) of predominant boulder (BO) morphologies from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 405). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Variable	0% LS		3% LS		15.5% LS		38% LS		63% LS		88% LS		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
D (m)	0.14	0.08	0.12	0.10	0.12	0.08	0.12	0.07	0.14	0.07	0.16	0.15	NS
K (m^{-1})	2.70	0.20	2.54	0.25	2.70	0.20	2.58	0.17	2.58	0.18	2.45	0.38	NS
$Z_{eu}^{1\%}$ (m)	0.28	0.21	0.31	0.29	0.26	0.22	0.29	0.19	0.27	0.22	0.35	0.38	NS
Z_{eu}:$D^{1\%}$	2.00	0.26	2.46	0.37	2.25	0.25	2.53	0.22	1.98	0.23	2.17	0.46	NS
$Z_{eu}^{3\%}$ (m)	0.98	0.21	1.07	0.29	0.88	0.22	1.03	0.18	0.92	0.23	1.23	0.39	NS
Z_{eu}:$D^{3\%}$	6.93	0.25	8.63	0.36	7.62	0.24	8.93	0.22	6.72	0.22	7.63	0.45	NS
pH	6.98	0.10	6.96	0.12	6.91	0.09	6.97	0.09	6.92	0.07	7.10	0.14	NS
Conductivity ($\mu S\ cm^{-1}$)	71.7	0.08	74.2	0.11	66.7	0.07	73.6	0.06	64.5	0.05	72.0	0.14	NS
Water Temperature (^oC)	10.5	0.08	9.4	0.10	10.4	0.09	10.4	0.07	9.7	0.08	9.9	0.21	NS
Flow (ms^{-1})	0.193	0.03	0.284	0.04	0.226	0.03	0.227	0.03	0.256	0.03	0.270	0.05	NS
Substrate diversity (Simpson's D)	1.42^a	0.06	1.49^a	0.07	2.18^b	0.08	2.55^c	0.05	2.05^b	0.07	1.41^a	0.07	P<0.001***
Substrate dominance (Berger-Parker)	0.84^a	0.02	0.82^a	0.02	0.63^b	0.02	0.50^c	0.01	0.67^b	0.02	0.84^a	0.03	P<0.001***
Hydromorphological diversity (Simpson's D)	1.47^a	0.06	1.56^a	0.07	2.27^b	0.08	2.66^c	0.05	2.13^b	0.07	1.46^a	0.08	P<0.001***

Table 2.12 Mean values (± 1 standard error) of normally distributed environmental habitat variables (data back-transformed where necessary): response to variation in the abundance (median % cover) of predominant large stone (LS) morphologies from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 405). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Variable	0% SS		3% SS		15.5% SS		38% SS		63% SS		88% SS		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
D (m)	0.13	0.06	0.14	0.07	0.13	0.08	0.12	0.09	0.11	0.09	0.10	0.33	NS
K (m^{-1})	2.93	0.18	2.43	0.20	2.59	0.17	2.47	0.21	2.67	0.22	2.54	0.55	NS
$Z_{eu}^{1\%}$ (m)	0.25	0.20	0.30	0.21	0.28	0.19	0.31	0.22	0.28	0.27	0.32	0.63	NS
Z_{eu}:$D^{1\%}$	1.86^{a}	0.23	2.05^{ab}	0.23	2.22^{ab}	0.23	2.61^{ab}	0.28	2.53^{ab}	0.30	3.32^{b}	0.76	P<0.05*
$Z_{eu}^{3\%}$ (m)	0.87	0.20	1.03	0.21	0.97	0.20	1.05	0.23	0.97	0.27	1.14	0.64	NS
Z_{eu}:$D^{3\%}$	7.48^{a}	0.22	7.54^{a}	0.23	7.58^{a}	0.23	8.95^{ab}	0.27	9.04^{ab}	0.29	10.15^{b}	0.72	P<0.05*
pH	6.70^{a}	0.10	6.78^{a}	0.08	7.05^{ab}	0.08	7.01^{ab}	0.08	7.21^{b}	0.08	7.53^{b}	0.17	P<0.001***
Conductivity ($\mu S\ cm^{-1}$)	61.9^{a}	0.07	60.9^{a}	0.06	70.4^{ab}	0.06	68.8^{ab}	0.07	85.3^{b}	0.08	136.8^{b}	0.17	P<0.001***
Water Temperature (^{o}C)	10.4	0.08	9.7	0.09	10.5	0.08	9.7	0.10	10.4	0.11	8.9	0.17	NS
Flow (ms^{-1})	0.273^{a}	0.03	0.292^{a}	0.03	0.205^{b}	0.03	0.179^{b}	0.03	0.229^{b}	0.03	0.209^{b}	0.05	P<0.05*
Substrate diversity (Simpson's D)	1.33^{a}	0.04	1.72^{b}	0.05	2.27^{c}	0.07	2.69^{d}	0.07	2.14^{c}	0.07	1.70^{ab}	0.18	P<0.001***
Substrate dominance (Berger-Parker)	0.86^{a}	0.02	0.74^{b}	0.02	0.61^{c}	0.02	0.49^{d}	0.01	0.62^{c}	0.02	0.76^{ab}	0.06	P<0.001***
Hydromorphological diversity (Simpson's D)	1.40^{a}	0.04	1.81^{b}	0.05	2.36^{c}	0.07	2.77^{d}	0.07	2.21^{c}	0.07	1.76^{ab}	0.18	P<0.001***

Table 2.13 Mean values (± 1 standard error) of normally distributed environmental habitat variables (data back-transformed where necessary): response to variation in the abundance (median % cover) of predominant small stone (SS) morphologies from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 405). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Variable	0% GR		3% GR		15.5% GR		38% GR		63% GR		88% GR		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
D (m)	0.12	0.05	0.14	0.07	0.13	0.08	0.12	0.11	0.12	0.16	0.15	0.17	NS
K (m^{-1})	2.72	0.14	2.48	0.18	2.43	0.19	2.51	0.24	2.56	0.28	2.37	0.45	NS
$Z_{eu}^{1\%}$ (m)	0.26	0.17	0.30	0.18	0.27	0.24	0.31	0.26	0.32	0.30	0.40	0.44	NS
Z_{eu}:$D^{1\%}$	2.16	0.20	2.26	0.21	2.19	0.26	2.43	0.28	2.67	0.46	2.72	0.51	NS
$Z_{eu}^{3\%}$ (m)	0.98	0.17	1.01	0.19	0.94	0.24	1.05	0.28	1.12	0.30	1.37	0.44	NS
Z_{eu}:$D^{3\%}$	7.33	0.19	7.40	0.20	7.24	0.25	8.51	0.29	9.75	0.44	9.83	0.50	NS
pH	6.73^{a}	0.08	6.80^{a}	0.08	7.15^{b}	0.06	7.22^{b}	0.07	7.07^{ab}	0.14	7.56^{b}	0.15	P<0.01**
Conductivity (μS cm^{-1})	61.3^{a}	0.05	60.7^{a}	0.05	75.9^{ab}	0.08	86.1^{ab}	0.09	75.7^{ab}	0.11	120.6^{b}	0.15	P<0.001***
Water Temperature (^{o}C)	10.6	0.06	9.6	0.08	9.7	0.08	10.3	0.12	10.5	0.13	9.9	0.18	NS
Flow (ms^{-1})	0.290^{a}	0.02	0.283^{a}	0.02	0.187^{b}	0.03	0.193^{b}	0.03	0.133^{b}	0.04	0.129^{b}	0.06	P<0.001***
Substrate diversity (Simpson's D)	1.51^{a}	0.04	1.86^{b}	0.06	2.49^{c}	0.07	2.78^{d}	0.09	2.41^{c}	0.09	1.58^{ab}	0.11	P<0.001***
Substrate dominance (Berger-Parker)	0.80^{a}	0.02	0.70^{b}	0.02	0.56^{c}	0.02	0.48^{d}	0.02	0.55^{c}	0.02	0.78^{ab}	0.04	P<0.001***
Hydromorphological diversity (Simpson's D)	1.59^{a}	0.04	1.95^{b}	0.06	2.57^{c}	0.07	2.86^{d}	0.09	2.47^{c}	0.09	1.62^{ab}	0.11	P<0.001***

Table 2.14 Mean values (± 1 standard error) of normally distributed environmental habitat variables (data back-transformed where necessary): response to variation in the abundance (median % cover) of predominant gravel (GR) morphologies from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 405). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Variable	0% SA		3% SA		15.5% SA		38% SA		63% SA		88% SA		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
D (m)	0.13	0.03	0.11	0.26	0.12	0.28	0.11	0.49	0.10	0.39	0.37	0.12	NS
K (m^{-1})	2.59	0.10	2.80	0.38	2.58	0.45	3.01	0.58	3.69	0.85	2.64	0.73	NS
$Z_{eu}^{1\%}$ (m)	0.28	0.10	0.31	0.38	0.34	0.44	0.30	0.56	0.24	1.07	0.33	0.71	NS
Z_{eu}:$D^{1\%}$	2.09	0.12	2.72	0.64	2.60	0.56	2.753	1.08	2.65	0.92	1.84	0.72	NS
$Z_{eu}^{3\%}$ (m)	0.97	0.11	1.08	0.40	1.12	0.46	1.00	0.60	0.80	0.98	1.13	0.77	NS
Z_{eu}:$D^{3\%}$	7.57	0.12	8.09	0.61	8.45	0.55	8.19	0.98	8.59	0.83	7.91	0.70	NS
pH	6.89^{a}	0.04	7.39^{b}	0.06	7.40^{b}	0.07	7.54^{b}	0.18	7.32^{b}	0.03	7.39^{b}	0.11	P<0.001***
Conductivity (μS cm^{-1})	64.6^{a}	0.03	124.9^{b}	0.07	128.7^{b}	0.08	151.6^{b}	0.15	122.1^{b}	0.23	127.4^{b}	0.14	P<0.001***
Water Temperature (°C)	10.1	0.04	9.5	0.15	9.9	0.17	10.7	0.25	9.0	0.57	9.5	0.36	NS
Flow (ms^{-1})	0.250^{a}	0.01	0.196^{a}	0.05	0.087^{b}	0.06	0.055^{b}	0.06	0.009^{b}	0.02	0.005^{b}	0.08	P<0.001***
Substrate diversity (Simpson's D)	1.94^{a}	0.04	2.02^{a}	0.16	2.94^{b}	0.17	2.81^{b}	0.31	2.32^{ab}	0.72	1.58^{a}	0.19	P<0.01**
Substrate dominance (Berger-Parker)	0.69^{a}	0.01	0.67^{a}	0.05	0.47^{b}	0.04	0.48^{b}	0.06	0.62^{ab}	0.15	0.75^{a}	0.09	P<0.01**
Hydromorphological diversity (Simpson's D)	2.03^{a}	0.04	2.07^{a}	0.16	3.01^{b}	0.18	2.85^{b}	0.32	2.36^{ab}	0.72	1.62^{a}	0.19	P<0.01**

Table 2.15 Mean values (± 1 standard error) of normally distributed environmental habitat variables (data back-transformed where necessary): response to variation in the abundance (median % cover) of predominant sand (SA) morphologies from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 405). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

2.6.5 Relationships between environmental habitat conditions

Output of Pearson product-moment correlations can be found in Appendix 2a (note that only significant relationships are shown and discussed).

2.6.5.1 Underlying geology and substrate morphology

In terms of substrate morphology, granite and mica schist geologies were significantly positively correlated with high boulder cover, and negatively correlated with streambed particles of smaller size. Large stones tended to occur in streambeds underlain with granodiorite, diorite, amphibolite, serpentinite, QP, DA, QPP and mixed limestone, wherein boulder cover was markedly reduced. Moine Thrust geologies (Durness limestone, Moine schist, ESG, ACF and An-T'Sron) were significantly positively correlated with finer substrate particles: small stones, gravel and sand, and generally lacking significant cover of boulders and large stones. Streambeds characterised by a predominance of large-sized particle substrates (e.g. boulders, large stones) had low physical habitat diversity. Conversely, smaller-sized particles (e.g. small stones, gravel, sand) and assorted substrate morphologies increased physical habitat diversity. Substrate dominance was inversely correlated with the physical habitat indices, substrate and hydromorphological diversity, which were highly positively correlated with one another.

2.6.5.2 Underlying geology and water physico-chemistry

Boulder-dominated streambeds underlain with granite and granodiorite exhibited significant negative correlations with pH, conductivity and alkalinity, compared to mica schist, diorite, amphibolite, serpentinite, QP, DA, QPP, mixed limestone and Moine Thrust geologies, often had diverse streambed substrate morphologies and showed significant positive relationships to these physico-chemical

parameters. There were no significant correlations between depth, water temperature, stream flow and underlying geology.

2.6.5.3 Underlying geology and water chemistry

Sulphate, cadmium, chromium, copper, lead, nickel, zinc, aluminium, vanadium, iron and manganese were significantly positively correlated with granite and granodiorite geologies, and negatively related to mica schist, diorite, amphibolite, serpentinite, QP, DA, QPP, mixed limestone and Moine Thrust geologies. The opposite relationship was found for potassium, calcium and magnesium, which exhibited a negative relationship with base-poor geologies such as granite and granodiorite, and increased with the occurrence of other more base-rich geologies. Sodium chloride showed a negative relationship with R. Dee sub-catchment geologies and a positive relationship with the Moine Thrust Zone. This is probably due to the regional variation in these sub-catchments, with Knockan Burn situated closer to the sea and therefore more exposed to atmospheric sea salt deposition, rather than reflecting the predominant underlying geologies present.

2.6.5.4 Other environmental habitat relationships

Increased streamwater depths were negatively correlated with Z_{eu}:D, pH, conductivity, and alkalinity levels as well as temperature: characteristic of high discharge events during the winter and spring months. Light regime factors (K, Z_{eu} and Z_{eu}:D) were highly correlated with one another. As expected, increased light attenuation exerted a negative effect on the euphotic depth and Z_{eu}:D ratio. Thus highly coloured waters (typical of acid-sensitive storm flow events: characterised by low pH, conductivities and alkalinities, with accentuated heavy metal availability) limited the proportion of light penetrating into the benthic habitat by absorbing wavelengths of incoming solar radiation. Values for depth of euphotic zone and Z_{eu}:D generally increased during summer groundwater

baseflows (and were positively correlated with pH, conductivity and alkalinity etc.), though these values were restrained by shade and the height of riparian vegetation. Increasing pH, conductivity and alkalinity showed strong significant positive correlation to increased streamwater temperatures and to each other. Increasing pH, conductivities and alkalinities were also strongly significantly positively correlated to increasing streamwater concentrations of potassium, calcium and magnesium, but demonstrated negative relationships with sulphate and the abundance of other heavy metals (Cd, Cr, Cu, Pb, Ni, Zn, Al, V, As, Fe and Mn).

Similarly, NaCl was negatively related to SO_4 and heavy metals, except potassium, calcium and magnesium, to which a positive correlation was found. However, this was to do with differences in regional distribution of salty deposition from the Atlantic Ocean and the North Sea (affecting the NW and NE of the country respectively) and natural variation in water chemistry between the three streams.

Phosphate levels rose significantly in the spring in response to major flush events, characterised by increased streamwater depth, and significant reductions in streamwater conductivity, alkalinity, temperature, Ca and Mg.

The majority of heavy metal cations (e.g. Cd, Cr, Cu, Pb, Ni, Zn, Al, V, Fe and Mn) were strongly significantly positively correlated to the abundance of sulphate and to one another, but were significantly negatively related to the occurrence of K^+, Ca, and Mg. Potassium, calcium and magnesium were strongly significantly positively correlated to each other and increased water temperatures.

Increased water temperatures showed a negative relationship with flow, and a positive relationship with shade. Shade was significantly positively correlated with the height of riparian vegetation.

High flows were positively correlated with coarse, robust substrates such as boulders, and had negative relationship with the abundance of finer streambed

particles (small stones, gravel and sand). Thus, high current velocities exerted a negative effect on physical habitat diversity, favouring the predominance of large-sized substrates and reduced the occurrence of smaller substrate particles, which prevailed under low flow conditions.

2.7 Discussion

2.7.1 Habitat characteristics of the Water of Dye, River Girnock and Knockan Burn; their catchments and sites

As expected, geological composition varied significantly between the Water of Dye, River Girnock and Knockan Burn sub-basins. This indicates that the study has captured at least part of the range of geologies characteristic of the Scottish Highlands. The incorporation of RHS guidelines and predominant geologies into this study has proven invaluable in explaining natural variation in streambed substrate features and environmental habitat conditions between the three streams sampled. Brocky Burn, Charr Flume, Bogendreip (Water of Dye), and Iron Bridge (an upper tributary of the River Girnock), are all granite dominated streams (Cluster 1). Hampshire's Bridge and Littlemill of the River Girnock possess more base-rich strata (e.g. amphibolite, serpentinite, calcareous limestone) in their underlying geology (Cluster 2). The three sites along Knockan Burn are characterised by Moine Thrust Zone geologies and form their own grouping (Cluster 3). The three streams thus cover a reasonable sub-sample of the rock catchment types of Highland Scotland.

Extensive boulder cover characterised stable streambed morphology of the Water of Dye and upper Girnock (Cluster 1). The predominance of streambed boulder morphology at Brocky Burn, Charr Flume, Bogendreip and Iron Bridge coincides with resistant granitic formations: the principal geology occurring in the Cairngorm region of Scotland (Trewin 2002). Low geochemical erosion of granite favours formation of bouldery streambed architecture. Therefore, stable

streambed morphology is a direct product of the predominance of hard-weathering, resistant rock types such as granite in the underlying geology (Gordon *et al*. 2004). Differing from Cluster 1 sites, the main channel of the River Girnock (Cluster 2: HB and LM) featured a significantly higher proportion of large stones, as well as the occurrence of many small stones. The cobbled streambed feature observed here reflects the incidence of softer geologies in this part of the basin wherein metamorphic (e.g. amphibolite, serpentinite) and sedimentary (mixed limestone) strata occurred. Softer, more weatherable rock types are naturally eroded by flow and downsized into cobbled substrates (Gordon *et al*. 2004). The highly calcareous geologies of the Moine Thrust Zone are more vulnerable to the effects of geochemical weathering processes. Consequently, these rock types are easily fragmented into much finer substrates. This explains the notable lack of larger streambed structures and predominance of smaller sized streambed particles such as cobbles, gravel and sands characterising Knockan Burn (Cluster 3: UK, MK and LK), and generally greater physical habitat diversity of this stream. Overall, the observed substrate distributions between the three multivariate clusters were not unexpected. Streambed substrate particle composition was clearly related to the predominant geologies and their varying predisposition to naturally erode.

The three sub-catchment streams were also comparable in terms of depth, but varied in underwater light climate. This indicated that an environmental factor other than streamwater depth was affecting underwater light availability. The extent of peat cover varies between the three sub-catchments, with the largest expanse (c. 65%) associated with the Water of Dye, which drains terrain dominated by carbon-rich peat moorland (Dawson *et al.* 2001, 2004, Soulsby *et al.* 2003). The River Girnock is essentially peat poor (c. 5%) with most of the sub-catchment covered with peat podzols and peaty gleys (Soulsby *et al.* 2007, Tetzlaff *et al.* 2007a). Peat occupies c. 25% of the Knockan sub-catchment, along with peaty podzols and brown rendzinas (The Macaulay Institute for Soil Research, 1981). Thus Water of Dye streamwaters are predisposed to stronger light attenuations

(K) through the high cover of peaty soils and blanket bog habitat, releasing organic materials into the streams which colour the water (Soulsby *et al.* 2003, Dawson *et al*. 2004). Overland drainage produces highly whisky-brown coloured streamwaters consistent with this and explains the significantly lower values of Z_{eu} and Z_{eu}:D obtained. Brocky Burn had significantly higher light attenuation values compared to the other eight sites of the study, almost certainly because this stream drains peat bog dominated headlands of the Water of Dye sub-catchment, with a high abundance of organic matter (OM), derived from peaty soil water, explaining the strong streamwater colouration here. Previous research has demonstrated these streamwaters as having high OM content (Dawson *et al*. 2001, 2004). It would be anticipated that the River Girnock and Knockan Burn streamwaters will experience reduced concentrations of in-stream OM as these two basins score lower on peat cover and are thus expected to have lower light attenuation coefficients. It is perhaps relevant to note that accurate light measurements can be difficult to obtain in fast-flowing shallow streams such as those sampled in this study (reviewed in Westlake 1965). Given the associated practical difficulties (see Westlake 1965) then I accept that the method used to measure light in field may have incurred considerable errors. Having said this, the main focus was to determine the strength of underwater light attenuation (due to water colouration, not light intensity) and whether benthic light ever approached limiting reductions.

Significant differences in streamwater physico-chemistry between the three target streams coincide with natural variation in basin geology and proportion of peat cover (as described by Langan *et al.* 1997, Smart *et al.* 1998, Dawson *et al.* 2001, 2004, Soulsby *et al.* 2003, Rogers *et al*. 2005, Tetzlaff *et al*. 2007b). Water of Dye geology is relatively homogenous wherein c. 85% of the basin is granite dominated (Dawson *et al.* 2001, 2004, Soulsby *et al.* 2003, Rogers *et al.* 2005). Some metamorphic mica schist occurs in the southern region of the sub-catchment (Dawson *et al.* 2004, Soulsby *et al.* 2003). Peatland occupies 65% of the Water of Dye drainage basin. The remainder of soils are mostly peaty podzols and an

assortment of gleys and humic-ferric iron podzols (Dawson *et al.* 2001, 2004, Soulsby *et al.* 2003). Overland flows through acidic soil horizons are known to profoundly affect streamwater hydrochemistry (Soulsby *et al.* 2002b, 2003, 2007, Tetzlaff *et al*. 2007b). The acidic streamwaters of the Water of Dye are strongly influenced by the high proportion of base-poor granite combined with overland drainage of acidic peaty soils occupying the sub-catchment (Langan *et al.* 1997, Dawson *et al.* 2001, 2004, Tetzlaff *et al*. 2007b). This is most discernible in the upper regions of the drainage basin at Brocky Burn where the lowest pH, conductivities and alkalinities were observed. The Water of Dye is characteristic of an acid-sensitive upland river system. Igneous geologies such as granite exhibit poor-weathering properties. This explains the low concentrations of calcium and magnesium, and low alkalinities recorded in this stream: other recent work has reported similar findings for the Water of Dye (Langan *et al.* 1997, Smart *et al.* 1998, Dawson *et al.* 2001, Soulsby *et al.* 2003, Rogers *et al*. 2005, Tetzlaff *et al*. 2007b). The underlying geologies of Charr Flume and Bogendreip in the Water of Dye contain varying proportions of base-rich mica-schist (Soulsby *et al.* 2003, Dawson *et al.* 2004, Rogers *et al*. 2005). Base-rich parental rock types such as mica schist contribute higher concentrations of base cations (e.g. Ca^{2+}) through biogeochemical weathering processes. This accounts for the circumneutral pH and higher alkalinities observed at these two sites compared to Brocky Burn, again consistent with other research (Langan *et al.* 1997, Smart *et al.* 1998, Soulsby *et al.* 2003, Dawson *et al.* 2004, Tetzlaff *et al*. 2007b). In the River Girnock, underlying rock types deviate from granite to varying proportions of base-rich strata. Granite accounts for approximately 50% of the geology in the River Girnock, a substantial proportion of which is concentrated in the upper South Western region of the basin allied to Lochnagar and upstream of Iron Bridge (Soulsby *et al.* 2007). The upper Girnock sampling site, Iron Bridge, is characterised by low pH, conductivities and alkalinities (e.g. c. 200-300 $\mu eq\ l^{-1}$: Soulsby *et al.* 2007) at base flow. This reflects the predominance of granite (c. 86%) in this region of the sub-catchment and acid leaching properties of the peaty soils, reducing alkalinities during storm events (Dawson *et al.* 2001, Soulsby *et al.* 2007, Tetzlaff *et al*. 2007a).

By comparison, the main stem of the River Girnock has a lower proportion of granite and catchment soils are relatively peat poor. Base-rich strata such as calcareous limestone and amphibolite as well as magnesium-rich serpentinite occur in these parts (Soulsby *et al.* 2003, 2007, Tetzlaff *et al*. 2007a). Base-rich groundwater sources derived from adjoining tributaries, the East and Camlet Burns, exert a strong influence on stream physico-chemistry (Soulsby *et al.* 2007, Tetzlaff *et al*. 2007a). Respectively, these streams are underlain with base-rich strata: amphibolite and ultra-basic Mg-rich serpentinite that contribute increased loads of Ca^{2+} and Mg^{2+} to the main channel of the River Girnock. This produces the high base flow alkalinities (c. 500-700 μeq l^{-1}) and pH values that characterise streamwaters at Hampshire's Bridge and Littlemill (Langan *et al.* 1997, Soulsby *et al.* 2007, Tetzlaff *et al*. 2007a). The Knockan Burn basin is dominated by Moine Thrust Zone geology, containing base-rich strata (e.g. dolomitic limestone) high in calcium magnesium carbonate minerals (Trewin 2002). Streamwater physico-chemistry is similar in the upper and mid basins. However, pH, conductivity, and alkalinity rise dramatically in the lower region of the Knockan sub-catchment. This reflects groundwater sources that have passed through a band of highly calcareous Ant-S'ron rocks containing fossilised shells of prehistoric marine fauna (Trewin 2002). Such rock types are prone to mineral weathering and contribute higher inputs of Ca^{2+} and Mg^{2+}, explaining the observations at Lower Knockan. The lack of significant variation in potassium concentrations between the three streams probably reflects the mineralogy of the different catchment geologies containing varying proportions of K^{+} feldspars.

Knockan Burn exhibited the coldest mean streamwater temperatures, and the River Girnock the warmest. This may not reflect the climatic differences one would expect to occur between the NW and NE Highlands of Scotland. Overall, Scotland experiences a high latitude temperate climate. A maritime climate prevails over the West coast of Scotland, which is strongly influenced by the Atlantic, tending to be wetter and generally warmer due to the influence of the Gulf Stream (see Appendix 3b and 3f, respectively). On the other hand, the North

East coast of Scotland tends to be drier and colder due to the North Sea influence (see Appendix 3a and 3e, respectively). Streamwater temperature is affected by a number of factors for example, variation in the incident light due to the effects of topography, aspect, slope and afforestation (Malcolm *et al.* 2004). The differences in mean streamwater temperature between the target streams can probably be attributed to temporal variation (e.g. prevailing weather conditions) between sampling dates. This would also explain strongly significant inter-site differences in mean streamwater temperature. Records may also have been affected by time of day when sampling was undertaken, as streamwater temperatures exhibit diurnal variation (Malcolm *et al.* 2004). For example, due to the sampling routine, Hampshire's Bridge was often sampled at midday when solar radiation was most intense, and purely by coincidence, also on brighter days with clearer skies.

The lack of marked variation in flow regime between the Water of Dye and River Girnock may not be surprising, as both streams are tributaries of the R. Dee, and lie in relative close proximity to one another. The sub-catchments experience similar microclimatic conditions (Appendix 3a), receiving of approximately 1110-1130 mm precipitation per annum although a significant proportion is often locked-up in snowpacks (Helliwell *et al.* 1998, Dawson *et al.* 2001, Malcolm *et al.* 2004, Soulsby *et al.* 2003, 2007, Tetzlaff *et al.* 2007b). However, stream velocities in the Knockan sub-catchment were notably fiercer in comparison. Typically, the West coast of Scotland experiences milder, wetter climatic conditions (Appendix 3b) than the East, due to the oceanic Atlantic influence with an average of c. 1900 mm annual precipitation (Gordon *et al*. 2004). This would certainly contribute to the rapid flows characterising Knockan Burn, but the steeper slope of Mid Knockan was undoubtedly also a contributory factor.

The low values obtained for nitrate-nitrogen, ammonia-nitrogen and phosphate indicate that the three target streams were of exceptionally high water quality, with oligotrophic status and in near-pristine condition. The R. Dee basin is classed as an oligotrophic system (Benzie *et al.* 1991), and findings of this study suggest

Knockan Burn is of similar trophic status although certainly richer in calcium and magnesium content.

Sodium and chloride concentrations were highest in Knockan Burn streamwaters. As primary components of sea-salt (Moldan & Černý 1994), Na and Cl ions were very highly correlated with each other. The findings suggest that atmospheric deposition of sodium chloride is similar between the two R. Dee sub-catchments, occurring approximately 25 and 60 km inland, respectively, from the North Sea coastline. However, Knockan Burn is situated relatively close to the North Atlantic coastline and is therefore likely to be more exposed to sea water components and expected to exhibit higher in-stream content of sodium chloride deposits. Note however that the sodium signal is dampened and not significantly different from concentrations occurring in the Water of Dye and River Girnock. Na^+ is susceptible to adsorption by soil cation exchange processes which curbs sodium seasonality (Neal & Kirchner 2000). In comparison, the chloride signal is apparent as anions are not readily bound to cation exchange sites (Neal & Kirchner 2000).

The observed differences in sulphate concentrations between the Water of Dye, River Girnock and Knockan Burn, suggests that the three streams exhibited differential buffering capacities in response to acid rain and overland run-off. This is accountable to spatial variation in basin geology and peat bog habitat. Water of Dye streamwaters are naturally acid-sensitive from draining an area dominated by base-poor geology and peat rich soils. In acid conditions SO_4 becomes highly available, and this explains its abundance in the Water of Dye. The calcium magnesium carbonate geologies of the River Girnock and Knockan Burn act as effective buffers against acidic rainfall and overland flows thereby reducing SO_4 availability (Moldan & Černý 1994, O'Neill 1998). This could account for the lower concentrations of sulphate recorded in these two streams. However, buffering capacity is more pronounced in the Knockan sub-catchment, given the extensive occurrence of calcareous dolomitic limestone and Ant-S'ron strata in this

region, both highly rich in Ca-Mg carbonates. Inter-site variation in streamwater SO_4 content was also significant, with the most acidic sites having higher sulphate concentrations. Refer to section 2.7.2 for further discussion of sulphate mobility and buffering capacities of streams.

Seven of the fifteen heavy metal determinands measured showed no significant variation between the three streams or PCA clusters. However, lead, zinc, aluminium, vanadium, iron and manganese became highly available in acid habitat conditions which characterised Cluster 1 sites (BB, CF, BD and IB), and were least soluble in streamwaters of high buffering capacity (e.g. Cluster 3). Concentrations of lead, zinc, aluminium, vanadium, iron and manganese were most abundant at Brocky Burn compared to all other sites (Table 2.2). This strongly implies an aspect of spatial variation in the availability of heavy metals related to geology and soil components affecting the chemistry of streamwaters. Base-poor granite and extensive peat bog habitat predominate in the headwaters of the Water of Dye, indicating that this site is particularly acid-sensitive and may be expected to exhibit elevated concentrations of heavy metal cations such as lead and aluminium (which become more available at low pH). Metals such as lead and aluminium are readily leached from peaty soils during run-off events and also occur in mineral constituents of granite (refer to section 2.7.2). Cluster 2 sites (HB and LM) of the River Girnock possess varying extents of base-rich geologies (e.g. amphibolite, serpentinite and metamorphic limestone) and have inherently greater buffering capacities. This probably explains the suppressed content of heavy metals and higher content of Ca^{2+} and Mg^{2+} occurring in these parts of the stream. Knockan Burn is underlain with highly calcareous base-rich strata comprised of calcium and magnesium carbonates. Weathering of these solutes from base-rich geologies such as dolomitic limestone and An-T'Sron indicate that the stream is highly buffered against the impacts of acid rain constituents. For further discussion on heavy metals refer to section 2.7.2.

Multivariate analyses indicated that the Water of Dye and upper Girnock were functionally similar in terms of stable streambed morphology and representative of base-poor acid-sensitive streams. Cluster 1 is characterised by high boulder cover, low pH, conductivity, Ca^{2+} and Mg^{2+} coupled to high sulphate and metal availability with particular emphasis on Pb, Zn, Al, V, Fe and Mn. Therefore Brocky Burn, Charr Flume, Bogendreip and Iron Bridge would be expected to support similar assemblages of aquatic flora based on their overlapping similarities in environmental habitat conditions. Cluster 2 contains two sites in the River Girnock (Hampshire's Bridge and Littlemill) exhibiting moderate buffering capacities and occupying habitat conditions intermediate between the Water of Dye and Knockan Burn, in terms of pH, conductivity, sulphate and metal cation concentrations. The streambed features large cobbles, and heavy shade from tall riparian vegetation is predominant in the lower basin (at LM). The aquatic biota occurring at HB and LM is expected to embody a transitional community between Clusters 1 and 3. Strongly buffered, relatively hard waters characterise Cluster 3 samples (Knockan Burn sites: UK, MK and LK) with high pH, and conductivity, and a predominance of calcium magnesium carbonates. The three sites of Knockan Burn are expected to host groups of aquatic vegetation communities more similar to one another, than to sampling sites associated with the other clusters.

2.7.2 Seasonal variation in environmental habitat conditions of the Water of Dye, River Girnock and Knockan Burn

In both the Water of Dye and River Girnock, most substrate particles did not respond significantly to seasonal changes in hydrological regime, except for small gravel sized particles. During the major flood event of May 2005, gravel was mobilised and transported downstream in the high flows. This probably accounts for the relatively low occurrence of gravel in these two streams after the heavy spring rains, and the significant negative relationship that gravel exhibits in

response to increased velocities. Conversely, the deposition of gravel occurred at reduced velocities similar to those experienced during summer base flows. Unlike gravel, larger streambed particles such as boulders are more resistant to the motional effects of high discharge events, for obvious reasons, explaining why no significant change in their distribution was observed between the seasons. These data agree with general theory on substrate stability and suggest that the 'lift off' thresholds of smaller streambed particles can be more easily overcome during periods of bedload movement (e.g. high discharges) than for bulkier immobile substrates (Gordon *et al*. 2004). Although changes in gravel cover were attributed to variation in flow regime, current velocities did not vary significantly between May and August 2005, with the highest velocities recorded in April 2006 when gravel cover was still high (for the Water of Dye) or between any of the sampling dates (for the River Girnock). One possible explanation for this is that these are snap-shot measurements and do not fully reflect long-term variations in discharge. In May 2005 sampling in the R. Dee sub-catchment occurred after a major spate event (brought on by prolonged heavy rainfall throughout the spring, during which time it would have been impossible to enter the rivers judging from the disturbance of the river banks and loss of artificial substrates) had subsided and flows associated with the flash flooding had dampened considerably. When sampling occurred in April 2006, weather conditions were generally dry and extremely cold. However, towards the end of sampling in the Water of Dye, winter weather conditions typical of Scottish Highlands had developed: blizzards of heavy sleet and snow storms which fed directly into the streams and began to cause a visible increase in surface flow. Gravel cover may not yet have responded to the increase in current velocity, explaining why the proportion recorded was similar to that occurring at lower discharges (August 2005). Perhaps flow response appears more subdued in the River Girnock in April 2006 because sampling in this sub-catchment had been completed prior to the drastic change in weather that occurred whilst sampling the Water of Dye. In the Knockan sub-catchment, fine sandy particles were highly responsive to variations in stream current velocities and were most abundant in the late summer (September) than in

the spring or winter seasons. Sands were probably most mobile under high flow conditions with spates limiting the occurrence of fine sandy substrates on the streambed. In Knockan Burn, gravel did not show the same significant responsiveness as that observed in the R. Dee catchment. This is probably attributed to the high variability associated with the standard errors of the mean values. Refer to section 2.7.3 for further discussion on streambed hydromorphological interactions.

Streamwater depth showed marked seasonal fluctuation in each of the three target streams. Deep streamwaters of April 2006 reflect inputs from spring snowmelt, typical of mountain streams in the Scottish Highlands (Langan *et al.* 1997, Helliwell *et al.* 1998, Smart *et al.* 2001, Soulsby *et al.* 2002a). Furthermore, heavy, variable and prolonged rainfall drives major spate events, explaining the frequent floods and variable stream depths maintained throughout the spring and autumn/winter which gradually become shallower as precipitation eases. Low streamwater levels in the summer are due to lower inputs from precipitation, and discharge, coupled to high climate (light, temperature and evaporation) associated with the summer solar maxima and represent base flow conditions recorded by other studies in the R. Dee catchment and similar highland basins (Langan *et al.* 1997, Helliwell *et al.* 1998, Smart *et al.* 1998, Soulsby *et al.* 2001, 2003, Malcolm *et al*. 2004).

Underwater light climate responded significantly to seasonal changes in the three sub-catchment streams. Z_{eu} is probably most restricted in the summer at sites experiencing heavy shade from riparian vegetation (e.g. trees, bracken), limiting the proportion of ambient light reaching the surface waters. Light attenuation (K) is strongly influenced by water colouration, which is a product of the amount of organic matter (OM) suspended or dissolved in the stream load. Precipitation events drive basin drainage, bringing in organic matter (especially from peaty soils) accounting for higher light attenuation values in May 2005 (after the heavy rains during which time the basin is still being leached). In the summer, lower

rainfall coupled to higher temperatures and evaporation rates produces base flow conditions. Lack of adequate precipitation arrests basin drainage reducing particulate organic matter input (Dawson *et al.* 2004). This results in improved light penetration and weaker light attenuation values. Thus Z_{eu}:D becomes significantly higher in summer base flow conditions in the Water of Dye and River Girnock. The high light attenuation values and low euphotic depth recorded in Knockan Burn during the summer can probably be attributed to fine sediments being resuspended into the water column. Nevertheless, Z_{eu}:D values did not vary between seasons indicating that Knockan Burn is not light limited by variations in water turbidity to the same extent as experienced in the R. Dee sub-catchment streams. However in these characteristically shallow streams it is unlikely light ever reached limiting conditions, except perhaps for Littlemill which was heavily shaded by trees (refer to relevant discussion in Chapter 3, section 3.6.1.2).

Low streamwater pH in the spring can be attributed to the acidifying effects of snowmelt and acid rain on stream physico-chemistry (Helliwell *et al.* 1998, Soulsby *et al*. 2001). Dry depositional processes contaminate upland snowpacks with atmospheric pollutants such as NO_2, SO_4 and Cl, derived from gaseous emissions originating from anthropogenic sources (Helliwell *et al.* 1998, Soulsby *et al.* 2002b). The acidic properties of snowmelt are attributed to the presence of these atmospheric pollutants, which are discharged in the initial stages as snow undergoes transition into meltwater (Helliwell *et al.* 1998, Bates 2000, Soulsby *et al.* 2002a). In milder climatic conditions these pollutants are deposited in precipitation. Acid rain has a pH <5.6 (O'Neill 1998). Sulphate (SO_4) is the major component of acid rain derived from dissociation of sulphuric acid (H_2SO_4) and oxidation of sulphur dioxide (SO_2). These are by-products of industry and fossil fuel consumption (Moldan & Černý 1994, O'Neill 1998, Bates 2000, vanLoon & Duffy 2000, Soulsby *et al.* 2002b). Like Na and Cl, sulphate also occurs naturally in vaporized seawater (Moldan & Černý 1994). In freshwaters the incidence of sulphate is generally low though large continental regions of Europe and North America are subject to acid rain problems from anthropogenic emissions (O'Neill

1998, vanLoon & Duffy 2000). SO_4^{2-} is a highly mobile anion showing strong significant precipitation in response to acidity. The sulphate present in acid rain displaces base cations from naturally acidic catchment soils (e.g. peat). The abundance of streamwater Al^{3+} is pH-dependent as at high pH, aluminium is mostly insoluble. Low pH conditions promote formation of soluble aluminium and facilitate leaching of Al^{3+} ions. Further acidification occurs as these cations are liberated to surface waters. This can have profound effects on stream hydrochemistry (Moldan & Černý 1994, Bates 2000, vanLoon & Duffy 2000, Soulsby *et al.* 2002b). In small pristine catchments stream hydrochemistry reflects underlying geology and natural buffering capacity to neutralize acids. The R. Dee basin is principally acid-sensitive (Soulsby *et al.* 2002b). This is attributable to the base-poor geology that underpins a large proportion of the catchment. Basins underlain with hard, weathering-resistant igneous rock types such as granite have low buffering capacity and are susceptible to SO_4-induced acidity. Input from snowmelt and acid rain exaggerates the characteristic acidic nature of these streamwaters. Metamorphic (e.g. amphibolite, serpentinite, schists) and sedimentary strata (e.g. limestones, dolomites) contain increased proportions of carbonates and have higher buffering capacities. Consequently, streamwaters with elevated pH and alkalinities usually have reduced SO_4 and Al content (Moldan & Černý 1994, O'Neill 1998, Bates 2000, Soulsby *et al.* 2002b). Increased calcium magnesium carbonate concentrations are products of base-rich geologies and are highly positively correlated with pH, conductivity and alkalinity. Reductions in streamwater pH are also attributable to dilution of groundwater chemistry by overland stormflows such as those often characteristic of the late spring in Scottish Highlands (Langan *et al.* 1997, Soulsby *et al.* 2002b, 2003, 2007). This is likely to be a function of the extensive peat cover in upper Water of Dye, which exerts an acidifying affect on upland streamwaters (Dawson *et al.* 2001, 2004, Soulsby *et al.* 2003, Tetzlaff *et al*. 2007b). High cover of both granite and granodiorite showed strong significant negative relationships with pH, conductivity and alkalinity. Conductivity is a measure of the contribution of dissolved mineral ions in solution. Minerals are derived from parental rock types

through biogeochemical weathering processes, together with any modifying sources of additional ions from, for example human catchment uses such as agriculture (Moldan & Černý 1994, O'Neill 1998). Conductivities are generally expected to be low for sub-catchments underlain with igneous parent material resistant to weathering (e.g. granite, granodiorite) that dominate the Water of Dye sub-catchment. Higher conductivities are generally associated with softer rock types (e.g. limestones) more vulnerable to the effects of weathering processes and readily liberate mineral carbonates into solution such as those occurring in River Girnock and Knockan Burn. Conductivity is a useful indicator of mineral nutrient status. Upland oligotrophic (nutrient-poor), acid-based streams typically have low ionic strength, characterised by low conductivities and alkalinities (Langan *et al.* 1997, Smart *et al.* 1998, Soulsby *et al.* 2002b). Like pH, streamwater conductivities and alkalinities increase during the summer, as during periods of reduced overland flows, cation concentrations in groundwaters increase. This explains why alkalinities associated with low base flows are usually high (Soulsby *et al.* 2002b, 2007, Tetzlaff *et al*. 2007ab). In contrast, pH, conductivities and alkalinities sharply reduce in response to dilution from overland flood events that affect stream hydrochemistry (Langan *et al.* 1997, Soulsby *et al.* 2001, 2002b, 2003, 2007, Tetzlaff *et al*. 2007ab). This reinforces the negative relationship of stream physico-chemistry to high discharges (e.g. storm run-off events) and variation in basin characteristics like underlying geology and peat cover. Dilution effects on stream hydro-chemistry are caused by increased discharge and acidification from soil water (Langan *et al.* 1997, Dawson *et al.* 2001, 2004, Soulsby *et al.* 2007). The negative effect that high flows exert on streamwater pH, conductivities and alkalinities, has been documented in a number of studies (e.g. Smart *et al*. 1998, Dawson *et al.* 2001, Soulsby *et al*. 2001, 2003, 2007, Tetzlaff *et al*. 2007ab). During summer baseflows (low discharges), concentrations of mineral cations are usually higher therefore alkalinity, conductivity and pH values increase (Soulsby *et al.* 2002b, 2003). Therefore, base flow conditions promote increased concentrations of K^+, Ca^{2+}, and Mg^{2+}.

A significant phosphate pulse was detected in each of the three target streams in April 2006, but levels remained near the detection limit (<0.003 mg l^{-1}) on all other dates sampled, indicating that habitat conditions were principally oligotrophic. The observed seasonal pulse in streamwater phosphate is consistent with other recent research that primarily focussed on the nutrient cycling of similarly oligotrophic upland streams in the UK (e.g. Turner *et al*. 2003). The findings of Turner *et al*. (2003), showed that spring streamwaters included in their study were also characterised by a distinct pulse of organic phosphorus containing an inorganic P fraction derived from soil water. This reflects catchment soil composition (e.g. blanket peat) and their propensity to release otherwise limiting, nutrients into aquatic systems during significant run-off events. Therefore, as PO_4-P was mostly limited at other times of the year, it was necessary to determine whether this spring phosphate pulse had a significant effect on the abundance and community structure of stream primary producers, when phosphate suddenly became more available for potential utilisation.

Streamwater temperatures are a direct function of solar heating (Malcolm *et al.* 2004). Significant variation in streamwater temperatures between sampling dates reflects seasonal variation in the solar pattern (Appendix 3c, 3d) and ambient air temperatures (Appendix 3e, 3f). Close-to-freezing in-stream temperatures often associated with the early spring (e.g. March-April) in Highland Scotland are a result of low light intensities (short daylength), and low air temperatures (c. 0^{o}C and below), coupled to extremely cold climatic conditions of that time of year with icy meltwaters and snow feeding into the system. In the late-spring (e.g. May), ambient air temperatures begin to rise and daylight is lengthening, raising stream temperatures although input from drainage and groundwater discharges is cool. Warm stream temperatures are a product of the summer maxima and improved thermal insolation. In the winter (e.g. November) lowering light levels and air temperatures cause streamwater temperatures to fall. Variation in air temperature is a known function of topographical positioning, with higher altitudes experiencing colder temperatures comparable to that of a sub-artic climate and

prolonged snowdrift (Helliwell *et al.* 1998, Smart *et al.* 2001, Soulsby *et al.* 2002a, Malcolm *et al.* 2004). Air temperature has been used as a reasonable predictor of streamwater temperature, although a multitude of factors including the extent of riparian cover are also known to affect energy budgets of stream ecosystems (Malcolm *et al.* 2004). Lower streamwater temperatures are expected to occur at upland sites of heightened elevation, subjected to extreme sub-zero air temperatures during the winter (Dawson *et al.* 2001). Low altitude sites located in the valley bottom may be sheltered from the effects of wind by the presence of riparian vegetation, although the effects on streamwater temperature are more pronounced during the solar maxima (Malcolm *et al.* 2004). Furthermore, streamwater temperatures are also influenced by aspect and solar angle (Malcolm *et al.* 2004). Therefore, the time of day at which sampling occurred is highly likely to have affected the results. Note that climatic conditions in the West coast of Scotland are often milder (and wetter), compared to the North East which experiences regular cold polar weather fronts. This possibly explains why streamwater temperatures were colder in April 2006 for both the Water of Dye and River Girnock compared to Knockan Burn (Appendix 3e and 3f, respectively).

Stream flows in the Water of Dye were higher in the early spring (April 2006) than in May or August 2005. However, changes in current velocity showed no significant variation between sampling dates in the River Girnock. This is surprising because flows were observed as being considerably reduced in the summer, and accentuated during spates. Previous work in the R. Dee catchment has shown that these streams are characterised by heightened and more variable 'flashy' flow regimes characterising the winter and spring months, and curbed velocities in the summer (Dawson *et al.* 2001, Soulsby *et al.* 2001, 2003, Tetzlaff *et al.* 2007ab). Thus a subdued flow regime response is to be expected after prolonged deficiency in precipitation, such as would occur during a warm summer season (Dawson *et al.* 2001, Soulsby *et al.* 2003). The results obtained were probably affected by the fact the data was based on snap-shot measurements, rather than long-term monitoring (logger) records. However, the higher than average rainfall

of the summer 2005 (particularly in August) could explain why differences in current velocity were not as prominent as may have been expected. The Western Highlands experience a wetter climate (Appendix 3b) compared to the East coast of Scotland (Appendix 3a). Therefore it would be expected that stream flows were more responsive to wetter climatic conditions in Knockan Burn. In agreement with this: at the time of sampling (April 2006), weather conditions were particularly arduous with heavy rains and sleet associated with this region of Scotland, and can most certainly account for the high flows recorded during fieldwork (particularly evident at MK).

The seasonal variation in the abundance of shade was most marked in the Water of Dye and River Girnock. This can be explained by the abundance of riparian vegetation bordering and overhanging these two streams during the summer months. The development of a dense bankside canopy of riparian bracken fronds (at Brocky Burn and, to a lesser extent, Charr Flume) and tree canopy foliage (at Bogendreip and, especially, Littlemill) exerts a major effect on ambient light availability at these four sites during the summer. This is potentially limiting to photosynthetic production as the presence of encroaching riverside vegetation may intercept >95% of the incident photosynthetically active radiation reducing both the quantity and quality of light penetrating down into the benthic stream habitat (Hill *et al.* 1995, Hill *et al.* 2001). Of these sites, Littlemill on the lower River Girnock, is subjected to the most severe shade throughout the year and receives the lowest incident light (at stream surface) of any of the sites studied. Light penetration into this stream is restricted because the site is enclosed by tall pine trees bordering the stream and branches of broadleaf woodland vegetation projecting overhead restricting light penetration. Dieback of bankside vegetation like bracken and riparian leaf drop alleviates shade pressure on the stream channel from the late autumn-early winter throughout the spring, although ambient daylight levels are shorter during this time.

The lack of significant variation in ammonia-nitrogen and nitrate-nitrogen between sampling dates, sub-catchments and sites, with concentrations below the limit of detection would be expected as these streams form pristine, nutrient-poor habitats located in relatively undisturbed upland environments.

Streamwater sulphate (SO_4) concentrations were reduced in base flow conditions but the difference between sampling dates was not significant. Indicating that although highly available in acid conditions there was little seasonal variation in streamwater SO_4 content. This suggests that atmospherically derived SO_4 did not vary significantly between seasons. One possible explanation is that although common to snowmelt and acid rain, dry deposition (e.g. salt sea air deposits) is another source of SO_4. Temperate-cold regions often experience protracted wet deposition in the summer months from heavy cloud and precipitation. Furthermore, upland systems are exposed to variable and extreme climatic conditions (Moldan & Černý 1994).

Heavy metals like Cadmium (Cd), Chromium (Cr), Copper (Cu), Lead (Pb), Nickel (Ni), Zinc (Zn), Aluminium (Al), Vanadium (V), Arsenic (As), Iron (Fe) and Manganese (Mn) are common combustion products polluting the atmosphere (Moldan & Černý 1994, Soulsby *et al.* 2002b). Some are also minor constituents of minerals occurring naturally in rocks and released by geochemical weathering processes. Granite is mainly comprised of silicate quartz, and feldspar (Na, K and Al). Subsidiary mafic minerals like biotite rich in Fe and Mg are often present. Vanadite belongs to the apatite group, another common accessory mineral of granite containing Pb and V. Granodiorite and Diorite have reduced quartz content and increased proportions of feldspar. The former is most similar to Granite. Calcium and magnesium carbonates are weathering products derived from metamorphic rocks (Amphibolite, Serpentinite) and sedimentary limestones (Lapidus & Winstanley 1990, O'Neill 1998, Smart *et al.* 1998, 2001, Allaby & Allaby 1999, Samecka-Cymerman *et al.* 2002, Soulsby *et al.* 2007). Other trace metals have also marine origin (e.g. Na, K, Ca and Mg). Most heavy metal concentrations

dwindled naturally as soil drainage diminished during summer base flows and pH levels rose. In more acid conditions, concentrations of lead, zinc, aluminium, vanadium, iron and manganese became highly available. However, these changes were not significant for the entire suite of metal cations measured. The mobility of some metal cations is strongly pH-dependent (e.g. lead, zinc, aluminium etc.) whilst other cation species are less affected by variation in stream chemistry.

In general, potassium, calcium and magnesium concentrations increased in the summer. Hence there was a strong seasonal effect of temperature on the abundance of these minerals. Low rainfall coupled to high evaporation rates creates low discharge, base flow conditions in which the aforementioned solutes become highly concentrated. In effect increased concentrations of these mineral cations drives significant changes in water chemistry by raising pH, conductivities and alkalinities and refers back to prior discussion on this topic (section 2.7.1). Sodium chloride concentrations peaked in April 2006 in Knockan Burn, coinciding with peak rainfall (Appendix 3b) and strong influence of marine derived deposition in this region. Na^+ variability in the Water of Dye was probably masked by the adhering cation exchange effect from the abundance of peaty soils in this sub-catchment, which were lacking in the River Girnock and Knockan Burn.

2.7.3 Response of habitat characteristics in the Water of Dye, River Girnock and Knockan Burn to variation in flow regime and substrate morphology

Unsurprisingly, riffles tended to occur in shallower regions of the streams, and pools were generally deeper. This flow-depth-substrate pattern may not have been observed in Knockan Burn as substrate morphology was finer and more homogenous with fewer boulders. Furthermore, riffles were not as abundant in this stream compared to the Water of Dye and River Girnock.

On the whole, boulders occurred largely in riffle zones. It is highly probable that basic flow patterns do not shape boulder cover, moreover observed flow patterns are an offset of streambed morphology (e.g. riffles occur when boulders are a predominant feature). This emphasizes boulders as stable substrates that are not readily dislodged and resist translocation under high velocity conditions. Therefore the flow regime patterns produced appear to depend on the presence or absence of boulder morphologies in the streambed. Overall, highest cover of large stones tended to occur in glides. This indicated that large stone particles did not exert as profound an effect on flow patterns as did boulders. In general, there was a significant declination in the proportion of small stones from pool through to riffle habitats. Clearly, the abundance of small stone was significantly negatively correlated to increasing current velocity. Similarly, significantly higher proportions of gravel and sands occurred in pools, than in glides and riffles. This identifies small stones, gravel and sand particles as unstable substrates subject to movement in high flow conditions. To summarise, natural variation in predominant substrate morphologies exerts a profound effect on those current velocities and consequently, flow patterns detected at the water surface

In general underwater light climate, pH, conductivity and temperature were not significantly affected by variation in flow regime. This suggests that these streams were well mixed from turbulence and mostly homogenous in the distribution of these habitat variables (Gordon *et al*. 2004). Furthermore, any differences in the aquatic vegetation (e.g. abundance, species diversity) arising between pool, glide and riffle zones is most likely to relate to variation in depth, substrate morphology, and/or current velocity and will be determined in subsequent chapters of this thesis. Riffles were characterised by the highest velocities, whilst glides had moderate flows, and pools consisted of the slowest currents, agreeing with the basic three range category of Ali *et al.* (1999) and Gordon *et al*. (2004).

In general, and agreeing with former discussion higher current velocities were linked to higher proportions of streambed boulder cover and low occurrences of

small sized substrates. Current velocities displayed a similar response to variation in large stone cover as to boulder zones, though these differences were not significant. Low current velocities tended to occur in habitats characterised by an abundance of small stones, gravel and sand particles. A predominance of boulder morphology in the streambed appears to exert a significant effect on basic flow patterns. Riffle zones were dominated by the occurrence of boulders. In these areas, boulders tended to protrude through the water column producing a frictional drag effect and thus notable turbulence creating characteristic riffles at the surface. Low flow, pool habitats occurred where boulder cover was considerably lacking. Finer particles also exhibited a significant relationship with flow patterns due to their discrete size, showing marked reductions at high velocities. Small stones, gravel and sand particles are readily transported under high flows, and deposited in subdued low velocity conditions. Therefore low energy pools are characterised by an abundance of unstable, highly motile substrates. These data pinpoint natural variation in boulder cover as the decisive feature shaping flow regime patterns in upland streams. These data concur with the descriptions of pool, glide (run) and riffle habitats as documented in Gordon *et al*. (2004). Some other aspects of stream habitat conditions (e.g. Z_{eu}:D, pH and conductivity) exhibited significant responses to variation in substrate morphology. This is most likely to be due the fact that high pH and conductivities were attributed to Knockan Burn, characterised by finer substrate particles and lacked boulder features.

2.8 Conclusions

In summary this chapter demonstrates that using a methodology largely following the River Habitat Survey approach, the environmental habitat conditions of the three upland streams in this study can be characterised in detail. An overview of the findings of this chapter is provided below:

- Three primary clusters of stream habitat conditions emerged from the abiotic data set collected using multivariate approaches (Principal Components Analysis and Variable Clustering) to effectively categorise sites (or sample observations) based on their intrinsic similarities. Cluster 1 sites; Brocky Burn, Charr Flume, Bogendreip (all Water of Dye) plus Iron Bridge (River Girnock) were characterised as typically base-poor, acid-sensitive upland streams of low ionic strength, low buffering capacities, with highly stable streambed morphologies predominated by exposed bedrock and large boulders. Cluster 2 sites; Hampshire's Bridge and Littlemill (both River Girnock) were representative of a moderately buffered, cobble-dominated stream of intermediate pH and ionic strength. Cluster 3 sites; Upper, Mid and Lower Knockan Burn comprise a base-rich calcareous stream of high pH and ionic strength, high buffering capacity, and a streambed featuring mostly finer substrates: small stones, gravel and sand. The influential factors driving these separations were identified as: geology, substrate particle composition, pH and conductivity, as well as the abundance of sulphate and some heavy metal cations (specifically; lead, zinc, aluminium, vanadium, iron, manganese, calcium and magnesium).

- Drawdown of streamwaters during summer base flow conditions explains the seasonality observed in many of the physico-chemical parameters measured such as depth, and improved light penetration (Z_{eu}:D). Furthermore, increased concentrations of Ca and Mg result in higher pH, conductivities and alkalinities typical of low discharge periods such as summer base flows. The lack of sufficient drainage and change in pH explain reduced availability of streamwater sulphate and some heavy metals (e.g. aluminium, etc).

- Riffle habitats were characterised as shallow regions of the streams exhibiting high current velocities, and dominated by boulders (responsible for the 'riffle effect'). Smaller substrates such as small stones, gravel and sand were scarce in riffle zones and identified as unstable particles susceptible to scouring under high flow conditions. Glides occupied intermediate habitats of moderate velocity (amid the extremes of high and low flows), and a substrate morphology was

comprised principally of large stones with modest proportions of smaller substrate particles. Pools formed in deeper areas of extremely low flows, characterised by fine particle morphologies (e.g. small stones, gravel, sand), and lacked larger substrate features.

- This information outlined in this chapter provides the basis required to determine relationships between environmental habitat variation and variation in the abundance, diversity and community composition of periphyton (principally diatoms), aquatic bryophytes and submerged vascular macrophytes between sampling sites and the three target streams, in subsequent chapters of this thesis.

Chapter 3. Upland Stream Freshwater Plant Production

3.1 Objectives

- To quantify natural variation in freshwater primary production (as biomass and chlorophyll content), as well as other vegetation state variables (% plant cover) and un-vegetated (% bare) area between the three target streams over one full growing season, for three groups of aquatic plants: periphytic algae, bryophytes and (where present) vascular submerged macrophytes.

- To determine the effectiveness of various types of artificial substrate samplers in acting as surrogate microhabitat for periphyton production compared to naturally-occurring substrata.

- To determine potential environmental factors driving differences in freshwater primary production in response to variation in habitat characteristics and seasonality, for each of periphyton, aquatic bryophytes and (where present) vascular submerged macrophytes.

- To determine the nature, strength and significance of any associations between freshwater primary production and these habitat conditions, for each of periphyton, aquatic bryophytes and (where present) vascular submerged macrophytes.

- To demonstrate the potential of the project outcomes in helping implement the biomonitoring of upland stream water quality in Scotland by using multiple regression modelling procedures to determine the relative predictive strength of combinations of environmental factors in acting as drivers of functional attributes (community production) of stream vegetation.

3.2 Introduction

The introduction of the Water Framework Directive (WFD; EC 2000) has led to renewed interest in the relationships between aquatic plants and habitat conditions. Particularly, the environmental drivers which control assemblage and functional attributes of river plant communities (e.g. Ali *et al*. 1999, Sabbatini *et al*. 2002, Garbey *et al*. 2004).

Compared to freshwater vegetation characterising standing waters, less is understood of the interacting environmental processes controlling primary production in upland headwater streams usually dominated by growth of periphytic algae, aquatic bryophytes and (where present) a handful of specialised vascular macrophytes. The standing crop of aquatic vegetation is integral to the ecosystem support functioning of inland water habitats. Thus it is implemented as an integrative indicator of water quality status. Though this is usually practiced more effectively for lochs (e.g. phytoplankton) than for streams or rivers in Scotland.

It is of fundamental interest to improve knowledge of the environmental constraints affecting various freshwater plants in upland streams. This to ensure appropriate environmental protection of reference condition sites through sustainable river management. Therefore it is imperative to determine critical thresholds of nutrients to manage nuisance growths of algae (e.g. *Cladophora*) or aquatic weeds (e.g. *Ranunculus*) in enriched habitats, or manage flood risk and encourage ecological restoration to resemble closely-natural conditions in physically-disturbed (e.g. channelised, dredged) or hydraulically-altered (e.g. impeded flow due to excessive macrophyte growth of *Ranunculus*) watercourses.

3.3 Methods

3.3.1 Field sampling of periphyton

Periphyton was sampled routinely from artificial substrates to assess short-term colonisation (section 3.3.1.1), and also collected less frequently during field survey campaigns to assess long-term colonisation (section 3.3.1.2). Periphyton material was also removed from the surfaces of naturally-occurring substrata: mineral streambed particles (section 3.3.1.3), aquatic bryophytes and (where present) vascular submerged macrophytes (section 3.3.1.4). Thereby, the overall survey design facilitated a comparative study of periphyton production (section 3.5.5) and community composition (Chapter 4, section 4.5.5) between artificial and naturally-occurring substrata.

3.3.1.1 Artificial substrata: short-term routine periphyton sampling

A standard sampling regime was undertaken at each of the nine sampling sites (refer back to Chapter 2, section 2.3) using artificial substrate samplers. At each site, replicate 20 x 20 cm (= 400 cm^2) linoleum tiles (Figure 3.1) were inset as sampling stations (n = 6 samplers per site; n = 18 samplers per subcatchment stream) to capture periphyton colonisation. Linoleum was selected as the principal artificial substrate because its appearance (sand-coloured with a lightly-pitted texture: Figure 3.2) was considered comparable to that of the naturally-occurring bedrock material that predominated the streambed geomorphology. These short-term linoleum substrates were harvested and replaced on a monthly-bimonthly basis, to obtain an estimate of the periphyton standing crop that had occurred during the intervals of exposure (refer to section 3.3.2.1 for methodology explaining how periphyton material was gathered and processed in the lab). It was anticipated that this approach would provide an overall representation of net annual lotic periphyton production (this Chapter) and perhaps also ecological shifts in community composition (Chapter 4) in response to natural variation in

environmental habitat conditions. Sampling stations were usually harvested and replaced at approximately monthly intervals during the spring-summer period and approximately every two months over the autumn-winter period.

The pre-sized samplers were horizontally loaded and adhered onto heavy weighted objects (pair of monoblocks) using cable ties. The samplers were then randomly distributed and fully submerged in the streams at each of the nine sites. Short-term periphyton samplers were placed in the Water of Dye in September 2004; sampling was initiated in October 2004, and thereafter conducted on a regular basis until cessation of sampling in April 2006. Short-term periphyton samplers were placed in the River Girnock in March 2005; sampling was initiated in April 2005, and thereafter conducted on a regular basis until cessation of sampling in April 2006. Short-term periphyton samplers were placed in Knockan Burn in November 2005; sampling was initiated in December 2005, and thereafter conducted on a regular basis until cessation of sampling in November 2006. Sampling in Knockan Burn overlapped with that of the two R. Dee sub-catchment streams in April 2006. Timelines for short-term samplers are given in Table 3.2.

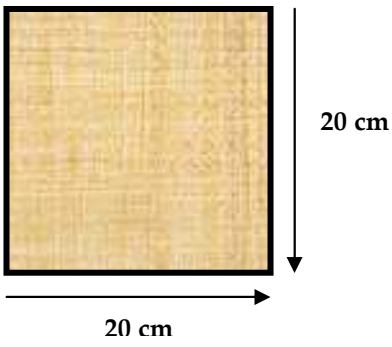


Figure 3.1 (Above left) Short-term linoleum sampler textile and dimensions

Figure 3.2 (Above right) Short-term linoleum sampler operational instream

After the short-term interval of colonisation had elapsed (e.g. 1 – 2 months), the 20 x 20 cm linoleum samplers were harvested (hand-collected in the field) from their

weighted anchor and replaced with a new set of pre-cut, sterile samplers for the subsequent colonisation period (those to be collected during the next harvest).

Harvested samplers were stored separately in individual re-sealable polythene bags to avoid sample contamination and preserve the periphyton by keeping the substrates (and therefore live specimens) as moist and fresh as possible, these bags were kept chilled and darkened in a cool box during their transport from the field back to the lab (Saunders & Eaton 1976, Ali *et al*. 1999). Short-term artificial periphyton samplers were processed in the lab according to 3.3.2.1.

Environmental variables measured at each sample station (n = 6 per site) comprised snapshot indications of streamwater depth, underwater light availability: K, Z_{eu} (1% for algae), Z_{eu}:D and water physico-chemistry: pH, conductivity, water temperature and mean flow (averaged from three readings measured across the area of each individual artificial sampler per site), as detailed in Chapter 2, section 2.4.2. All measurements were taken prior to harvesting the samplers to avoid disturbing stream conditions, and minimize sample error.

In addition to the aforementioned environmental variables recorded prior to the harvest, measurements of alkalinity, nutrient and heavy metal concentrations were also recorded during survey periods from water samples collected during sampling visits in the field, and analysed by SEPA East Kilbride (refer back to Chapter 2, section 2.4.2).

There were two occasions during fieldwork due to either or a combination of technical difficulties and ardous weather conditions where it was not possible to gather a sufficient data set of physico-chemical variables (e.g. flow etc.). This happened in October 2004 and January 2005, during routine sampling of the Water of Dye sub-catchment stream. Since there were gaps associated with the environmental measurements, these particular samples were omitted from multivariate ordinations (e.g. CCA) but could still be classified by TWINSPAN to determine whether the composition of these samples was comparable or

dissimilar to other periphyton populations sampled from the same stream on other dates (for details refer to Chapter 4, section 4.5.6.1).

There were also some occasions whereby the complete set of artificial periphyton samplers could not be recovered due to loss of samplers as a result of major spate events (often occurring in the spring); details provided in Table 3.2 and Table 3.3. Nevertheless, the weighted monoblocks generally acted as effective anchors for the artificial periphyton samplers and most were retrieved as planned from their designated stations.

3.3.1.2 Artificial substrata: long-term periphyton sampling

Two sets of long-term artificial samplers, sized as 10 x 30 cm strips were placed in each of the nine sampling sites and nested along with the pre-located short-term periphyton samplers. Long-term periphyton samplers were placed in the Water of Dye and River Girnock in March 2005 and were sampled in May 2005, August 2005 and April 2006 (coinciding with sampling of natural substrata). In Knockan Burn, long-term periphyton samplers were set in place in December 2005 and sampling undertaken in April, September and November 2006 (coinciding with sampling of natural substrata). Sampling in Knockan Burn overlapped with that of the two R. Dee subcatchment streams in April 2006. To mimic mineral particle streambed material, one set of long-term samplers was composed of linoleum (n = 6 samplers per site; n = 18 samplers per subcatchment stream) using the same source material as described in 3.3.1.1: Figure 3.4. The other set utilised long-term plastic Astroturf samplers (n = 6 samplers per site; n = 18 samplers per subcatchment stream), sized as 10 x 30 cm strips but with a larger overall surface area than for long-term linoleum substrates (refer to Table 3.1). The Astroturf samplers were dark-green, coarse and bristly in texture (Figure 3.6) chosen to mimic the complex microhabitat offered by aquatic bryophyte vegetation present in the sample streams. An additional set of long-term samplers (n = 8 samplers per site) comprised of plastic aquarium plants were set in place in November 2005

(stationed with the short-term and other long-term artificial substrates) in the upper and lower reaches of Knockan Burn to reflect the vascular submerged macrophyte vegetation occurring therein. The plastic aquarium plant samplers were of two differing forms strapped to the same weighted object: *Potamogeton*-like samplers (n = 4 per site): Figure 3.8, and *Myriophyllum*-like samplers (n = 4 per site): Figure 3.10. The plastic aquarium plant sampler 'root' end was positioned facing the direction of flow because macrophytes adopt this orientation in flowing waters. The long-term artificial periphyton samplers were nested together instream with the short-term linoleum substrates (Figure 3.11).

The function of the long-term artificial samplers was to permit cumulative studies of periphyton biomass (the focus of this Chapter), community architecture and succession (addressed in Chapter 4) on ranging substrata, in parallel with the short-term analyses. This was achieved by dividing the underside area of the long-term linoleum (Figure 3.3) and Astroturf (Figure 3.5) samplers into three contiguous sub-samplers, each comprising a 10 x 10 cm base segment, using permanent (water resistant) marker pen. The unit area available for periphyton colonisation on individual segments of long-term linoleum and Astroturf samplers equated to 100 cm^2 and 1440 cm^2, respectively (refer to Table 3.1). Individual plastic aquarium plant samplers were also divided into three sectors similar in unit area, and could be easily detached during sampling (refer to Figure 3.7 and Figure 3.9). Segments of the long-term samplers were harvested by hand (using shearing scissors or scalpel) at the following intervals: segment 1 removed after approximately 2 - 4 months colonisation; segment 2 removed after approximately 6 - 8 months colonisation; segment 3 after approximately 10 - 12 months colonisation (refer to Table 3.3 for sampling timelines). Harvested long-term artificial samplers were stored in individual resealable polythene bags kept cool and dark, until lab processing could be undertaken (refer to section 3.3.2.1). The functional (e.g. chlorophyll content) and structural (e.g. diversity) characteristics of the periphyton material harvested were calculated on a per unit area basis using the total surface area available for colonisation during the exposure interlude

(refer also to Table 3.1 for details). This meant that natural variation in periphyton production (this Chapter) and community structure (Chapter 4) could be assessed over an entire growing season.

Sub-catchment Stream	Survey Date Sampled	Segment No.	Long-term Artificial Substrate Type	Unit Area Sampled (cm^2)
Water of Dye	May 2005	1	Linoleum	100
Water of Dye	May 2005	1	Astroturf	1440
River Girnock	May 2005	1	Linoleum	100
River Girnock	May 2005	1	Astroturf	1440
Water of Dye	August 2005	2	Linoleum	100
Water of Dye	August 2005	2	Astroturf	1440
River Girnock	August 2005	2	Linoleum	100
River Girnock	August 2005	2	Astroturf	1440
Water of Dye	April 2006	3	Linoleum	100
Water of Dye	April 2006	3	Astroturf	1440
River Girnock	April 2006	3	Linoleum	100
River Girnock	April 2006	3	Astroturf	1440
Knockan Burn	April 2006	1	Linoleum	100
Knockan Burn	April 2006	1	Astroturf	1440
Knockan Burn	April 2006	1	*Potamogeton*-like plastic plant	95.8
Knockan Burn	April 2006	1	*Myriophyllum*-like plastic plant	26.1
Knockan Burn	September 2006	2	Linoleum	100
Knockan Burn	September 2006	2	Astroturf	1440
Knockan Burn	September 2006	2	*Potamogeton*-like plastic plant	83.8
Knockan Burn	September 2006	2	*Myriophyllum*-like plastic plant	26.1
Knockan Burn	November 2006	3	Linoleum	100
Knockan Burn	November 2006	3	Astroturf	1440
Knockan Burn	November 2006	3	*Potamogeton*-like plastic plant	83.4
Knockan Burn	November 2006	3	*Myriophyllum*-like plastic plant	26.1

Table 3.1 Artificial sampler type, unit area available for periphyton colonisation, and dates of segment removal during surveys.

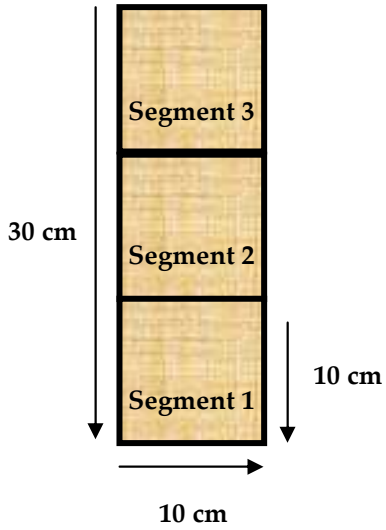


Figure 3.3 (Above left) Diagram of long-term linoleum sampler textile, dimensions and sub-divisions indicated

Figure 3.4 (Above right) Long-term linoleum sampler operational instream

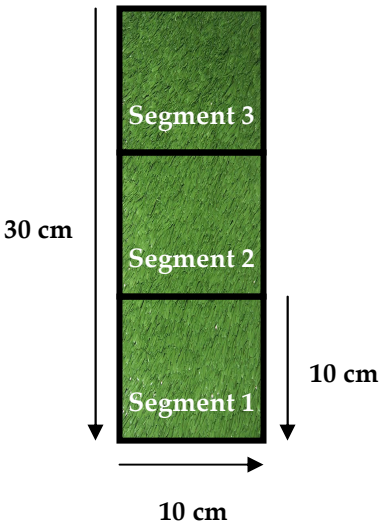


Figure 3.5 (Above left) Diagram of long-term Astroturf sampler textile, dimensions and sub-divisions indicated

Figure 3.6 (Above right) Long-term Astroturf sampler operational instream

Figure 3.7 (Above left) Diagram of long-term plastic aquarium *Potamogeton*-like plant sampler with sub-divisions indicated

Figure 3.8 (Above right) Plastic aquarium plant type utilised as *Potamogeton* mimic

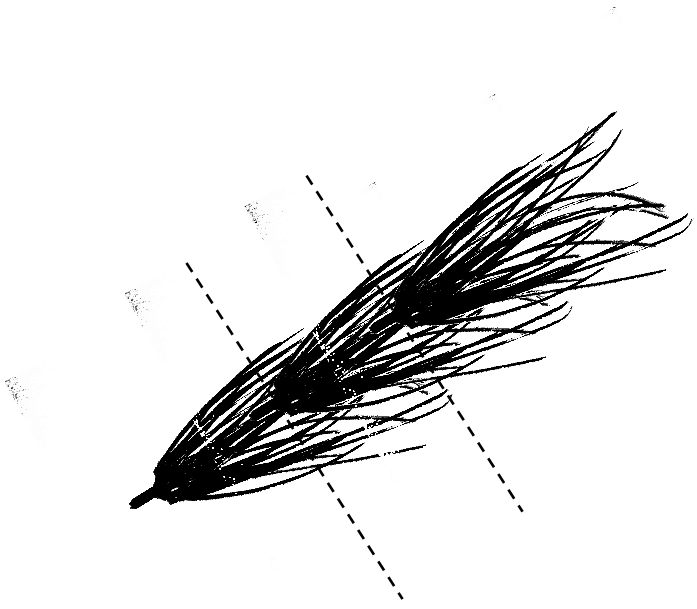


Figure 3.9 (Above left) Diagram of long-term plastic aquarium *Myriophyllum*-like plant sampler with subdivisions indicated

Figure 3.10 (Above right) Plastic aquarium plant type utilised as *Myriophyllum* mimic

Figure 3.11 Artificial periphyton samplers nested instream, from L-R: long-term plastic aquarium plant samplers, long-term Astroturf sampler, short-term linoleum sampler, and long-term linoleum sampler.

Date placed	Date sampled	Sub-catchment	% Recovery	Sampling Site	% Recovery
September 2004	October 2004	Water of Dye	50%	Brocky Burn	83%
				Charr Flume	33%
				Bogendreip	33%
October 2004	November 2004	Water of Dye	67%	Brocky Burn	100%
				Charr Flume	50%
				Bogendreip	50%
November 2004	January 2005	Water of Dye	67%	Brocky Burn	100%
				Charr Flume	33%
				Bogendreip	67%
January 2005	March 2005	Water of Dye	78%	Brocky Burn	100%
				Charr Flume	50%
				Bogendreip	83%
March 2005	April 2005	Water of Dye	94%	Brocky Burn	100%
				Charr Flume	100%
				Bogendreip	83%
March 2005	April 2005	River Girnock	100%	Iron Bridge	N/A
				Hamp. Bridge	100%
				Littlemill	100%
April 2005	May 2005	Water of Dye	50%	Brocky Burn	83%
				Charr Flume	33%
				Bogendreip	33%
April 2005	May 2005	River Girnock	100%	Iron Bridge	100%
				Hamp. Bridge	100%
				Littlemill	100%
May 2005	July 2005	Water of Dye	94%	Brocky Burn	83%
				Charr Flume	100%
				Bogendreip	100%
May 2005	July 2005	River Girnock	94%	Iron Bridge	83%
				Hamp. Bridge	100%
				Littlemill	100%
July 2005	August 2005	Water of Dye	100%	Brocky Burn	100%
				Charr Flume	100%
				Bogendreip	100%
July 2005	August 2005	River Girnock	100%	Iron Bridge	100%
				Hamp. Bridge	100%
				Littlemill	100%

November 2005	December 2005	Knockan Burn	100%	Upper Knockan	100%
				Mid-Knockan	100%
				Lower Knockan	100%
August 2005	April 2006	Water of Dye	78%	Brocky Burn	67%
				Charr Flume	83%
				Bogendreip	83%
August 2005	April 2006	River Girnock	94%	Iron Bridge	83%
				Hamp. Bridge	100%
				Littlemill	100%
December 2005	April 2006	Knockan Burn	100%	Upper Knockan	100%
				Mid-Knockan	100%
				Lower Knockan	100%
April 2006	July 2006	Knockan Burn	83%	Upper Knockan	50%
				Mid-Knockan	100%
				Lower Knockan	100%
July 2006	September 2006	Knockan Burn	100%	Upper Knockan	100%
				Mid-Knockan	100%
				Lower Knockan	100%
September 2006	November 2006	Knockan Burn	94%	Upper Knockan	100%
				Mid-Knockan	83%
				Lower Knockan	100%

Table 3.2 Percent successful recovery of short-term linoleum samplers retrieved from each sub-catchment stream and their respective sampling sites, indicating dates substrate samplers were placed in the rivers and later sampled.

Date placed	Date sampled	Sub-catchment	% Recovery			Sampling Site	% Recovery		
			LL	AS	PAP		LL	AS	PAP
March 2005	May 2005	Water of Dye	50%	50%	-	Brocky Burn	83%	50%	-
						Charr Flume	33%	67%	-
						Bogendreip	33%	33%	-
March2005	May 2005	River Girnock	100%	100%	-	Iron Bridge	100%	100%	-
						Hamp. Bridge	100%	100%	-
						Littlemill	100%	100%	-
March2005	August 2005	Water of Dye	100%	100%	-	Brocky Burn	100%	100%	-
						Charr Flume	100%	100%	-
						Bogendreip	100%	100%	-
March2005	August 2005	River Girnock	100%	100%	-	Iron Bridge	100%	100%	-
						Hamp. Bridge	100%	100%	-
						Littlemill	100%	100%	-
March2005	April 2006	Water of Dye	72%	67%	-	Brocky Burn	83%	50%	-
						Charr Flume	83%	67%	-
						Bogendreip	50%	83%	-
March2005	April 2006	River Girnock	89%	72%	-	Iron Bridge	100%	67%	-
						Hamp. Bridge	83%	50%	-
						Littlemill	83%	100%	-
December 2005	April 2006	Knockan Burn	94%	50%	88%	Upper Knockan	83%	50%	100%
						Mid-Knockan	100%	50%	-
						Lower Knockan	100%	50%	75%
December 2005	September 2006	Knockan Burn	100%	50%	88%	Upper Knockan	100%	50%	100%
						Mid-Knockan	100%	50%	-
						Lower Knockan	100%	50%	75%
December 2006	November 2006	Knockan Burn	100%	44%	75%	Upper Knockan	100%	50%	100%
						Mid-Knockan	100%	33%	-
						Lower Knockan	100%	50%	75%

Table 3.3 Percent successful recovery of long-term linoleum (LL), Astroturf (AS), and plastic aquarium plant (PAP) samplers retrieved from each sub-catchment stream and their respective sampling sites, indicating dates substrate samplers were placed in the rivers and later sampled.

3.3.1.3 Naturally-occurring substrata: sampling of epilithic periphyton from mineral particle surfaces

During the field surveys, within each hydromorphological unit (refer to Chapter 2, section 2.4.2) three regions of mineral particle surfaces were selected at random and scraped to remove periphyton from the surface. This was performed using a cylindrical sampling device to provide a given sampling unit area and a pastry brush to harvest the attached periphyton directly from the mineral substrata (see Figure 3.12). An isolated bottle-neck (Douglas 1958, Sládečková 1962) with a diameter of 2.5 cm (= 4.91 cm^2) was adhered to the mineral surface by firmly pressing the device against the natural substratum. An outer ring of plasticine was also used as a sealant to prevent loss of periphyton material from the sampling arena (Ertl 1971). The entire procedure was conducted underwater to avoid loss of attached periphyton at the water surface. Periphyton (from the inner sampling arena) was dislodged from mineral substrata using a combination of jet-spraying distilled water and scrubbing with a clean pastry brush. This approach was repeated until the sampling arena was rinsed clear and one was satisfied that the periphyton present had been successfully detached (Douglas 1958, Ertl 1971, Sherwood *et al*. 2000). The isolated periphyton material (in solution) was collected using a sterile syringe and decanted into a clean, airtight 50 ml centrifuge tube. Samples were kept dark, and stored in chilled conditions prior to lab processing. Periphyton material was processed as detailed in 3.3.2.2, and data expressed as the mean value per unit area sampled. Within each hydromorphological unit, environmental variables were measured according to Chapter 2, section 2.4.2.

3.3.1.4 Naturally-occurring substrata: sampling of epiphytic periphyton from the surfaces of aquatic bryophytes and other submerged macrophytes

Refer to 3.3.3 and 3.3.5 respectively, for methodology detailing how aquatic bryophytes and vascular submerged macrophyte vegetation were sampled from

the target streams. Periphyton colonising the surfaces of aquatic bryophytes and other submerged macrophytes was removed and processed according to 3.3.2.2.

Small sub-samples of aquatic bryophytes were collected from each hydromorphological unit (refer to 3.3.3) representative of the population present and stored in labelled resealable polythene bags, separately from the core samples. These were also kept dark and chilled until specimens could be properly identified in the lab (refer to Chapter 4, section 4.3.2).

Aquatic bryophyte core samples were taken only during survey periods (and not during routine sampling efforts) to avoid denuding areas of attached vegetation throughout the course of fieldwork, and minimise impact on the stream ecosysytems targeted in this project.

Figure 3.12 Sampling periphyton from the surfaces of naturally-occurring mineral substrata

3.3.2 Laboratory analyses of periphyton material

3.3.2.1 Processing of artificial substrates

A clean glass microscope slide was used as a scraping device to slough-off periphyton that had successfully colonised the surface of the linoleum-based substrates. A combination of toothbrushes and nail brushes were used to remove periphyton from the plastic fronds of the Astroturf and aquarium plant samplers.

To avoid loss of material, periphyton removal was conducted within the polythene bag used to transport the hand-collected substrates from the field to the lab. Distilled water was added intermittently to the polythene bag to aid thorough removal and collection of periphyton material. The bag was thoroughly rinsed and the contents were emptied into a large clean 1000ml beaker. Tweezers were then used to remove evident contaminants from the algal suspension (e.g. pieces of gravel, plant fragments, or benthic macroinvertebrates that had also colonised the sampler) to reduce sample error. The beaker contents (algal suspension) were decanted into pre-labelled 50ml screw-cap centrifuge tubes and centrifuged for 15 minutes at 4,000 r.p.m. to concentrate the organic material at the tube bottom (Vollenweider 1969). Centrifuging was repeated when required. After centrifugation, the clear supernatant was siphoned-off and the solid algal matter retained at the bottom of the tube was re-suspended in (<30ml) distilled water. A priority was to minimize the volume of water in suspension to increase the efficiency of freeze-drying. The concentrated algal suspension was transferred to a sterile, pre-labelled and pre-weighed 100ml plastic beaker. A 10ml syringe was used to distribute the algal material into suspension (by hand mixing and drawing the mixture up and down several times) to remove coherent lumps that had gelled together forming a solid mass and ensure a liquid format. This action in conjunction with vigorous stirring mixed the suspension thoroughly to enable a 2 ml representative sub-sample of periphyton to be collected for formal species identification and analyses of community composition (refer to Chapter 4, section 4.3.1.2). The plastic beaker containing the remainder of the periphyton batch was

capped with a sheet of tin-foil (pierced to allow escape of water vapour during freeze-drying), sealed with an elastic band and transferred to a -20°C freezer and stored for at least 48 h, or until samples were completely frozen for freeze-drying. After freeze drying, biomass and chlorophyll content analyses were undertaken according to 3.3.7.1 and 3.3.7.2 respectively, to obtain measures of periphyton production per unit area sampled.

3.3.2.2 Processing of naturally-occurring substrata

Sample tubes containing periphyton material harvested directly from mineral substrata (refer back to 3.3.1.3) in the field, were centrifuged and sub-sampled as in 3.3.2.1. These tubes were then capped with pierced tin foil and allowed to freeze for at least 48 h for freeze drying. Biomass and chlorophyll content analyses were undertaken according to 3.3.7 to obtain measures of epilithic periphyton production per unit area sampled.

Bryophyte cores and vascular submerged macrophyte specimens were gently brushed to remove epiphytic periphyton material from the surface, using a clean toothbrush and/or nailbrush. This procedure was conducted within the resealable polythene bags in which the samples were collected, to avoid loss of periphyton. The material was then processed and sub-sampled for periphyton according to 3.3.2.1, and capped samples were frozen for at least 48 h prior to freeze drying. Biomass and chlorophyll content analyses were undertaken according to 3.3.7 to obtain measures of epiphytic periphyton production per unit area sampled.

3.3.3 Field sampling of aquatic bryophytes

A stratified random sampling quadrat procedure (using a sub-divided 1 m^2 quadrat) was used to sample naturally-occurring aquatic vegetation: periphyton (refer to Chapter 2, section 2.4.2) and aquatic bryophytes to determine standing

crop (this Chapter), community composition and species diversity (Chapter 4) within 6 sub-samples, for each of "low", "intermediate" and "high" abundance strata of aquatic vegetation, in each of three habitat flow-types present in the river (R: riffle; G: glide; P: pool). A 5 cm diameter metal corer (= 19.64 cm^2) was used as a sampler to extract or 'punch' cores from bryophyte vegetation (where present), as described by Douglas (1958). Re-sealable polythene bags were used to transport core specimens to the lab for processing (section 3.3.4), and smaller sub-samples for formal species identification (see Chapter 4, section 4.3.2).

The environmental sampling regime for aquatic bryophytes was as detailed previously (refer to Chapter 2, section 2.4.2).

The area occupied by each plant group: periphyton (Sládečková 1962, Vollenweider 1969, Dennis & Isom 1984, Sherwood *et al.* 2000) and aquatic bryophytes (Vollenweider 1969) were assessed visually as percent (%) cover, an estimate of the proportion held within the sample unit (quadrat) boundaries (Saunders & Eaton 1976). Bare (unvegetated) area was scored also using this approach (Vollenweider 1969). Median % cover of each plant group within each hydromorphological unit was categorised on a five-point scale: scarce, ≤3%; occasional, 15.5%; frequent, 38%; highly abundant, 63%; and dominant, ≥88%. Such % cover estimates provide a simple and rapid approach to obtaining many indirect determinations of biomass over a much larger area, as opposed to a few, precise, more time-consuming analyses (Whitton 1975, Saunders & Eaton 1976, Dennis & Isom 1984).

3.3.4 Laboratory analyses of aquatic bryophyte material

Aquatic bryophyte core samples were frozen for at least 48 h prior to freeze drying. Biomass and chlorophyll content analyses were performed on freeze dried material according to section 3.3.7 to obtain a measure of aquatic bryophyte production per unit area sampled.

Sub-samples of aquatic bryophyte specimens collected in the field were kept chilled until formal species identification could be undertaken (refer to Chapter 4, section 4.3.2).

3.3.5 Field sampling of vascular submerged macrophytes

Field sampling of vascular submerged macrophytes was conducted following a similar approach to that for aquatic bryophytes (see section 3.3.3), with the following exceptions: a 20 x 20 cm (= 400 cm^2) sub-section of a 1 m^2 quadrat was used to sample vascular macrophyte vegetation and plant material was removed (cut) at substrate level of the stem, above the root system for direct measurements of biomass. In keeping with the sampling protocol for the other two plant groups (periphyton and aquatic bryophytes) pre-cut estimates were also taken of vascular submerged macrophyte % cover, from within the hydromorphological unit. Plant material was stored in individually labelled, resealable polythene bags and kept in the fridge until lab processing could be conducted. Vascular submerged macrophyte vegetation was present only in Knockan Burn, and not found in the R. Dee sub-catchment streams.

3.3.6 Laboratory analyses of vascular submerged macrophyte material

Vascular submerged macrophyte sample tissue was frozen for at least 48 h and then freeze dried. After freeze drying, biomass and chlorophyll content analyses were carried out according to section 3.3.7 to obtain measures of vascular submerged macrophyte production per unit area sampled.

Sub-samples of vascular submerged macrophytes obtained from Knockan Burn were returned to the lab to confirm species identity (refer to Chapter 4, section 4.3.3).

3.3.7 Laboratory analyses of freshwater vegetation: measuring primary production

3.3.7.1 DW biomass measures of freshwater vegetation

Processed samples were kept in a -20°C freezer for at least 48 h to ensure specimens were frozen thoroughly. Frozen samples were then freeze dried under darkened vacuum conditions, at ice-condenser temperatures (in the region of -40oC) for at least 48 h. This is usually a satisfactory exposure time to ensure samples were fully freeze-dried, but in the instance they were not, then extra freeze-drying time was allocated.

Freeze-drying samples removed the moisture content of plant tissue to obtain an accurate measure of Dry Weight (DW) biomass using a digital balance (weighing precision to 0.1 mg). Samples exposed to freeze-drying process are unaffected by the problems encountered with oven-drying such as the loss of organic substances due to increased temperatures (Vollenweider 1969). Further still, chlorophyll analysis can be performed afterwards. Although DW biomass (expressed as per unit area) provides a useful index and comparative measure of the organic matter allocated to growth from photosynthesis allowing the standing crop of primary production to be estimated (Vollenweider 1969, Barnes & Mann 1980, Dennis & Isom 1984). A possible source of inaccuracy arises from the presence of accumulated, contaminant organic material such as sediment and organic detritus collected in-situ along with the sample can influence the weight of biomass estimate obtained (NRC 1969, Vollenweider 1969, Whitton 1975, Dennis & Isom 1984). The quantification of biomass per unit area if substrate provides an estimate of Net Primary Production, that is, Gross Primary Production minus carbon losses to respiration, mortality, decomposition, scouring effects of flow and grazing pressure (Vollenweider 1969, Barnes & Mann 1980, Dennis & Isom 1984, Dickinson & Murphy 2007).

After the DW biomass of each sample had been measured and recorded (3.3.7.1), the plant material was then allocated to chlorophyll analysis (3.3.7.2). On

occasions, for example when primary production was low, there was insufficient material available for chlorophyll analysis.

3.3.7.2 Chlorophyll a analyses of freshwater vegetation

Chlorophyll a is the primary photosynthetic pigment that utilises light energy to drive photosynthesis and is ubiquitous to producer organisms such as macrophytes, algae and prokaryotic cyanobacteria (Vollenweider 1969, Kirk 1994, Buchanan *et al*. 2000, Uno *et al*. 2001, Taiz & Zeiger 2002). Although primary producers are unified by chlorophyll a biosynthesis, their composition of additional photosynthetic pigments (other chlorophylls and accessory pigments) may vary (Sathyendranath *et al*. 1987, Buchanan *et al*. 2000, Uno *et al*. 2001, Taiz & Zeiger 2002). Furthermore, photosynthetic pigment composition may vary with physiological cell state and prevailing environmental conditions (Sathyendranath *et al*. 1987).

Chlorophyll a exhibits principal absorption in the blue and red regions of the visible light spectrum at specific wavelengths of 430nm and 665nm, respectively (Moss 1967b). The spectral properties of the chlorophyll a pigment are attributed to its molecular structure: comprised of a tetrapyrrole (porphyrin) ring attached to a lengthy hydrophobic hydrocarbon (phytol) tail. A central magnesium atom (Mg^{2+}) is chelated to the core region of the ringed structure forming the hydrophilic head-group of the pigment molecule (Kirk 1994, Buchanan *et al*. 2000, Uno *et al*. 2001, Taiz & Zeiger 2002).

Chlorophyll a determination is useful measure of producer biomass (Moss 1967a, Vollenweider 1969, Tett *et al*. 1977, Whitney & Darley 1979) and photosynthetic capacity (Chang & Rossman 1982). Chlorophyll a quantifies the living proportion of photosynthesising cells present in plant populations and often provides a more accurate measure of plant standing crop than direct biomass measurements which can contain contaminants (e.g. dead cells, adhering debris and organic matter)

contributing error to the estimate (Grzenda & Brehmer 1960, NRC 1969, Whitton 1975, Antoine & Benson-Evans 1985).

In preparation for pigment extraction and spectrophotometric analysis, grinding of source plant material (periphyton, aquatic bryophyte or vascular submerged macrophyte tissue) was performed using either a mortar and pestle, or automated grinder, to lyse cells and aid complete pigment extraction (Yentsch & Menzel 1963, Lorenzen 1967, Chang & Rossman 1982). Chlorophyll a pigment extraction of ground plant material was performed in 50 ml chemically-resistant, screw-cap centrifuge tubes with at least 10 ml volume of 90% aqueous acetone: complying with the methods of Parsons & Strickland (1963), Patterson & Parsons (1963), Lorenzen (1965, 1967), NRC (1969), Vollenweider (1969), Chang & Rossman (1982). Acetone is a polar lipid solvent that dissolves the membrane bilayer in which chloroplastic pigments are embedded. Samples were centrifuged at 4,000 r.p.m. for 15 minutes to separate the source plant material from the chlorophyll pigment extract and to reduce turbidity of the supernatant. Centrifuging helped to minimise error associated with light absorption and/or scattering by suspended organic material, thereby improving the accuracy of the results obtained by spectrophotometric analysis (Lorenzen 1965). Samples were stored in a -20^{o}C in the freezer for a minimum of 24 h prior to spectrophotometric analysis, in accordance with the methods of Patterson & Parsons (1963), Tett *et al.* (1977), Marker & Jinks (1982). Storing extracts in a chilled and darkened environmental prevents chlorophyll degradation from the effects of light and temperature (Daley & Brown 1973). Chlorophyll pigment extracts were removed only from cold storage only immediately prior to spectrophotometric analysis.

A sterile pipette tip was used to dispense 3ml of 90% acetone-extract into a 4ml glass cuvette of 1-cm path length for spectrophotometric chlorophyll analysis. A short path length was chosen to minimise diffraction of light to scattering (a potential source of error: Yentsch & Menzel 1963). To avoid introducing error,

caution was taken to avoid disturbing the source plant material concentrated at the centrifuge bottom, whilst the chlorophyll extract was being siphoned-off.

Chlorophyll a analysis was conducted using a UV-1201 Shimadzu UV-VIS spectrophotometer. Prior to measuring light absorbance of the chlorophyll extract at both wavelengths (665nm and 750nm), the spectrophotometer was calibrated to zero to correct light absorbance for the control (90% acetone only). This ensured that light absorbance measurements obtained from the pigment extract were due to variation in chlorophyll a concentration and not to properties attributed to the solvent. Light absorbance of the extract was recorded at wavelengths of 665nm (to capture peak chlorophyll a absorption in the red region of the visible light spectra), and 750nm (to correct for non-selective background absorption and light scattering attributed to organic matter) following the recommendations of Parsons & Strickland (1963), Yentsch & Menzel (1963), Moss (1967a), Lorenzen (1967), Vollenweider (1969), and Marker & Jinks (1982).

Furthermore, light absorption at 665nm and 750nm were re-recorded post acidification (approximately 1 minute after treatment with 0.1ml 6M HCl). Acidification of the extract is an important step to conduct as chlorophyll degradation products (mainly pheophytin and pheophorbide) present in pigment extracts interfere with specific absorption (Richards & Thompson 1952, Patterson & Parsons 1963, Yentsch & Menzel 1963, Lorenzen 1965, 1967, Moss 1967a, Vollenweider 1969, Marker & Jinks 1982). Scenescence is marked by chlorophyll catabolism and the formation of degradation products (Buchanan *et al*. 2000). Each chlorophyll molecule has its own version of degradation pigment (Lorenzen 1967). Pheophytin a shares the same molecular foundations as chlorophyll a, but the central magnesium (Mg^{2+}) of the porphyrin ring is replaced with two hydrogens ($2H^{+}$) (Taiz & Zeiger 2002). Chlorophyllide a is formed during cleavage of the phytol tail by the enzyme chlorophyllase from the chlorophyll a molecule. Further to this, pheophorbide a is a product of Mg-dechelatase activity on the catabolic product chlorophyllide a, which removes the chelated Mg^{2+}-core from the

ringed unit (Buchanan *et al*. 2000). The major chlorophyll a degradation products are considered to be pheophytin and pheophorbide, which are often grouped collectively as the 'pheopigments' (Lorenzen 1967). These pheopigments exhibit similar, yet differential, absorption spectra to chlorophyll a. Inactive forms of chlorophyll typically absorb light in the red region of the visible spectrum and are not readily discernible from chlorophyll absorption (Lorenzen 1967). However, the addition of a dilute hydrochloric acid (HCl) forces rapid conversion (<1 minute) of chlorophyll a to its corresponding pheopigment(s) through displacement of the core Mg^{2+}, thereby reducing absorbancy of the solution (Holm-Hansen *et al*. 1965, Moss 1967a, Lorenzen 1967). Based on this discrepancy in the specific absorption properties of chlorophyll a and its corresponding pheopigments, Lorenzen (1965, 1967) described the importance of the 'acidification step' in minimizing error in chlorophyll analysis. The acidification step allows distinction between active chlorophyll (before acidification) and its derived degradation products (after acidification). Chlorophyll a content was calculated according to Lorenzen (1967) and the mean value expressed per unit area of substrate sampled (refer to Equation 3.1 for details).

$$\textbf{Chlorophyll a } \mu g\ cm^{-2} = 665^{o} - 665^{a} * A * K * V / \text{unit area sampled } (cm^{2})$$

665^{o} = Corrected chlorophyll a absorbance *before* acidification

665^{a} = Corrected chlorophyll a absorbance *after* acidification

A = Chlorophyll a absorption coefficient = 11.0

K = Factor used to equate the reduction in absorbancy to initial chlorophyll concentration = 2.43

V = Volume of acetone used for chlorophyll a extraction (ml)

Equation 3.1 Calculation used to determine chlorophyll a concentration of plant material per unit area (μg cm^{-2}), adapted from Lorenzen (1967).

3.4 Data Analysis

Normality of the response data was examined visually in the form of histograms and analysed statistically using the Ryan-Joiner test. Data with a p-value >0.05 were considered normal, confirming the null hypothesis that a dataset was not significantly different from normal distribution and could be analysed using parametric tests as appropriate. Most response variables were either normally distributed or those that were skewed could be normalised by natural log transformation. Arcsine transformations were applied to normalise proportional or percentage data. All statistical analyses were conducted using Minitab version 15.1.0. One-way ANOVA was performed on response variables with normal distribution, and Tukey's multiple comparison method was used to identify where significant differences occurred. Logistic values were back-transformed where necessary to display original data. Data that was not considered to be normally distributed ($p<0.05$) and could not be normalised by transformation were analysed using the Kruskal-Wallis test (non-parametric equivalent of one-way ANOVA) and the median value(s) quoted where relevant for non-normal variables.

Pearson product-moment correlation coefficients (r) were used to analyse normally distributed data to determine the nature and strength of relationships (if any) between pairs of variables. A strong negative relationship is indicated by a value close to -1, whilst +1 indicates a strong positive relationship. Values closer to 0 suggest no relationship between variables. Correlations were considered significant if $P < 0.05$. The non-parametric alternative, Spearman rank-order correlation was used in the instance data could not be normalised, and is based on the same principle as the Pearson correlation but instead utilises ranked median data to find any relationships that may be present.

Regression analysis examines the relationship between a dependent y ('response') variable such as aquatic plant biomass or species diversity, with one or more independent x ('predictor') variables, such as the environmental variables measured. Linear regression describes the relationship between the response and

predictor variables by a straight line, and assumes that the data are not best explained by some other curve. Regression analysis produces a straight line regression equation and depicts the value of y (the response variable) for a given value of x (the predictor variable): refer to Equation 3.2.

$$\mathbf{y = a + b\,x}$$

***y**, response variable; **a,** constant or intercept; **b**, slope; **x**, predictor variable*

Equation 3.2 Linear regression equation

Regression output also produces an r^2 value indicating the proportion of variation in y, the response variable, explained by the regression with predictor variable(s), and a p-value indicating whether or not the relationship is significantly different from zero. However, the 'r^2 adjusted' value was the preferenced descriptor as this is considered more conservative measure of fit. This is the r^2 coefficient adjusted for the number of predictor variables in the regression model, and may decrease if variables inserted into the regression equation do not contribute significantly to the model fit. If $p<0.05$, then the null hypothesis can be rejected, indicating that a proportion of the variance observed is explained by a significant relationship between the variables.

Multiple linear regression analysis was used to investigate combinations of predictor variables with the intention of producing more effective predictive models to determine the principal environmental factors driving the functional attributes of stream vegetation. This method helps identify predictor variables that are most influential on the response variable, and also avoids the weaknesses associated with stepwise modelling (Whittingham *et al*. 2006).

Initially full models were developed utilising 100% of the data set available for each specific plant group, where sufficient data existed (e.g. periphyton, aquatic bryophytes) and all potential predictor variables (chosen from correlation

matrices, and/or longest arrows on CCA diagrams). The response variables chosen were highly relevant in addressing the primary theme of the project (to establish the '*processes driving freshwater plant production and diversity in upland streams*'). Plant chlorophyll content (this Chapter) and species diversity (Chapter 4) were selected as the appropriate response variables and potential predictor variables were identified from correlation and CCA analyses as those factors showing a significant relationship with each of the response variables. In order to broaden the envelope of applicability, general minimal models were adopted that were constructed from fewer predictor variables, rather than employing specific models whose envelope of applicability would therefore be considerably more limited (Scheffer & Beets 1994, Murphy & Hootsmans 2002). Only those minimal models which had the highest predictive power (explained a high proportion of the variation in the response variable: usually ≥60% r^2-adj value, or alternatively the best available option when r^2-adj was lower than the recommended value), with a single or combination of predictor variables were selected to test the potential effectiveness of these models in predicting the response of key functional attributes of stream vegetation. To do this, an internal test procedure was conducted which retained c. 90-95% of the original data set to re-build the minimal models yielding reduced versions that utilised the remaining c.10% as 'test data'. The effectiveness of these reduced (minimal) models was determined by plotting observed values against the predicted values of each model for the test data set, as appropriate for each of the chosen response variables. Furthermore, a two-sample t-test was used to determine whether the mean observed and predicted response values were the same; thus enabling the effectiveness of each minimal model to be evaluated. If $p<0.05$, then the null hypothesis can be rejected, indicating that the sample population means differ significantly and the model is not a good fit for accurately predicting the response variable.

Minitab version 15.1.0 was used to conduct the regression analyses, and Microsoft Excel 2003 was used to plot the observed and predicted values of each response variable for the test data.

3.5 Results

3.5.1 Variation in periphyton production and environmental habitat conditions in the Water of Dye, River Girnock and Knockan Burn; their sub-catchments and sites

3.5.1.1 Periphyton production of artificial substrata

The use of short-term linoleum substrates (Table 3.4) indicated that significantly higher quantities of periphyton biomass were harvested from Knockan Burn compared to the Water of Dye and River Girnock (which did not differ significantly from each other) but periphyton chlorophyll content did not vary significantly between the three streams. Long-term linoleum and long-term Astroturf substrates showed similar patterns (as for short-term linoleum substrates) of periphyton production between the three streams (Table 3.6 and Table 3.8, respectively).

3.5.1.2 Periphyton production of naturally-occurring substrata

Periphyton biomass harvested from naturally-occurring mineral substrata was significantly lower in the Water of Dye compared to the River Girnock and Knockan Burn (which did not vary significantly from one another). However, periphyton chlorophyll content did not vary significantly between the streams (Table 3.14). Of the three sub-catchment streams, the River Girnock had significantly higher periphyton cover and lower unvegetated area on the surface of naturally-occurring mineral substrata.

Aquatic bryophytes harvested from Knockan Burn had significantly higher quantities of periphyton biomass and chlorophyll content compared to the other two streams. Furthermore, periphyton production was significantly higher in the Water of Dye than on aquatic bryophytes occurring in the River Girnock (Table 3.16).

Mean Variable	**Water of Dye**		**River Girnock**		**Knockan Burn**		**P**ANOVA
	Mean	***S.E***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **short-term linoleum** substrates	0.38^{a}	0.18	0.29^{a}	0.20	0.60^{b}	0.22	P<0.01**
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **short-term linoleum** substrates	0.21	0.17	0.18	0.18	0.31	0.20	NS
D (m)	0.14	0.09	0.11	0.11	0.10	0.11	NS
K (m^{-1})	3.06^{a}	0.18	2.50^{b}	0.22	2.31^{b}	0.24	P<0.01**
$Z_{eu}^{1\%}$ (m)	0.24^{a}	0.18	0.23^{a}	0.29	0.40^{b}	0.24	P<0.001***
Z_{eu}:$D^{1\%}$	1.60^{a}	0.20	2.33^{b}	0.30	3.86^{c}	0.26	P<0.001***
pH	6.34^{a}	0.08	6.85^{b}	0.05	7.52^{c}	0.04	P<0.001***
Conductivity (μS cm^{-1})	46.0^{a}	0.13	50.8^{b}	0.16	146.8^{c}	0.06	P<0.001***
Water Temperature (^{o}C)	8.1^{a}	0.08	11.0^{b}	0.05	8.5^{a}	0.06	P<0.01**
Flow (m s^{-1})	0.144^{a}	0.03	0.178^{a}	0.04	0.269^{b}	0.03	P<0.01**

Table 3.4 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 293), periphyton chlorophyll content per unit area (n = 242) and environmental habitat variables (n = 263) of short-term linoleum substrates between study stream sub-catchments. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

	Water of Dye						River Girnock						Knockan Burn						
Mean Variable	**BB**		**CF**		**BD**		**IB**		**HB**		**LM**		**UK**		**MK**		**LK**		**P_{ANOVA}**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **short-term linoleum** substrates	0.28^{a}	0.25	0.41^{a}	0.34	0.45^{a}	0.31	0.20^{a}	0.42	0.38^{a}	0.42	0.29^{a}	0.25	0.79^{b}	0.37	0.15^{a}	0.29	0.86^{b}	0.38	P<0.001***
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **short-term linoleum** substrates	0.19^{a}	0.32	0.21^{a}	0.38	0.24^{a}	0.31	0.12^{a}	0.29	0.19^{a}	0.33	0.23^{a}	0.28	0.20^{a}	0.38	0.12^{a}	0.24	0.62^{b}	0.26	P<0.001***
D (m)	0.11^{ab}	0.12	0.19^{b}	0.15	0.17^{b}	0.14	0.13^{ab}	0.15	0.08^{a}	0.23	0.12^{ab}	0.18	0.11^{ab}	0.13	0.08^{a}	0.11	0.12^{ab}	0.14	P<0.001***
K (m^{-1})	4.58^{a}	0.25	2.27^{b}	0.29	2.42^{b}	0.31	2.21^{b}	0.37	2.57^{b}	0.34	2.65^{b}	0.41	2.53^{b}	0.38	2.35^{b}	0.45	2.10^{b}	0.40	P<0.001***
$Z_{eu}{}^{1\%}$ (m)	0.16^{a}	0.25	0.36^{bc}	0.29	0.28^{c}	0.31	0.40^{b}	0.37	0.38^{b}	0.34	0.09^{d}	0.41	0.34^{bc}	0.38	0.38^{b}	0.45	0.47^{b}	0.40	P<0.001***
Z_{eu}:$D^{1\%}$	1.44^{a}	0.30	1.87^{ab}	0.40	1.59^{a}	0.32	3.06^{b}	0.40	5.39^{c}	0.52	0.80^{d}	0.40	3.24^{b}	0.45	4.57^{c}	0.47	3.83^{c}	0.41	P<0.001***
pH	5.72^{a}	0.13	6.91^{bc}	0.05	6.61^{b}	0.07	6.57^{b}	0.07	6.96^{c}	0.07	6.97^{c}	0.08	7.26^{d}	0.04	7.38^{d}	0.03	7.88^{e}	0.04	P<0.001***
Conductivity (μS cm^{-1})	39.9^{a}	0.02	47.2^{b}	0.04	53.6^{bc}	0.03	42.2^{ab}	0.08	51.7^{c}	0.07	57.2^{c}	0.07	109.7^{d}	0.07	127.3^{d}	0.07	217.1^{e}	0.06	P<0.001***
Water Temperature (^{o}C)	7.4^{a}	0.11	8.4^{ab}	0.17	8.8^{ab}	0.16	11.0^{b}	0.07	12.0^{b}	0.07	10.2^{ab}	0.11	8.1^{ab}	0.10	8.2^{ab}	0.10	9.0^{ab}	0.09	P<0.001***
Flow ($m\ s^{-1}$)	0.190^{a}	0.04	0.194^{a}	0.05	0.06^{b}	0.04	0.208^{a}	0.06	0.191^{a}	0.06	0.146^{a}	0.04	0.182^{a}	0.04	0.422^{c}	0.04	0.221^{a}	0.04	P<0.001***

Table 3.5 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 293), chlorophyll content per unit area (n = 242) and environmental habitat variables (n = 263) of short-term linoleum substrates between sampling sites. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Mean Variable	**Water of Dye**		**River Girnock**		**Knockan Burn**		**P**ANOVA
	Mean	***S.E***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **long-term linoleum** substrates	0.31^{a}	0.23	0.41^{a}	0.22	0.65^{b}	0.24	P<0.01**
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **long-term linoleum** substrates	0.34	0.25	0.29	0.19	0.31	0.21	NS
D (m)	0.14	0.13	0.11	0.14	0.10	0.10	NS
K (m^{-1})	2.96^{a}	0.32	2.60^{ab}	0.34	2.25^{b}	0.22	P<0.05*
$Z_{eu}^{1\%}$ (m)	0.28	0.32	0.29	0.34	0.36	0.25	NS
Z_{eu}:$D^{1\%}$	1.85^{a}	0.33	2.79^{ab}	0.39	3.36^{b}	0.26	P<0.05*
pH	6.28^{a}	0.13	6.76^{b}	0.07	7.47^{c}	0.05	P<0.001***
Conductivity (μS cm^{-1})	45.6^{a}	0.04	49.9^{b}	0.06	136.8	0.06	P<0.001***
Water Temperature (^{o}C)	9.5^{ab}	0.13	10.9^{b}	0.07	8.5^{a}	0.05	P<0.01**
Flow (m s^{-1})	0.180^{a}	0.04	0.181^{a}	0.04	0.395^{b}	0.03	P<0.01**

Table 3.6 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 144), periphyton chlorophyll content per unit area (n = 112) and environmental habitat variables (n = 144) of long-term linoleum substrates between study stream sub-catchments. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

	Water of Dye						River Girnock						Knockan Burn						
Mean Variable	**BB**		**CF**		**BD**		**IB**		**HB**		**LM**		**UK**		**MK**		**LK**		**P_{ANOVA}**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **long-term linoleum** substrates	0.28^{a}	0.35	0.24^{a}	0.92	0.40^{a}	0.39	0.35^{a}	0.34	0.48^{a}	0.57	0.39^{a}	0.22	0.68^{ab}	0.38	0.22^{a}	0.33	1.04^{b}	0.45	P<0.05*
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **long-term linoleum** substrates	0.34^{a}	0.36	0.30^{ab}	0.46	0.38^{a}	0.29	0.26^{ab}	0.44	0.35^{ab}	0.26	0.27^{ab}	0.21	0.24^{ab}	0.32	0.15^{b}	0.33	0.55^{a}	0.30	P<0.05*
D (m)	0.12^{ab}	0.17	0.21^{b}	0.20	0.16^{b}	0.24	0.12^{ab}	0.25	0.09^{a}	0.25	0.11^{ab}	0.20	0.11^{ab}	0.19	0.09^{a}	0.10	0.11^{ab}	0.20	P<0.05*
K (m^{-1})	4.45^{a}	0.40	1.78^{b}	0.41	1.92^{b}	0.60	2.34^{ab}	0.55	2.03^{b}	0.77	1.78^{b}	0.47	2.92^{ab}	0.38	2.97^{ab}	0.33	2.81^{ab}	0.43	P<0.01**
$Z_{eu}^{1\%}$ (m)	0.16^{a}	0.40	0.46^{b}	0.41	0.35^{b}	0.60	0.35^{b}	0.55	0.48^{b}	0.77	0.14^{a}	0.47	0.29^{b}	0.38	0.30^{b}	0.33	0.35^{b}	0.43	P<0.001***
Z_{eu}:$D^{1\%}$	1.41^{a}	0.38	2.21^{ab}	0.66	2.20^{ab}	0.64	3.05^{b}	0.50	5.66^{c}	0.88	1.25^{a}	0.74	2.52^{ab}	0.44	3.47^{b}	0.39	3.26^{b}	0.48	P<0.001***
pH	5.58^{a}	0.22	6.93^{bc}	0.11	6.55^{b}	0.09	6.47^{b}	0.09	6.94^{c}	0.11	6.87^{c}	0.13	7.18^{d}	0.03	7.28^{d}	0.02	7.87^{e}	0.06	P<0.001***
Conductivity (μS cm^{-1})	37.7^{a}	0.05	48.5^{ab}	0.09	55.9^{b}	0.04	38.3^{a}	0.09	51.7^{b}	0.10	56.6^{b}	0.09	91.6^{c}	0.03	101.9^{c}	0.05	176.5^{d}	0.06	P<0.001***
Water Temperature (^{o}C)	8.0^{a}	0.18	10.3^{ab}	0.29	11.0^{ab}	0.21	10.5^{ab}	0.08	12.1^{b}	0.10	10.2^{ab}	0.15	8.4^{a}	0.11	8.2^{a}	0.10	9.1^{ab}	0.06	P<0.01**
Flow (m s^{-1})	0.179^{ab}	0.06	0.299^{a}	0.09	0.067^{b}	0.06	0.195^{ab}	0.08	0.216^{ab}	0.07	0.138^{ab}	0.06	0.264^{ab}	0.05	0.422^{c}	0.05	0.514^{c}	0.06	P<0.001***

Table 3.7 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 144), chlorophyll content per unit area (n = 112) and environmental habitat variables (n = 144) of long-term linoleum substrates between sampling sites. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Mean Variable	**Water of Dye**		**River Girnock**		**Knockan Burn**		**P**ANOVA
	Mean	***S.E***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **long-term Astroturf** substrates	0.52^{a}	0.20	0.57^{a}	0.14	2.68^{b}	0.22	P<0.001***
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **long-term Astroturf** substrates	0.15	0.19	0.18	0.11	0.23	0.20	NS
D (m)	0.13	0.17	0.11	0.16	0.12	0.14	NS
K (m^{-1})	2.98^{a}	0.35	2.25^{b}	0.33	2.21^{b}	0.39	P<0.05*
$Z_{eu}^{1\%}$ (m)	0.25	0.34	0.26	0.33	0.38	0.40	NS
Z_{eu}:$D^{1\%}$	1.88^{a}	0.35	2.99^{ab}	0.47	3.31^{b}	0.44	P<0.05*
pH	6.39^{a}	0.11	6.79^{b}	0.07	7.45^{c}	0.06	P<0.001***
Conductivity (μS cm^{-1})	46.5^{a}	0.09	50.6^{b}	0.08	137.8^{c}	0.07	P<0.001***
Water Temperature (^{o}C)	9.6^{ab}	0.13	11.1^{a}	0.07	8.4^{b}	0.07	P<0.01**
Flow (m s^{-1})	0.155^{a}	0.05	0.147^{a}	0.04	0.401^{b}	0.05	P<0.001***

Table 3.8 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 113), periphyton chlorophyll content per unit area (n = 112) and environmental habitat variables (n = 113) of long-term Astroturf substrates between study stream sub-catchments. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

	Water of Dye						River Girnock						Knockan Burn						
Mean Variable	**BB**		**CF**		**BD**		**IB**		**HB**		**LM**		**UK**		**MK**		**LK**		**P_{ANOVA}**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **long-term Astroturf** substrates	0.43^{a}	0.22	0.80^{a}	0.36	0.34^{a}	0.36	0.60^{a}	0.30	0.72^{a}	0.24	0.38^{a}	0.13	5.22^{b}	0.19	1.34^{c}	0.21	1.47^{c}	0.25	P<0.001***
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **long-term Astroturf** substrates	0.18^{a}	0.25	0.10^{a}	0.37	0.11^{a}	0.27	0.19^{a}	0.21	0.22^{a}	0.19	0.14^{a}	0.14	0.07^{a}	0.21	0.20^{a}	0.27	0.41^{b}	0.12	P<0.01**
D (m)	0.10^{ab}	0.28	0.11^{ab}	0.30	0.24^{a}	0.24	0.08^{b}	0.28	0.06^{b}	0.28	0.13^{ab}	0.25	0.11^{ab}	0.27	0.11^{ab}	0.19	0.15^{ab}	0.23	P<0.05*
K (m^{-1})	4.60^{a}	0.54	2.78^{ab}	0.65	2.09^{ab}	0.45	2.81^{ab}	0.49	2.69^{ab}	0.63	1.41^{b}	0.50	3.28^{ab}	0.63	2.11^{ab}	0.58	1.78^{ab}	0.72	P<0.01**
$Z_{eu}^{1\%}$ (m)	0.16^{a}	0.54	0.30^{ab}	0.65	0.32^{ab}	0.45	0.32^{ab}	0.49	0.38^{b}	0.63	0.17^{a}	0.50	0.26^{ab}	0.63	0.43^{b}	0.58	0.55^{b}	0.72	P<0.01**
Z_{eu}:$D^{1\%}$	1.67^{a}	0.52	2.75^{ab}	0.59	1.36^{a}	0.64	4.20^{b}	0.56	6.26^{c}	0.76	1.32^{a}	0.68	2.51^{ab}	0.65	4.06^{b}	0.56	3.63^{b}	1.08	P<0.001***
pH	5.83^{a}	0.25	6.87^{bc}	0.10	6.46^{b}	0.09	6.53^{b}	0.09	6.98^{c}	0.12	6.85^{c}	0.13	7.14^{d}	0.04	7.27^{d}	0.03	7.89^{e}	0.08	P<0.001***
Conductivity (μS cm^{-1})	40.2^{a}	0.06	48.2^{ab}	0.07	55.2^{b}	0.05	40.5^{a}	0.09	54.6^{b}	0.10	55.1^{b}	0.09	91.9^{c}	0.04	95.9^{c}	0.06	176.2^{d}	0.09	P<0.001***
Water Temperature (oC)	9.2^{ab}	0.21	10.0^{ab}	0.23	10.3^{ab}	0.25	10.9^{ab}	0.08	12.8^{a}	0.10	9.9^{ab}	0.15	8.2^{b}	0.15	7.8^{b}	0.14	9.1^{ab}	0.09	P<0.05*
Flow (m s^{-1})	0.142^{a}	0.06	0.220^{a}	0.09	0.105^{a}	0.09	0.140^{a}	0.07	0.134^{a}	0.06	0.164^{a}	0.07	0.162^{a}	0.07	0.521^{b}	0.05	0.539^{b}	0.05	P<0.001***

Table 3.9 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 113), chlorophyll content per unit area (n = 112) and environmental habitat variables (n = 113) of long-term Astroturf substrates between sampling sites. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Mean Variable	**Water of Dye**		**River Girnock**		**Knockan Burn**		**P**ANOVA
	Mean	***S.E***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **long-term plastic aquarium *Potamogeton*-like** substrates	N/A		N/A		0.19	0.25	N/A
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **long-term plastic aquarium *Potamogeton*-like** substrates	N/A		N/A		0.16	0.27	N/A
D (m)	N/A		N/A		0.11	0.18	N/A
K (m^{-1})	N/A		N/A		2.75	0.41	N/A
$Z_{eu}^{1\%}$ (m)	N/A		N/A		0.33	0.42	N/A
Z_{eu}:$D^{1\%}$	N/A		N/A		3.03	0.38	N/A
pH	N/A		N/A		7.48	0.09	N/A
Conductivity (μS cm^{-1})	N/A		N/A		133.8	0.08	N/A
Water Temperature (^{o}C)	N/A		N/A		8.5	0.10	N/A
Flow (m s^{-1})	N/A		N/A		0.310	0.04	N/A

Table 3.10 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 22), periphyton chlorophyll content per unit area (n = 20) and environmental habitat variables (n = 22) of long-term plastic aquarium *Potamogeton*-like substrates between study stream sub-catchments. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. [Note: N/A represents data wherein records do not exist and therefore one-way ANOVA could not be performed].

	Water of Dye								River Girnock						Knockan Burn					
Mean Variable	**BB**		**CF**		**BD**		**IB**		**HB**		**LM**		**UK**		**MK**		**LK**		**PANOVA**	
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***		
Periphyton biomass per unit area (mg cm^{-2}) harvested from **long-term plastic *Potamogeton*-like** substrates	N/A		N/A		N/A		N/A		N/A		N/A		0.05^{a}	0.23	N/A		0.33^{b}	0.28	P<0.001***	
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **long-term plastic *Potamogeton*-like** substrates	N/A		N/A		N/A		N/A		N/A		N/A		0.05^{a}	0.27	N/A		0.27^{b}	0.19	P<0.001***	
D (m)	N/A		N/A		N/A		N/A		N/A		N/A		0.10	0.21	N/A		0.12	0.31	NS	
K (m^{-1})	N/A		N/A		N/A		N/A		N/A		N/A		3.36	0.48	N/A		2.16	0.65	NS	
$Z_{eu}^{1\%}$ (m)	N/A		N/A		N/A		N/A		N/A		N/A		0.26	0.48	N/A		0.46	0.65	NS	
Z_{eu}:$D^{1\%}$	N/A		N/A		N/A		N/A		N/A		N/A		2.50	0.50	N/A		3.62	0.56	NS	
pH	N/A		N/A		N/A		N/A		N/A		N/A		7.14^{a}	0.04	N/A		7.84^{b}	0.09	P<0.001***	
Conductivity (μS cm^{-1})	N/A		N/A		N/A		N/A		N/A		N/A		92.1^{a}	0.04	N/A		175.9^{b}	0.08	P<0.001***	
Water Temperature (^{o}C)	N/A		N/A		N/A		N/A		N/A		N/A		8.2	0.13	N/A		8.9	0.08	NS	
Flow (m s^{-1})	N/A		N/A		N/A		N/A		N/A		N/A		0.239	0.07	N/A		0.405	0.08	NS	

Table 3.11 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 22), chlorophyll content per unit area (n = 20) and environmental habitat variables (n = 22) of long-term plastic aquarium *Potamogeton*-like substrates between sampling sites. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Mean Variable	**Water of Dye**		**River Girnock**		**Knockan Burn**		**P**ANOVA
	Mean	***S.E***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **long-term plastic aquarium *Myriophyllum*-like** substrates	N/A		N/A		0.39	0.30	N/A
Periphyton chlorophyll content per unit area (µg cm^{-2}) harvested from **long-term plastic aquarium *Myriophyllum*-like** substrates	N/A		N/A		0.26	0.30	N/A
D (m)	N/A		N/A		0.11	0.18	N/A
K (m^{-1})	N/A		N/A		2.75	0.41	N/A
$Z_{eu}^{1\%}$ (m)	N/A		N/A		0.33	0.42	N/A
Z_{eu}:$D^{1\%}$	N/A		N/A		3.03	0.38	N/A
pH	N/A		N/A		7.48	0.09	N/A
Conductivity (µS cm^{-1})	N/A		N/A		133.8	0.08	N/A
Water Temperature (^{o}C)	N/A		N/A		8.5	0.10	N/A
Flow (m s^{-1})	N/A		N/A		0.310	0.04	N/A

Table 3.12 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 22), periphyton chlorophyll content per unit area (n = 20) and environmental habitat variables (n = 22) of long-term plastic aquarium *Myriophyllum*-like substrates between study stream sub-catchments. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. [Note: N/A represents data wherein records do not exist and therefore one-way ANOVA could not be performed].

	Water of Dye								River Girnock						Knockan Burn						
Mean Variable	**BB**		**CF**		**BD**		**IB**		**HB**		**LM**		**UK**		**MK**		**LK**		**P**ANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **long-term plastic *Myriophyllum*-like** substrates	N/A		N/A		N/A		N/A		N/A		N/A		0.13^{a}	0.39	N/A		0.64^{b}	0.23	P<0.001***
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **long-term plastic *Myriophyllum*-like** substrates	N/A		N/A		N/A		N/A		N/A		N/A		0.16^{a}	0.43	N/A		0.35^{b}	0.11	P<0.01**
D (m)	N/A		N/A		N/A		N/A		N/A		N/A		0.10	0.21	N/A		0.12	0.31	NS
K (m^{-1})	N/A		N/A		N/A		N/A		N/A		N/A		3.36	0.48	N/A		2.16	0.65	NS
$Z_{eu}^{1\%}$ (m)	N/A		N/A		N/A		N/A		N/A		N/A		0.26	0.48	N/A		0.46	0.65	NS
Z_{eu}:$D^{1\%}$	N/A		N/A		N/A		N/A		N/A		N/A		2.50	0.50	N/A		3.62	0.56	NS
pH	N/A		N/A		N/A		N/A		N/A		N/A		7.14^{a}	0.04	N/A		7.84^{b}	0.09	P<0.001***
Conductivity (μS cm^{-1})	N/A		N/A		N/A		N/A		N/A		N/A		92.1^{a}	0.04	N/A		175.9^{b}	0.08	P<0.001***
Water Temperature (^{o}C)	N/A		N/A		N/A		N/A		N/A		N/A		8.2	0.13	N/A		8.9	0.08	NS
Flow (m s^{-1})	N/A		N/A		N/A		N/A		N/A		N/A		0.239	0.07	N/A		0.405	0.08	NS

Table 3.13 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 22), chlorophyll content per unit area (n = 20) and environmental habitat variables (n = 22) of long-term plastic aquarium *Myriophyllum*-like substrates between sampling sites. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Mean Variable	**Water of Dye**		**River Girnock**		**Knockan Burn**		**P**ANOVA
	Mean	***S.E***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **mineral** substrata	0.76^{a}	0.16	2.56^{b}	0.21	2.93^{b}	0.19	P<0.001***
Periphyton chlorophyll content per unit area (µg cm^{-2}) harvested from **mineral** substrata	1.55	0.29	1.46	0.28	1.23	0.23	NS
Periphyton cover (%)	18.2^{a}	1.65	38.5^{b}	1.89	19.5^{a}	1.59	P<0.001***
Bare area (%)	38.6^{a}	1.95	28.1^{b}	1.75	42.9^{a}	2.09	P<0.001***
D (m)	0.14	0.17	0.12	0.16	0.13	0.19	NS
K (m^{-1})	2.99^{a}	0.15	2.32^{b}	0.15	2.57^{b}	0.14	P<0.001***
$Z_{eu}^{1\%}$ (m)	0.25^{a}	0.15	0.26^{a}	0.19	0.36^{b}	0.14	P<0.001***
Z_{eu}:$D^{1\%}$	1.81^{a}	0.17	2.14^{a}	0.22	2.84^{b}	0.17	P<0.001***
pH	6.33^{a}	0.07	6.93^{b}	0.05	7.56^{c}	0.03	P<0.001***
Conductivity (µS cm^{-1})	45.8^{a}	0.11	51.8^{b}	0.13	138.5^{c}	0.12	P<0.001***
Water Temperature (^{o}C)	10.2^{ab}	0.05	11.2^{a}	0.07	9.0^{b}	0.02	P<0.01**
Flow (m s^{-1})	0.218^{a}	0.02	0.203^{a}	0.02	0.290^{b}	0.03	P<0.01**

Table 3.14 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 405), periphyton chlorophyll content per unit area (n = 173), periphyton cover (n = 405), bare area (n = 405) and environmental habitat variables (n = 405) of naturally-occurring mineral substrata between study stream sub-catchments. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of underlying geology, substrate morphology, alkalinity, nutrient status and heavy metal composition refer to Table 2.1 in Chapter 2.

	Water of Dye						River Girnock						Knockan Burn						
Mean Variable	**BB**		**CF**		**BD**		**IB**		**HB**		**LM**		**UK**		**MK**		**LK**		**P_{ANOVA}**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **mineral** substrata	0.57^{a}	0.21	0.96^{b}	0.23	0.75^{ab}	0.32	4.32^{c}	0.18	2.56^{cd}	0.15	0.81^{b}	0.15	4.26^{cd}	0.20	1.64^{d}	0.33	2.88^{d}	0.40	P<0.001***
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **mineral** substrata	1.67^{a}	0.28	1.44^{a}	0.16	1.55^{a}	0.13	1.53^{a}	0.21	1.85^{a}	0.22	1.01^{b}	0.33	0.72^{b}	0.24	1.82^{a}	0.26	1.16^{b}	0.21	P<0.01**
Periphyton cover (%)	19.4^{a}	2.69	22.9^{a}	3.41	12.2^{b}	2.18	42.1^{c}	3.43	43.0^{c}	3.15	30.5^{ac}	3.01	18.7^{a}	2.62	16.3^{ab}	2.41	23.4^{a}	3.22	P<0.001***
Bare area (%)	33.3^{ab}	2.83	44.7^{a}	3.74	37.7^{ab}	3.34	25.5^{b}	3.02	25.0^{b}	2.95	33.9^{ab}	3.01	42.0^{a}	3.27	47.0^{a}	3.51	39.8^{a}	4.04	P<0.001***
D (m)	0.09^{a}	0.30	0.17^{b}	0.27	0.18^{b}	0.24	0.12^{ab}	0.25	0.11^{a}	0.28	0.13^{ab}	0.30	0.13^{ab}	0.38	0.11^{a}	0.29	0.13^{ab}	0.30	P<0.001***
K (m^{-1})	4.39^{a}	0.22	2.37^{b}	0.22	2.58^{b}	0.26	2.50^{b}	0.25	2.43^{b}	0.24	2.05^{b}	0.28	2.64^{b}	0.22	2.35^{b}	0.22	2.64^{b}	0.29	P<0.001***
$Z_{eu}^{1\%}$ (m)	0.17^{a}	0.22	0.35^{bd}	0.22	0.26^{d}	0.26	0.36^{b}	0.25	0.40^{b}	0.24	0.12^{c}	0.28	0.33^{bd}	0.22	0.37^{b}	0.22	0.37^{b}	0.29	P<0.001***
Z_{eu}:$D^{1\%}$	1.91^{a}	0.26	2.08^{ab}	0.31	1.48^{a}	0.30	2.87^{b}	0.25	3.66^{b}	0.31	0.93^{c}	0.36	2.46^{b}	0.32	3.34^{b}	0.27	2.81^{b}	0.30	P<0.001***
pH	5.89^{a}	0.13	6.80^{b}	0.07	6.29^{c}	0.12	6.51^{c}	0.08	7.15^{d}	0.06	7.13^{d}	0.06	7.31^{d}	0.01	7.43^{d}	0.02	7.95^{e}	0.03	P<0.001***
Conductivity (μS cm^{-1})	38.4^{a}	0.18	48.1^{b}	0.19	52.1^{bc}	0.19	39.0^{a}	0.22	58.6^{c}	0.22	60.9^{c}	0.21	116.8^{d}	0.19	110.9^{d}	0.18	205.2^{e}	0.19	P<0.001***
Water Temperature (^{o}C)	9.9^{ab}	0.10	10.7^{ab}	0.06	10.1^{ab}	0.10	10.9^{ab}	0.12	12.8^{a}	0.16	9.9^{ab}	0.05	8.8^{b}	0.05	8.2^{b}	0.03	10.1^{ab}	0.02	P<0.001***
Flow (m s^{-1})	0.228^{a}	0.06	0.216^{a}	0.06	0.209^{a}	0.06	0.224^{a}	0.07	0.198^{a}	0.07	0.188^{a}	0.06	0.168^{a}	0.08	0.475^{b}	0.08	0.267^{a}	0.08	P<0.001***

Table 3.15 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 405), chlorophyll content per unit area (n = 173), periphyton cover (n = 405), bare area (n = 405) and environmental habitat variables (n = 405) of naturally-occurring mineral substrata between sampling sites. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of underlying geology, substrate morphology, alkalinity, nutrient status and heavy metal composition refer to Table 2.2 in Chapter 2.

Mean Variable	**Water of Dye**		**River Girnock**		**Knockan Burn**		**PANOVA**
	Mean	***S.E***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **aquatic bryophytes**	1.45^{a}	0.17	0.67^{b}	0.19	11.52^{c}	0.33	P<0.001***
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **aquatic bryophytes**	1.28^{a}	0.16	0.85^{b}	0.11	2.06^{c}	0.24	P<0.001***
Periphyton cover (%)	18.2^{a}	1.65	38.5^{b}	1.89	19.5^{a}	1.59	P<0.001***
Bare area (%)	38.6^{a}	1.95	28.1^{b}	1.75	42.9^{a}	2.09	P<0.001***
D (m)	0.14	0.17	0.12	0.16	0.13	0.19	NS
K (m^{-1})	2.99^{a}	0.15	2.32^{b}	0.15	2.57^{b}	0.14	P<0.001***
$Z_{eu}^{1\%}$ (m)	0.25^{a}	0.15	0.26^{a}	0.19	0.36^{b}	0.14	P<0.001***
Z_{eu}:$D^{1\%}$	1.81^{a}	0.17	2.14^{a}	0.22	2.84^{b}	0.17	P<0.001***
pH	6.33^{a}	0.07	6.93^{b}	0.05	7.56^{c}	0.03	P<0.001***
Conductivity (μS cm^{-1})	45.8^{a}	0.11	51.8^{b}	0.13	138.5^{c}	0.12	P<0.001***
Water Temperature (^{o}C)	10.2^{ab}	0.05	11.2^{a}	0.07	9.0^{b}	0.02	P<0.01**
Flow (m s^{-1})	0.218^{a}	0.02	0.203^{a}	0.02	0.290^{b}	0.03	P<0.01**

Table 3.16 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 306), periphyton chlorophyll content per unit area (n = 176), periphyton cover (n = 306), bare area (n = 306) and environmental habitat variables (n = 306) of naturally-occurring aquatic bryophytes between study stream sub-catchments. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of underlying geology, substrate morphology, alkalinity, nutrient status and heavy metal composition refer to Table 2.1 in Chapter 2.

	Water of Dye						River Girnock						Knockan Burn						
Mean Variable	**BB**		**CF**		**BD**		**IB**		**HB**		**LM**		**UK**		**MK**		**LK**		**P_{ANOVA}**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **aquatic bryophytes**	1.55^{a}	0.23	1.49^{a}	0.26	1.31^{a}	0.21	1.23^{a}	0.16	0.47^{b}	0.21	0.30^{b}	0.51	11.76^{c}	0.25	9.58^{c}	0.26	13.22^{c}	0.16	P<0.001***
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **aquatic bryophytes**	1.68^{a}	0.16	1.01^{b}	0.31	1.16^{b}	0.17	0.95^{b}	0.12	0.83^{b}	0.16	0.78^{b}	0.39	1.44^{ab}	0.17	1.80^{b}	0.16	2.93^{c}	0.17	P<0.001***
Periphyton cover (%)	19.4^{a}	2.69	22.9^{a}	3.41	12.2^{b}	2.18	42.1^{c}	3.43	43.0^{c}	3.15	30.5^{ac}	3.01	18.7^{a}	2.62	16.3^{ab}	2.41	23.4^{a}	3.22	P<0.001***
Bare area (%)	33.3^{ab}	2.83	44.7^{a}	3.74	37.7^{ab}	3.34	25.5^{b}	3.02	25.0^{b}	2.95	33.9^{ab}	3.01	42.0^{a}	3.27	47.0^{a}	3.51	39.8^{a}	4.04	P<0.001***
D (m)	0.09^{a}	0.30	0.17^{b}	0.27	0.18^{b}	0.24	0.12^{ab}	0.25	0.11^{a}	0.28	0.13^{ab}	0.30	0.13^{ab}	0.38	0.11^{a}	0.29	0.13^{ab}	0.30	P<0.001***
K (m^{-1})	4.39^{a}	0.22	2.37^{b}	0.22	2.58^{b}	0.26	2.50^{b}	0.25	2.43^{b}	0.24	2.05^{b}	0.28	2.64^{b}	0.22	2.35^{b}	0.22	2.64^{b}	0.29	P<0.001***
$Z_{eu}{}^{1\%}$ (m)	0.17^{a}	0.22	0.35^{bd}	0.22	0.26^{d}	0.26	0.36^{b}	0.25	0.40^{b}	0.24	0.12^{c}	0.28	0.33^{bd}	0.22	0.37^{b}	0.22	0.37^{b}	0.29	P<0.001***
Z_{eu}:$D^{1\%}$	1.91^{a}	0.26	2.08^{ab}	0.31	1.48^{a}	0.30	2.87^{b}	0.25	3.66^{b}	0.31	0.93^{c}	0.36	2.46^{b}	0.32	3.34^{b}	0.27	2.81^{b}	0.30	P<0.001***
pH	5.89^{a}	0.13	6.80^{b}	0.07	6.29^{c}	0.12	6.51^{c}	0.08	7.15^{d}	0.06	7.13^{d}	0.06	7.31^{d}	0.01	7.43^{d}	0.02	7.95^{e}	0.03	P<0.001***
Conductivity (μS cm^{-1})	38.4^{a}	0.18	48.1^{b}	0.19	52.1^{bc}	0.19	39.0^{a}	0.22	58.6^{c}	0.22	60.9^{c}	0.21	116.8^{d}	0.19	110.9^{d}	0.18	205.2^{e}	0.19	P<0.001***
Water Temperature (^{o}C)	9.9^{ab}	0.10	10.7^{ab}	0.06	10.1^{ab}	0.10	10.9^{ab}	0.12	12.8^{a}	0.16	9.9^{ab}	0.05	8.8^{b}	0.05	8.2^{b}	0.03	10.1^{ab}	0.02	P<0.001***
Flow (m s^{-1})	0.228^{a}	0.06	0.216^{a}	0.06	0.209^{a}	0.06	0.224^{a}	0.07	0.198^{a}	0.07	0.188^{a}	0.06	0.168^{a}	0.08	0.475^{b}	0.08	0.267^{a}	0.08	P<0.001***

Table 3.17 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 306), chlorophyll content per unit area (n = 176), periphyton cover (n = 306), bare area (n = 306) and environmental habitat variables (n = 306) of naturally-occurring aquatic bryophytes between sampling sites. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of underlying geology, substrate morphology, alkalinity, nutrient status and heavy metal composition refer to Table 2.2 in Chapter 2.

Mean Variable	**Water of Dye**		**River Girnock**		**Knockan Burn**		**P**ANOVA
	Mean	***S.E***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **vascular submerged macrophytes**	N/A		N/A		0.44	0.40	N/A
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **vascular submerged macrophytes**	N/A		N/A		0.11	0.24	N/A
Periphyton cover (%)	N/A		N/A		18.7	4.23	N/A
Bare area (%)	N/A		N/A		42.5	3.13	N/A
D (m)	N/A		N/A		0.15	0.17	N/A
K (m^{-1})	N/A		N/A		2.26	0.33	N/A
$Z_{eu}^{1\%}$ (m)	N/A		N/A		0.42	0.33	N/A
Z_{eu}:$D^{1\%}$	N/A		N/A		2.50	0.32	N/A
pH	N/A		N/A		7.68	0.08	N/A
Conductivity (μS cm^{-1})	N/A		N/A		164.7	0.09	N/A
Water Temperature (^{o}C)	N/A		N/A		9.4	0.08	N/A
Flow (m s^{-1})	N/A		N/A		0.215	0.03	N/A

Table 3.18 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 24), periphyton chlorophyll content per unit area (n = 24), periphyton cover (n = 24), bare area (n = 24) and environmental habitat variables (n = 24) of naturally-occurring vascular submerged macrophytes between study stream sub-catchments. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of underlying geology, substrate morphology, alkalinity, nutrient status and heavy metal composition refer to Table 2.1 in Chapter 2.

	Water of Dye						River Girnock						Knockan Burn						
Mean Variable	**BB**		**CF**		**BD**		**IB**		**HB**		**LM**		**UK**		**MK**		**LK**		**PANOVA**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **vascular submerged macrophytes**	N/A		N/A		N/A		N/A		N/A		N/A		0.72^{a}	0.17	N/A		0.16^{b}	0.21	P<0.001***
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **vascular submerged macrophytes**	N/A		N/A		N/A		N/A		N/A		N/A		0.05^{a}	0.20	N/A	N/A	0.17^{b}	0.32	P<0.05*
Periphyton cover (%)	N/A		N/A		N/A		N/A		N/A		N/A		11.2	3.93	N/A	N/A	25.3	6.02	NS
Bare area (%)	N/A		N/A		N/A		N/A		N/A		N/A		40.2	4.76	N/A	N/A	46.8	4.02	NS
D (m)	N/A		N/A		N/A		N/A		N/A		N/A		0.15	0.27	N/A		0.15	0.22	NS
K (m^{-1})	N/A		N/A		N/A		N/A		N/A		N/A		2.35	0.66	N/A		2.21	0.35	NS
$Z_{eu}^{1\%}$ (m)	N/A		N/A		N/A		N/A		N/A		N/A		0.37	0.66	N/A		0.45	0.35	NS
Z_{eu}:$D^{1\%}$	N/A		N/A		N/A		N/A		N/A		N/A		2.44	0.57	N/A		2.54	0.39	NS
pH	N/A		N/A		N/A		N/A		N/A		N/A		7.34^{a}	0.02	N/A		7.95^{b}	0.06	P<0.001***
Conductivity (μS cm^{-1})	N/A		N/A		N/A		N/A		N/A		N/A		118.5^{a}	0.05	N/A		210.7^{b}	0.07	P<0.001***
Water Temperature (^{o}C)	N/A		N/A		N/A		N/A		N/A		N/A		8.2	0.15	N/A		10.1	0.10	NS
Flow (m s^{-1})	N/A		N/A		N/A		N/A		N/A		N/A		0.176	0.05	N/A		0.245	0.04	NS

Table 3.19 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 24), chlorophyll content per unit area (n = 24), periphyton cover (n = 24), bare area (n = 24) and environmental habitat variables (n = 24) of naturally-occurring vascular submerged macrophytes between sampling sites. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of underlying geology, substrate morphology, alkalinity, nutrient status and heavy metal composition refer to Table 2.2 in Chapter 2.

3.5.2 Temporal and seasonal variation in periphyton production and environmental habitat conditions in the Water of Dye, River Girnock and Knockan Burn

3.5.2.1 Periphyton production of artificial substrata in the Water of Dye

In the Water of Dye, periphyton biomass showed significant temporal variation on short-term linoleum substrates ranging between 0.02 – 2.04 mg cm^{-2} (Table 3.20). During October 2004 through to May 2005 periphyton biomass exhibited minor fluctuations but did not vary significantly between these sampling dates and remained at a minimal level (0.02 – 0.16 mg cm^{-2}). However, in July and August 2005, periphyton biomass harvested was significantly higher compared to previous sampling occasions. Periphyton biomass had returned to a significantly low background level of 0.12 mg cm^{-2} when short-term linoleum substrates were sampled in April 2006. In general, periphyton chlorophyll content reflected temporal variation observed for periphyton biomass on short-term linoleum substrates (Figure 3.13). Periphyton chlorophyll content ranged between 0.01 – 0.80 μg cm^{-2}, peaking significantly in the summer of 2005 and remained at a low background level during October 2004 – May 2005, and also in April 2006.

Streamwater depth varied significantly between sampling dates, ranging between 0.09 – 0.32 m in the Water of Dye (Table 3.20 and Figure 3.14). There was no significant variation in depth between November 2004 and May 2005 samplings in the Water of Dye. However, streamwaters were significantly shallower in July and August 2005, and deepest in April 2006.

Table 3.20 indicates that light regime factors (K, Zeu, and Zeu:D) showed significant temporal variation in the Water of Dye (see also Figure 3.15, Figure 3.16 and Figure 3.17, respectively). Light attenuation varied between 2.06 – 4.48 m^{-1}, Zeu ranged between 0.16 – 0.36 m, and Zeu:D ranged between 1.12 and 2.75, the latter peaking significantly in July and August 2005, and being lowest in November 2004, March 2005 and April 2006.

Streamwater pH exhibited strong temporal variation, ranging between pH 5.60 – 6.94 in the Water of Dye (Table 3.20 and Figure 3.18). In March and May 2005, streamwater pH was significantly lower compared to November 2004 and April 2005 records (which were similar to one another). In July and August 2005, pH reached a significantly high level (pH ˜6.9) compared to all other sampling dates. In April 2006, streamwater pH was comparable to that of March and May 2005.

Temporal variation of streamwater conductivity reflected that of pH in the Water of Dye, and ranged between 35.9 and 59.1 μScm^{-1} (Table 3.20 and Figure 3.19). Most marked, was the significant rise in streamwater conductivity in the summer of 2005.

Streamwater temperatures in the Water of Dye exhibited a strong temporal response, varying between 4.1 and 15.6^{o}C (Table 3.20 and Figure 3.20). Streamwaters were coldest in March 2005, April 2005 and April 2006, and were warmest in July and August 2005.

Streamwater flow varied between 0.049 and 0.571 ms^{-1} in the Water of Dye (Table 3.20 and Figure 3.21). Current velocities were recorded as occurring highest in April 2006 compared to all other dates sampled.

Seasonal variation in periphyton production and environmental habitat conditions is shown from short-term linoleum (Table 3.21), long-term linoleum (Table 3.22) and long-term Astroturf substrates (Table 3.23) sampled during surveys: May 2005, August 2005 and April 2006 in the Water of Dye sub-catchment.

Periphyton production (both biomass and chlorophyll content) harvested from short-term linoleum substrates exhibited significant seasonal variation between survey dates in the Water of Dye (Table 3.21). Periphyton production was significantly higher in August 2005 compared to May 2005 and April 2006 (which did not differ significantly from each other).

Periphyton biomass harvested from long-term linoleum substrates was also significantly higher in August 2005, compared to May 2005 and April 2006 in the Water of Dye (Table 3.22). Periphyton chlorophyll content showed a similar pattern.

Periphyton biomass harvested from from long-term Astroturf substrates in August 2005 was significantly higher compared to May 2005, but the April 2006 harvest did not vary significantly from either preceding survey date sampled (Table 3.23). Periphyton chlorophyll content showed a similar trend, but did not vary significantly between survey dates.

3.5.2.2 Periphyton production of naturally-occurring substrata in the Water of Dye

Periphyton production (biomass and chlorophyll) harvested from naturally-occurring mineral substrata was significantly lower in May 2005 compared to August 2005 and April 2006 in the Water of Dye (Table 3.24). Furthermore, harvested periphyton production was significantly higher in August 2005 than in April 2006. This pattern was reflected in seasonal variation of periphyton cover. However, bare (or unvegetated) area did not vary significantly between surveys.

The same pattern was observed for periphyton biomass and chlorophyll content harvested from aquatic bryophytes in the Water of Dye (Table 3.25).

Variation in environmental habitat conditions associated with naturally-occurring substrata in the Water of Dye is described in detail elsewhere (refer to Chapter 2, section 2.6.2.1).

3.5.2.3 Periphyton production of artificial substrata in the River Girnock

In the River Girnock, both periphyton biomass and chlorophyll content harvested from short-term linoleum substrates showed similar patterns of significant temporal variation, ranging between 0.02 – 0.63 mg cm^{-2} and 0.02 – 0.33 μg cm^{-2}, respectively (Table 3.26). Minimal periphyton production characterised the April and May 2005 harvests, and was significantly higher during the summer of 2005 and also in April 2006. Overall, periphyton chlorophyll content mirrored temporal variation observed for periphyton biomass on short-term linoleum substrates (Figure 3.22).

There was significant variation in benthic depth in the River Girnock between sampling dates, ranging between 0.04 – 0.31 m (Table 3.26 and Figure 3.23). Streamwater depth was similar in April and May 2005, but significantly shallower by comparison in July and August 2005. Streamwaters in the River Girnock were significantly deeper in April 2006 compared to all other dates sampled.

In the River Girnock, light attenuation (Table 3.26 and Figure 3.24) varied significantly between sampling dates (1.95 – 3.35 m^{-1}), but Zeu did not (Table 3.26 and Figure 3.25). Zeu:D ranged between 1.00 and 4.51, peaking in the summer of 2005 and being lowest in April 2005 and April 2006 (Table 3.26 and Figure 3.26).

In the River Girnock, streamwater pH ranged significantly between pH 6.30 – 7.22 (Table 3.26 and Figure 3.27). In April and May 2005, streamwater pH was similarly and lower compared to July and August 2005 during which levels had peaked significantly to pH ˜7.2. In April 2006, streamwater pH was significantly lower compared to all other dates sampled.

Streamwater conductivity exhibited a similar response to that of pH in the River Girnock, and varied between 31.2 and 76.6 μScm^{-1} (Table 3.26 and Figure 3.28). Compared to all other dates sampled, streamwater conductivity peaked significantly in July and August 2005, and was lowest in April 2006.

Table 3.26 and Figure 3.29 show that streamwater temperatures varied significantly between sampling dates in the River Girnock (7.2 – 14.3°C). Streamwaters were coldest in April 2005 and April 2006, and were warmest in July and August 2005.

In the River Girnock, streamwater flow varied between 0.062 and 0.676 ms^{-1} (Table 3.26 and Figure 3.30). The highest flows were recorded during the April 2006 harvest, and were significantly different from all other dates sampled.

Periphyton production (biomass and chlorophyll content) harvested from short-term linoleum substrates was significantly higher in August 2005 and April 2006 (which did not differ significantly from one another) compared to May 2005 in the River Girnock (Table 3.27).

In the River Girnock, periphyton production (biomass and chlorophyll content) harvested from long-term linoleum substrates was significantly lower in May 2005, compared to August 2005 and April 2006 (which were similar): Table 3.28.

Periphyton biomass harvested from long-term Astroturf substrates was significantly lower in May 2005 than in August 2005, but neither varied significantly from the April 2006 harvest. Periphyton chlorophyll content showed no significant variation between dates sampled in the River Girnock (Table 3.29).

3.5.2.4 Periphyton production of naturally-occurring substrata in the River Girnock

In the River Girnock, naturally-occurring mineral substrata (Table 3.30) and aquatic bryophytes (Table 3.31) showed similar trends in periphyton biomass and chlorophyll content as for the linoleum substrates. Periphyton production harvested (from both mineral particles and aquatic bryophytes) during May 2005 was significantly lower compared to August 2005 and April 2006 (which were similar). Significant variation in periphyton cover occurring on naturally-

occurring mineral substrata showed a similar pattern. However, bare area did not exhibit significant seasonal variation.

Variation in environmental habitat conditions associated with naturally-occurring substrata in the River Girnock is described in detail elsewhere (refer to Chapter 2, section 2.6.2.2).

3.5.2.5 Periphyton production of artificial substrata in Knockan Burn

In Knockan Burn, temporal variation in periphyton production (both biomass and chlorophyll content) harvested from short-term linoleum substrates exhibited similar trends that ranged between 0.07 – 1.11 mg cm^{-2} and 0.04 – 0.50 μg cm^{-2}, respectively (Table 3.32, and also Figure 3.31). Minimal periphyton production occurred in December 2006, compared to all other dates sampled. Periphyton biomass increased in the April 2006 harvest, continuing to do so through July 2006, peaking significantly in September 2006, and thereafter declining in November 2006. However, periphyton chlorophyll content did not vary significantly between the April, July, September and November 2006 harvests.

Benthic depth varied significantly between sampling dates in Knockan Burn (0.05 – 0.17 m): Table 3.32 and Figure 3.32. Streamwater depth was shallowest in July 2006, moderate in December 2005 and September 2006, and deepest in April and November 2006.

Underwater light regime showed significant temporal variation in Knockan Burn. Light attenuation (1.50 – 4.01 m^{-1}) was lowest in July 2006 and highest in September 2006 (Table 3.32 and Figure 3.33). Zeu ranged between 0.23 – 0.62 m, being highest in July 2006 and lowest in September 2006 (Table 3.32 and Figure 3.34). Zeu:D varied between 2.50 – 11.62, and was significantly higher in July 2006 compared to any other date sampled (Table 3.32 and Figure 3.35).

Streamwater pH ranged between pH 7.39 – 7.71 in Knockan Burn (Table 3.32 and Figure 3.36). In April and November 2006 streamwater pH was lowest and did not differ significantly between these sampling dates. In December 2005, July and September 2006 streamwater pH was similarly high.

In Knockan Burn, streamwater conductivity varied significantly between 94.5 - 253.9 μScm^{-1} (Table 3.32 and Figure 3.37). In April 2006, conductivity fell significantly lower than recorded during the December 2005 harvest. Streamwater conductivity peaked in July 2006, declining significantly thereafter in September and again in November 2006 (to levels comparable with April 2006).

Table 3.32 and Figure 3.38 show that streamwater temperatures ranged significantly between sampling dates in Knockan Burn (5.5 – 13.3^{o}C). Streamwaters were coldest in December 2005, increasing in April 2006, becoming warmest in July 2006, to gradually cooling in September and again in November 2006.

Streamwater flow varied between 0.179 and 0.458 ms^{-1} in Knockan Burn (Table 3.32 and Figure 3.39). Moderate flows characterised December 2005 and November 2006 harvests, with velocities peaking in April 2006 and becoming more subdued in July and September 2006.

Short-term linoleum, long-term linoleum and long-term Astroturf substrates supported similar trends of periphyton production in Knockan Burn (Table 3.33, Table 3.34 and Table 3.35, respectively). Periphyton biomass harvested in April 2006 was significantly lower than in September 2006, with the November 2006 harvest not differing significantly from either of the previous, but chlorophyll content showed no significant variation between the three survey dates sampled. Although exhibiting a similar pattern, the long-term plastic aquarium plants showed no significant variation in periphyton production between survey dates (Table 3.36 and Table 3.37).

3.5.2.6 Periphyton production of naturally-occurring substrata in Knockan Burn

Periphyton biomass on naturally-occurring mineral substrata (Table 3.38) was significantly lower in April 2006 compared to harvests in September and November 2006 (which also differed significantly from one another). However, periphyton chlorophyll content did not differ significantly between survey dates. Periphyton cover on naturally-occurring mineral substrata was significantly higher in September 2006 than in April and November 2006 (which were similar). The area of unvegetated streambed was significantly higher in April 2006 than observed in September 2006, but neither varied significantly from November 2006.

Periphyton biomass on naturally-occurring aquatic bryophyte (Table 3.39) and vascular submerged macrophytes (Table 3.40) was significantly lower in April 2006 than in September but did not differ significantly from material harvested in November 2006 (which was also similar to the September 2006 harvest). However, periphyton chlorophyll content showed no significant variation between dates sampled.

Variation in environmental habitat conditions associated with naturally-occurring substrata in Knockan Burn is described in detail elsewhere (refer to Chapter 2, section 2.6.2.3).

Mean Variable	October 2004		November 2004		January 2005		March 2005		April 2005		May 2005		July 2005		August 2005		April 2006		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **short-term linoleum** substrates	0.14^{a}	0.33	0.11^{a}	0.29	0.06^{a}	0.40	0.13^{a}	0.37	0.16^{a}	0.34	0.02^{a}	0.10	2.04^{b}	0.27	0.67^{b}	0.35	0.12^{a}	0.20	P<0.001***
Periphyton chlorophyll content per unit area (µg cm^{-2}) harvested from **short-term linoleum** substrates	0.12^{a}	0.15	0.03^{a}	0.19	0.05^{a}	0.25	0.10^{a}	0.28	0.13^{a}	0.35	0.01^{a}	0.03	0.80^{b}	0.23	0.53^{b}	0.29	0.09^{a}	0.19	P<0.001***
D (m)	N/A		0.19^{a}	0.18	N/A		0.23^{ac}	0.15	0.23^{ac}	0.17	0.17^{a}	0.28	0.09^{b}	0.21	0.12^{b}	0.14	0.32^{c}	0.13	P<0.001***
K (m^{-1})	N/A		3.77^{ab}	0.59	N/A		2.79^{ab}	0.37	2.48^{a}	0.42	4.43^{b}	0.68	4.48^{b}	0.42	2.93^{ab}	0.42	2.06^{a}	0.38	P<0.01**
$Z_{eu}^{1\%}$ (m)	N/A		0.20^{ab}	0.58	N/A		0.26^{ab}	0.37	0.30^{a}	0.43	0.16^{b}	0.66	0.16^{b}	0.44	0.25^{ab}	0.41	0.36^{a}	0.37	P<0.01**
Z_{eu}:$D^{1\%}$	N/A		1.13^{a}	0.55	N/A		1.21^{a}	0.38	1.48^{ab}	0.41	1.55^{ab}	0.59	2.75^{b}	0.50	2.21^{b}	0.45	1.12^{a}	0.41	P<0.001***
pH	N/A		6.58^{a}	0.10	N/A		5.60^{b}	0.24	6.27^{a}	0.14	5.64^{b}	0.17	6.94^{c}	0.08	6.90^{c}	0.06	5.84^{b}	0.24	P<0.001***
Conductivity (µS cm^{-1})	N/A		40.5^{ab}	0.03	N/A		45.9^{b}	0.01	43.0^{ab}	0.03	35.9^{a}	0.08	53.0^{c}	0.04	59.1^{c}	0.03	37.8^{a}	0.04	P<0.001***
Water Temperature (^{o}C)	N/A		6.0^{a}	0.01	N/A		4.1^{b}	0.03	4.8^{b}	0.02	7.5^{c}	0.05	14.9^{d}	0.03	15.6^{d}	0.04	4.8^{b}	0.03	P<0.001***
Flow (m s^{-1})	N/A		0.129^{a}	0.06	N/A		0.166^{a}	0.06	0.049^{a}	0.03	0.251^{a}	0.11	0.087^{a}	0.04	0.079^{a}	0.04	0.571^{b}	0.05	P<0.001***

Table 3.20 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 126), periphyton chlorophyll content per unit area (n = 105) and environmental habitat variables (n = 96) of short-term linoleum substrates between sampling dates (October 2005 – April 2006) in the Water of Dye sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

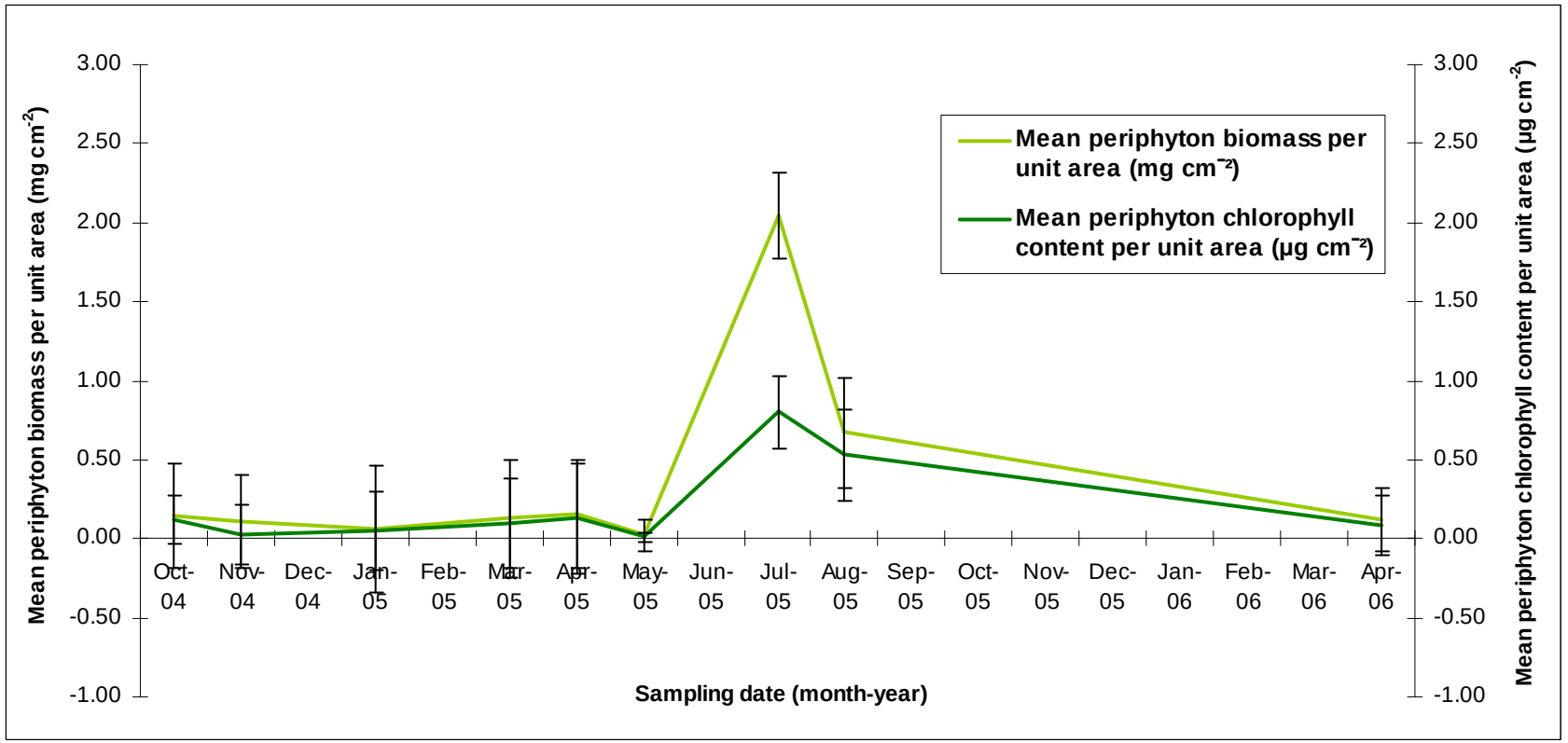


Figure 3.13 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) periphyton biomass (n = 126) and chlorophyll content (n = 105) per unit area harvested from short-term linoleum substrates in the Water of Dye sub-catchment between October 2004 and April 2006.

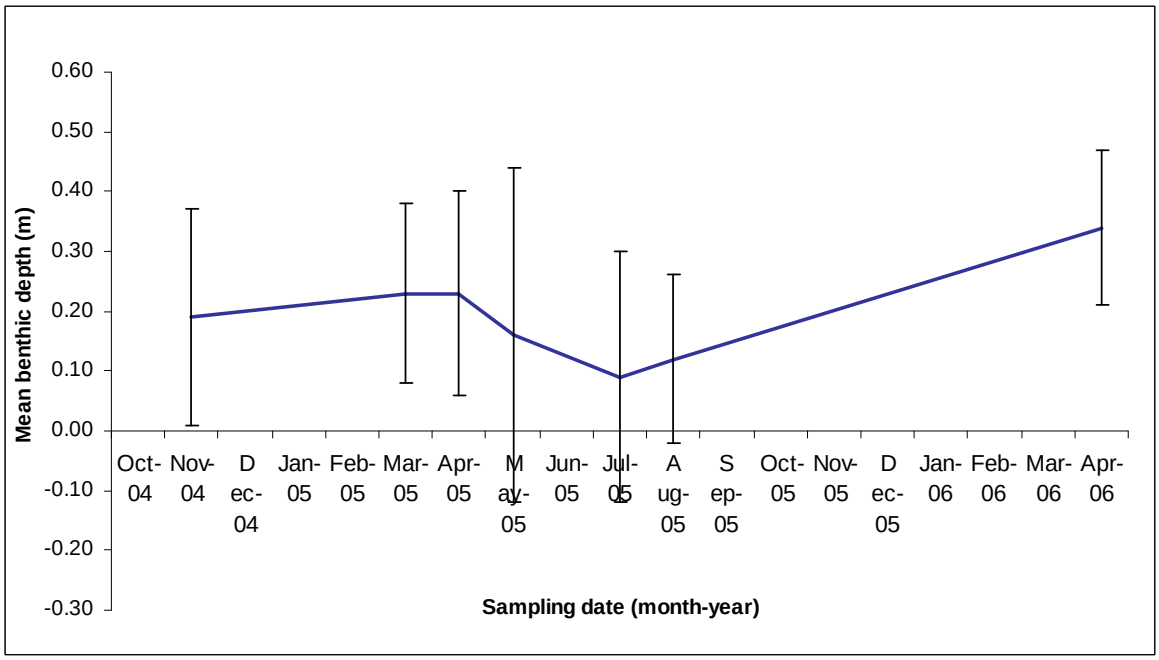


Figure 3.14 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) benthic depth of short-term linoleum substrates in the Water of Dye sub-catchment between October 2004 and April 2006 (n = 96).

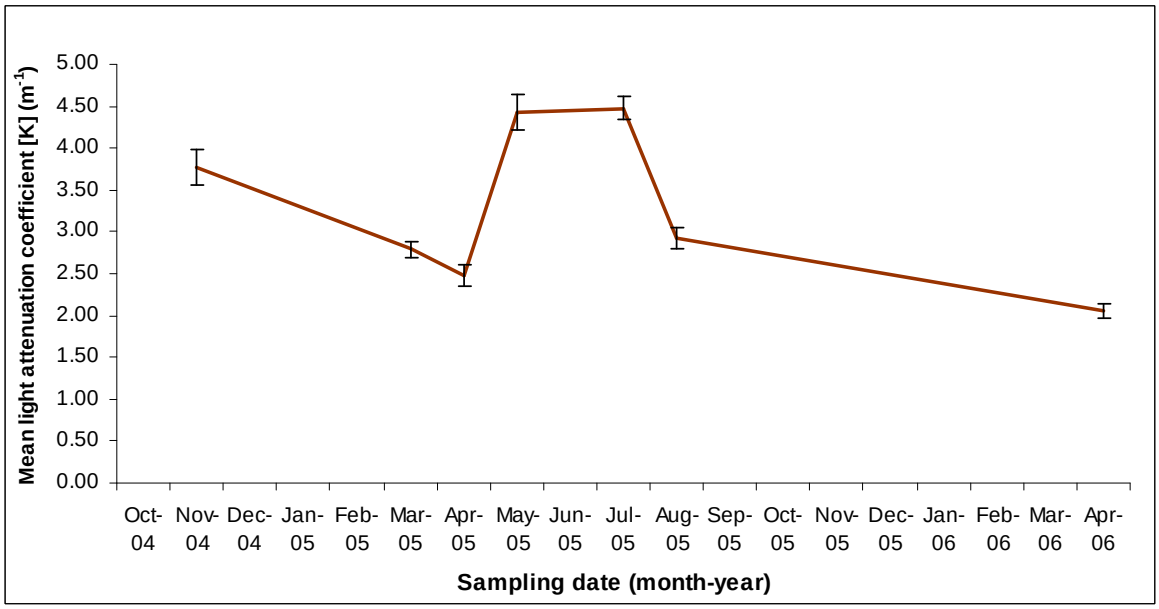


Figure 3.15 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) light attenuation coefficient [K] of short-term linoleum substrates in the Water of Dye sub-catchment between October 2004 and April 2006 (n = 96).

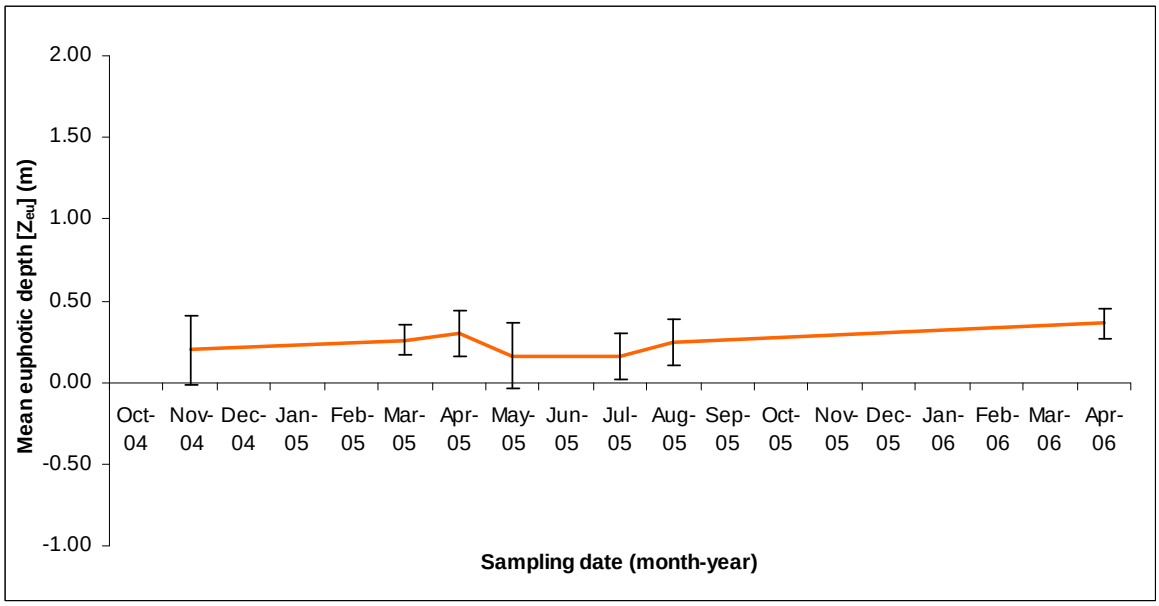


Figure 3.16 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) euphotic depth [Z_{eu}] of short-term linoleum substrates in the Water of Dye sub-catchment between October 2004 and April 2006 (n = 96).

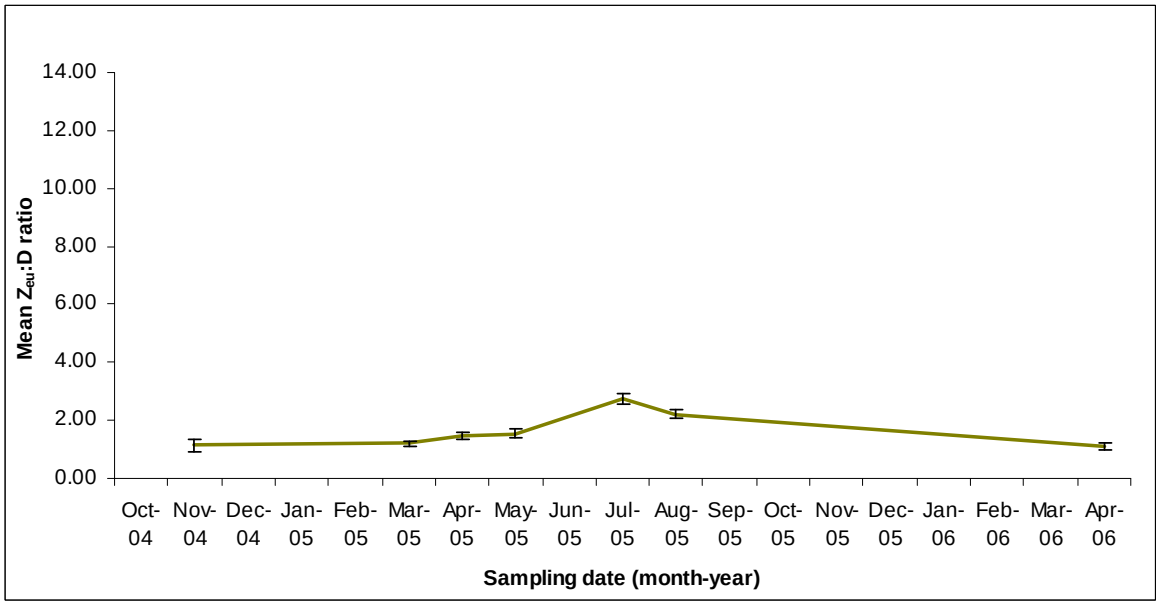


Figure 3.17 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) Z_{eu}:D ratio of short-term linoleum substrates in the Water of Dye sub-catchment between October 2004 and April 2006 (n = 96). Note[1]: standard error bars barely visible.

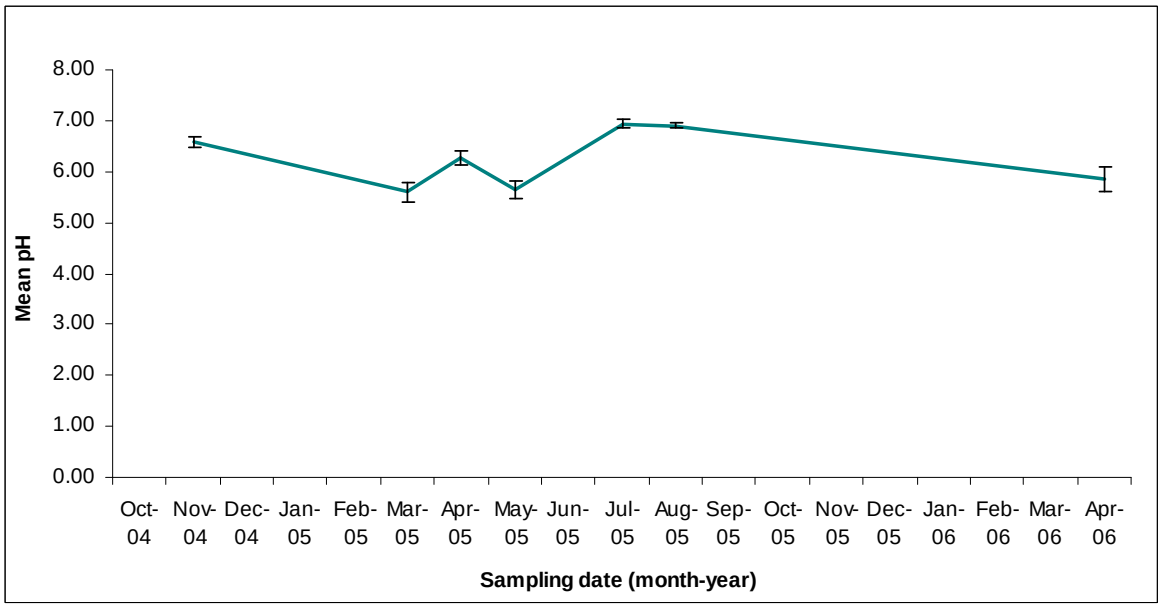


Figure 3.18 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) pH of short-term linoleum substrates in the Water of Dye sub-catchment between October 2004 and April 2006 (n = 96). Note[1]: standard error bars barely visible.

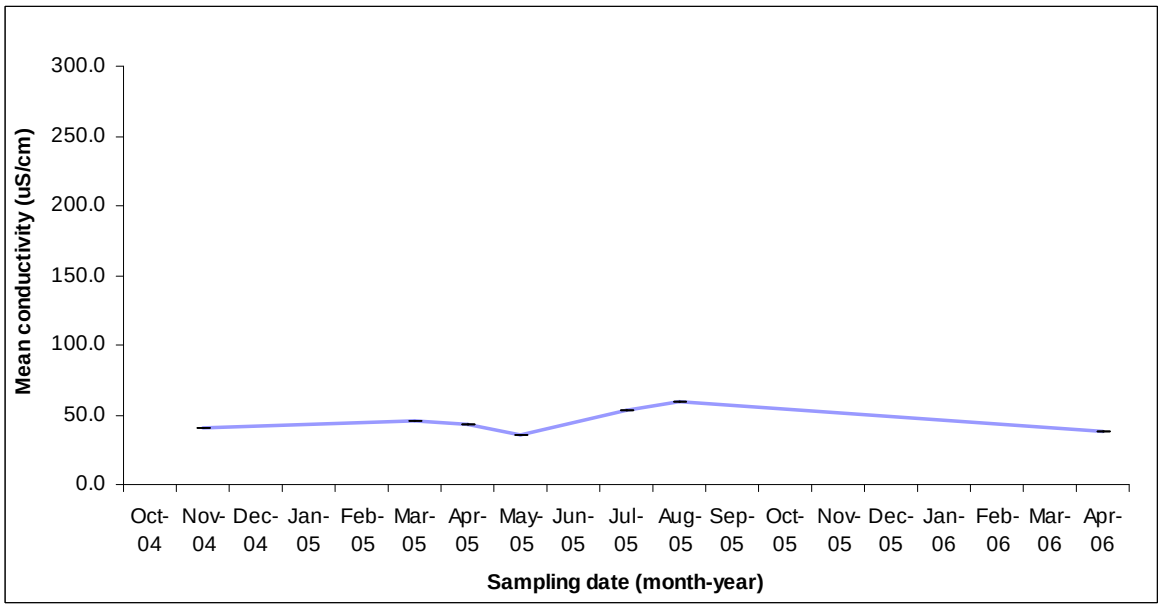


Figure 3.19 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) conductivity of short-term linoleum substrates in the Water of Dye sub-catchment between October 2004 and April 2006 (n = 96). Note[1]: standard error bars barely visible.

Mean Variable	May 2005	August 2005	April 2006	PANOVA

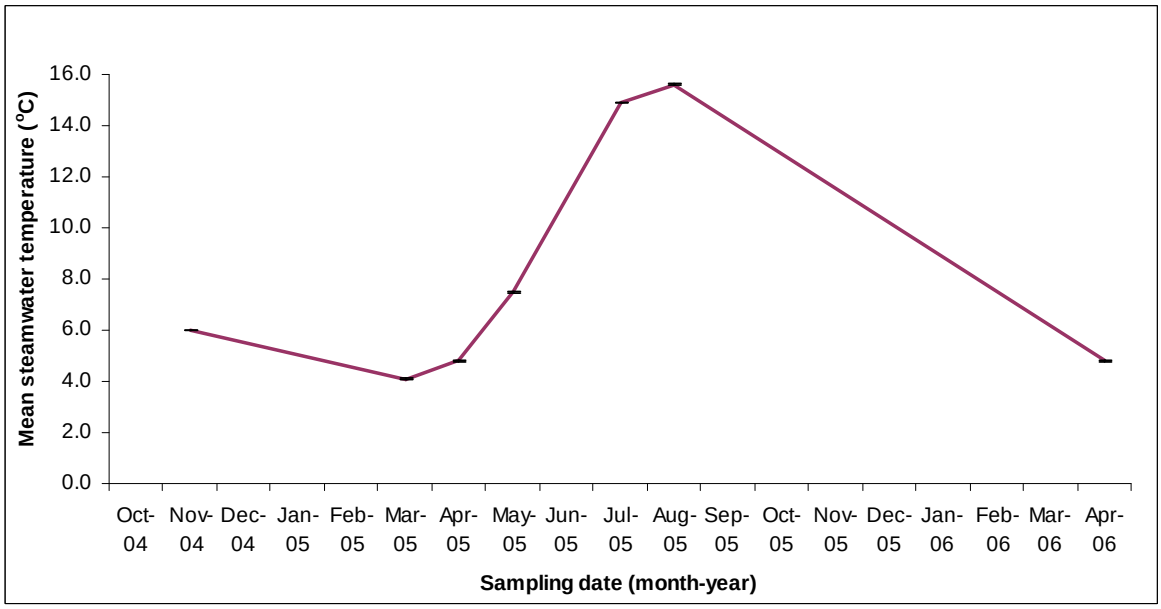


Figure 3.20 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) streamwater temperature of short-term linoleum substrates in the Water of Dye sub-catchment between October 2004 and April 2006 (n = 96). Note[1]: standard error bars barely visible.

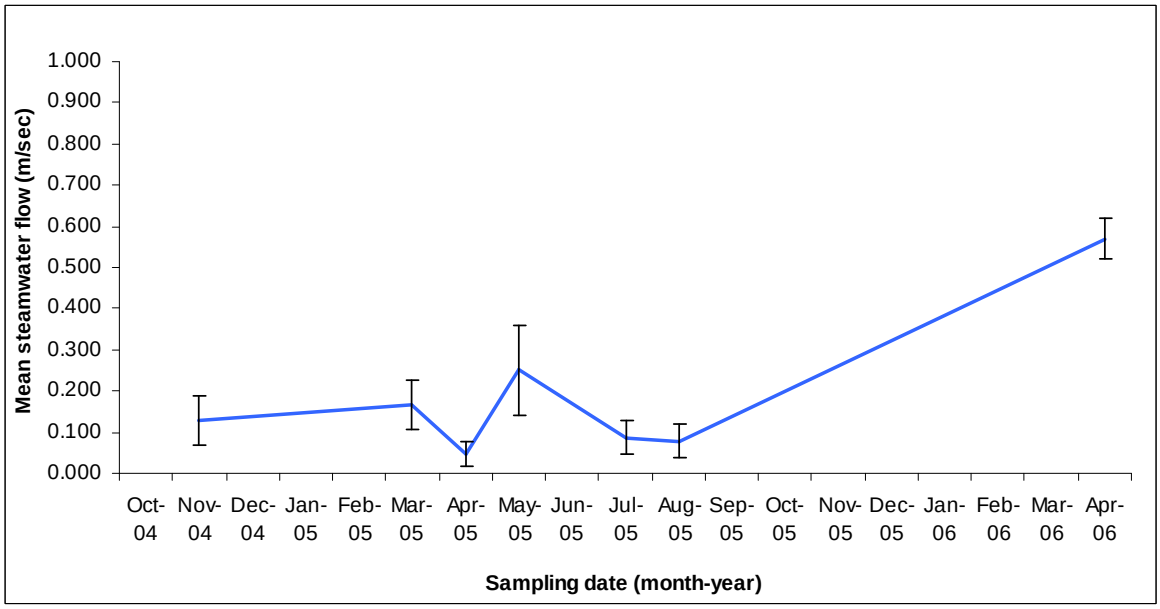


Figure 3.21 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) streamwater flow of short-term linoleum substrates in the Water of Dye sub-catchment between October 2004 and April 2006 (n = 96).

	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **short-term linoleum** substrates	0.02^{a}	0.10	0.67^{b}	0.35	0.12^{a}	0.20	P<0.001***
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **short-term linoleum** substrates	0.01^{a}	0.03	0.53^{b}	0.29	0.09^{a}	0.19	P<0.01**
D (m)	0.17^{a}	0.28	0.12^{b}	0.14	0.32^{c}	0.13	P<0.001***
K (m^{-1})	4.43^{b}	0.68	2.93^{ab}	0.42	2.06^{a}	0.38	P<0.01**
$Z_{eu}^{1\%}$ (m)	0.16^{b}	0.66	0.25^{ab}	0.41	0.36^{a}	0.37	P<0.01**
Z_{eu}:$D^{1\%}$	1.55^{ab}	0.59	2.21^{b}	0.45	1.12^{a}	0.41	P<0.01**
pH	5.64^{b}	0.17	6.90^{c}	0.06	5.84^{b}	0.24	P<0.001***
Conductivity (μS cm^{-1})	35.9^{a}	0.08	59.1^{c}	0.03	37.5^{a}	0.04	P<0.001***
Water Temperature (^{o}C)	7.5^{c}	0.05	15.6^{d}	0.04	4.8^{b}	0.03	P<0.001***
Flow (m s^{-1})	0.251^{a}	0.11	0.079^{a}	0.04	0.571^{b}	0.05	P<0.001***

Table 3.21 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 37), chlorophyll content per unit area (n = 31) and environmental habitat variables (n = 37) of short-term linoleum substrates between sampling dates in the Water of Dye sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Mean Variable	**May**	**August**	**April**	**P**ANOVA

	2005		**2005**		**2006**		
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **long-term linoleum** substrates	0.05^{a}	0.13	0.79^{b}	0.29	0.10^{a}	0.22	P<0.001***
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **long-term linoleum** substrates	0.09^{a}	0.17	0.75^{b}	0.33	0.17^{a}	0.25	P<0.001***
D (m)	0.15^{ab}	0.16	0.09^{a}	0.20	0.30^{b}	0.14	P<0.001***
K (m^{-1})	3.01	0.70	2.63	0.57	2.32	0.40	NS
$Z_{eu}^{1\%}$ (m)	0.23	0.67	0.28	0.58	0.32	0.41	NS
Z_{eu}:$D^{1\%}$	1.57^{a}	0.55	3.01^{b}	0.48	1.11^{a}	0.37	P<0.001***
pH	5.89^{a}	0.18	6.92^{b}	0.07	5.73^{a}	0.25	P<0.001***
Conductivity (μS cm^{-1})	40.4^{a}	0.08	59.4^{b}	0.03	36.4^{a}	0.04	P<0.001***
Water Temperature (^{o}C)	7.9^{a}	0.05	15.7^{b}	0.04	4.8^{c}	0.02	P<0.001***
Flow (m s^{-1})	0.118^{a}	0.06	0.062^{a}	0.05	0.513^{b}	0.06	P<0.001***

Table 3.22 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 39), chlorophyll content per unit area (n = 24) and environmental habitat variables (n = 39) of long-term linoleum substrates (n = 39) between sampling dates in the Water of Dye sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Mean Variable	**May**	**August**	**April**	**P_{ANOVA}**

	2005		**2005**		**2006**		
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **long-term Astroturf** substrates	0.31^{a}	0.19	0.76^{b}	0.26	0.50^{ab}	0.23	P<0.05*
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **long-term Astroturf** substrates	0.09	0.15	0.21	0.24	0.14	0.22	NS
D (m)	0.11^{a}	0.23	0.07^{a}	0.22	0.36^{b}	0.11	P<0.001***
K (m^{-1})	3.30	0.54	3.53	0.64	2.15	0.35	NS
$Z_{eu}^{1\%}$ (m)	0.23	0.52	0.21	0.64	0.34	0.37	NS
Z_{eu}:$D^{1\%}$	2.05^{ab}	0.64	2.87^{a}	0.50	0.94^{b}	0.40	P<0.001***
pH	6.14^{a}	0.20	6.88^{b}	0.07	5.90^{a}	0.23	P<0.001***
Conductivity (μS cm^{-1})	40.9^{a}	0.07	58.2^{b}	0.03	38.6^{a}	0.05	P<0.001***
Water Temperature (^{o}C)	7.6^{a}	0.03	15.5^{b}	0.05	4.9^{c}	0.03	P<0.001***
Flow (m s^{-1})	0.090^{a}	0.05	0.047^{a}	0.04	0.529^{b}	0.06	P<0.001***

Table 3.23 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 38), chlorophyll content per unit area (n = 37) and environmental habitat variables (n = 38) of long-term Astroturf substrates between sampling dates in the Water of Dye sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Mean Variable	**May 2005**		**August 2005**		**April 2006**		**PANOVA**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **mineral** substrata	0.26^{a}	0.20	1.34^{b}	0.29	0.68^{c}	0.21	P<0.001***
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **mineral** substrata	0.04^{a}	0.23	3.11^{b}	0.43	1.49^{c}	0.67	P<0.001***
Periphyton cover (%)	16.4^{a}	1.99	24.9^{b}	3.29	13.4^{a}	2.11	P<0.001***
Bare area (%)	36.8	2.38	39.7	3.54	39.4	4.81	NS
D (m)	0.15^{a}	0.27	0.10^{b}	0.24	0.23^{c}	0.30	P<0.001***
K (m^{-1})	3.73^{a}	0.21	2.76^{b}	0.24	2.25^{b}	0.30	P<0.001***
$Z_{eu}^{1\%}$ (m)	0.20^{a}	0.22	0.26^{ab}	0.24	0.33^{b}	0.29	P<0.001***
Z_{eu}:$D^{1\%}$	1.37^{a}	0.27	2.68^{b}	0.25	1.43^{a}	0.28	P<0.001***
pH	5.56^{a}	0.08	7.07^{b}	0.03	6.37^{c}	0.11	P<0.001***
Conductivity (μS cm^{-1})	37.6^{a}	0.15	59.5^{b}	0.16	40.4^{a}	0.24	P<0.001***
Water Temperature (^{o}C)	9.8^{a}	0.02	15.4^{b}	0.02	3.7^{c}	0.03	P<0.001***
Flow (m s^{-1})	0.217^{a}	0.02	0.172^{a}	0.03	0.326^{b}	0.03	P<0.01**

Table 3.24 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 135), chlorophyll content per unit area (n = 17), periphyton cover (n = 135), bare area (n = 135) and environmental habitat variables (n = 135) of naturally-occurring mineral substrata between sampling dates in the Water of Dye sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of substrate morphology, alkalinity, nutrient status and heavy metal composition refer to Table 2.4 in Chapter 2.

Mean Variable	**May 2005**		**August 2005**		**April 2006**		**PANOVA**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **aquatic bryophytes**	0.33^{a}	0.15	2.37^{b}	0.20	1.66^{c}	0.22	P<0.001**
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **aquatic bryophytes**	0.19^{a}	0.21	2.44^{b}	0.24	1.22^{c}	0.29	P<0.001***
Periphyton cover (%)	16.4^{a}	1.99	24.9^{b}	3.29	13.4^{a}	2.11	P<0.001***
Bare area (%)	36.8	2.38	39.7	3.54	39.4	4.81	NS
D (m)	0.15^{a}	0.27	0.10^{b}	0.24	0.23^{c}	0.30	P<0.001***
K (m^{-1})	3.73^{a}	0.21	2.76^{b}	0.24	2.25^{b}	0.30	P<0.001***
$Z_{eu}^{1\%}$ (m)	0.20^{a}	0.22	0.26^{ab}	0.24	0.33^{b}	0.29	P<0.001***
Z_{eu}:$D^{1\%}$	1.37^{a}	0.27	2.68^{b}	0.25	1.43^{a}	0.28	P<0.001***
pH	5.56^{a}	0.08	7.07^{b}	0.03	6.37^{c}	0.11	P<0.001***
Conductivity (μS cm^{-1})	37.6^{a}	0.15	59.5^{b}	0.16	40.4^{a}	0.24	P<0.001***
Water Temperature (^{o}C)	9.8^{a}	0.02	15.4^{b}	0.02	3.7^{c}	0.03	P<0.001***
Flow (m s^{-1})	0.217^{a}	0.02	0.172^{a}	0.03	0.326^{b}	0.03	P<0.01**

Table 3.25 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 120), chlorophyll content per unit area (n = 41), periphyton cover (n = 120), bare area (n = 120) and environmental habitat variables (n = 120) of naturally-occurring aquatic bryophytes between sampling dates in the Water of Dye sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of substrate morphology, alkalinity, nutrient status and heavy metal composition refer to Table 2.4 in Chapter 2.

Mean Variable	April 2005		May 2005		July 2005		August 2005		April 2006		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **short-term linoleum** substrates	0.07^{a}	0.12	0.02^{a}	0.07	0.45^{b}	0.28	0.30^{b}	0.23	0.63^{b}	0.34	P<0.001***
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **short-term linoleum** substrates	0.04^{a}	0.09	0.02^{a}	0.04	0.29^{b}	0.17	0.24^{b}	0.21	0.33^{b}	0.30	P<0.01**
D (m)	0.14^{a}	0.10	0.10^{a}	0.13	0.04^{b}	0.24	0.07^{b}	0.22	0.31^{c}	0.17	P<0.001***
K (m^{-1})	3.29^{a}	0.43	2.34^{ab}	0.42	3.35^{a}	0.65	2.09^{b}	0.42	1.95^{b}	0.38	P<0.05*
$Z_{eu}^{1\%}$ (m)	0.15	0.56	0.25	0.65	0.17	0.95	0.29	0.47	0.31	0.53	NS
Z_{eu}:$D^{1\%}$	1.06^{a}	0.65	2.68^{ab}	0.69	4.51^{b}	0.81	3.38^{b}	0.68	1.00^{a}	0.62	P<0.001***
pH	6.82^{a}	0.02	6.74^{a}	0.04	7.16^{b}	0.06	7.22^{b}	0.07	6.30^{c}	0.08	P<0.001***
Conductivity (μS cm^{-1})	43.0^{a}	0.02	45.1^{a}	0.04	69.0^{b}	0.04	76.6^{b}	0.04	31.2^{c}	0.04	P<0.001***
Water Temperature (^{o}C)	8.8^{a}	0.07	11.6^{b}	0.04	13.5^{c}	0.05	14.3^{c}	0.03	7.2^{d}	0.06	P<0.001***
Flow (m s^{-1})	0.168^{a}	0.05	0.062^{a}	0.02	0.107^{a}	0.04	0.100^{a}	0.04	0.676^{b}	0.04	P<0.001***

Table 3.26 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 82), periphyton chlorophyll content per unit area (n = 64) and environmental habitat variables (n = 82) of short-term linoleum substrates between sampling dates (April 2005 – April 2006) in the River Girnock sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

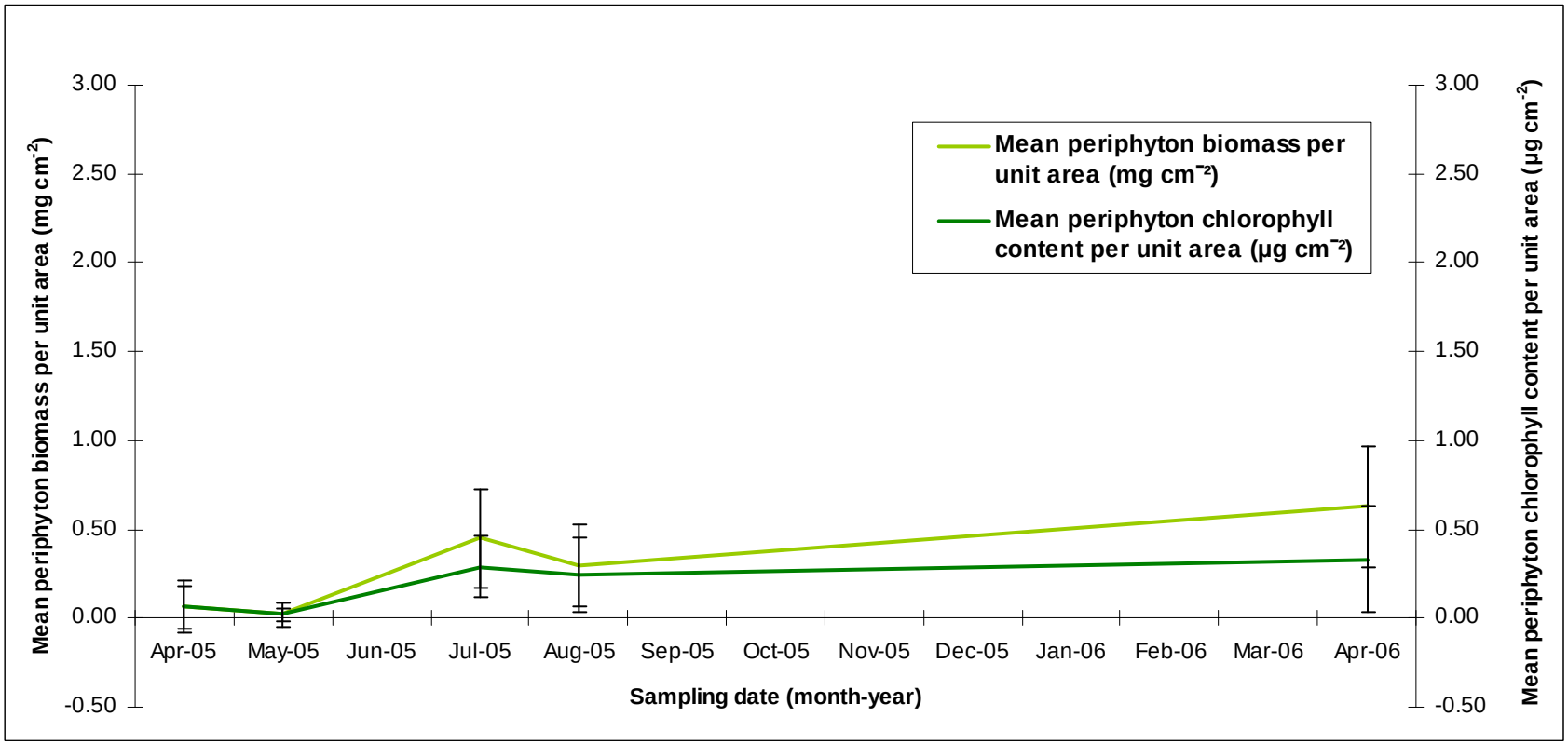


Figure 3.22 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) periphyton biomass (n = 82) and chlorophyll content (n = 64) per unit area harvested from short-term linoleum substrates in the River Girnock sub-catchment between April 2005 and April 2006.

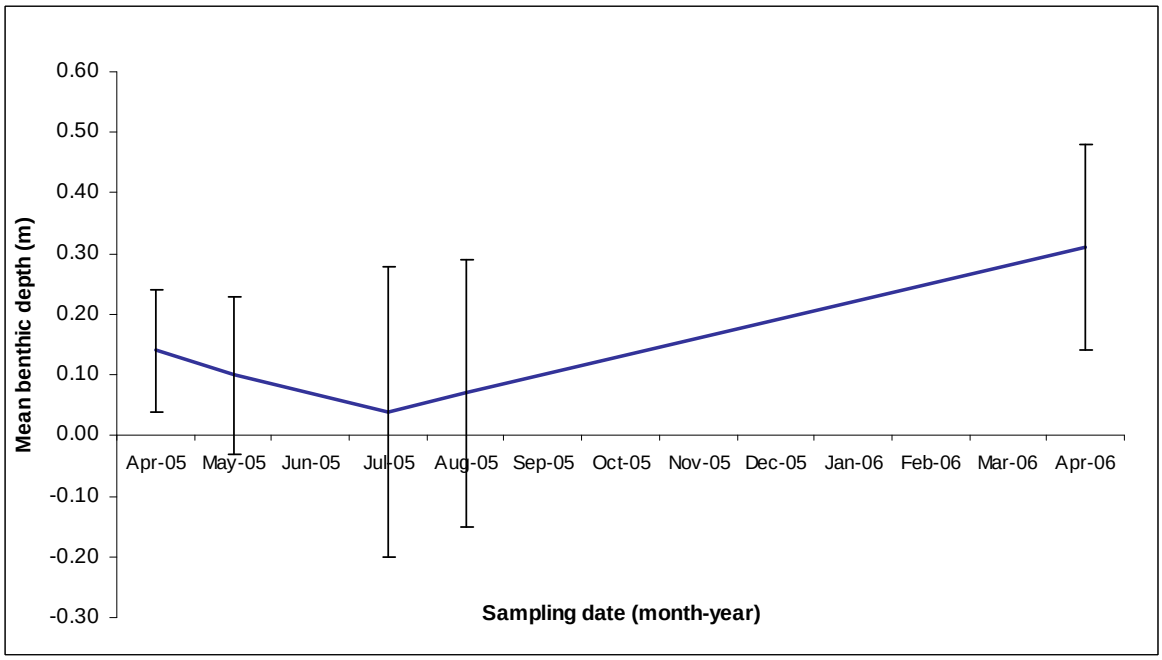


Figure 3.23 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) benthic depth of short-term linoleum substrates in the River Girnock sub-catchment between April 2005 and April 2006 (n = 82).

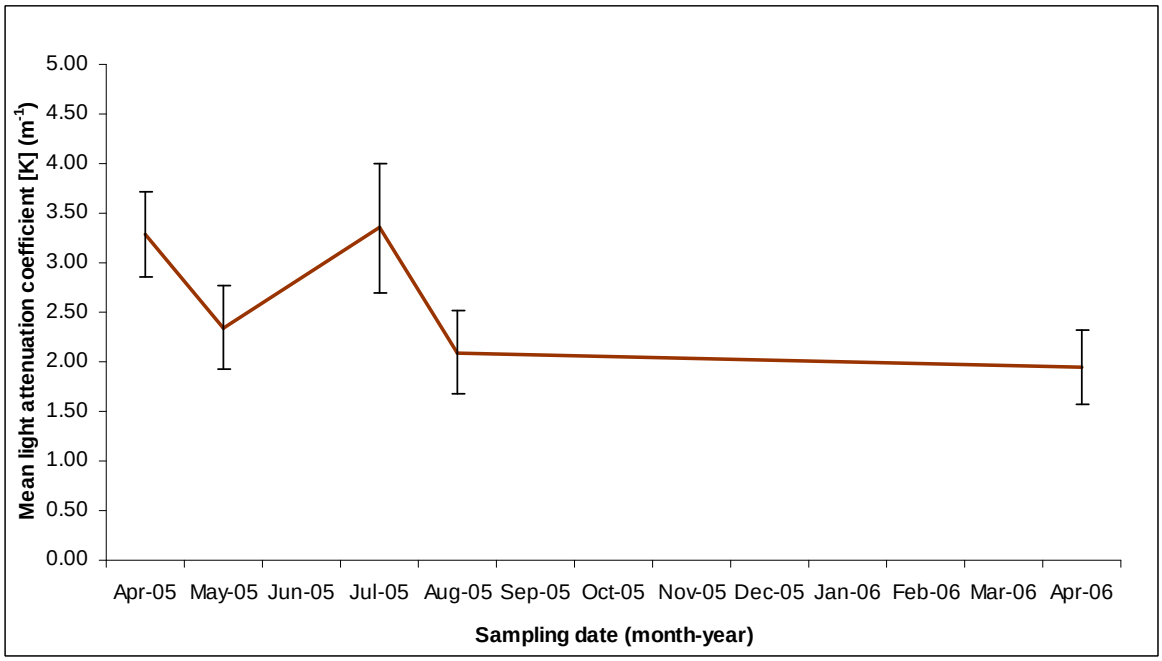


Figure 3.24 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) light attenuation coefficient [K] of short-term linoleum substrates in the River Girnock sub-catchment between April 2005 and April 2006 (n = 82).

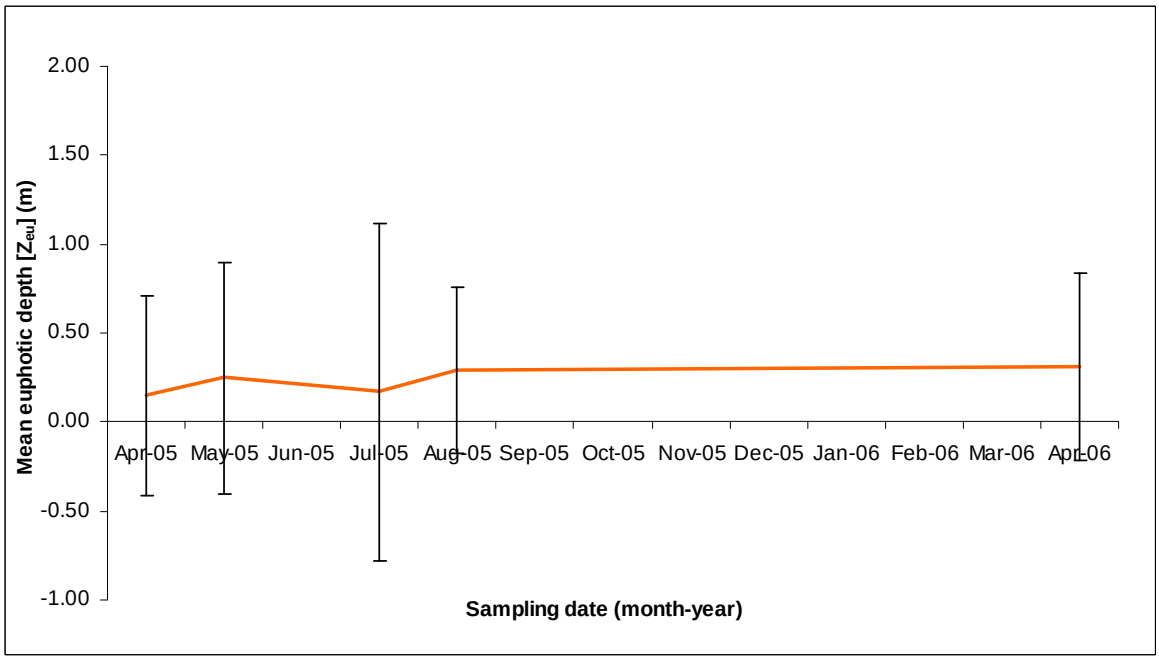


Figure 3.25 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) euphotic depth [Z_{eu}] of short-term linoleum substrates in the River Girnock sub-catchment between April 2005 and April 2006 (n = 82).

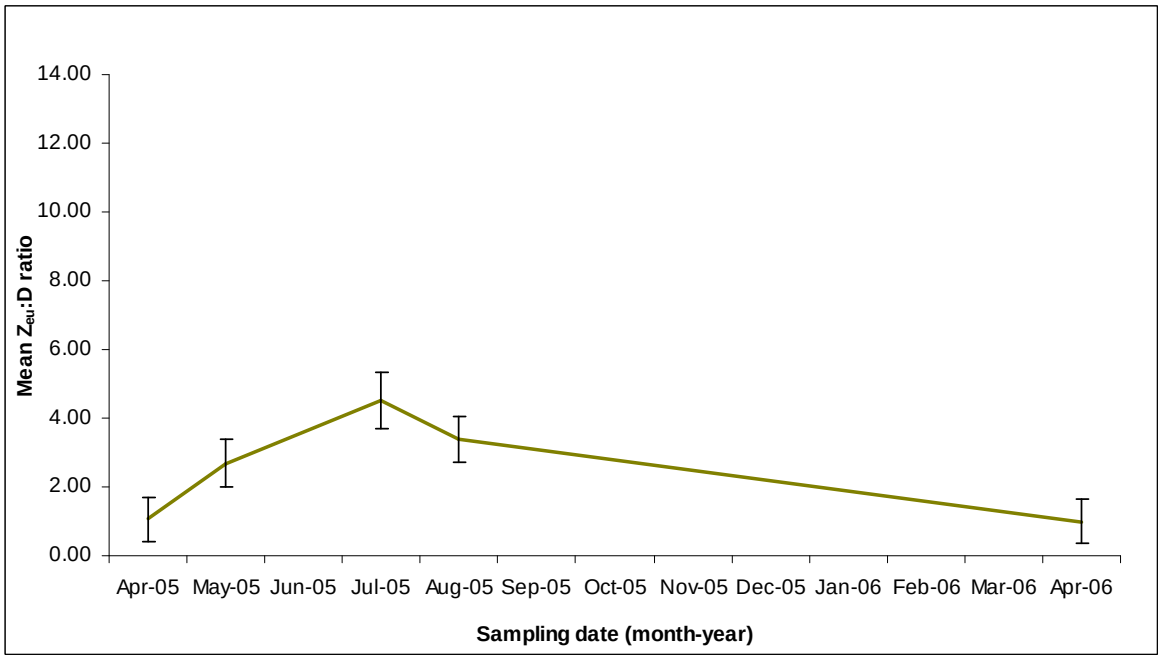


Figure 3.26 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) Z_{eu}:D ratio of short-term linoleum substrates in the River Girnock sub-catchment between April 2005 and April 2006 (n = 82).

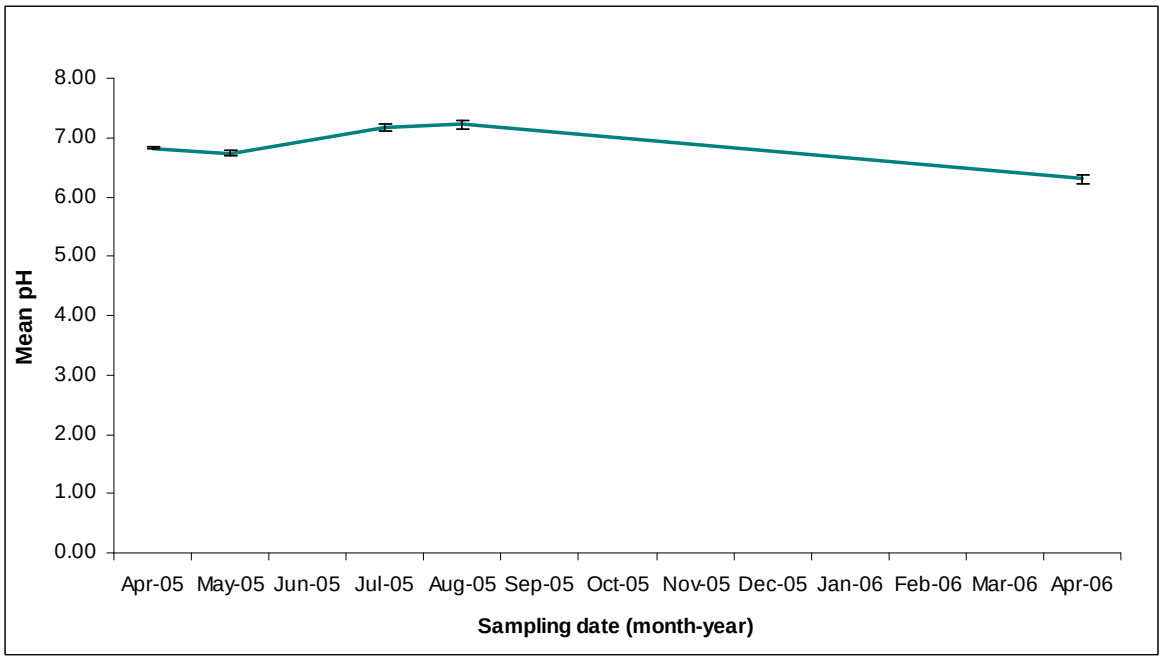


Figure 3.27 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) pH of short-term linoleum substrates in the River Girnock sub-catchment between April 2005 and April 2006 (n = 82). Note[1]: standard error bars barely visible.

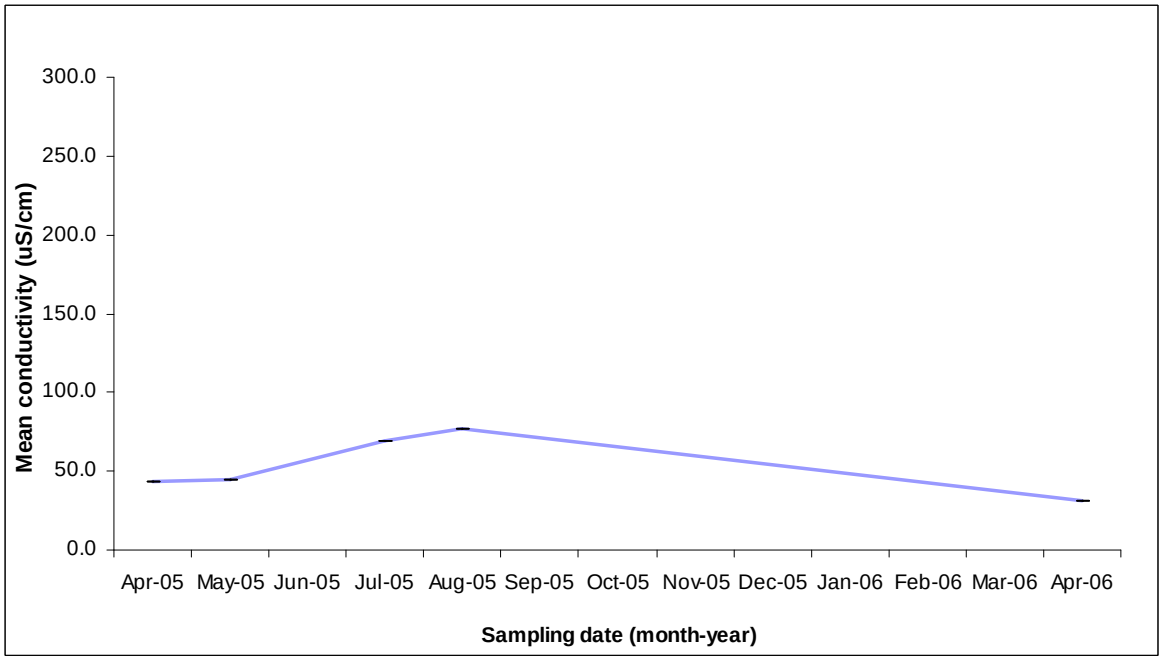


Figure 3.28 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) conductivity of short-term linoleum substrates in the River Girnock sub-catchment between April 2005 and April 2006 (n = 82). Note[1]: standard error bars barely visible.

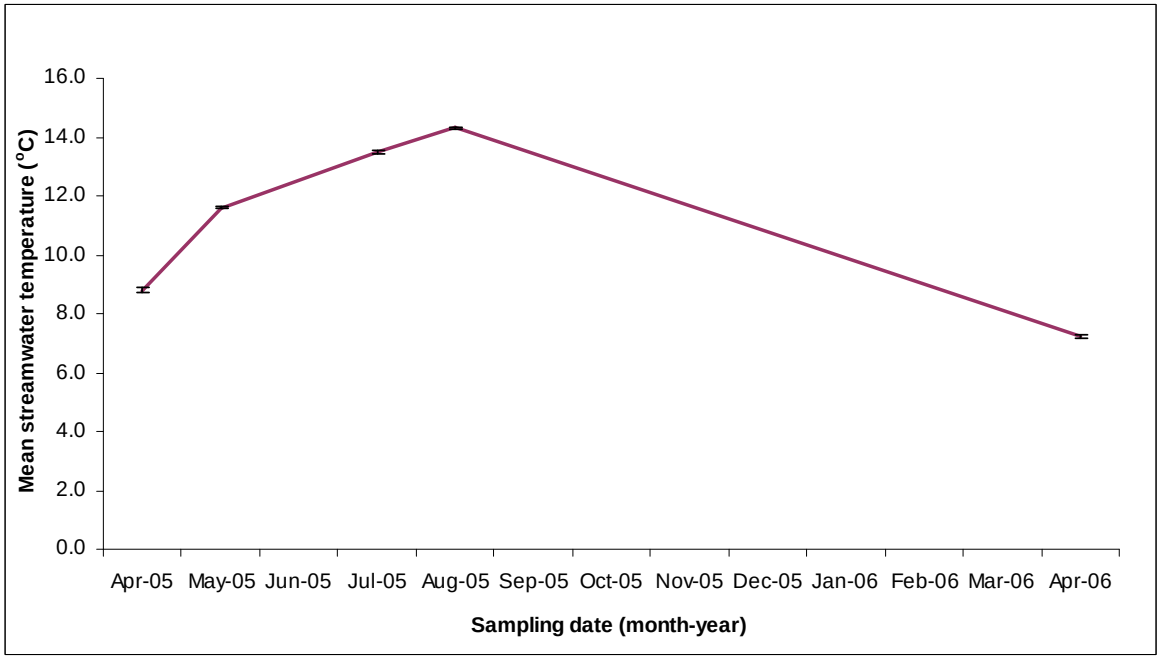


Figure 3.29 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) streamwater temperature of short-term linoleum substrates in the River Girnock sub-catchment between April 2005 and April 2006 (n = 82). Note[1]: standard error bars barely visible.

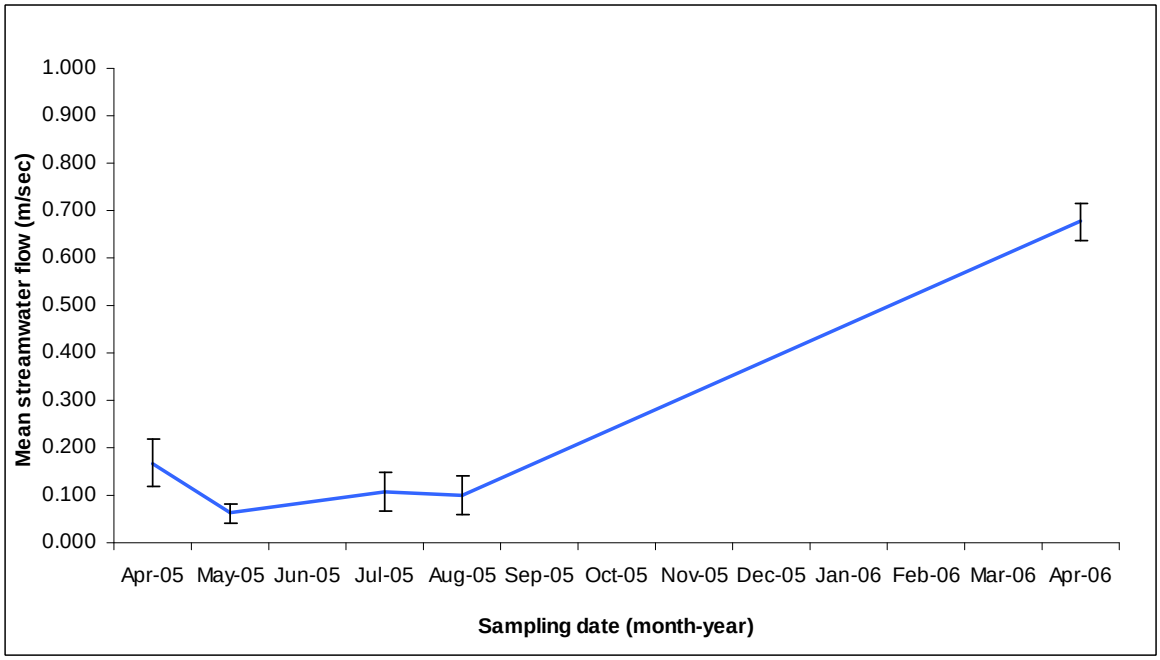


Figure 3.30 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) streamwater flow of short-term linoleum substrates in the River Girnock sub-catchment between April 2005 and April 2006 (n = 82).

Mean Variable	**May 2005**		**August 2005**		**April 2006**		**PANOVA**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **short-term linoleum** substrates	0.02^{a}	0.07	0.30^{b}	0.23	0.63^{b}	0.34	P<0.01**
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **short-term linoleum** substrates	0.02^{a}	0.04	0.24^{b}	0.21	0.33^{b}	0.30	P<0.01**
D (m)	0.10^{ab}	0.13	0.07^{b}	0.22	0.31^{c}	0.17	P<0.001***
K (m^{-1})	2.34	0.42	2.09	0.42	1.95	0.38	NS
$Z_{eu}^{1\%}$ (m)	0.25	0.65	0.29	0.47	0.31	0.53	NS
Z_{eu}:$D^{1\%}$	2.68^{ab}	0.69	3.38^{b}	0.68	1.00^{a}	0.62	P<0.01**
pH	6.74^{a}	0.04	7.22^{b}	0.07	6.30^{c}	0.08	P<0.001***
Conductivity (μS cm^{-1})	45.1^{a}	0.04	76.6^{c}	0.04	31.2^{c}	0.04	P<0.001***
Water Temperature (^{o}C)	11.6^{b}	0.04	14.3^{c}	0.03	7.2^{d}	0.06	P<0.001***
Flow (m s^{-1})	0.062^{a}	0.02	0.100^{a}	0.04	0.676^{b}	0.04	P<0.001***

Table 3.27 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 53), chlorophyll content per unit area (n = 39) and environmental habitat variables (n = 53) of short-term linoleum substrates between sampling dates in the River Girnock sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Mean Variable	**May 2005**		**August 2005**		**April 2006**		**PANOVA**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **long-term linoleum** substrates	0.06^{a}	0.12	0.46^{b}	0.22	0.72^{b}	0.28	P<0.01**
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **long-term linoleum** substrates	0.06^{a}	0.13	0.35^{b}	0.25	0.47^{b}	0.17	P<0.01**
D (m)	0.11^{a}	0.13	0.04^{b}	0.22	0.25^{c}	0.14	P<0.001***
K (m^{-1})	2.38	0.37	2.05	0.76	1.88	0.65	NS
$Z_{eu}^{1\%}$ (m)	0.25	0.59	0.29	0.64	0.32	0.83	NS
Z_{eu}:$D^{1\%}$	2.27^{a}	0.55	6.62^{b}	0.62	1.33^{a}	0.88	P<0.001***
pH	6.76^{a}	0.04	7.24^{b}	0.08	6.20^{c}	0.07	P<0.001***
Conductivity (μS cm^{-1})	45.1^{a}	0.04	77.2^{c}	0.04	30.2^{c}	0.04	P<0.001***
Water Temperature (^{o}C)	11.7^{a}	0.04	14.1^{b}	0.03	7.2^{c}	0.06	P<0.001***
Flow (m s^{-1})	0.048^{a}	0.03	0.089^{a}	0.05	0.651^{b}	0.04	P<0.001***

Table 3.28 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 52), chlorophyll content per unit area (n = 37) and environmental habitat variables (n = 52) of long-term linoleum substrates between sampling dates in the River Girnock sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Mean Variable	**May 2005**		**August 2005**		**April 2006**		**PANOVA**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **long-term Astroturf** substrates	0.33^{a}	0.20	0.82^{b}	0.23	0.57^{ab}	0.17	P<0.05*
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **long-term Astroturf** substrates	0.15	0.16	0.22	0.19	0.18	0.18	NS
D (m)	0.09^{a}	0.13	0.04^{a}	0.26	0.29^{b}	0.10	P<0.001***
K (m^{-1})	1.99	0.48	2.77	0.63	1.73	0.59	NS
$Z_{eu}^{1\%}$ (m)	0.30	0.60	0.22	0.46	0.29	0.64	NS
Z_{eu}:$D^{1\%}$	3.54^{ab}	0.59	6.11^{b}	0.59	1.02^{b}	0.30	P<0.001***
pH	6.75^{a}	0.04	7.26^{b}	0.07	6.19^{c}	0.06	P<0.001***
Conductivity (μS cm^{-1})	45.1^{a}	0.04	76.5^{c}	0.04	31.3^{c}	0.05	P<0.001***
Water Temperature (^{o}C)	11.7^{a}	0.04	14.2^{b}	0.03	6.8^{c}	0.08	P<0.001***
Flow (m s^{-1})	0.058^{a}	0.03	0.076^{a}	0.04	0.530^{b}	0.06	P<0.001***

Table 3.29 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 49), chlorophyll content per unit area (n = 49) and environmental habitat variables (n = 49) of long-term Astroturf substrates between sampling dates in the River Girnock sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Mean Variable	**May 2005**		**August 2005**		**April 2006**		**P**ANOVA
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **mineral** substrata	1.56^{a}	0.19	2.92^{b}	0.22	3.21^{b}	0.21	P<0.001***
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **mineral** substrata	0.14^{a}	0.22	2.27^{b}	0.17	1.98^{b}	0.22	P<0.001***
Periphyton cover (%)	33.3^{a}	3.07	43.7^{b}	3.01	38.6^{ab}	4.07	P<0.05*
Bare area (%)	27.3	2.88	30.7	2.85	26.4	3.12	NS
D (m)	0.14^{a}	0.20	0.08^{b}	0.25	0.20^{c}	0.32	P<0.001***
K (m^{-1})	2.90^{a}	0.21	2.10^{b}	0.26	1.80^{b}	0.26	P<0.001***
$Z_{eu}^{1\%}$ (m)	0.21^{a}	0.29	0.28^{ab}	0.28	0.34^{b}	0.42	P<0.01*
Z_{eu}:$D^{1\%}$	1.51^{a}	0.33	3.46^{b}	0.30	1.62^{a}	0.46	P<0.001***
pH	6.50^{a}	0.06	7.38^{b}	0.04	6.90^{c}	0.09	P<0.001***
Conductivity (μS cm^{-1})	39.4^{a}	0.18	78.5^{b}	0.16	39.0^{a}	0.23	P<0.001***
Water Temperature (^{o}C)	10.3^{a}	0.29	17.2^{b}	0.40	3.9^{c}	0.22	P<0.001***
Flow (m s^{-1})	0.217	0.02	0.167	0.03	0.253	0.03	NS

Table 3.30 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 135), chlorophyll content per unit area (n = 56), periphyton cover (n = 135), bare area (n = 135) and environmental habitat variables (n = 135) of naturally-occurring mineral substrata between sampling dates in the River Girnock sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of substrate morphology, alkalinity, nutrient status and heavy metal composition refer to Table 2.5 in Chapter 2.

Mean Variable	**May 2005**		**August 2005**		**April 2006**		**PANOVA**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **aquatic bryophytes**	0.46^{a}	0.22	0.89^{b}	0.24	0.70^{b}	0.30	P<0.01**
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **aquatic bryophytes**	0.22^{a}	0.25	1.24^{b}	0.12	1.08^{b}	0.27	P<0.001***
Periphyton cover (%)	33.3^{a}	3.07	43.7^{b}	3.01	38.6^{ab}	4.07	P<0.05*
Bare area (%)	27.3	2.88	30.7	2.85	26.4	3.12	NS
D (m)	0.14^{a}	0.20	0.08^{b}	0.25	0.20^{c}	0.32	P<0.001***
K (m^{-1})	2.90^{a}	0.21	2.10^{b}	0.26	1.80^{b}	0.26	P<0.001***
$Z_{eu}^{1\%}$ (m)	0.21^{a}	0.29	0.28^{ab}	0.28	0.34^{b}	0.42	P<0.01*
Z_{eu}:$D^{1\%}$	1.51^{a}	0.33	3.46^{b}	0.30	1.62^{a}	0.46	P<0.001***
pH	6.50^{a}	0.06	7.38^{b}	0.04	6.90^{c}	0.09	P<0.001***
Conductivity (μS cm^{-1})	39.4^{a}	0.18	78.5^{b}	0.16	39.0^{a}	0.23	P<0.001***
Water Temperature (^{o}C)	10.3^{a}	0.29	17.2^{b}	0.40	3.9^{c}	0.22	P<0.001***
Flow (m s^{-1})	0.217	0.02	0.167	0.03	0.253	0.03	NS

Table 3.31 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 95), chlorophyll content per unit area (n = 47), periphyton cover (n = 95), bare area (n = 95) and environmental habitat variables (n = 95) of naturally-occurring aquatic bryophytes between sampling dates in the River Girnock sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of substrate morphology, alkalinity, nutrient status and heavy metal composition refer to Table 2.5 in Chapter 2.

Mean Variable	**December 2005**		**April 2006**		**July 2006**		**September 2006**		**November 2006**		**P**ANOVA
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **short-term linoleum** substrates	0.07^{a}	0.08	0.37^{b}	0.22	0.58^{bc}	0.30	1.11^{c}	0.41	0.89^{bc}	0.47	P<0.05*
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **short-term linoleum** substrates	0.04^{a}	0.03	0.25^{b}	0.20	0.37^{b}	0.29	0.50^{b}	0.39	0.39^{b}	0.32	P<0.01**
D (m)	0.09^{a}	0.14	0.14^{b}	0.13	0.05^{c}	0.24	0.09^{a}	0.11	0.17^{b}	0.07	P<0.001***
K (m^{-1})	2.17^{ab}	0.45	2.23^{ab}	0.45	1.50^{a}	0.76	4.01^{b}	0.35	2.05^{ab}	0.56	P<0.01**
$Z_{eu}^{1\%}$ (m)	0.42^{ab}	0.45	0.41^{ab}	0.45	0.62^{a}	0.77	0.23^{b}	0.36	0.45^{ab}	0.58	P<0.05*
Z_{eu}:$D^{1\%}$	4.65^{a}	0.41	3.12^{a}	0.37	11.62^{b}	0.64	2.50^{a}	0.41	2.54^{a}	0.64	P<0.001***
pH	7.71^{a}	0.06	7.42^{b}	0.06	7.53^{ab}	0.08	7.61^{a}	0.12	7.39^{b}	0.06	P<0.05*
Conductivity (μS cm^{-1})	185.6^{a}	0.06	117.8^{cd}	0.06	253.9^{b}	0.08	143.4^{c}	0.09	94.5^{d}	0.06	P<0.001***
Water Temperature (^{o}C)	5.5^{a}	0.03	6.4^{b}	0.05	13.3^{c}	0.04	11.5^{d}	0.02	7.9^{e}	0.01	P<0.001***
Flow (m s^{-1})	0.238^{ab}	0.04	0.458^{a}	0.05	0.189^{b}	0.06	0.179^{b}	0.05	0.305^{ab}	0.04	P<0.001***

Table 3.32 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 85), periphyton chlorophyll content per unit area (n = 73) and environmental habitat variables (n = 85) of short-term linoleum substrates between sampling dates in the Knockan Burn sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

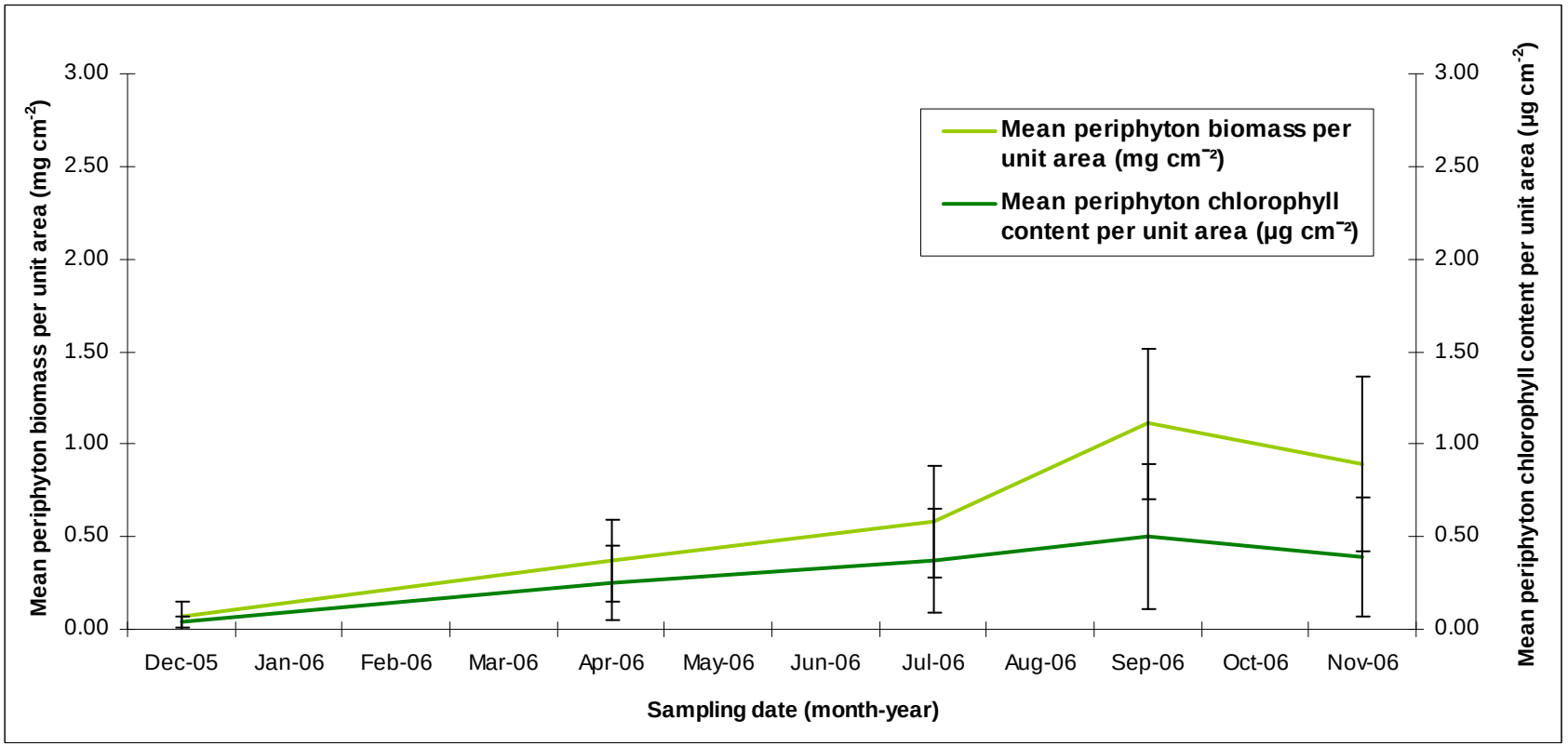


Figure 3.31 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) periphyton biomass (n = 85) and chlorophyll content (n = 73) per unit area harvested from short-term linoleum substrates in the Knockan Burn sub-catchment between December 2005 and November 2006.

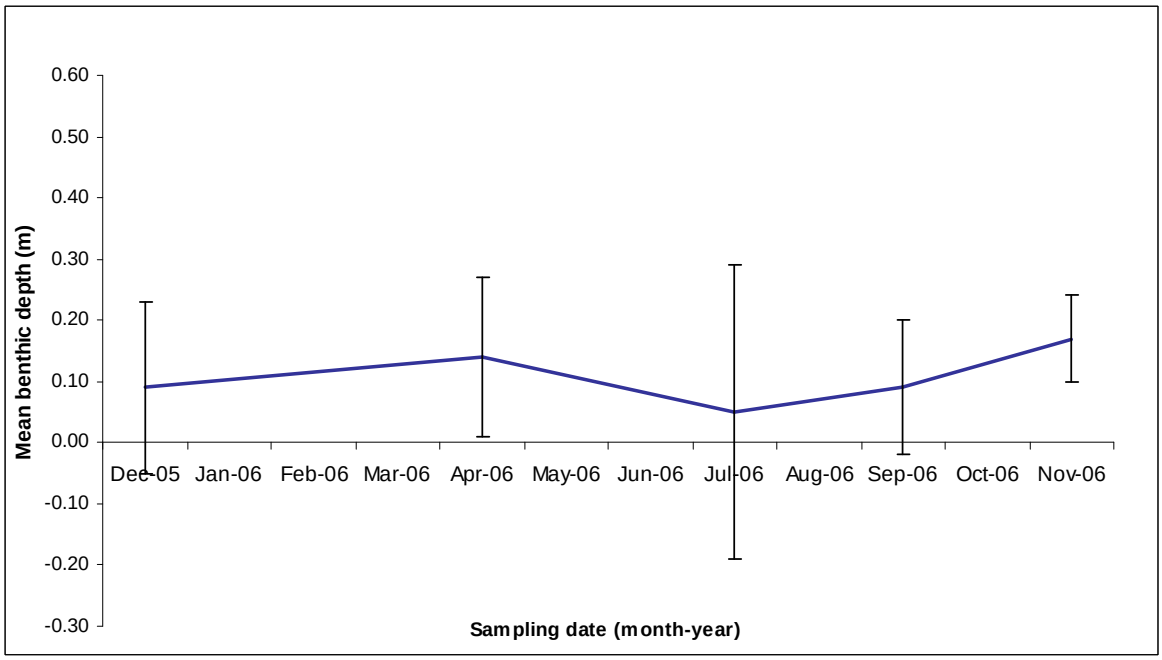


Figure 3.32 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) benthic depth of short-term linoleum substrates in the Knockan Burn sub-catchment between December 2005 and November 2006 (n = 85).

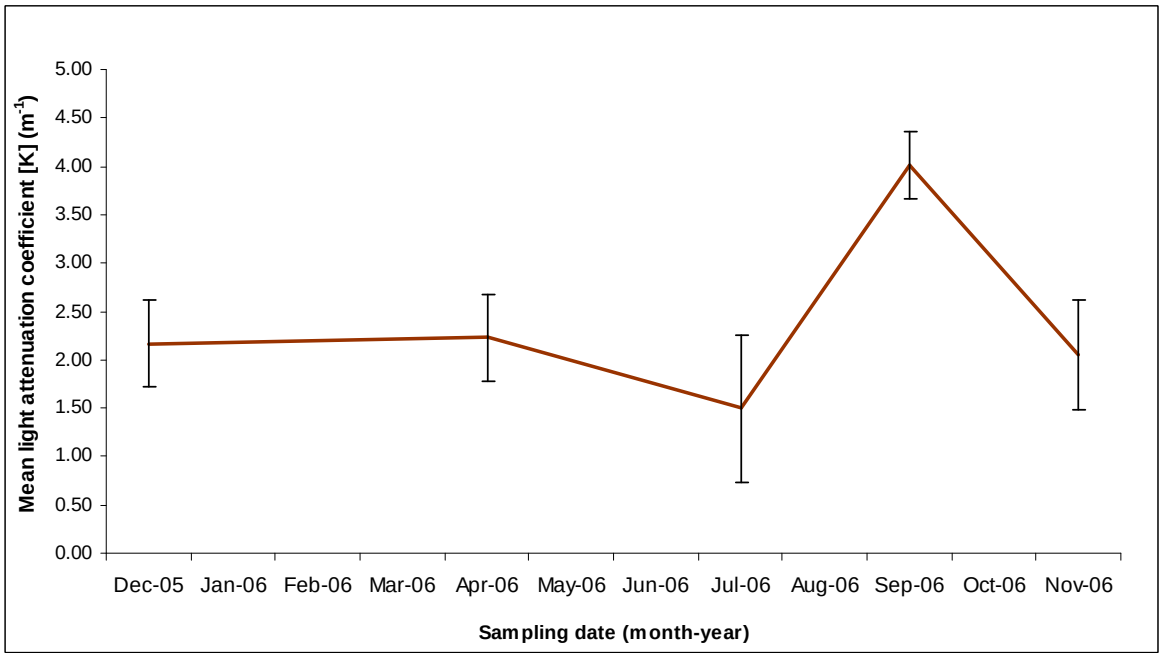


Figure 3.33 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) light attenuation coefficient [K] of short-term linoleum substrates in the Knockan Burn sub-catchment between December 2005 and November 2006 (n = 85).

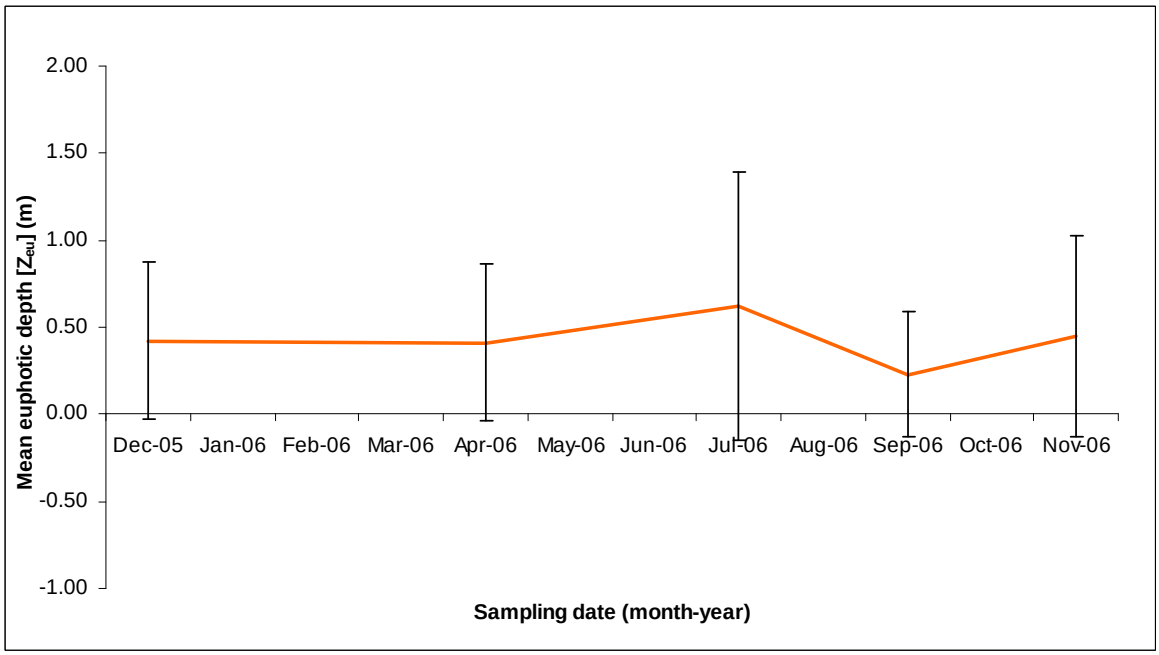


Figure 3.34 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) euphotic depth [Z_{eu}] of short-term linoleum substrates in the Knockan Burn sub-catchment between December 2005 and November 2006 (n = 85).

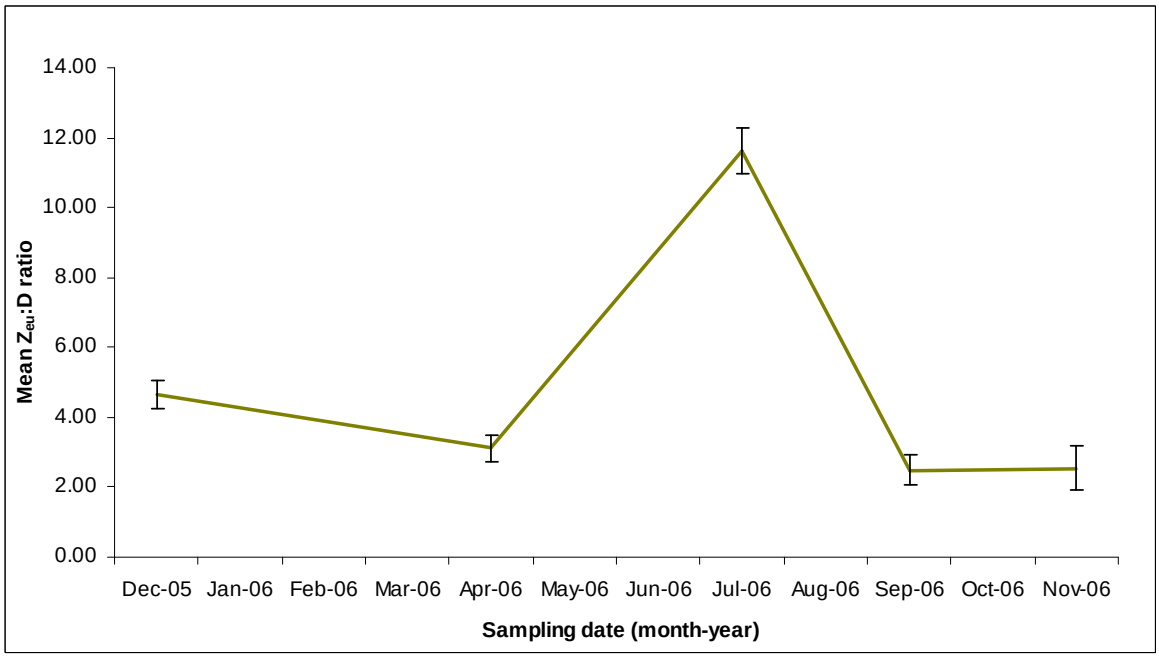


Figure 3.35 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) Z_{eu}:D ratio of short-term linoleum substrates in the Knockan Burn sub-catchment between December 2005 and November 2006 (n = 85).

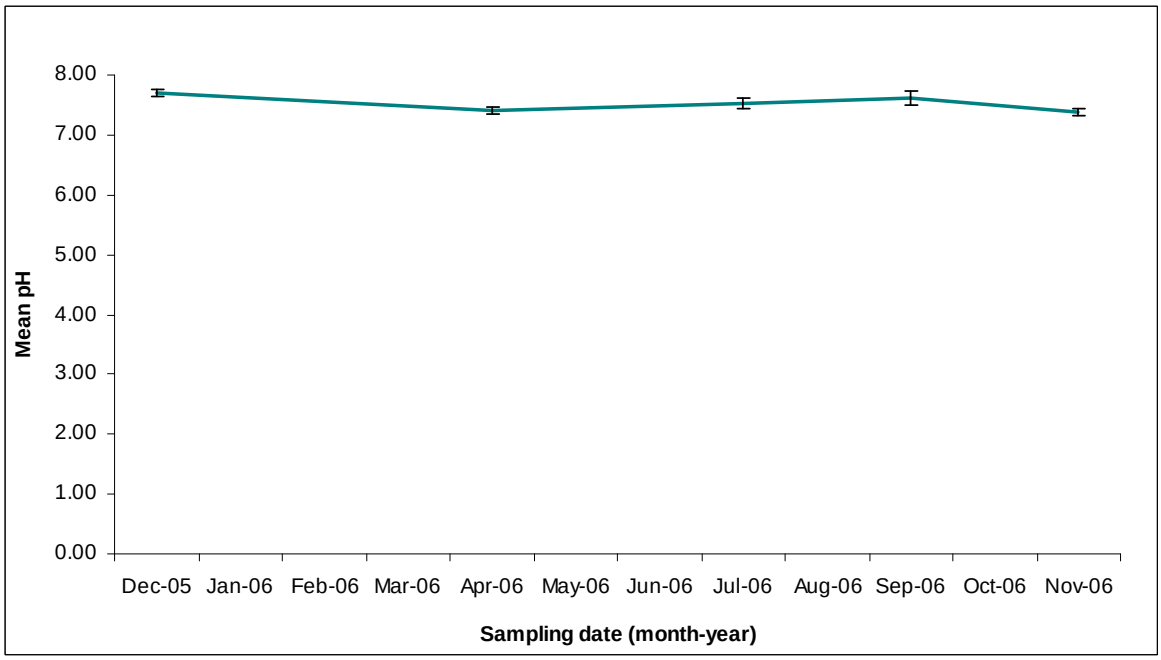


Figure 3.36 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) pH of short-term linoleum substrates in the Knockan Burn sub-catchment between December 2005 and November 2006 (n = 85). Note[1]: standard error bars barely visible.

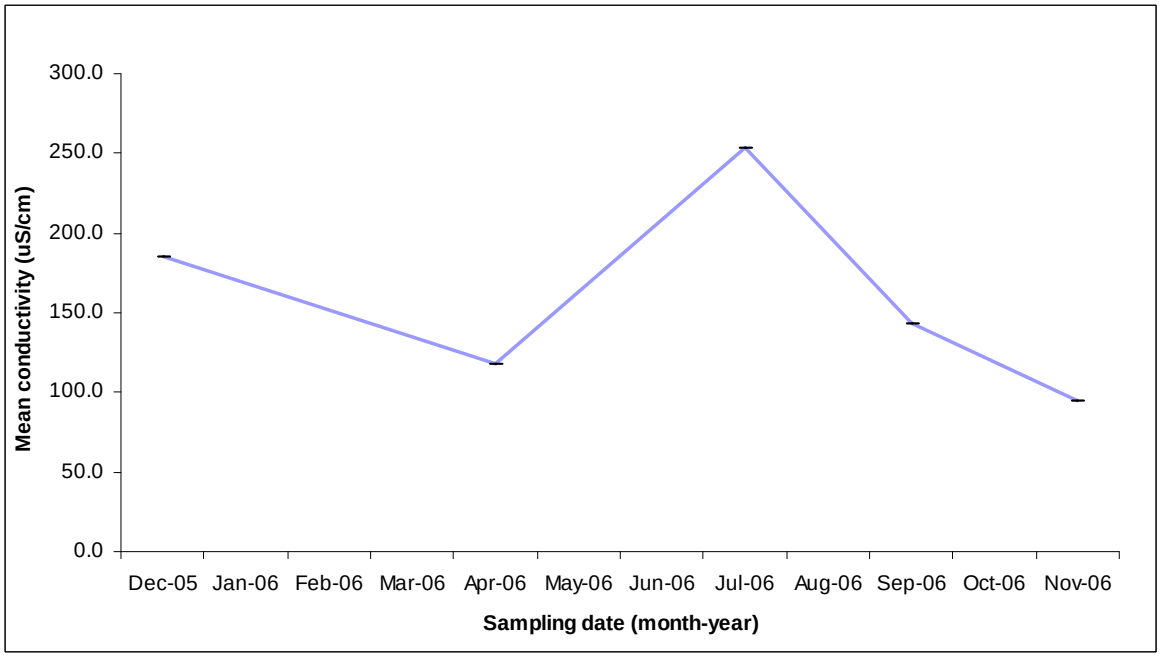


Figure 3.37 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) conductivity of short-term linoleum substrates in the Knockan Burn sub-catchment between December 2005 and November 2006 (n = 85). Note[1]: standard error bars barely visible.

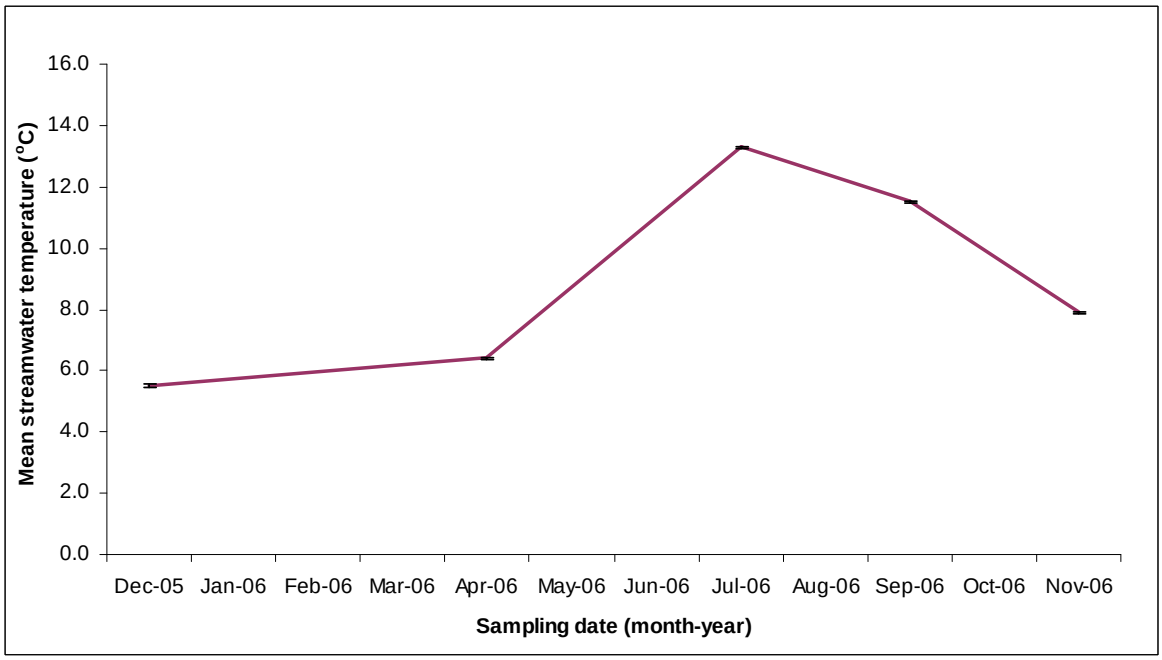


Figure 3.38 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) streamwater temperature of short-term linoleum substrates in the Knockan Burn sub-catchment between December 2005 and November 2006 (n = 85). Note[1]: standard error bars barely visible.

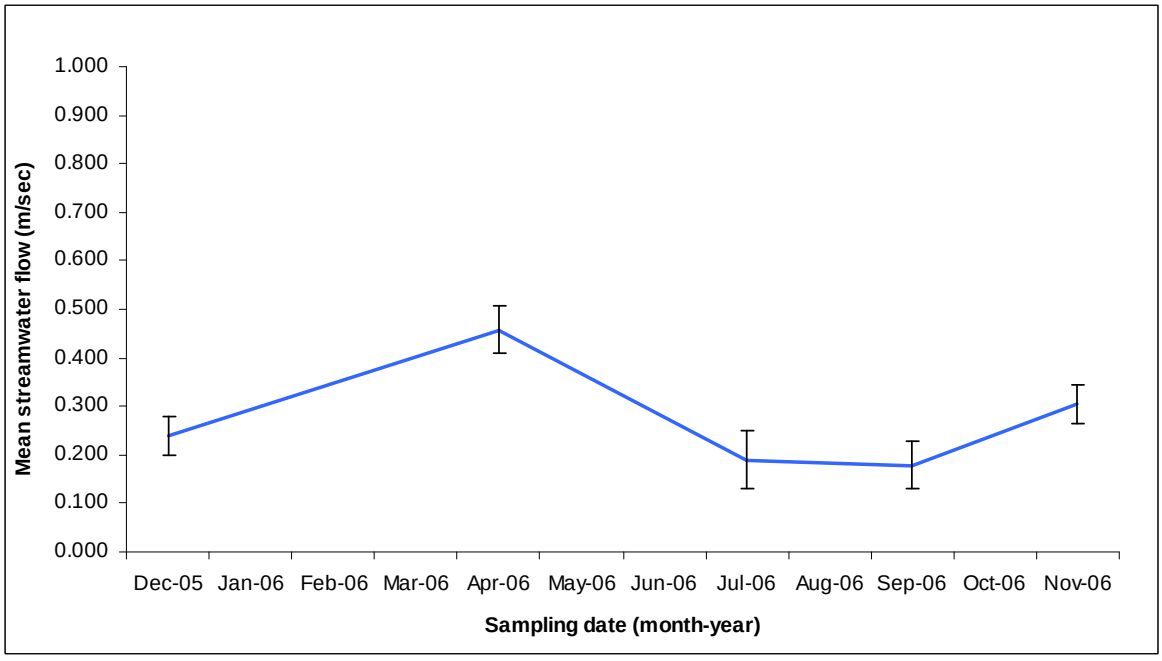


Figure 3.39 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) streamwater flow of short-term linoleum substrates in the Knockan Burn sub-catchment between December 2005 and November 2006 (n = 85).

Mean Variable	**April 2006**		**September 2006**		**November 2006**		**PANOVA**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **short-term linoleum** substrates	0.37^{a}	0.22	1.11^{b}	0.41	0.89^{ab}	0.47	P<0.05*
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **short-term linoleum** substrates	0.25	0.20	0.50	0.39	0.39	0.32	NS
D (m)	0.14^{b}	0.13	0.09^{a}	0.11	0.17^{b}	0.07	P<0.01**
K (m^{-1})	2.23	0.45	4.01	0.35	2.05	0.56	NS
$Z_{eu}^{1\%}$ (m)	0.41	0.45	0.23	0.36	0.45	0.58	NS
Z_{eu}:$D^{1\%}$	3.12	0.37	2.50	0.41	2.54	0.64	NS
pH	7.42^{b}	0.06	7.61^{a}	0.12	7.39^{b}	0.06	P<0.01**
Conductivity (μS cm^{-1})	117.8^{cd}	0.06	143.4^{c}	0.09	94.5^{d}	0.06	P<0.01**
Water Temperature (^{o}C)	6.4^{b}	0.05	11.5^{d}	0.02	7.9^{e}	0.01	P<0.001***
Flow (m s^{-1})	0.458^{a}	0.05	0.179^{b}	0.05	0.305^{ab}	0.04	P<0.01**

Table 3.33 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 53), chlorophyll content per unit area (n = 53) and environmental habitat variables (n = 53) of short-term linoleum substrates between sampling dates in the Knockan Burn sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Mean Variable	**April 2006**		**September 2006**		**November 2006**		**P**ANOVA
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **long-term linoleum** substrates	0.42^{a}	0.23	0.88^{b}	0.35	0.64^{ab}	0.33	P<0.05*
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **long-term linoleum** substrates	0.25	0.24	0.37	0.30	0.31	0.29	NS
D (m)	0.12^{a}	0.14	0.06^{b}	0.14	0.15^{a}	0.11	P<0.001***
K (m^{-1})	2.25^{a}	0.37	4.03^{b}	0.36	2.65^{a}	0.33	P<0.001***
$Z_{eu}^{1\%}$ (m)	0.41^{a}	0.38	0.23^{b}	0.36	0.34^{a}	0.33	P<0.001***
Z_{eu}:$D^{1\%}$	3.31^{ab}	0.38	3.88^{a}	0.50	2.26^{b}	0.38	P<0.05*
pH	7.44^{a}	0.06	7.62^{b}	0.10	7.37^{a}	0.04	P<0.01**
Conductivity (μS cm^{-1})	118.9^{ab}	0.06	144.0^{a}	0.11	93.3^{b}	0.06	P<0.01**
Water Temperature (^{o}C)	6.4^{a}	0.06	11.5^{b}	0.05	7.9^{c}	0.02	P<0.001***
Flow (m s^{-1})	0.470^{a}	0.05	0.237^{b}	0.06	0.512^{a}	0.06	P<0.001***

Table 3.34 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 53), chlorophyll content per unit area (n = 51) and environmental habitat variables (n = 53) of long-term linoleum substrates between sampling dates in the Knockan Burn sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Mean Variable	**April 2006**		**September 2006**		**November 2006**		**PANOVA**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **long-term Astroturf** substrates	1.94^{a}	0.40	3.39^{b}	0.60	2.72^{ab}	0.48	P<0.05*
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **long-term Astroturf** substrates	0.18	0.24	0.28	0.33	0.22	0.29	NS
D (m)	0.17^{a}	0.15	0.05^{b}	0.21	0.18^{a}	0.09	P<0.001***
K (m^{-1})	1.55^{a}	0.48	3.77^{b}	0.90	2.24^{ab}	0.46	P<0.05*
$Z_{eu}^{1\%}$ (m)	0.59^{a}	0.50	0.24^{b}	0.96	0.41^{a}	0.46	P<0.05*
Z_{eu}:$D^{1\%}$	3.56^{ab}	0.48	4.61^{a}	0.93	2.29^{b}	0.48	P<0.05*
pH	7.42^{a}	0.09	7.59^{b}	0.17	7.37^{a}	0.08	P<0.05*
Conductivity (μS cm^{-1})	117.8^{ab}	0.08	146.1^{a}	0.16	93.5^{b}	0.09	P<0.01**
Water Temperature (^{o}C)	6.4^{a}	0.08	11.4^{b}	0.02	8.0^{c}	0.02	P<0.001***
Flow (m s^{-1})	0.574^{a}	0.05	0.203^{b}	0.09	0.451^{ab}	0.08	P<0.01***

Table 3.35 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 26), chlorophyll content per unit area (n = 26) and environmental habitat variables (n =26) of long-term Astroturf substrates between sampling dates in the Knockan Burn sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Mean Variable	**April 2006**		**September 2006**		**November 2006**		**PANOVA**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **long-term plastic *Potamogeton*-like** substrates	0.10	0.22	0.29	0.33	0.17	0.26	NS
Periphyton chlorophyll content per unit area (µg cm^{-2}) harvested from **long-term plastic *Potamogeton*-like** substrates	0.11	0.17	0.21	0.26	0.15	0.16	NS
D (m)	0.12^{a}	0.19	0.05^{b}	0.24	0.15^{a}	0.09	P<0.001***
K (m^{-1})	1.92	0.57	3.69	0.53	2.65	0.56	NS
$Z_{eu}^{1\%}$ (m)	0.44	0.58	0.22	0.54	0.34	0.59	NS
Z_{eu}:$D^{1\%}$	2.81^{ab}	0.49	4.32^{a}	0.63	2.02^{b}	0.58	P<0.05*
pH	7.42^{a}	0.08	7.60^{b}	0.13	7.37^{a}	0.08	P<0.05*
Conductivity (µS cm^{-1})	122.6^{ab}	0.07	149.9^{a}	0.12	98.5^{b}	0.09	P<0.01**
Water Temperature (^{o}C)	6.4^{a}	0.07	11.4^{b}	0.02	7.9^{c}	0.01	P<0.001***
Flow (m s^{-1})	0.348^{a}	0.08	0.185^{b}	0.07	0.403^{a}	0.06	P<0.01***

Table 3.36 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 22), chlorophyll content per unit area (n = 20) and environmental habitat variables (n = 22) of long-term plastic aquarium *Potamogeton*-like substrates between sampling dates in the Knockan Burn sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Mean Variable	**April 2006**		**September 2006**		**November 2006**		**PANOVA**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **long-term plastic aquarium *Myriophyllum*-like** substrates	0.22	0.29	0.57	0.35	0.39	0.33	NS
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **long-term plastic aquarium *Myriophyllum*-like** substrates	0.18	0.18	0.36	0.31	0.25	0.29	NS
D (m)	0.12^{a}	0.19	0.05^{b}	0.24	0.15^{a}	0.09	P<0.001***
K (m^{-1})	1.92	0.57	3.69	0.53	2.65	0.56	NS
$Z_{eu}^{1\%}$ (m)	0.44	0.58	0.22	0.54	0.34	0.59	NS
Z_{eu}:$D^{1\%}$	2.81^{ab}	0.49	4.32^{a}	0.63	2.02^{b}	0.58	P<0.05*
pH	7.42^{a}	0.08	7.60^{b}	0.13	7.37^{a}	0.08	P<0.05*
Conductivity (μS cm^{-1})	122.6^{ab}	0.07	149.9^{a}	0.12	98.5^{b}	0.09	P<0.01**
Water Temperature (°C)	6.4^{a}	0.07	11.4^{b}	0.02	7.9^{c}	0.01	P<0.001***
Flow (m s^{-1})	0.348^{a}	0.08	0.185^{b}	0.07	0.403^{a}	0.06	P<0.01***

Table 3.37 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 22), chlorophyll content per unit area (n = 20) and environmental habitat variables (n = 22) of long-term plastic aquarium *Myriophyllum*-like substrates between sampling dates in the Knockan Burn sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Mean Variable	April 2006		September 2006		November 2006		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **mineral** substrata	0.66^{a}	0.34	5.68^{b}	0.25	3.32^{c}	0.30	P<0.001***
Periphyton chlorophyll content per unit area (µg cm^{-2}) harvested from **mineral** substrata	1.07	0.22	1.41	0.24	1.20	0.26	NS
Periphyton cover (%)	14.4^{a}	2.15	28.2^{b}	2.79	15.8^{a}	2.58	P<0.001***
Bare area (%)	50.1^{a}	3.36	36.2^{b}	2.97	42.3^{ab}	4.80	P<0.05*
D (m)	0.21^{a}	0.23	0.08^{b}	0.26	0.11^{b}	0.46	P<0.001***
K (m^{-1})	1.76^{a}	0.20	3.61^{b}	0.20	2.77^{c}	0.28	P<0.001***
$Z_{eu}^{1\%}$ (m)	0.52^{a}	0.20	0.25^{b}	0.20	0.33^{c}	0.29	P<0.001***
Z_{eu}:$D^{1\%}$	2.50	0.25	3.19	0.28	2.92	0.42	NS
pH	7.45^{a}	0.03	7.70^{b}	0.05	7.52^{a}	0.05	P<0.001***
Conductivity (µS cm^{-1})	116.9^{a}	0.17	173.4^{b}	0.18	124.3^{a}	0.29	P<0.001***
Water Temperature (^{o}C)	6.4^{a}	0.04	12.5^{b}	0.02	8.3^{c}	0.02	P<0.001***
Flow (m s^{-1})	0.514^{a}	0.04	0.141^{b}	0.04	0.259^{b}	0.05	P<0.001***

Table 3.38 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 135), chlorophyll content per unit area (n = 100), periphyton cover (n = 135), bare area (n = 135) and environmental habitat variables (n = 135) of naturally-occurring mineral substrata between sampling dates in the Knockan Burn sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of substrate morphology, alkalinity, nutrient status and heavy metal composition refer to Table 2.6 in Chapter 2.

Mean Variable	April 2006		September 2006		November 2006		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **aquatic bryophytes**	10.36^{a}	0.55	12.76^{b}	0.65	11.44^{ab}	0.59	P<0.05*
Periphyton chlorophyll content per unit area (µg cm^{-2}) harvested from **aquatic bryophytes**	1.82	0.39	2.30	0.37	2.08	0.40	NS
Periphyton cover (%)	14.4^{a}	2.15	28.2^{b}	2.79	15.8^{a}	2.58	P<0.001***
Bare area (%)	50.1^{a}	3.36	36.2^{b}	2.97	42.3^{ab}	4.80	P<0.05*
D (m)	0.21^{a}	0.23	0.08^{b}	0.26	0.11^{b}	0.46	P<0.001***
K (m^{-1})	1.76^{a}	0.20	3.61^{b}	0.20	2.77^{c}	0.28	P<0.001***
$Z_{eu}^{1\%}$ (m)	0.52^{a}	0.20	0.25^{b}	0.20	0.33^{c}	0.29	P<0.001***
Z_{eu}:$D^{1\%}$	2.50	0.25	3.19	0.28	2.92	0.42	NS
pH	7.45^{a}	0.03	7.70^{b}	0.05	7.52^{a}	0.05	P<0.001***
Conductivity (µS cm^{-1})	116.9^{a}	0.17	173.4^{b}	0.18	124.3^{a}	0.29	P<0.001***
Water Temperature (^{o}C)	6.4^{a}	0.04	12.5^{b}	0.02	8.3^{c}	0.02	P<0.001***
Flow (m s^{-1})	0.514^{a}	0.04	0.141^{b}	0.04	0.259^{b}	0.05	P<0.001***

Table 3.39 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 91), chlorophyll content per unit area (n = 88), periphyton cover (n = 91), bare area (n = 91) and environmental habitat variables (n = 91) of naturally-occurring aquatic bryophytes between sampling dates in the Knockan Burn sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of substrate morphology, alkalinity, nutrient status and heavy metal composition refer to Table 2.6 in Chapter 2.

Mean Variable	April 2006		September 2006		November 2006		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **vascular submerged macrophytes**	0.18^{a}	0.15	0.74^{b}	0.29	0.40^{ab}	0.43	P<0.05*
Periphyton chlorophyll content per unit area (µg cm^{-2}) harvested from **vascular submerged macrophytes**	0.09	0.22	0.13	0.17	0.11	0.35	NS
Periphyton cover (%)	14.4^{a}	2.15	28.2^{b}	2.79	15.8^{a}	2.58	P<0.001***
Bare area (%)	50.1^{a}	3.36	36.2^{b}	2.97	42.3^{ab}	4.80	P<0.05*
D (m)	0.21^{a}	0.13	0.08^{b}	0.16	0.15^{ab}	0.17	P<0.05*
K (m^{-1})	1.78	0.48	2.39	0.54	2.66	0.66	NS
$Z_{eu}^{1\%}$ (m)	0.53	0.48	0.38	0.54	0.34	0.66	NS
Z_{eu}:$D^{1\%}$	2.39	0.41	2.95	0.62	2.18	0.64	NS
pH	7.57^{a}	0.08	7.91^{b}	0.15	7.58^{a}	0.07	P<0.01**
Conductivity (µS cm^{-1})	138.9^{a}	0.09	204.3^{b}	0.11	151.2^{a}	0.21	P<0.001***
Water Temperature (^{o}C)	7.2^{a}	0.10	12.5^{b}	0.02	8.5^{c}	0.02	P<0.001***
Flow (m s^{-1})	0.323^{a}	0.05	0.106^{b}	0.03	0.216^{b}	0.05	P<0.001***

Table 3.40 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 24), chlorophyll content per unit area (n = 24), periphyton cover (n = 24), bare area (n = 24) and environmental habitat variables (n = 24) of naturally-occurring vascular submerged macrophytes between sampling dates in the Knockan Burn sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of substrate morphology, alkalinity, nutrient status and heavy metal composition refer to Table 2.6 in Chapter 2.

3.5.3 Response of periphyton production and environmental habitat conditions in the Water of Dye, River Girnock and Knockan Burn to variation in flow regime: pool, glide and riffle zones

3.5.3.1 Periphyton production of naturally-occurring substrata

Overall, the amalgamated data set indicated that periphyton biomass and abundance (% cover) harvested from naturally-occurring mineral substrata was significantly higher in pools than in glides or riffles, and furthermore was significantly lower in riffle zones than in glides (Table 3.44). However, periphyton chlorophyll content did not exhibit a significant response to variation in flow pattern. Bare area did not tend to vary significantly between flow regimes. These observed trends were consistent with those found in each individual sub-catchment stream: Water of Dye (Table 3.41), River Girnock (Table 3.42) and Knockan Burn (Table 3.43).

Overall, the amalgamated data set showed that although periphyton biomass harvested from naturally-occurring aquatic bryophytes followed a similar trend as for the aforementioned mineral substrata and tended to decrease in response to increasing current velocity, periphyton production (biomass and chlorophyll content) did not vary significantly between pool, glide and riffle habitats (Table 3.48). This was reflected in each of the sub-catchment streams: Water of Dye (Table 3.45), River Girnock (Table 3.46) and Knockan Burn (Table 3.47).

There was no significant difference in periphyton production (biomass and chlorophyll content) between vascular submerged macrophytes occurring in pools or glides (note: vascular submerged macrophytes were markedly absent from riffle zones) in Knockan Burn (Table 3.49). Periphyton cover was however, significantly higher in pools than in glides. The proportion of unvegetated streambed did not appear to vary significantly between pools and glide habitats, wherein vascular submerged macrophytes were present.

Variable	Pool		Glide		Riffle		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **mineral** substrata	1.37^{a}	0.25	0.70^{b}	0.23	0.22^{c}	0.22	P<0.001***
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **mineral** substrata	1.28	0.62	1.77	0.23	1.61	0.22	NS
Periphyton cover (%)	25.4^{a}	3.50	17.9^{b}	2.71	11.4^{c}	1.63	P<0.001***
Bare area (%)	38.4	2.98	38.5	3.52	38.8	3.66	NS
D (m)	0.15^{a}	0.26	0.15^{a}	0.29	0.11^{b}	0.31	P<0.05*
K (m^{-1})	2.78	0.24	2.84	0.25	3.40	0.26	NS
$Z_{eu}^{1\%}$ (m)	0.27	0.24	0.26	0.26	0.23	0.27	NS
Z_{eu}:$D^{1\%}$	1.74	0.32	1.78	0.27	1.90	0.30	NS
pH	6.33	0.34	6.33	0.34	6.32	0.34	NS
Conductivity (μS cm^{-1})	45.9	0.19	45.8	0.19	45.7	0.19	NS
Water Temperature (^{o}C)	10.1	0.08	10.3	0.09	10.3	0.09	NS
Flow (m s^{-1})	0.009^{a}	0.01	0.066^{b}	0.02	0.164^{c}	0.02	P<0.001***

Table 3.41 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 135), chlorophyll content per unit area (n = 17), periphyton cover (n = 135), bare area (n = 135) and environmental habitat variables (n = 135) of naturally-occurring mineral substrata between flow regime (pool, glide, riffle habitats) in the Water of Dye sub-catchment (n = 135). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Table 2.7 in Chapter 2.

Variable	Pool		Glide		Riffle		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **mineral** substrata	3.23^{a}	0.22	2.48^{b}	0.24	1.96^{c}	0.21	P<0.001***
Periphyton chlorophyll content per unit area (µg cm^{-2}) harvested from **mineral** substrata	1.71	0.29	1.28	0.25	1.40	0.27	NS
Periphyton cover (%)	49.5^{a}	3.56	37.7^{b}	3.20	28.2^{c}	3.15	P<0.001***
Bare area (%)	26.8	3.17	27.1	3.06	30.4	2.91	NS
D (m)	0.14^{a}	0.26	0.13^{a}	0.28	0.10^{b}	0.27	P<0.05*
K (m^{-1})	2.25	0.28	2.27	0.23	2.43	0.26	NS
$Z_{eu}^{1\%}$ (m)	0.26	0.32	0.26	0.32	0.25	0.34	NS
Z_{eu}:$D^{1\%}$	1.90	0.35	2.02	0.34	2.50	0.44	NS
pH	7.04	0.09	6.93	0.08	6.84	0.08	NS
Conductivity (µS cm^{-1})	53.1	0.23	50.9	0.22	51.5	0.22	NS
Water Temperature (^{o}C)	11.2	0.12	11.1	0.11	11.2	0.11	NS
Flow (m s^{-1})	0.069^{a}	0.02	0.205^{b}	0.02	0.389^{c}	0.02	P<.0001***

Table 3.42 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 135), chlorophyll content per unit area (n = 56), periphyton cover (n = 135), bare area (n = 135) and environmental habitat variables (n = 135) of naturally-occurring mineral substrata between flow regime (pool, glide, riffle habitats) in the River Girnock sub-catchment (n = 135). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Table 2.8 in Chapter 2.

Variable	Pool		Glide		Riffle		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **mineral** substrata	4.77^{a}	0.25	2.54^{b}	0.30	1.47^{c}	0.35	P<0.001***
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **mineral** substrata	1.18	0.23	1.30	0.25	1.22	0.33	NS
Periphyton cover (%)	26.3^{a}	3.08	18.9^{b}	2.07	12.3^{c}	3.22	P<0.001***
Bare area (%)	39.9	3.11	44.0	3.23	44.7	5.09	NS
D (m)	0.12	0.36	0.14	0.26	0.11	0.38	NS
K (m^{-1})	2.79	0.23	2.40	0.22	2.60	0.30	NS
$Z_{eu}^{1\%}$ (m)	0.32	0.24	0.38	0.22	0.36	0.29	NS
Z_{eu}:$D^{1\%}$	2.90	0.33	2.64	0.25	3.22	0.33	NS
pH	7.54	0.20	7.57	0.18	7.59	0.26	NS
Conductivity (μS cm^{-1})	148.1	0.20	134.2	0.19	133.6	0.26	NS
Water Temperature (^{o}C)	9.5	0.07	8.7	0.06	8.4	0.09	NS
Flow (m s^{-1})	0.001^{a}	0.01	0.208^{b}	0.04	0.589^{c}	0.05	P<0.001***

Table 3.43 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 135), chlorophyll content per unit area (n = 100), periphyton cover (n = 135), bare area (n = 135) and environmental habitat variables (n = 135) of naturally-occurring mineral substrata between flow regime (pool, glide, riffle habitats) in the Knockan Burn sub-catchment (n = 135). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Table 2.9 in Chapter 2.

Variable	Pool		Glide		Riffle		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **mineral** substrata	3.12^{a}	0.15	1.91^{b}	0.16	1.22^{c}	0.18	P<0.001***
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **mineral** substrata	1.38	0.19	1.45	0.17	1.40	0.21	NS
Periphyton cover (%)	33.7^{a}	2.02	24.8^{b}	1.74	17.3^{c}	1.89	P<0.001***
Bare area (%)	35.0	1.85	36.5	1.99	38.0	2.19	NS
D (m)	0.14^{a}	0.17	0.14^{a}	0.16	0.11^{b}	0.18	P<0.01**
K (m^{-1})	2.60	0.15	2.48	0.14	2.80	0.16	NS
$Z_{eu}^{1\%}$ (m)	0.28	0.16	0.30	0.15	0.26	0.18	NS
Z_{eu}:$D^{1\%}$	2.13	0.20	2.18	0.17	2.40	0.22	NS
pH	6.97	0.07	7.01	0.06	6.82	0.08	NS
Conductivity (μS cm^{-1})	71.5	0.16	72.8	0.15	62.0	0.16	NS
Water Temperature (^{o}C)	10.6	0.06	9.7	0.06	10.1	0.07	NS
Flow (m s^{-1})	0.062^{a}	0.01	0.289^{b}	0.02	0.465^{c}	0.03	P<0.001***

Table 3.44 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 405), chlorophyll content per unit area (n = 173), periphyton cover (n = 405), bare area (n = 405) and environmental habitat variables (n = 405) of naturally-occurring mineral substrata between flow regime (pool, glide, riffle habitats) for amalgamated sub-catchment data (the Water of Dye, River Girnock, and Knockan Burn, n = 405). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Table 2.10 in Chapter 2.

Variable	Pool		Glide		Riffle		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **aquatic bryophytes**	1.56	0.25	1.42	0.24	1.38	0.23	NS
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **aquatic bryophytes**	1.09	0.27	1.44	0.28	1.30	0.23	NS
Periphyton cover (%)	25.4^{a}	3.50	17.9^{b}	2.71	11.4^{c}	1.63	P<0.001***
Bare area (%)	38.4	2.98	38.5	3.52	38.8	3.66	NS
D (m)	0.15^{a}	0.26	0.15^{a}	0.29	0.11^{b}	0.31	P<0.05*
K (m^{-1})	2.78	0.24	2.84	0.25	3.40	0.26	NS
$Z_{eu}^{1\%}$ (m)	0.27	0.24	0.26	0.26	0.23	0.27	NS
Z_{eu}:$D^{1\%}$	1.74	0.32	1.78	0.27	1.90	0.30	NS
pH	6.33	0.34	6.33	0.34	6.32	0.34	NS
Conductivity (μS cm^{-1})	45.9	0.19	45.8	0.19	45.7	0.19	NS
Water Temperature (^{o}C)	10.1	0.08	10.3	0.09	10.3	0.09	NS
Flow (m s^{-1})	0.009^{a}	0.01	0.066^{b}	0.02	0.164^{c}	0.02	P<0.001***

Table 3.45 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 120), chlorophyll content per unit area (n = 41), periphyton cover (n = 120), bare area (n = 120) and environmental habitat variables (n = 120) of naturally-occurring aquatic bryophytes between flow regime (pool, glide, riffle habitats) in the Water of Dye sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Table 2.7 in Chapter 2.

Variable	Pool		Glide		Riffle		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **aquatic bryophytes**	0.73	0.25	0.68	0.24	0.62	0.27	NS
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **aquatic bryophytes**	0.86	0.16	0.90	0.15	0.80	0.14	NS
Periphyton cover (%)	49.5^{a}	3.56	37.7^{b}	3.20	28.2^{c}	3.15	P<0.001***
Bare area (%)	26.8	3.17	27.1	3.06	30.4	2.91	NS
D (m)	0.14^{a}	0.26	0.13^{a}	0.28	0.10^{b}	0.27	P<0.05*
K (m^{-1})	2.25	0.28	2.27	0.23	2.43	0.26	NS
$Z_{eu}^{1\%}$ (m)	0.26	0.32	0.26	0.32	0.25	0.34	NS
Z_{eu}:$D^{1\%}$	1.90	0.35	2.02	0.34	2.50	0.44	NS
pH	7.04	0.09	6.93	0.08	6.84	0.08	NS
Conductivity (μS cm^{-1})	53.1	0.23	50.9	0.22	51.5	0.22	NS
Water Temperature (^{o}C)	11.2	0.12	11.1	0.11	11.2	0.11	NS
Flow (m s^{-1})	0.069^{a}	0.02	0.205^{b}	0.02	0.389^{c}	0.02	P<.0001***

Table 3.46 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 95), chlorophyll content per unit area (n = 47), periphyton cover (n = 95), bare area (n = 95) and environmental habitat variables (n = 95) of naturally-occurring aquatic bryophytes between flow regime (pool, glide, riffle habitats) in the River Girnock sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Table 2.8 in Chapter 2.

Variable	Pool		Glide		Riffle		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **aquatic bryophytes**	12.09	0.59	11.58	0.47	10.89	0.63	NS
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **aquatic bryophytes**	1.96	0.41	2.12	0.36	2.09	0.50	NS
Periphyton cover (%)	26.3^{a}	3.08	18.9^{b}	2.07	12.3^{c}	3.22	P<0.001***
Bare area (%)	39.9	3.11	44.0	3.23	44.7	5.09	NS
D (m)	0.12	0.36	0.14	0.26	0.11	0.38	NS
K (m^{-1})	2.79	0.23	2.40	0.22	2.60	0.30	NS
$Z_{eu}^{1\%}$ (m)	0.32	0.24	0.38	0.22	0.36	0.29	NS
Z_{eu}:$D^{1\%}$	2.90	0.33	2.64	0.25	3.22	0.33	NS
pH	7.54	0.20	7.57	0.18	7.59	0.26	NS
Conductivity (μS cm^{-1})	148.1	0.20	134.2	0.19	133.6	0.26	NS
Water Temperature (^{o}C)	9.5	0.07	8.7	0.06	8.4	0.09	NS
Flow (m s^{-1})	0.001^{a}	0.01	0.208^{b}	0.04	0.589^{c}	0.05	P<0.001***

Table 3.47 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 91), chlorophyll content per unit area (n = 88), periphyton cover (n = 91), bare area (n = 91) and environmental habitat variables (n = 91) of naturally-occurring aquatic bryophytes between flow regime (pool, glide, riffle habitats) in the Knockan Burn sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Table 2.9 in Chapter 2.

Variable	Pool		Glide		Riffle		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **aquatic bryophytes**	4.79	0.32	4.56	0.29	4.30	0.29	NS
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **aquatic bryophytes**	1.30	0.24	1.49	0.22	1.40	0.25	NS
Periphyton cover (%)	33.7^{a}	2.02	24.8^{b}	1.74	17.3^{c}	1.89	P<0.001***
Bare area (%)	35.0	1.85	36.5	1.99	38.0	2.19	NS
D (m)	0.14^{a}	0.17	0.14^{a}	0.16	0.11^{b}	0.18	P<0.01**
K (m^{-1})	2.60	0.15	2.48	0.14	2.80	0.16	NS
$Z_{eu}^{1\%}$ (m)	0.28	0.16	0.30	0.15	0.26	0.18	NS
Z_{eu}:$D^{1\%}$	2.13	0.20	2.18	0.17	2.40	0.22	NS
pH	6.97	0.07	7.01	0.06	6.82	0.08	NS
Conductivity (μS cm^{-1})	71.5	0.16	72.8	0.15	62.0	0.16	NS
Water Temperature (^{o}C)	10.6	0.06	9.7	0.06	10.1	0.07	NS
Flow (m s^{-1})	0.062^{a}	0.01	0.289^{b}	0.02	0.465^{c}	0.03	P<0.001***

Table 3.48 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 306), chlorophyll content per unit area (n = 176), periphyton cover (n = 306), bare area (n = 306) and environmental habitat variables (n = 306) of naturally-occurring aquatic bryophytes between flow regime (pool, glide, riffle habitats) for amalgamated sub-catchment data (the Water of Dye, River Girnock, and Knockan Burn). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Table 2.10 in Chapter 2.

Variable	Pool		Glide		Riffle		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **vascular submerged macrophytes**	0.30	0.72	0.57	0.44	N/A		NS
Periphyton chlorophyll content per unit area (µg cm^{-2}) harvested from **vascular submerged macrophytes**	0.04	0.88	0.18	0.24	N/A		NS
Periphyton cover (%)	26.3	3.08	18.9	2.07	N/A		P<0.01**
Bare area (%)	39.9	3.11	44.0	3.23	N/A		NS
D (m)	0.12	0.36	0.14	0.26	N/A		NS
K (m^{-1})	2.79	0.23	2.40	0.22	N/A		NS
$Z_{eu}^{1\%}$ (m)	0.32	0.24	0.38	0.22	N/A		NS
Z_{eu}:$D^{1\%}$	2.90	0.33	2.64	0.25	N/A		NS
pH	7.54	0.20	7.57	0.18	N/A		NS
Conductivity (µS cm^{-1})	148.1	0.20	134.2	0.19	N/A		NS
Water Temperature (^oC)	9.5	0.07	8.7	0.06	N/A		NS
Flow (m s^{-1})	0.001	0.01	0.208	0.04	N/A		P<0.001***

Table 3.49 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 24), chlorophyll content per unit area (n = 24), periphyton cover (n = 24), bare area (n = 24) and environmental habitat variables (n = 24) of naturally-occurring vascular submerged macrophytes between flow regime (pool, glide, riffle habitats) in the Knockan Burn sub-catchment. Significance testing: one-way ANOVA. For details of environmental habitat conditions refer to Table 2.9 in Chapter 2.

3.5.4 Response of periphyton production and environmental habitat conditions in the Water of Dye, River Girnock and Knockan Burn to variation in substrate morphology

3.5.4.1 Periphyton production of naturally-occurring substrata

Periphyton production (biomass and chlorophyll content) and abundance (% cover) on naturally-occurring mineral substrata did not exhibit a significant response to variation in the proportion of boulders (Table 3.50). However, the proportion of unvegetated streambed (bare area) increased significantly in response to a reduction in boulder cover.

Although periphyton production on naturally-occurring mineral substrata tended to increase as the proportion of large stones increased, this was not significant, unlike that of periphyton abundance (Table 3.51). Bare area increased as the proportion of large stones decreased.

Periphyton production (biomass and chlorophyll content) and abundance on naturally-occurring mineral substrata did not respond significantly to variation in the proportion of small stones occupying the streambed (Table 3.52). However, there was a significant response of increasing bare area to increasing cover of small stones.

Periphyton biomass and abundance on naturally-occurring mineral substrata increased significantly in response to high gravel cover, but chlorophyll content did not (Table 3.53). Bare area responded similarly, becoming more abundant with increased proportions of gravely substrates.

There was no significant response of periphyton production, abundance or bare area on naturally-occurring mineral substrata to variation in streambed sand cover (Table 3.54).

Variable	0% BO		3% BO		15.5% BO		38% BO		63% BO		88% BO		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **mineral** substrata	2.37	0.59	2.22	0.89	2.16	0.67	2.27	1.06	1.89	0.77	1.56	1.29	NS
Periphyton chlorophyll content per unit area (μg cm^{-2}) harvested from **mineral** substrata	1.14	0.39	1.38	0.95	1.31	0.49	1.40	0.83	1.57	0.80	1.65	2.14	NS
Periphyton cover (%)	24.1	1.83	26.1	2.93	23.9	2.68	25.9	3.07	26.0	2.95	26.4	3.95	NS
Bare area (%)	56.4^{a}	1.98	37.8^{b}	2.99	37.0^{b}	2.82	33.5^{b}	3.25	26.7^{c}	2.47	25.5^{c}	3.95	P<0.001***

Table 3.50 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): response of periphyton biomass per unit area (n = 405), chlorophyll content per unit area (n = 173), periphyton cover (n = 405) and bare area of naturally-occurring mineral substrata to variation in the abundance (median % cover) of boulders (BO) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 405). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Table 2.11 in Chapter 2.

Variable	0% LS		3% LS		15.5% LS		38% LS		63% LS		88% LS		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **mineral** substrata	1.58	0.88	2.17	1.27	1.83	0.93	2.05	1.14	1.71	0.89	3.11	1.29	NS
Periphyton chlorophyll content per unit area (µg cm^{-2}) harvested from **mineral** substrata	1.46	0.87	1.53	0.60	1.39	0.92	1.24	0.56	1.51	0.54	1.32	0.72	NS
Periphyton cover (%)	16.2^{a}	2.81	18.3^{a}	3.32	25.6^{b}	2.40	26.4^{b}	1.88	28.7^{b}	2.36	37.4^{c}	4.75	P<0.001***
Bare area (%)	41.1^{a}	3.14	39.6^{a}	3.78	38.1^{a}	2.54	37.3^{a}	2.12	36.8^{a}	2.26	26.1^{b}	4.78	P<0.01**

Table 3.51 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): response of periphyton biomass per unit area (n = 405), chlorophyll content per unit area (n = 173), periphyton cover (n = 405) and bare area of naturally-occurring mineral substrata to variation in the abundance (median % cover) of large stones (LS) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 405). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Table 2.12 in Chapter 2.

Variable	0% SS		3% SS		15.5% SS		38% SS		63% SS		88% SS		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **mineral** substrata	1.68	0.79	2.11	0.72	2.58	0.67	2.36	0.71	1.97	0.96	1.75	1.17	NS
Periphyton chlorophyll content per unit area (µg cm^{-2}) harvested from **mineral** substrata	1.34	1.01	1.49	0.70	1.37	0.51	1.42	0.64	1.53	0.59	1.28	1.16	NS
Periphyton cover (%)	26.8	2.72	26.9	2.55	25.8	2.14	25.6	2.49	24.3	2.24	23.1	4.59	NS
Bare area (%)	23.3^{a}	2.32	21.7^{a}	2.44	36.5^{b}	2.21	38.7^{b}	2.83	40.2^{b}	2.76	58.8^{c}	5.00	P<0.001***

Table 3.52 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): response of periphyton biomass per unit area (n = 405), chlorophyll content per unit area (n = 173), periphyton cover (n = 405) and bare area of naturally-occurring mineral substrata to variation in the abundance (median % cover) of small stones (SS) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 405). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Table 2.13 in Chapter 2.

Variable	0% GR		3% GR		15.5% GR		38% GR		63% GR		88% GR		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **mineral** substrata	1.27^{a}	0.54	1.19^{a}	0.72	1.34^{a}	0.81	2.38^{ab}	1.10	2.52^{ab}	0.74	3.80^{b}	1.43	P<0.05*
Periphyton chlorophyll content per unit area (µg cm^{-2}) harvested from **mineral** substrata	1.82	0.58	1.78	0.53	1.42	0.72	1.27	0.91	1.03	0.89	1.11	0.68	NS
Periphyton cover (%)	22.6^{a}	1.97	22.1^{a}	2.31	23.7^{a}	2.34	22.3^{a}	2.41	22.2^{a}	2.91	34.3^{b}	5.39	P<0.01**
Bare area (%)	24.2^{a}	1.77	35.6^{b}	2.42	36.4^{b}	2.38	38.8^{b}	3.35	40.7^{b}	3.52	43.2^{b}	7.52	P<0.001***

Table 3.53 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): response of periphyton biomass per unit area (n = 405), chlorophyll content per unit area (n = 173), periphyton cover (n = 405) and bare area of naturally-occurring mineral substrata to variation in the abundance (median % cover) of gravel (GR) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 405). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Table 2.14 in Chapter 2.

Variable	0% SA		3% SA		15.5% SA		38% SA		63% SA		88% SA		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton biomass per unit area (mg cm^{-2}) harvested from **mineral** substrata	1.22	0.34	1.77	1.07	2.76	1.35	3.04	1.87	1.74	4.89	1.96	6.19	NS
Periphyton chlorophyll content per unit area (µg cm^{-2}) harvested from **mineral** substrata	1.59	0.49	1.78	0.97	1.53	1.19	2.61	1.95	0.44	3.48	0.48	5.56	NS
Periphyton cover (%)	22.7	1.17	24	3.87	28.6	4.74	32.9	9.63	28.3	6.25	15.7	10.83	NS
Bare area (%)	35.9	1.22	38.5	5.79	38	5.12	29	11.56	39.5	12.51	37.9	13.72	NS

Table 3.54 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): response of periphyton biomass per unit area (n = 405), chlorophyll content per unit area (n = 173), periphyton cover (n = 405) and bare area of naturally-occurring mineral substrata to variation in the abundance (median % cover) of sand (SA) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 405). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Table 2.15 in Chapter 2.

3.5.5 Comparison of periphyton production and environmental habitat conditions between artificial and naturally-occurring substrata: do artificial substrates make good surrogates for naturally-occurring microhabitats?

3.5.5.1 Water of Dye

In the Water of Dye there was no significant difference in periphyton production (biomass and chlorophyll content) between short-term linoleum and long-term linoleum substrates (Table 3.55). However, periphyton biomass and chlorophyll content was significantly higher on naturally-occurring mineral substrata compared to both short-term and long-term linoleum substrates. There was no significant difference in any of the environmental habitat variables measured between the linoleum and mineral substrates.

There was significant variation in periphyton production between long-term Astroturf and naturally-occurring aquatic bryophytes, the latter exhibited significantly higher periphyton biomass and chlorophyll content in the Water of Dye (Table 3.56). There was no significant difference in any of the environmental habitat variables measured between the Astroturf and aquatic bryophyte substrates.

3.5.5.2 River Girnock

Also shown in the River Girnock was that periphyton biomass and chlorophyll content did not vary significantly between short-term linoleum and long-term linoleum substrates (Table 3.57). However, periphyton production (biomass and chlorophyll content) was significantly lower on both short-term and long-term linoleum substrates in comparison to naturally-occurring mineral substrata. There was no significant difference in any of the environmental habitat variables measured between the linoleum and mineral substrates.

Periphyton biomass between long-term Astroturf and naturally-occurring aquatic bryophytes was similar in the River Girnock. However, significantly higher periphyton chlorophyll content was harvested from the surfaces of aquatic bryophytes (Table 3.58). There was no significant difference in any of the environmental habitat variables measured between the Astroturf and aquatic bryophyte substrates.

3.5.5.3 Knockan Burn

In Knockan Burn periphyton production (biomass and chlorophyll content) was similar on both short-term and long-term linoleum substrates, which were significantly lower than harvested from naturally-occurring mineral substrata (Table 3.59). There was no significant difference in any of the environmental habitat variables measured between the linoleum and mineral substrates.

Periphyton biomass and chlorophyll content harvested from naturally-occurring aquatic bryophytes were significantly higher compared to periphyton occurring in long-term Astroturf substrates in Knockan Burn (Table 3.60). There was no significant difference in any of the environmental habitat variables measured between the Astroturf and aquatic bryophyte substrates.

In Knockan Burn, there was no significant variation in periphyton production (biomass and chlorophyll content) between the artificial plant samplers and naturally-occurring vascular submerged macrophytes (Table 3.61). However, there were significant differences in environmental habitat conditions between the artificial aquarium plant samplers and naturally-occurring plants, with mean streamwater pH and conductivity tending to be higher within the vascular submerged macrophyte stands than for the surrogate plastic plants.

3.5.5.4 Amalgamated data

Overall, naturally-occurring mineral substrata had higher periphyton production (biomass and chlorophyll content) than did either short-term or long-term linoleum substrates, which possessed similar quantities (see Table 3.62, and also Figure 3.40). Furthermore, periphyton biomass and chlorophyll content was also higher in naturally-occurring aquatic bryophytes than in Astroturf substrates.

Overall, periphyton biomass harvested from Astroturf substrates was significantly higher than obtained from short-term and long-term linoleum substrates, as well as long-term plastic plants but chlorophyll content did not vary between these four types of artificial substrates (Table 3.62, and also Figure 3.40).

Periphyton production (biomass and chlorophyll content) on plastic plants reflected quantities harvested from vascular submerged macrophyte surfaces, and did not differ significantly from periphyton occurring on short-term and long-term linoleum substrates (Table 3.62, and also Figure 3.40).

Mostly, there was no significant variation in any of the environmental habitat conditions between the artificial substrates and their naturally-occurring counterparts (Table 3.62). Notable exceptions were however, the significant differences detected in mean streamwater pH and conductivity between the naturally-occurring vascular submerged macrophyte zones and plastic plant samplers in the Knockan Burn sub-catchment.

Mean Variable	Short-term linoleum		Long-term linoleum		Mineral substrata		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton biomass per unit area (mg cm^{-2})	0.38^{a}	0.18	0.31^{a}	0.23	0.76^{b}	0.16	P<0.001***
Periphyton chlorophyll content per unit area (μg cm^{-2})	0.21^{a}	0.17	0.34^{a}	0.25	1.55^{b}	0.29	P<0.001***
D (m)	0.16	0.12	0.15	0.13	0.14	0.07	NS
K (m^{-1})	2.77	0.28	2.60	0.32	2.99	0.15	NS
$Z_{eu}^{1\%}$ (m)	0.27	0.33	0.28	0.31	0.25	0.16	NS
Z_{eu}:$D^{1\%}$	1.64	0.31	1.85	0.32	1.81	0.17	NS
pH	6.32	0.13	6.28	0.12	6.33	0.07	NS
Conductivity (μS cm^{-1})	46.4	0.05	46.1	0.04	45.8	0.03	NS
Water Temperature (^{o}C)	9.8	0.14	9.6	0.13	10.2	0.07	NS
Flow (m s^{-1})	0.233	0.05	0.185	0.04	0.219	0.02	NS

Table 3.55 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 211), periphyton chlorophyll content per unit area (n = 72), and environmental habitat variables (n = 211) between short-term linoleum, long-term linoleum and naturally-occurring mineral substrata in the Water of Dye sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Mean Variable	**Long-term Astroturf**		**Aquatic Bryophytes**		**P**ANOVA
	Mean	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2})	0.52	0.20	1.45	0.17	P<0.001***
Periphyton chlorophyll content per unit area (μg cm^{-2})	0.15	0.19	1.28	0.16	P<0.001***
D (m)	0.13	0.17	0.14	0.07	NS
K (m^{-1})	2.98	0.34	2.99	0.15	NS
$Z_{eu}^{1\%}$ (m)	0.25	0.34	0.25	0.16	NS
Z_{eu}:$D^{1\%}$	1.88	0.35	1.81	0.17	NS
pH	6.40	0.12	6.33	0.07	NS
Conductivity (μS cm^{-1})	47.5	0.04	45.8	0.03	NS
Water Temperature (^oC)	9.9	0.13	10.2	0.07	NS
Flow (m s^{-1})	0.156	0.06	0.219	0.02	NS

Table 3.56 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 158), periphyton chlorophyll content per unit area (n = 78), and environmental habitat variables (n = 158) between long-term Astroturf and naturally-occurring aquatic bryophytes in the Water of Dye sub-catchment. Significance testing: one-way ANOVA.

Mean Variable	**Short-term linoleum**		**Long-term linoleum**		**Mineral substrata**		**P_{ANOVA}**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2})	0.29^{a}	0.20	0.41^{a}	0.22	2.56^{b}	0.21	P<0.001***
Periphyton chlorophyll content per unit area (μg cm^{-2})	0.18^{a}	0.18	0.29^{a}	0.19	1.46^{b}	0.28	P<0.001***
D (m)	0.13	0.13	0.11	0.14	0.12	0.05	NS
K (m^{-1})	2.13	0.24	2.10	0.34	2.32	0.15	NS
$Z_{eu}^{1\%}$ (m)	0.28	0.31	0.29	0.39	0.26	0.19	NS
Z_{eu}:$D^{1\%}$	2.25	0.44	2.79	0.47	2.14	0.22	NS
pH	6.78	0.07	6.77	0.08	6.89	0.06	NS
Conductivity (μS cm^{-1})	47.9	0.06	48.0	0.06	51.7	0.04	NS
Water Temperature (^{o}C)	10.8	0.07	10.9	0.08	11.1	0.08	NS
Flow (m s^{-1})	0.208	0.05	0.182	0.04	0.202	0.02	NS

Table 3.57 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 240), periphyton chlorophyll content per unit area (n = 132), and environmental habitat variables (n = 240) between short-term linoleum, long-term linoleum and naturally-occurring mineral substrata in the River Girnock sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Mean Variable	**Long-term Astroturf**		**Aquatic Bryophytes**		**P**ANOVA
	Mean	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2})	0.57	0.14	0.68	0.19	NS
Periphyton chlorophyll content per unit area (μg cm^{-2})	0.18	0.11	0.85	0.11	P<0.001***
D (m)	0.10	0.16	0.12	0.05	NS
K (m^{-1})	2.15	0.33	2.32	0.15	NS
$Z_{eu}^{1\%}$ (m)	0.26	0.33	0.26	0.19	NS
Z_{eu}:$D^{1\%}$	2.68	0.49	2.14	0.22	NS
pH	6.81	0.07	6.89	0.06	NS
Conductivity (μS cm^{-1})	49.7	0.07	51.7	0.04	NS
Water Temperature (^oC)	11.1	0.11	11.1	0.08	NS
Flow (m s^{-1})	0.155	0.04	0.202	0.02	NS

Table 3.58 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 144), periphyton chlorophyll content per unit area (n = 96), and environmental habitat variables (n = 144) between long-term Astroturf and naturally-occurring aquatic bryophytes in the River Girnock sub-catchment. Significance testing: one-way ANOVA.

Mean Variable	Short-term linoleum		Long-term linoleum		Mineral substrata		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton biomass per unit area (mg cm^{-2})	0.60^{a}	0.22	0.65^{a}	0.24	2.93^{b}	0.19	P<0.001***
Periphyton chlorophyll content per unit area (μg cm^{-2})	0.31^{a}	0.20	0.31^{a}	0.21	1.23^{b}	0.23	P<0.001***
D (m)	0.13	0.07	0.11	0.09	0.13	0.07	NS
K (m^{-1})	2.65	0.28	2.90	0.21	2.58	0.14	NS
$Z_{eu}^{1\%}$ (m)	0.35	0.27	0.32	0.22	0.36	0.15	NS
Z_{eu}:$D^{1\%}$	2.71	0.27	3.06	0.25	2.84	0.17	NS
pH	7.47	0.05	7.46	0.05	7.56	0.04	NS
Conductivity (μS cm^{-1})	117.3	0.05	116.8	0.05	118.5	0.03	NS
Water Temperature (^{o}C)	8.5	0.05	8.5	0.05	9.0	0.04	NS
Flow (m s^{-1})	0.304	0.03	0.395	0.05	0.294	0.03	NS

Table 3.59 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 241), periphyton chlorophyll content per unit area (n = 204), and environmental habitat variables (n = 241) between short-term linoleum, long-term linoleum and naturally-occurring mineral substrata in the Knockan Burn sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Mean Variable	**Long-term Astroturf**		**Aquatic Bryophytes**		**P**ANOVA
	Mean	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2})	2.68	0.22	11.52	0.33	P<0.001***
Periphyton chlorophyll content per unit area (μg cm^{-2})	0.23	0.20	2.06	0.24	P<0.001***
D (m)	0.12	0.14	0.13	0.07	NS
K (m^{-1})	2.32	0.40	2.58	0.14	NS
$Z_{eu}^{1\%}$ (m)	0.39	0.39	0.36	0.15	NS
Z_{eu}:$D^{1\%}$	3.30	0.46	2.84	0.17	NS
pH	7.49	0.09	7.56	0.04	NS
Conductivity (μS cm^{-1})	116.2	0.08	118.5	0.03	NS
Water Temperature (^oC)	8.4	0.07	9.0	0.04	NS
Flow (m s^{-1})	0.401	0.07	0.294	0.03	NS

Table 3.60 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 117), periphyton chlorophyll content per unit area (n = 114), and environmental habitat variables (n = 117) between long-term Astroturf and naturally-occurring aquatic bryophytes in the Knockan Burn sub-catchment. Significance testing: one-way ANOVA.

Mean Variable	**Long-term plastic *Potamogeton***		**Long-term plastic *Myriophyllum***		**Vascular submerged macrophytes**		**P**ANOVA
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2})	0.19	0.25	0.39	0.30	0.44	0.40	NS
Periphyton chlorophyll content per unit area (μg cm^{-2})	0.16	0.27	0.26	0.30	0.11	0.24	NS
D (m)	0.11	0.18	0.11	0.18	0.15	0.17	NS
K (m^{-1})	2.75	0.41	2.75	0.41	2.26	0.33	NS
$Z_{eu}^{1\%}$ (m)	0.33	0.42	0.33	0.42	0.42	0.33	NS
Z_{eu}:$D^{1\%}$	3.03	0.38	3.03	0.38	2.50	0.32	NS
pH	7.48^{a}	0.09	7.48^{a}	0.09	7.68^{b}	0.08	P<0.01**
Conductivity (μS cm^{-1})	133.8^{a}	0.08	133.8^{a}	0.08	164.7^{b}	0.09	P<0.001***
Water Temperature (^{o}C)	8.5	0.10	8.5	0.10	9.4	0.08	NS
Flow (m s^{-1})	0.310	0.04	0.310	0.04	0.215	0.03	NS

Table 3.61 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 68), periphyton chlorophyll content per unit area (n = 64), and environmental habitat variables (n = 68) between long-term plastic *Potamogeton*-like, long-term plastic *Myriophyllum*-like and naturally-occurring vascular submerged macrophytes in the Knockan Burn sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Mean Variable	Short-term linoleum		Long-term linoleum		Mineral substrata		Long-term Astroturf		Aquatic bryophytes		Long-term plastic *Potamogeton*		Long-term plastic *Myriophyllum*		Vascular submerged macrophytes		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton biomass per unit area (mg cm^{-2})	0.42^{a}	0.18	0.46^{a}	0.16	2.08^{b}	0.11	1.26^{c}	0.22	4.55^{d}	0.40	0.19^{a}	0.25	0.39^{a}	0.30	0.44^{a}	0.40	P<0.001***
Periphyton chlorophyll content per unit area (μg cm^{-2})	0.23^{a}	0.16	0.31^{a}	0.14	1.41^{b}	0.10	0.19^{a}	0.15	1.40^{b}	0.14	0.16^{a}	0.27	0.26^{a}	0.30	0.11^{a}	0.24	P<0.001***
D (m)	0.14	0.06	0.12	0.07	0.13	0.04	0.11	0.11	0.13	0.04	0.11	0.18	0.11	0.18	0.15	0.17	NS
K (m^{-1})	2.47	0.20	2.51	0.21	2.60	0.14	2.44	0.21	2.60	0.14	2.75	0.41	2.75	0.41	2.26	0.33	NS
$Z_{eu}^{1\%}$ (m)	0.30	0.17	0.30	0.19	0.28	0.11	0.28	0.20	0.28	0.11	0.33	0.42	0.33	0.42	0.42	0.33	NS
Z_{eu}:$D^{1\%}$	2.21	0.21	2.66	0.23	2.32	0.12	2.65	0.26	2.32	0.12	3.03	0.38	3.03	0.38	2.50	0.32	NS
pH	6.91^{a}	0.06	6.89^{a}	0.06	6.94^{a}	0.05	6.82^{a}	0.06	6.94^{a}	0.05	7.48^{b}	0.09	7.48^{b}	0.09	7.68^{c}	0.08	P<0.001***
Conductivity (μS cm^{-1})	66.2^{a}	0.05	65.9^{a}	0.05	68.0^{a}	0.04	63.3^{a}	0.06	68.0^{a}	0.04	133.8^{b}	0.08	133.8^{b}	0.08	164.7^{c}	0.09	P<0.001***
Water Temperature (^{o}C)	9.7	0.05	9.7	0.05	10.1	0.04	10.0	0.06	10.1	0.04	8.5	0.10	8.5	0.10	9.4	0.08	NS
Flow (m s^{-1})	0.248	0.02	0.251	0.03	0.236	0.03	0.198	0.04	0.236	0.03	0.310	0.04	0.310	0.04	0.215	0.03	NS

Table 3.62 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton biomass per unit area (n = 1179), periphyton chlorophyll content per unit area (n = 760), and environmental habitat variables (n = 1179) between short-term linoleum, long-term linoleum, naturally-occurring mineral substrata, long-term Astroturf, aquatic bryophytes, long-term plastic *Potamogeton*-like, long term plastic *Myriophyllum*-like and vascular submerged macrophytes from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 1179). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

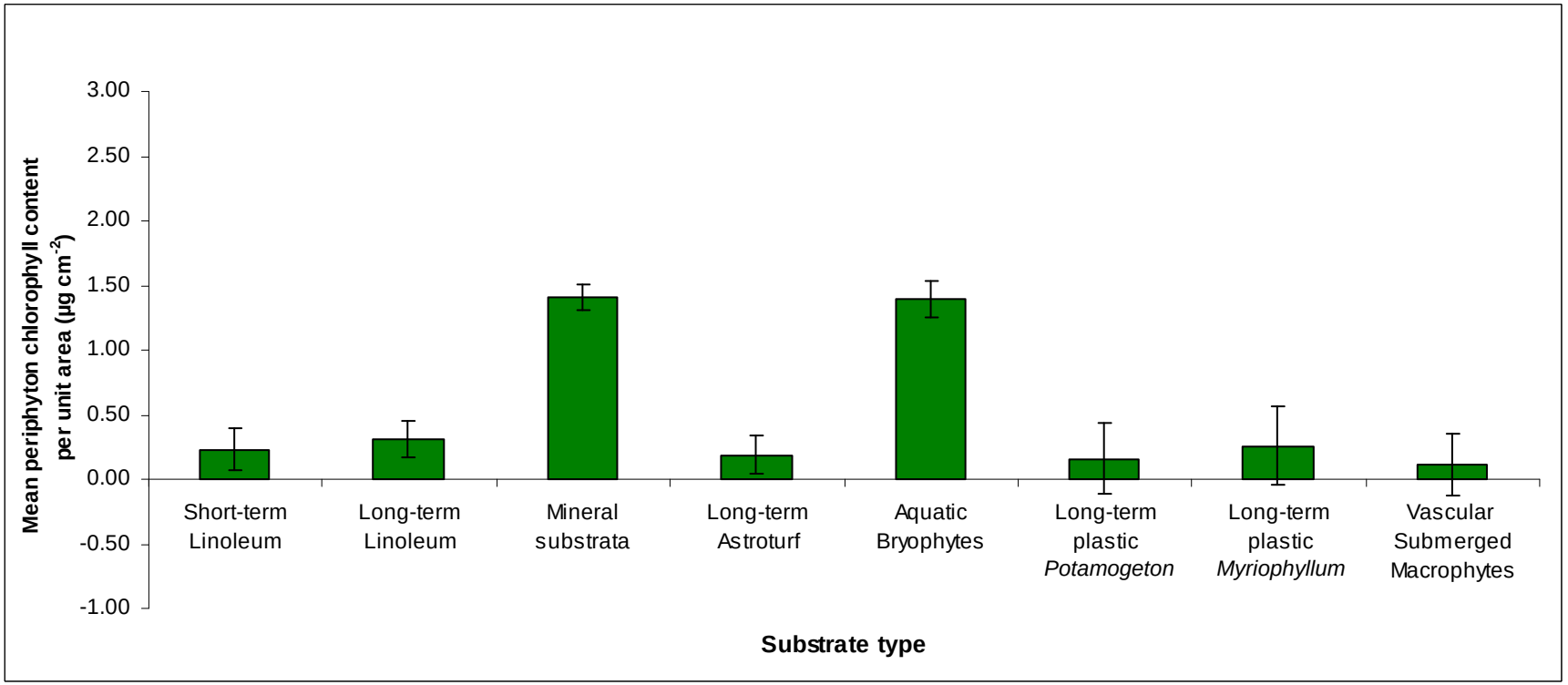


Figure 3.40 Comparison of mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) periphyton chlorophyll content per unit area (n = 760) between the various types of substrates utilised: short-term linoleum, long-term linoleum, naturally-occurring mineral particles, long-term Astroturf, aquatic bryophytes, long-term plastic *Potamogeton*-like, long term plastic *Myriophyllum*-like and vascular submerged macrophytes from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn).

3.5.6 Periphyton production and environmental habitat conditions in the Water of Dye, River Girnock and Knockan Burn as determined by TWINSPAN classification

3.5.6.1 Periphyton production of short-term linoleum substrates only

There was no significant difference in either periphyton biomass or chlorophyll content between the three TWINSPAN sample-groups identified from short-term artificial substrates (refer to Table 3.63, and also Figure 3.41). Consult Chapter 4, Figure 4.11 for details of the TWINSPAN classification here cited. See also Chapter 4, Table 4.61 and section 4.5.6.1 for details and discussion of variation in environmental habitat conditions between the TWINSPAN sample-groups.

3.5.6.2 Periphyton production of all artificial substrata

Group I (comprised exclusively of Knockan Burn samples) had significantly higher periphyton biomass compared to assemblages II and III identified from all artifical substrates combined, but chlorophyll content did not vary significantly between the three TWINSPAN sample-groups (refer to Table 3.64, and also Figure 3.42). Consult Chapter 4, Figure 4.15 for details of the TWINSPAN classification here cited. See also Chapter 4, Table 4.62 and section 4.5.6.2 for details and discussion of variation in environmental habitat conditions between the TWINSPAN sample-groups.

3.5.6.3 Periphyton production of all naturally-occurring substrata

Similarly, those TWINSPAN sample-groups identified from all naturally-occurring substrata combined indicated that although Group I (comprised exclusively of Knockan Burn samples) had significantly higher periphyton biomass compared to assemblages II and III, chlorophyll content did not differ significantly between the three communities (refer to Table 3.65, and also Figure

3.43). Group II had the highest periphyton cover and lowest bare area, compared to the other two assemblages. Consult Chapter 4, Figure 4.19 for details of the TWINSPAN classification here cited. See also Chapter 4, Table 4.63 and section 4.5.6.3 for details and discussion of variation in environmental habitat conditions between the TWINSPAN sample-groups.

	TWINSPAN sample-group						
Variable	**I**		**II**		**III**		**PANOVA**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2})	0.60	0.37	0.23	0.24	0.41	0.25	NS
Periphyton chlorophyll content per unit area (µg cm^{-2})	0.31	0.33	0.15	0.20	0.24	0.21	NS

Table 3.63 Mean values (± 1 standard error) of normally distributed periphyton biomass per unit area and periphyton chlorophyll content per unit area (data back-transformed where necessary) between TWINSPAN sample-groups I (n = 15), II (n = 13), and III (n = 28): for short-term linoleum substrates (n = 56). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Refer to Chapter 4, section 4.5.6 for TWINSPAN output (Figure 4.11) and details of environmental habitat conditions (Table 4.61).

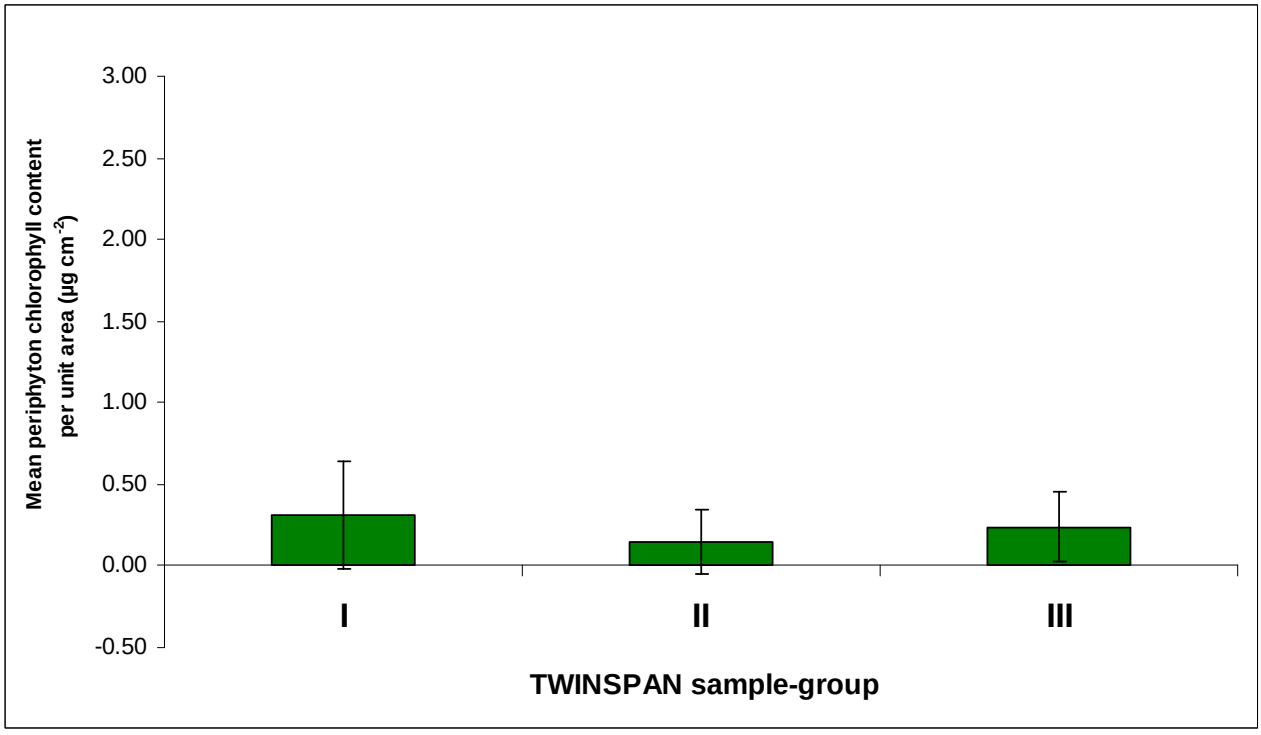


Figure 3.41 Comparison of mean values (± 1 standard error) of normally distributed periphyton chlorophyll content per unit area (data back-transformed where necessary) between TWINSPAN sample-groups I (n = 15), II (n = 13), and III (n = 28) of short-term linoleum substrates (n = 56).

	TWINSPAN sample-group						
Variable	**I**		**II**		**III**		**PANOVA**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton biomass per unit area (mg cm^{-2})	0.94^{a}	0.24	0.37^{b}	0.27	0.43^{b}	0.28	P<0.01**
Periphyton chlorophyll content per unit area (µg cm^{-2})	0.26	0.18	0.17	0.24	0.28	0.25	NS

Table 3.64 Mean values (± 1 standard error) of normally distributed periphyton biomass per unit area and periphyton chlorophyll content per unit area (data back-transformed where necessary) between TWINSPAN sample-groups I (n = 39), II (n = 21), and III (n = 33) of all artificial substrates sampled on survey dates only (n = 93): short-term linoleum, long-term linoleum, long-term Astroturf and plastic aquarium plants. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Refer to Chapter 4, section 4.5.6 for TWINSPAN output (Figure 4.15) and details of environmental habitat conditions (Table 4.62).

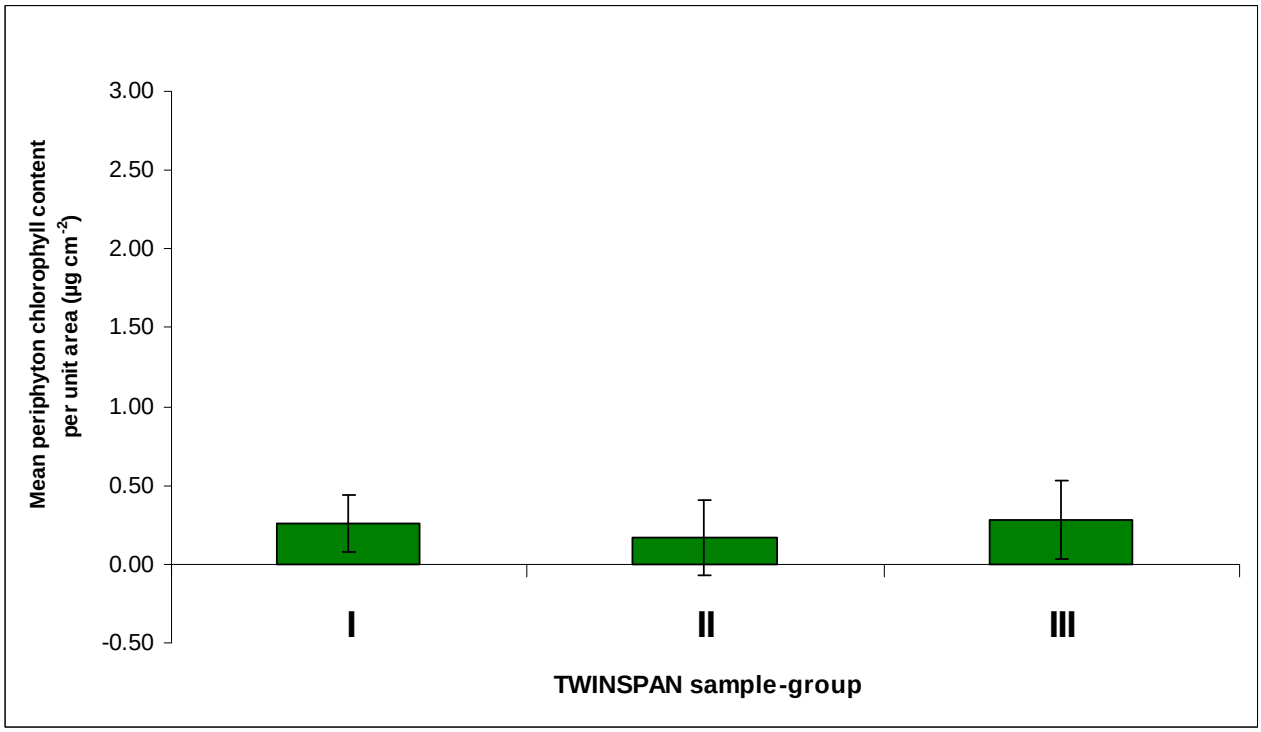


Figure 3.42 Comparison of mean values (± 1 standard error) of normally distributed periphyton chlorophyll content per unit area (data back-transformed where necessary) between TWINSPAN sample-groups I (n = 39), II (n = 21), and III (n = 33) of all artificial substrates sampled on survey dates only (n = 93): short-term linoleum, long-term linoleum, long-term Astroturf and plastic aquarium plants.

Variable	TWINSPAN sample-group I		II		III		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton biomass per unit area (mg cm^{-2})	4.96[a]	0.77	1.62[b]	0.34	1.11[b]	0.28	P<0.001***
Periphyton chlorophyll content per unit area (µg cm^{-2})	1.13	0.29	1.16	0.24	1.42	0.22	NS
Periphyton cover (%)	18.2[a]	1.65	38.5[b]	1.89	19.5[a]	1.59	P<0.001***
Bare area (%)	38.6[a]	1.95	28.1[b]	1.75	42.9[a]	2.09	P<0.001***

Table 3.65 Mean values (± 1 standard error) of normally distributed periphyton periphyton biomass per unit area, periphyton chlorophyll content per unit area, periphyton cover and bare area (data back-transformed where necessary) between TWINSPAN sample-groups I (n = 57), II (n = 52), and III (n = 54) of all naturally-occurring substrata sampled on survey dates only (n = 163): mineral particles, aquatic bryophytes and vascular submerged macrophytes. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Refer to Chapter 4, section 4.5.6 for TWINSPAN output (Figure 4.19) and details of environmental habitat conditions (Table 4.63).

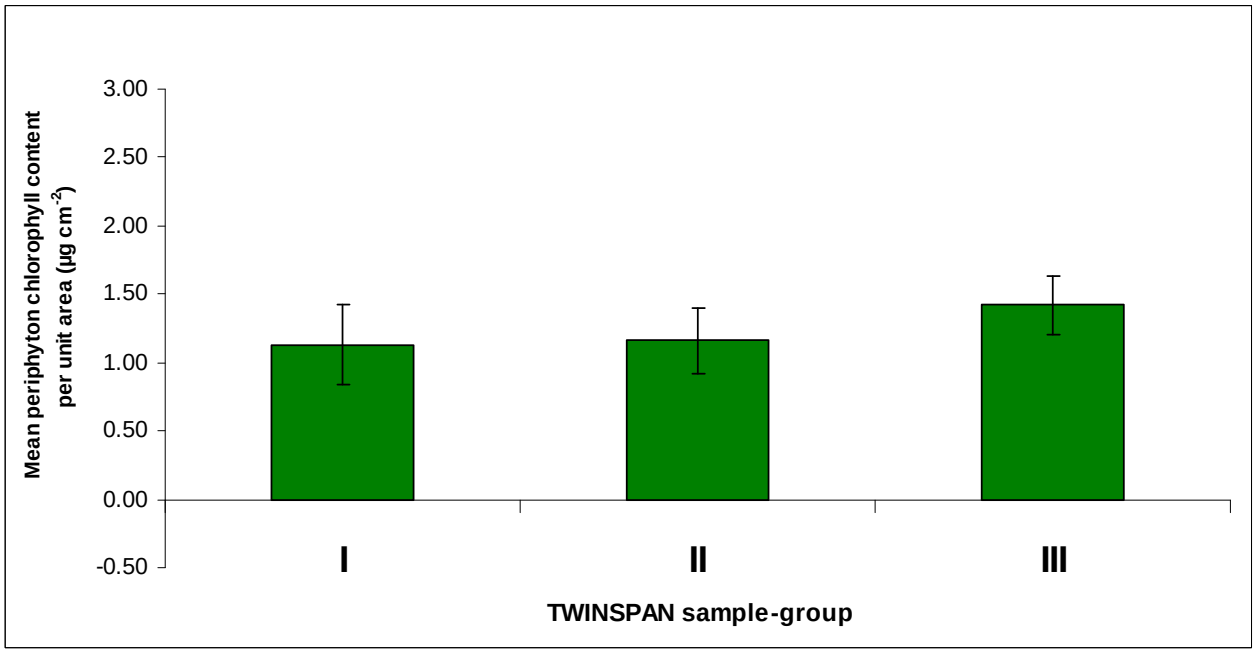


Figure 3.43 Comparison of mean values (± 1 standard error) of normally distributed periphyton periphyton chlorophyll content per unit area (data back-transformed where necessary) between TWINSPAN sample-groups I (n = 57), II (n = 52), and III (n = 54) of all naturally-occurring substrata sampled on survey dates only (n = 163): mineral particles, aquatic bryophytes and vascular submerged macrophytes.

3.5.7 Relationships between periphyton production and environmental habitat conditions

3.5.7.1 Periphyton production of short-term linoleum substrates only

Periphyton biomass and chlorophyll content were strongly and significantly positively correlated with each other from material harvested from short-term linoleum substrates (see Appendix 2b). Periphyton biomass and chlorophyll content were also positively correlated with increasing underwater light availability, streamwater pH, conductivity and temperature. Periphyton biomass was negatively correlated to increased flow conditions but periphyton chlorophyll content showed no significant relationship to current velocity.

3.5.7.2 Periphyton production of all artificial substrata

Agreeing with the former, periphyton biomass and chlorophyll content harvested from all artificial substrata (short-term linoleum, long-term linoleum, long-term Astroturf and plastic aquarium plants) during field survey campaigns (see Appendix 2c) showed similar relationships. Additionally, periphyton biomass was positively correlated with increasing calcium and magnesium concentrations, with chlorophyll content showing similar relationships. Whereas, increased concentrations of streamwater sulphate and heavy metals (e.g. lead, aluminium etc.) tended to be negatively correlated with periphyton biomass and chlorophyll production.

3.5.7.3 Periphyton production of all naturally-occurring substrata

Concurring with the aforementioned artificial substrates, similar relationships were found for periphyton production and abundance recorded from all naturally-occurring substrata (mineral particles, aquatic bryophytes, and where present vascular submerged macrophytes): see Appendix 2d. Furthermore,

periphyton production tended to increase in response to increasing proportions of finer sized mineral particles (e.g. gravel) associated with more basic geologies and more diverse streambed substrate morphology, but notably chlorophyll production did not consistently reflect these trends. The proportion of unvegetated streambed tended to increase as flow increased and was also positively associated with an abundance of small stones and gravel, associated with more weatherable geologies (e.g. Durness limestone). Bare area was negatively correlated with more stable streambeds predominated by boulders and large stones, which tended to be underlain with more resistant geologies often with acidic properties (e.g. Granite).

3.5.8 Predicting freshwater periphyton production

Data harvested from short-term linoleum substrate samplers were used to construct statistically significant full models using combinations of environmental predictor variables for predicting periphyton production ($\log_e$ chlorophyll content) of upland stream habitats (refer to Table 3.66). However, the only best-fitting model (PERIchlP1a) used a single environmental predictor variable and weakly predicted the response variable (r^2: 10.6%). From this, several minimal models were derived (see Table 3.67, Table 3.69, and Table 3.71) but their predictive power was similarly low. Therefore although these minimal models had some success in predicting periphyton production for certain months sampled, their ability to predict temporal variation for test data sets of the Water if Dye (Table 3.67, Figure 3.44), River Girnock (Table 3.70, Figure 3.45), and Knockan Burn (Table 3.72, Figure 3.46) was generally quite limited.

Full models	Regression equations	r^2-adj (%)	P_{value}
PERIchlP1a: Periphyton Production (log_e Chl)	log_e Chl = -3.78 + 0.649 (√ temp)	10.6	P<0.05*

Table 3.66 Statistically significant full model (n = 50) using environmental variable(s) for predicting temporal variation of freshwater periphyton production (measured as log_e chlorophyll content in μg cm^{-2}) of upland stream habitats. Model codes: loge Chl: log_e chlorophyll production (μg cm^{-2}); √ temp: √ water temperature (°C).

Reduced (minimal) models	Regression equations	r^2-adj (%)	P_{value}
PERIchlP1a: Periphyton Production (log_e Chl) excluding Water of Dye November 2005 test data set	log_e Chl = -3.19 + 0.497 (√ temp)	8.5	P<0.05*
PERIchlP1a: Periphyton Production (log_e Chl) excluding Water of Dye March 2005 test data set	log_e Chl = -4.08 + 0.736 (√ temp)	8.3	P<0.05*
PERIchlP1a: Periphyton Production (log_e Chl) excluding Water of Dye April 2005 test data set	log_e Chl = -4.35 + 0.812 (√ temp)	11.8	P<0.05*
PERIchlP1a: Periphyton Production (log_e Chl) excluding Water of Dye May 2005 test data set	log_e Chl = -3.71 + 0.647 (√ temp)	9.2	P<0.05*
PERIchlP1a: Periphyton Production (log_e Chl) excluding Water of Dye July 2005 test data set	log_e Chl = -3.46 + 0.521 (√ temp)	7.2	P<0.05*
PERIchlP1a: Periphyton Production (log_e Chl) excluding Water of Dye August 2005 test data set	log_e Chl = -3.55 + 0.557 (√ temp)	7.8	P<0.05*
PERIchlP1a: Periphyton Production (log_e Chl) excluding Water of Dye April 2006 test data set	log_e Chl = -3.72 + 0.635 (√ temp)	10.1	P<0.05*

Table 3.67 Statistically significant minimal models (n = 47) of PERIchlP1a for predicting temporal variation of freshwater periphyton production (log_e chl) of the Water of Dye test data set. For model codes refer to Table 3.66.

Mean test data	Observed log_e Chl: test data	Predicted log_e Chl: reduced model PERIchlP1a	t-statistic	P-value
Water of Dye November 2005	-3.51	-1.97	-3.36	P<0.05*
Water of Dye March 2005	-2.30	-2.57	1.13	NS
Water of Dye April 2005	-2.04	-2.58	2.50	NS
Water of Dye May 2005	-4.61	-1.90	5.24	P<0.01**
Water of Dye July 2005	-0.22	-1.45	4.32	P<0.05*
Water of Dye August 2005	-0.64	-1.35	2.36	NS
Water of Dye April 2006	-2.41	-2.32	-0.17	NS
Water of Dye Mean log_e Chl (cm^{-2})	**-2.25**	**-2.02**	**0.70**	**NS**

Table 3.68 Comparison of mean observed and predicted values of minimal model PERIchlP1a for predicting temporal variation in freshwater periphyton production (log_e chl) of the Water of Dye test data set (see also Figure 3.44).

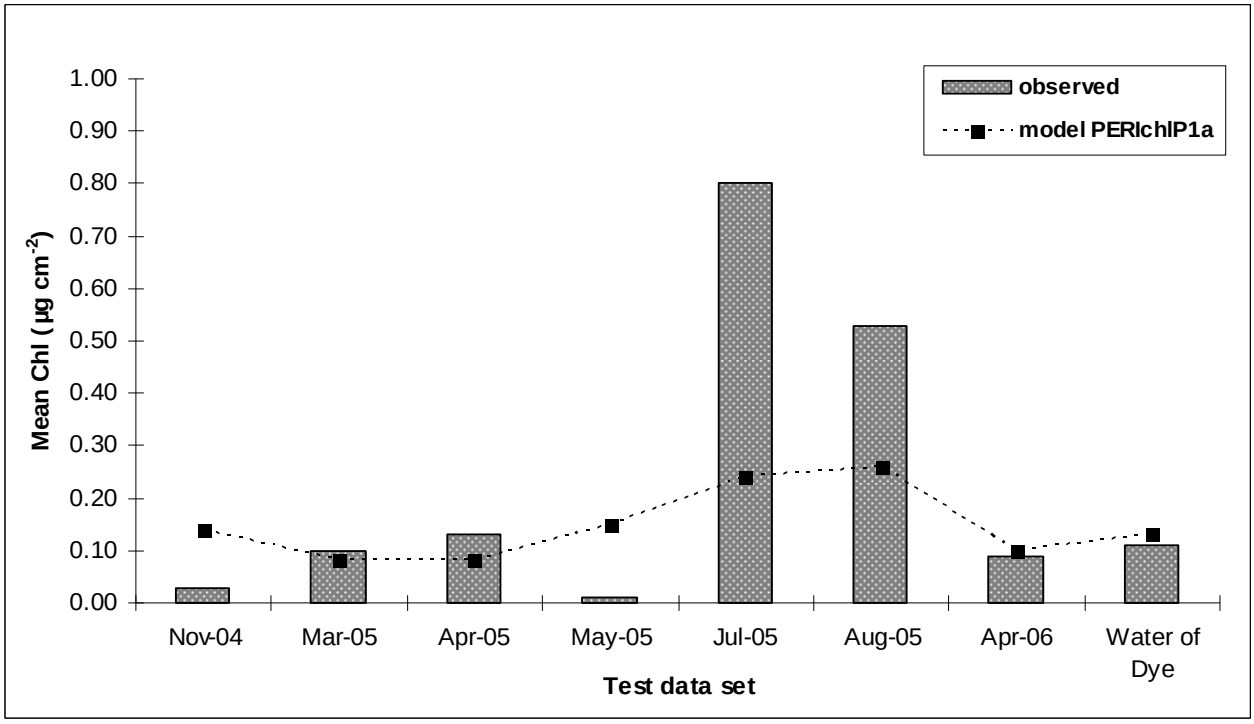


Figure 3.44 Comparison of mean observed and (back-transformed) predicted values of minimal model PERIchlP1a for predicting temporal variation in freshwater periphyton production (chl) of the Water of Dye test data set.

Reduced (minimal) models	Regression equations	r^2-adj (%)	P_{value}
PERIchlP1a: Periphyton Production (log_e Chl) excluding River Girnock April 2005 test data set	log_e Chl = -3.75 + 0.655 (√ temp)	10.8	P<0.05*
PERIchlP1a: Periphyton Production (log_e Chl) excluding River Girnock May 2005 test data set	log_e Chl = -3.94 + 0.725 (√ temp)	9.8	P<0.05*
PERIchlP1a: Periphyton Production (log_e Chl) excluding River Girnock July 2005 test data set	log_e Chl = -3.87 + 0.689 (√ temp)	9.9	P<0.05*
PERIchlP1a: Periphyton Production (log_e Chl) excluding River Girnock August 2005 test data set	log_e Chl = -4.09 + 0.780 (√ temp)	10.4	P<0.05*
PERIchlP1a: Periphyton Production (log_e Chl) excluding River Girnock April 2006 test data set	log_e Chl = -4.07 + 0.719 (√ temp)	10.8	P<0.05*

Table 3.69 Statistically significant minimal models (n = 47) of PERIchlP1a for predicting temporal variation in freshwater periphyton production (log_e chl) of the River Girnock test data set. For model codes refer to Table 3.66.

Mean test data	Observed log_e Chl: test data	Predicted log_e Chl: reduced model PERIchlP1a	t-statistic	P-value
River Girnock April 2005	-3.12	-1.80	-3.65	P<0.05*
River Girnock May 2005	-3.72	-1.47	-4.46	P<0.05*
River Girnock July 2005	-1.23	-1.35	1.57	NS
River Girnock August 2005	-1.41	-1.16	-1.12	NS
River Girnock April 2006	-1.10	-2.15	2.35	NS
River Girnock Mean log_e Chl (cm^{-2})	**-2.12**	**-1.59**	**-1.02**	**NS**

Table 3.70 Comparison of mean observed and predicted values of minimal model PERIchlP1a for predicting temporal variation in freshwater periphyton production (log_e chl) of the River Girnock test data set (see also Figure 3.45).

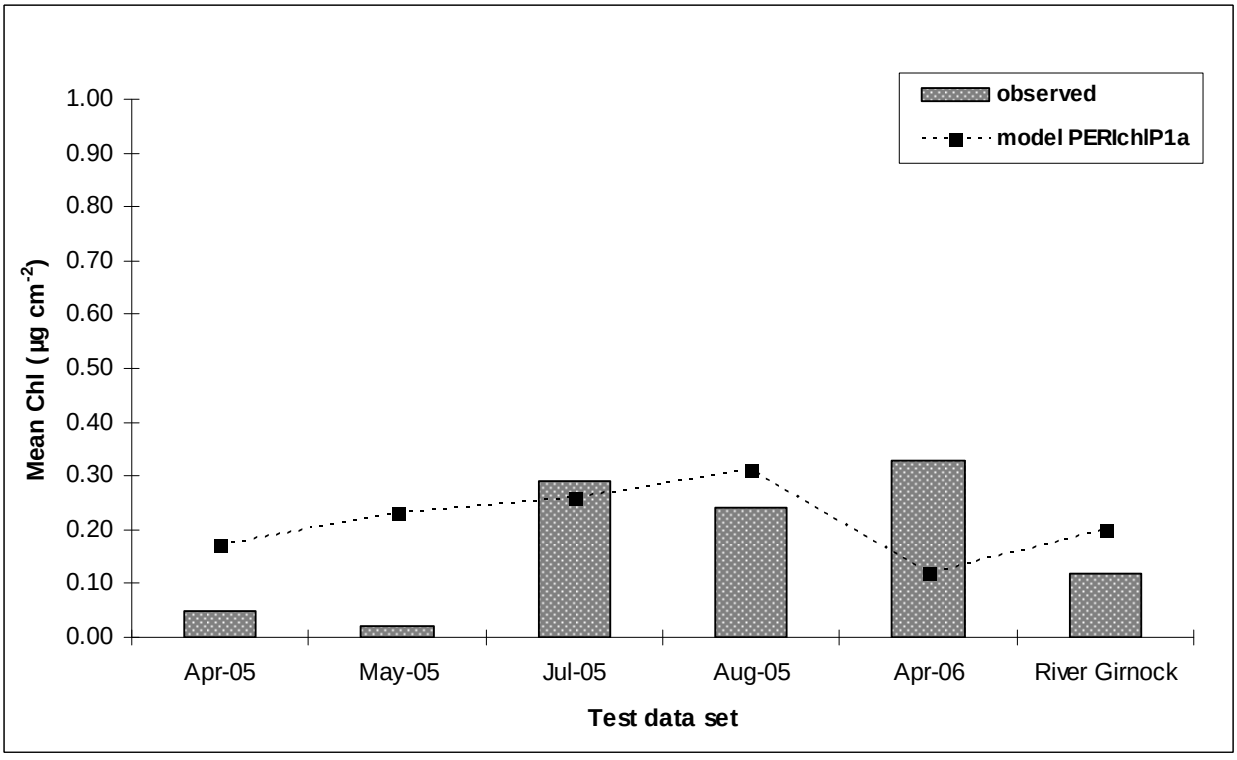


Figure 3.45 Comparison of mean observed and (back-transformed) predicted values of minimal model PERIchlP1a for predicting temporal variation in freshwater periphyton production (chl) of the River Girnock test data set.

Reduced (minimal) models	Regression equations	r^2-adj (%)	P_{value}
PERIchlP1a: Periphyton Production (log_e Chl) excluding Knockan Burn December 2005 test data set	log_e Chl = -3.20 + 0.491 (√ temp)	8.2	P<0.05*
PERIchlP1a: Periphyton Production (log_e Chl) excluding Knockan Burn April 2006 test data set	log_e Chl = - 3.96 + 0.695 (√ temp)	8.4	P<0.05*
PERIchlP1a: Periphyton Production (log_e Chl) excluding Knockan Burn July 2006 test data set	log_e Chl = - 3.59 + 0.568 (√ temp)	7.2	P<0.05*
PERIchlP1a: Periphyton Production (log_e Chl) excluding Knockan Burn September 2006 test data set	log_e Chl = - 3.74 + 0.624 (√ temp)	9.5	P<0.05*
PERIchlP1a: Periphyton Production (log_e Chl) excluding Knockan Burn November 2006 test data set	log_e Chl = - 3.95 + 0.678 (√ temp)	10.3	P<0.05*

Table 3.71 Statistically significant minimal models (n = 47) of PERIchlP1a for predicting temporal variation in freshwater periphyton production (log_e chl) of the Knockan Burn test data set. For model codes refer to Table 3.66.

Mean test data	Observed log_e Chl: test data	Predicted log_e Chl: reduced model PERIchlP1a	t-statistic	P-value
Knockan Burn December 2005	-3.12	-2.05	-1.99	NS
Knockan Burn April 2006	-1.37	-2.21	2.98	P<0.05*
Knockan Burn July 2006	-0.98	-1.51	2.27	NS
Knockan Burn September 2006	-0.69	-1.62	0.52	NS
Knockan Burn November 2006	-0.93	-2.04	3.25	P<0.05*
Knockan Burn Mean log_e Chl (cm^{-2})	**-1.42**	**-1.89**	**0.80**	**NS**

Table 3.72 Comparison of mean observed and predicted values of minimal model PERIchlP1a for predicting temporal variation in freshwater periphyton production (log_e chl) of the Knockan Burn test data set (see also Figure 3.46).

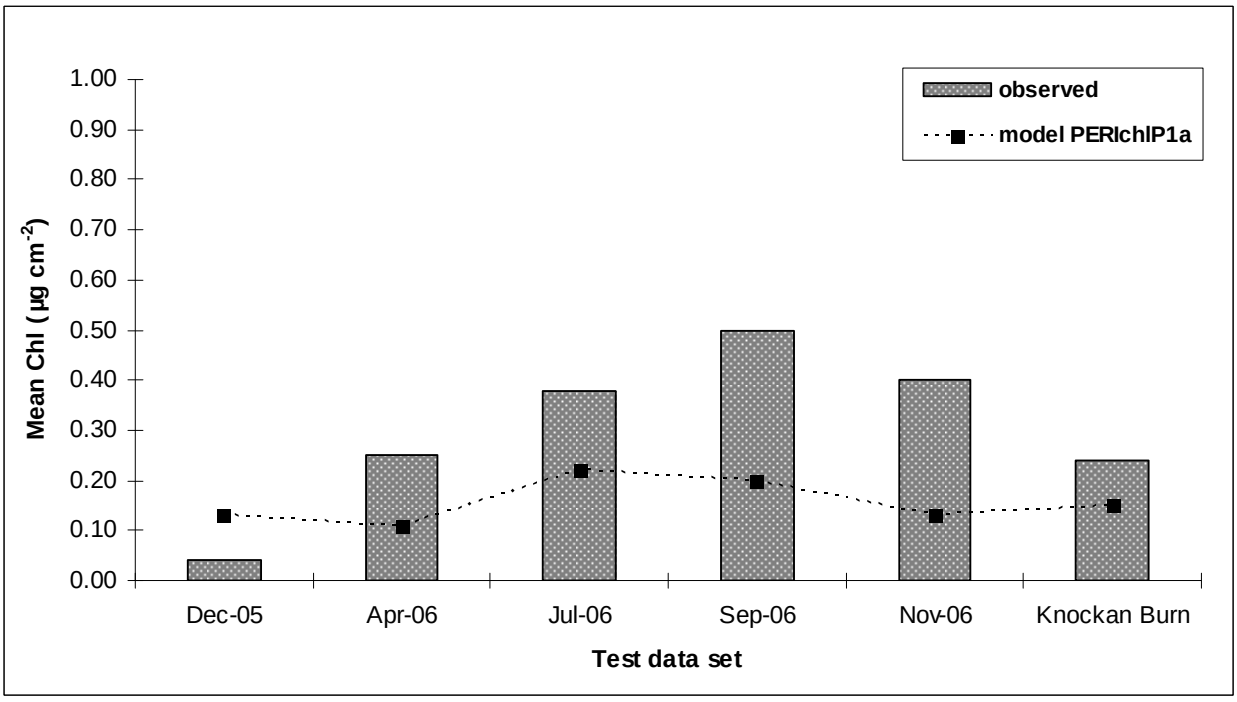


Figure 3.46 Comparison of mean observed and (back-transformed) predicted values of minimal model PERIchlP1a for predicting temporal variation in freshwater periphyton production (chl) of the Knockan Burn test data set.

3.5.9 Variation in aquatic bryophyte production and environmental habitat conditions in the Water of Dye, River Girnock and Knockan Burn; their sub-catchments and sites

Significant variation in aquatic bryophyte production was detected between the three sub-catchment streams (Table 3.73). Aquatic bryophyte biomass in Knockan Burn was significantly higher compared to that occurring in the Water of Dye and River Girnock. Furthermore, aquatic bryophyte production was lowest in the River Girnock, and differed significantly from the Water of Dye. Aquatic bryophyte chlorophyll content and % cover were significantly higher in Knockan Burn and the Water of Dye (which were similar), than in the River Girnock.

Mean Variable	Water of Dye		River Girnock		Knockan Burn		PANOVA
	Mean	*S.E*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Aquatic bryophyte biomass per unit area (mg cm^{-2})	10.00^{a}	0.43	4.22^{b}	0.48	24.13^{c}	1.06	P<0.001***
Aquatic bryophyte chlorophyll content per unit area (μg cm^{-2})	3.72^{a}	0.24	1.81^{b}	0.31	4.25^{a}	0.26	P<0.001***
Aquatic bryophyte cover (%)	25.2^{a}	2.05	10.7^{b}	1.33	21.7^{a}	1.99	P<0.001***
Bare area (%)	38.6^{a}	1.95	28.2^{b}	1.75	42.9^{a}	2.09	P<0.001***
D (m)	0.14	0.17	0.12	0.16	0.13	0.19	NS
K (m^{-1})	2.99^{a}	0.15	2.32^{b}	0.15	2.57^{b}	0.14	P<0.001***
$Z_{eu}^{3\%}$ (m)	0.84^{a}	0.15	0.89^{a}	0.19	1.24^{b}	0.14	P<0.001***
Z_{eu}:$D^{3\%}$	6.18^{a}	0.16	7.37^{a}	0.21	9.96^{b}	0.17	P<0.001***
pH	6.33^{a}	0.07	6.93^{b}	0.05	7.56^{c}	0.03	P<0.001***
Conductivity (μS cm^{-1})	45.8^{a}	0.11	51.8^{b}	0.13	138.5^{c}	0.12	P<0.001***
Water Temperature (^{o}C)	10.2^{ab}	0.05	11.2^{a}	0.07	9.0^{b}	0.02	P<0.01**
Flow (m s^{-1})	0.218^{a}	0.02	0.203^{a}	0.02	0.290^{b}	0.03	P<0.01**

Table 3.73 Mean values (± 1 standard error) of normally distributed plant variables (including '*no biomass*' zero values, and data back-transformed where necessary): aquatic bryophyte biomass per unit area (n = 405), aquatic bryophyte chlorophyll content per unit area (n = 405), aquatic bryophyte cover (n = 405), bare area (n = 405 and environmental habitat variables (n = 405) between study stream sub-catchments. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of underlying geology, substrate morphology, alkalinity, nutrient status and heavy metal composition refer to Table 2.1 in Chapter 2.

	Water of Dye								River Girnock						Knockan Burn				
Mean Variable	**BB**		**CF**		**BD**		**IB**		**HB**		**LM**		**UK**		**MK**		**LK**		**PANOVA**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Aquatic bryophyte biomass per unit area (mg cm^{-2})	13.36^{a}	0.53	5.17^{b}	1.09	11.47^{c}	0.58	8.94^{c}	0.49	2.10^{d}	0.59	1.63^{d}	0.63	23.95^{e}	2.13	38.63^{f}	1.22	9.82^{c}	1.84	P<0.001***
Aquatic bryophyte chlorophyll content per unit area (μg cm^{-2})	4.68^{a}	0.36	2.78^{b}	0.47	3.71^{b}	0.40	3.23^{b}	0.47	1.14^{c}	0.39	1.07^{c}	0.29	4.49^{a}	0.46	5.35^{a}	0.43	2.92^{b}	0.50	P<0.001***
Aquatic bryophyte cover (%)	34.3^{a}	3.43	19.2^{b}	3.58	22.2^{b}	3.29	21.0^{b}	2.89	6.9^{c}	1.31	4.3^{c}	1.63	19.6^{b}	3.15	24.2^{b}	3.19	21.3^{b}	4.00	P<0.001***
Bare area (%)	33.3^{ab}	2.83	44.7^{a}	3.74	37.7^{ab}	3.34	25.5^{b}	3.02	25.0^{b}	2.95	33.9^{ab}	3.01	42.0^{a}	3.27	47.0^{a}	3.51	39.8^{a}	4.04	P<0.001***
D (m)	0.09^{a}	0.30	0.17^{b}	0.27	0.18^{b}	0.24	0.12^{ab}	0.25	0.11^{a}	0.28	0.13^{ab}	0.30	0.13^{ab}	0.38	0.11^{a}	0.29	0.13^{ab}	0.30	P<0.001***
K (m^{-1})	4.39^{a}	0.22	2.37^{b}	0.22	2.58^{b}	0.26	2.50^{b}	0.25	2.43^{b}	0.24	2.05^{b}	0.28	2.64^{b}	0.22	2.35^{b}	0.22	2.64^{b}	0.29	P<0.001***
$Z_{eu}{}^{3\%}$ (m)	0.56^{a}	0.26	1.20^{bd}	0.22	0.90^{d}	0.25	1.25^{b}	0.25	1.41^{b}	0.24	0.39^{c}	0.27	1.14^{bd}	0.24	1.29^{b}	0.22	1.31^{b}	0.28	P<0.001***
Z_{eu}:$D^{3\%}$	6.42^{a}	0.26	7.21^{ab}	0.30	5.09^{a}	0.27	10.06^{b}	0.25	12.80^{b}	0.31	3.11^{c}	0.31	8.59^{b}	0.30	11.67^{b}	0.27	9.86^{b}	0.30	P<0.001***
pH	5.89^{a}	0.13	6.80^{b}	0.07	6.29^{c}	0.12	6.51^{c}	0.08	7.15^{d}	0.06	7.13^{d}	0.06	7.31^{d}	0.01	7.43^{d}	0.02	7.95^{e}	0.03	P<0.001***
Conductivity (μS cm^{-1})	38.4^{a}	0.18	48.1^{b}	0.19	52.1^{bc}	0.19	39.0^{a}	0.22	58.6^{c}	0.22	60.9^{c}	0.21	116.8^{d}	0.19	110.9^{d}	0.18	205.2^{e}	0.19	P<0.001***
Water Temperature (^{o}C)	9.9^{ab}	0.10	10.7^{ab}	0.06	10.1^{ab}	0.10	10.9^{ab}	0.12	12.8^{a}	0.16	9.9^{ab}	0.05	8.8^{b}	0.05	8.2^{b}	0.03	10.1^{ab}	0.02	P<0.001***
Flow (m s^{-1})	0.228^{a}	0.06	0.216^{a}	0.06	0.209^{a}	0.06	0.224^{a}	0.07	0.198^{a}	0.07	0.188^{a}	0.06	0.168^{a}	0.08	0.475^{b}	0.08	0.267^{a}	0.08	P<0.001***

Table 3.74 Mean values (± 1 standard error) of normally distributed plant variables (including '*no biomass*' zero values, and data back-transformed where necessary): aquatic bryophyte biomass per unit area (n = 405), aquatic bryophyte chlorophyll content per unit area (n = 405), aquatic bryophyte cover (n = 405), bare area (n = 405) and environmental habitat variables (n = 405) between sampling sites. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of underlying geology, substrate morphology, alkalinity, nutrient status and heavy metal composition refer to Table 2.2 in Chapter 2.

3.5.10 Seasonal variation in aquatic bryophyte production and environmental habitat conditions in the Water of Dye, River Girnock and Knockan Burn

3.5.10.1 Water of Dye

In the Water of Dye, aquatic bryophyte production (biomass and chlorophyll content) and abundance (% cover) were significantly lower in August 2005 than in May 2005 and April 2006 (which were similar): Table 3.75.

3.5.10.2 River Girnock

In the River Girnock, aquatic bryophyte production (biomass and chlorophyll content) and coverage were significantly lower in August 2005 compared to May 2005 and April 2006 (which were not significantly different): Table 3.76.

3.5.10.3 Knockan Burn

In Knockan Burn, aquatic bryophyte biomass, chlorophyll content and abundance (% cover) were significantly lower in September 2006 compared to April and November 2006 (which were similar): Table 3.77.

Mean Variable	**May 2005**		**August 2005**		**April 2006**		**PANOVA**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Aquatic bryophyte biomass per unit area (mg cm^{-2})	10.66^{a}	0.71	7.31^{b}	0.84	12.04^{a}	1.17	P<0.01**
Aquatic bryophyte chlorophyll content per unit area (µg cm^{-2})	3.89^{a}	0.34	2.92^{b}	0.40	4.36^{a}	0.67	P<0.01**
Aquatic bryophyte cover (%)	26.4^{a}	3.27	18.9^{b}	2.64	30.4^{a}	5.33	P<0.01**
Bare area (%)	36.8	2.38	39.7	3.54	39.4	4.81	NS
D (m)	0.15^{a}	0.27	0.10^{b}	0.24	0.23^{c}	0.30	P<0.001***
K (m^{-1})	3.73^{a}	0.21	2.76^{b}	0.24	2.25^{b}	0.30	P<0.001***
$Z_{eu}^{3\%}$ (m)	0.80^{a}	0.22	0.71^{a}	0.26	1.31^{b}	0.20	P<0.001***
Z_{eu}:$D^{3\%}$	5.55^{a}	0.27	7.10^{b}	0.26	5.80^{a}	0.28	P<0.05*
pH	5.56^{a}	0.08	7.07^{b}	0.03	6.37^{c}	0.11	P<0.001***
Conductivity (µS cm^{-1})	37.6^{a}	0.15	59.5^{b}	0.16	40.4^{a}	0.24	P<0.001***
Water Temperature (^{o}C)	9.8^{a}	0.02	15.4^{b}	0.02	3.7^{c}	0.03	P<0.001***
Flow (m s^{-1})	0.217^{a}	0.02	0.172^{a}	0.03	0.326^{b}	0.03	P<0.01**

Table 3.75 Mean values (± 1 standard error) of normally distributed plant variables (including '*no biomass*' zero values, and data back-transformed where necessary): aquatic bryophyte biomass per unit area (n = 135), aquatic bryophyte chlorophyll content per unit area (n = 135), aquatic bryophyte cover (n = 135), bare area (n = 135) and environmental habitat variables (n = 135) between sampling dates in the Water of Dye sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of substrate morphology, alkalinity, nutrient status and heavy metal composition refer to Table 2.4 in Chapter 2.

Mean Variable	**May 2005**		**August 2005**		**April 2006**		**PANOVA**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Aquatic bryophyte biomass per unit area (mg cm^{-2})	4.43^{a}	0.64	2.18^{b}	0.76	5.96^{a}	1.29	P<0.01**
Aquatic bryophyte chlorophyll content per unit area (µg cm^{-2})	1.87^{a}	0.37	1.08^{b}	0.30	2.49^{a}	0.53	P<0.01**
Aquatic bryophyte cover (%)	10.5^{a}	1.63	6.5^{b}	1.76	15.2^{a}	4.32	P<0.01**
Bare area (%)	27.3	2.88	30.7	2.85	26.4	3.12	NS
D (m)	0.14^{a}	0.20	0.08^{b}	0.25	0.20^{c}	0.32	P<0.001***
K (m^{-1})	2.90^{a}	0.21	2.10^{b}	0.26	1.80^{b}	0.26	P<0.001***
$Z_{eu}^{3\%}$ (m)	0.75^{a}	0.28	0.90^{a}	0.30	1.20^{b}	0.40	P<0.01**
Z_{eu}:$D^{3\%}$	5.51^{a}	0.31	11.01^{b}	0.32	5.91^{a}	0.47	P<0.001***
pH	6.50^{a}	0.06	7.38^{b}	0.04	6.90^{c}	0.09	P<0.001***
Conductivity (µS cm^{-1})	39.4^{a}	0.18	78.5^{b}	0.16	39.0^{a}	0.23	P<0.001***
Water Temperature (^{o}C)	10.3^{a}	0.29	17.2^{b}	0.40	3.9^{c}	0.22	P<0.001***
Flow (m s^{-1})	0.217	0.02	0.167	0.03	0.253	0.03	NS

Table 3.76 Mean values (± 1 standard error) of normally distributed plant variables (including '*no biomass*' zero values, and data back-transformed where necessary): aquatic bryophyte biomass per unit area (n = 135), aquatic bryophyte chlorophyll content per unit area (n = 135), aquatic bryophyte cover (n = 135), bare area (n = 135) and environmental habitat variables (n = 135) between sampling dates in the River Girnock sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of substrate morphology, alkalinity, nutrient status and heavy metal composition refer to Table 2.5 in Chapter 2.

Mean Variable	April 2006		September 2006		November 2006		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Aquatic bryophyte biomass per unit area (mg cm^{-2})	23.96^{a}	2.11	18.78^{b}	1.49	29.66^{a}	5.26	P<0.01**
Aquatic bryophyte chlorophyll content per unit area (µg cm^{-2})	4.37^{a}	0.56	3.15^{b}	0.48	5.12^{a}	1.19	P<0.01**
Aquatic bryophyte cover (%)	22.7^{a}	2.87	13.4^{b}	3.24	28.9^{a}	6.05	P<0.05*
Bare area (%)	50.1^{a}	3.36	36.2^{b}	2.97	42.3^{ab}	4.80	P<0.05*
D (m)	0.21^{a}	0.23	0.08^{b}	0.26	0.11^{b}	0.46	P<0.001***
K (m^{-1})	1.76^{a}	0.20	3.61^{b}	0.20	2.77^{c}	0.28	P<0.001***
$Z_{eu}^{3\%}$ (m)	1.87^{a}	0.20	0.85^{b}	0.20	1.19^{c}	0.28	P<0.001***
Z_{eu}:$D^{3\%}$	9.03	0.24	10.68	0.27	10.59	0.41	NS
pH	7.45^{a}	0.03	7.70^{b}	0.05	7.52^{a}	0.05	P<0.001***
Conductivity (µS cm^{-1})	116.9^{a}	0.17	173.4^{b}	0.18	124.3^{a}	0.29	P<0.001***
Water Temperature (^{o}C)	6.4^{a}	0.04	12.5^{b}	0.02	8.3^{c}	0.02	P<0.001***
Flow (m s^{-1})	0.514^{a}	0.04	0.141^{b}	0.04	0.259^{b}	0.05	P<0.001***

Table 3.77 Mean values (± 1 standard error) of normally distributed plant variables (including '*no biomass*' zero values, and data back-transformed where necessary): aquatic bryophyte biomass per unit area (n = 135), aquatic bryophyte chlorophyll content per unit area (n = 135), aquatic bryophyte cover (n = 135), bare area (n = 135) and environmental habitat variables (n = 135) between sampling dates in the Knockan Burn sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of substrate morphology, alkalinity, nutrient status and heavy metal composition refer to Table 2.6 in Chapter 2.

3.5.11 Response of aquatic bryophyte production and environmental habitat conditions in the Water of Dye, River Girnock and Knockan Burn to variation in flow regime: pool, glide and riffle zones

Overall, aquatic bryophyte production and abundance (% cover) responded significantly to variations in predominant flow pattern, as can be interpreted from the amalgamated data set: Table 3.81. Aquatic bryophyte biomass was significantly higher in riffle zones compared to glides or pools, and furthermore, was significantly lower in pool habitats than in glides (Table 3.81). Similarly, riffles were characterised by significantly higher quantities of aquatic bryophyte chlorophyll content and abundance, with least chlorophyll production and cover occurring in pools, and glides differing significantly from neither particularly fast, or slow flowing habitats. In general, these observed patterns were reflected in each of the individual sub-catchment streams: Water of Dye (Table 3.78), River Girnock (Table 3.79) and Knockan Burn (Table 3.80).

Variable	Pool		Glide		Riffle		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Aquatic bryophyte biomass per unit area (mg cm^{-2})	4.50^{a}	0.79	8.19^{b}	0.67	17.32^{c}	0.58	P<0.001***
Aquatic bryophyte chlorophyll content per unit area (μg cm^{-2})	2.88^{a}	0.36	3.62^{ab}	0.44	4.65^{b}	0.43	P<0.05*
Aquatic bryophyte cover (%)	17.4^{a}	2.61	25.9^{ab}	3.76	32.3^{b}	3.84	P<0.05*
Bare area (%)	38.4	2.98	38.5	3.52	38.8	3.66	NS
D (m)	0.15^{a}	0.26	0.15^{a}	0.29	0.11^{b}	0.31	P<0.05*
K (m^{-1})	2.78	0.24	2.84	0.25	3.40	0.26	NS
$Z_{eu}^{3\%}$ (m)	0.91	0.26	0.89	0.27	0.74	0.27	NS
Z_{eu}:$D^{3\%}$	5.95	0.30	6.07	0.26	6.52	0.29	NS
pH	6.33	0.34	6.33	0.34	6.32	0.34	NS
Conductivity (μS cm^{-1})	45.9	0.19	45.8	0.19	45.7	0.19	NS
Water Temperature (^{o}C)	10.1	0.08	10.3	0.09	10.3	0.09	NS
Flow (m s^{-1})	0.009^{a}	0.01	0.066^{b}	0.02	0.164^{c}	0.02	P<0.001***

Table 3.78 Mean values (± 1 standard error) of normally distributed plant variables (including '*no biomass*' zero values, and data back-transformed where necessary): aquatic bryophyte biomass per unit area (n = 135), aquatic bryophyte chlorophyll content per unit area (n = 135), aquatic bryophyte cover (n = 135), bare area (n = 135) and environmental habitat variables (n = 135) between flow regime (pool, glide, riffle habitats) in the Water of Dye sub-catchment (n = 135). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Table 2.7 in Chapter 2.

Variable	Pool		Glide		Riffle		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Aquatic bryophyte biomass per unit area (mg cm^{-2})	3.03^{a}	0.52	3.87^{ab}	1.09	5.75^{b}	0.85	P<0.01**
Aquatic bryophyte chlorophyll content per unit area (μg cm^{-2})	1.38^{a}	0.34	1.83^{ab}	0.44	2.26^{b}	0.38	P<0.05*
Aquatic bryophyte cover (%)	7.3^{a}	1.87	11.3^{ab}	2.78	13.6^{b}	2.10	P<0.05*
Bare area (%)	26.8	3.17	27.1	3.06	30.4	2.91	NS
D (m)	0.14^{a}	0.26	0.13^{a}	0.28	0.10^{b}	0.27	P<0.05*
K (m^{-1})	2.25	0.28	2.27	0.23	2.43	0.26	NS
$Z_{eu}^{3\%}$ (m)	0.90	0.31	0.91	0.32	0.85	0.34	NS
Z_{eu}:$D^{3\%}$	6.54	0.35	6.98	0.33	8.70	0.43	NS
pH	7.04	0.09	6.93	0.08	6.84	0.08	NS
Conductivity (μS cm^{-1})	53.1	0.23	50.9	0.22	51.5	0.22	NS
Water Temperature (^{o}C)	11.2	0.12	11.1	0.11	11.2	0.11	NS
Flow (m s^{-1})	0.069^{a}	0.02	0.205^{b}	0.02	0.389^{c}	0.02	P<0.001***

Table 3.79 Mean values (± 1 standard error) of normally distributed plant variables (including '*no biomass*' zero values, and data back-transformed where necessary): aquatic bryophyte biomass per unit area (n = 135), aquatic bryophyte chlorophyll content per unit area (n = 135), aquatic bryophyte cover (n = 135), bare area (n = 135) and environmental habitat variables (n = 135) between flow regime (pool, glide, riffle habitats) in the River Girnock sub-catchment (n = 135). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Table 2.8 in Chapter 2.

Variable	Pool		Glide		Riffle		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Aquatic bryophyte biomass per unit area (mg cm^{-2})	16.06^{a}	4.11	24.62^{ab}	4.35	31.71^{b}	6.38	P<0.05*
Aquatic bryophyte chlorophyll content per unit area (μg cm^{-2})	3.55^{a}	0.52	4.24^{ab}	0.48	4.86^{b}	0.60	P<0.05*
Aquatic bryophyte cover (%)	17.3^{a}	3.12	20.8^{ab}	3.06	27.1^{b}	4.49	P<0.05*
Bare area (%)	39.9	3.11	44.0	3.23	44.7	5.09	NS
D (m)	0.12	0.36	0.14	0.26	0.11	0.38	NS
K (m^{-1})	2.79	0.23	2.40	0.22	2.60	0.30	NS
$Z_{eu}{}^{3\%}$ (m)	1.09	0.25	1.36	0.22	1.26	0.30	NS
Z_{eu}:$D^{3\%}$	9.78	0.32	9.47	0.24	11.43	0.33	NS
pH	7.54	0.20	7.57	0.18	7.59	0.26	NS
Conductivity (μS cm^{-1})	148.1	0.20	134.2	0.19	133.6	0.26	NS
Water Temperature (^{o}C)	9.5	0.07	8.7	0.06	8.4	0.09	NS
Flow (m s^{-1})	0.001^{a}	0.01	0.208^{b}	0.04	0.589^{c}	0.05	P<0.001***

Table 3.80 Mean values (± 1 standard error) of normally distributed plant variables (including '*no biomass*' zero values, and data back-transformed where necessary): aquatic bryophyte biomass per unit area (n = 135), aquatic bryophyte chlorophyll content per unit area (n = 135), aquatic bryophyte cover (n = 135), bare area (n = 135) and environmental habitat variables (n = 135) between flow regime (pool, glide, riffle habitats) in the Knockan Burn sub-catchment (n = 135). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Table 2.9 in Chapter 2.

Variable	Pool		Glide		Riffle		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Aquatic bryophyte biomass per unit area (mg cm^{-2})	7.86^{a}	1.26	12.23^{b}	1.39	18.26^{c}	1.62	P<0.001***
Aquatic bryophyte chlorophyll content per unit area (μg cm^{-2})	2.61^{a}	0.39	3.24^{ab}	0.45	3.92^{b}	0.41	P<0.05*
Aquatic bryophyte cover (%)	14.0^{a}	2.12	19.3^{ab}	2.67	24.3^{b}	2.91	P<0.05*
Bare area (%)	35.0	1.85	36.5	1.99	38.0	2.19	NS
D (m)	0.14^{a}	0.17	0.14^{a}	0.16	0.11^{b}	0.18	P<0.01**
K (m^{-1})	2.60	0.15	2.48	0.14	2.80	0.16	NS
$Z_{eu}^{3\%}$ (m)	0.96	0.16	1.06	0.16	0.89	0.18	NS
Z_{eu}:$D^{3\%}$	7.26	0.19	7.57	0.16	8.34	0.22	NS
pH	6.97	0.07	7.01	0.06	6.82	0.08	NS
Conductivity (μS cm^{-1})	71.5	0.16	72.8	0.15	62.0	0.16	NS
Water Temperature (^{o}C)	10.6	0.06	9.7	0.06	10.1	0.07	NS
Flow (m s^{-1})	0.062^{a}	0.01	0.289^{b}	0.02	0.465^{c}	0.03	P<0.001***

Table 3.81 Mean values (± 1 standard error) of normally distributed plant variables (including '*no biomass*' zero values, and data back-transformed where necessary): aquatic bryophyte biomass per unit area (n = (n = 405), aquatic bryophyte chlorophyll content per unit area, aquatic bryophyte cover (n = 405), bare area (n = 405) and environmental habitat variables (n = 405) between flow regime (pool, glide, riffle habitats) for amalgamated sub-catchment data (the Water of Dye, River Girnock, and Knockan Burn, n = 405). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Table 2.10 in Chapter 2.

3.5.12 Response of aquatic bryophyte production and environmental habitat conditions in the Water of Dye, River Girnock and Knockan Burn to variation in substrate morphology

Aquatic bryophyte production (biomass and chlorophyll content) and abundance was highest in streambed habitats characterised by a high proportion of boulders. A scarcity of boulders tended to result in low production and abundance of aquatic bryophytes (Table 3.82).

There was no significant response of aquatic bryophyte production and abundance to variation in streambed cover of large stones (Table 3.83) or sand (Table 3.86).

The biomass, chlorophyll content and abundance of aquatic bryophytes showed a significant decline in response increasing proportions of unstable substrate particles such small stones and gravel (Table 3.84 and Table 3.85, respectively). Mostly, these streambed habitats (deposition zones) characterised by small-sized particles lacked aquatic bryophyte flora.

Variable	0% BO		3% BO		15.5% BO		38% BO		63% BO		88% BO		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Aquatic bryophyte biomass per unit area (mg cm^{-2})	6.03^{a}	0.39	7.59^{a}	0.45	7.19^{a}	0.29	13.81^{b}	0.38	21.46^{c}	0.31	20.58^{c}	0.46	P<0.001***
Aquatic bryophyte chlorophyll content per unit area (μg cm^{-2})	2.31^{a}	0.21	2.48^{a}	0.29	2.36^{a}	0.23	3.28^{b}	0.23	4.42^{c}	0.17	4.68^{c}	0.26	P<0.001***
Aquatic bryophyte cover (%)	8.4^{a}	1.26	13.6^{b}	2.15	16.1^{b}	2.69	18.9^{b}	3.27	28.2^{c}	2.94	30.2^{c}	4.33	P<0.001***

Table 3.82 Mean values (± 1 standard error) of normally distributed plant variables (including '*no biomass*' zero values, and data back-transformed where necessary): response of aquatic bryophyte biomass, chlorophyll content and abundance to variation in the abundance (median % cover) of boulders (BO) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 405). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Table 2.11 in Chapter 2.

Variable	0% LS		3% LS		15.5% LS		38% LS		63% LS		88% LS		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Aquatic bryophyte biomass per unit area (mg cm^{-2})	8.63	0.45	11.49	0.50	10.52	0.35	12.58	0.42	17.64	4.12	15.84	6.52	NS
Aquatic bryophyte chlorophyll content per unit area (μg cm^{-2})	3.28	0.23	3.47	0.27	3.25	0.24	3.13	0.23	3.33	0.22	3.11	0.36	NS
Aquatic bryophyte cover (%)	18.2	3.03	22.2	3.38	19.8	2.58	21	2.19	18.3	1.85	15.9	5.09	NS

Table 3.83 Mean values (± 1 standard error) of normally distributed plant variables (including '*no biomass*' zero values, and data back-transformed where necessary): response of aquatic bryophyte biomass, chlorophyll content and abundance to variation in the abundance (median % cover) of large stones (LS) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 405). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Table 2.12 in Chapter 2.

Variable	**0% SS**		**3% SS**		**15.5% SS**		**38% SS**		**63% SS**		**88% SS**		**PANOVA**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Aquatic bryophyte biomass per unit area (mg cm^{-2})	29.03^{a}	0.31	17.11^{b}	0.43	15.09^{b}	0.52	5.88^{c}	0.38	6.23^{c}	0.48	3.32^{d}	0.63	P<0.001***
Aquatic bryophyte chlorophyll content per unit area (μg cm^{-2})	4.92^{a}	0.33	3.92^{b}	0.22	3.74^{b}	0.21	2.67^{c}	0.22	2.55^{c}	0.36	1.73^{d}	0.35	P<0.001***
Aquatic bryophyte cover (%)	41.6^{a}	2.71	23.2^{b}	2.69	24.3^{b}	1.75	12.2^{c}	1.97	10.9^{c}	1.83	2.9^{d}	4.01	P<0.001***

Table 3.84 Mean values (± 1 standard error) of normally distributed plant variables (including '*no biomass*' zero values, and data back-transformed where necessary): response of aquatic bryophyte biomass, chlorophyll content and abundance to variation in the abundance (median % cover) of small stones (SS) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 405). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Table 2.13 in Chapter 2.

Variable	0% GR		3% GR		15.5% GR		38% GR		63% GR		88% GR		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Aquatic bryophyte biomass per unit area (mg cm^{-2})	26.01^{a}	0.56	18.91^{b}	0.39	17.45^{b}	0.48	6.53^{c}	0.42	5.67^{c}	0.57	2.08^{d}	0.49	P<0.001***
Aquatic bryophyte chlorophyll content per unit area (μg cm^{-2})	4.78^{a}	0.31	3.82^{b}	0.21	3.95^{b}	0.33	2.89^{c}	0.28	2.72^{c}	0.26	1.42^{d}	0.24	P<0.001***
Aquatic bryophyte cover (%)	36.7^{a}	2.09	24.6^{b}	2.11	26.8^{b}	2.32	13.0^{c}	2.42	10.3^{c}	2.09	3.9^{d}	2.66	P<0.001***

Table 3.85 Mean values (± 1 standard error) of normally distributed plant variables (including '*no biomass*' zero values, and data back-transformed where necessary): response of aquatic bryophyte biomass, chlorophyll content and abundance to variation in the abundance (median % cover) of gravel (GR) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 405). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Table 2.14 in Chapter 2.

Variable	0% SA		3% SA		15.5% SA		38% SA		63% SA		88% SA		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Aquatic bryophyte biomass per unit area (mg cm^{-2})	9.77	0.69	10.55	0.91	12.43	1.68	8.95	2.98	19.57	18.32	15.38	12.14	NS
Aquatic bryophyte chlorophyll content per unit area (μg cm^{-2})	3.59	0.37	3.25	0.47	3.45	0.43	3.32	0.49	3.1	0.68	2.87	0.74	NS
Aquatic bryophyte cover (%)	17.4	2.15	21.4	5.61	14.1	4.82	12.9	5.01	27.1	10.89	22.2	9.47	NS

Table 3.86 Mean values (± 1 standard error) of normally distributed plant variables (including '*no biomass*' zero values, and data back-transformed where necessary): response of aquatic bryophyte biomass, chlorophyll content and abundance to variation in the abundance (median % cover) of sand (SA) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 405). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Table 2.15 in Chapter 2.

3.5.13 Aquatic bryophyte production and environmental habitat conditions in the Water of Dye, River Girnock and Knockan Burn as determined by TWINSPAN classification

Of the five sample-groups identified from TWINSPAN analysis of the aquatic bryophyte dataset, assemblages III and IV were similarly characterised by a high abundance and production of aquatic bryophyte vegetation, and were significantly different from the other three communities (Table 3.87, and also Figure 3.47). Furthermore, the group II community had a significantly higher abundance of aquatic bryophytes than either assemblage I or V which were similar and contained either very modest quantities or entirely lacked aquatic bryophyte vegetation (often characterised by high periphyton cover or unvegetated regions of streambed). Consult Chapter 4, Figure 4.26 for details of the TWINSPAN classification here cited. See also Chapter 4, Table 4.86 and section 4.5.13 for details and discussion of variation in environmental habitat conditions between the TWINSPAN sample-groups.

	TWINSPAN sample-group										
Variable	**I**		**II**		**III**		**IV**		**V**		**P_{ANOVA}**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Aquatic bryophyte biomass per unit area (mg cm^{-2})	1.69^{a}	0.62	7.91^{b}	0.53	27.22^{c}	0.70	27.06^{c}	0.62	0.00^{a}	0.00	P<0.001***
Aquatic bryophyte chlorophyll content per unit area (μg cm^{-2})	1.20^{a}	0.50	3.36^{b}	0.45	5.87^{c}	0.58	5.94^{c}	0.54	0.00^{a}	0.00	P<0.001***
Aquatic bryophyte cover (%)	7.2^{a}	1.12	20.7^{b}	2.13	34.4^{c}	3.36	33.9^{c}	2.54	0.00^{a}	0.00	P<0.001***
Bare area (%)	23.1^{a}	2.40	32.3^{b}	2.06	36.7^{b}	2.79	38.5^{b}	2.84	51.8^{c}	4.83	P<0.001***
Periphyton cover (%)	41.1^{a}	3.65	25.0^{b}	2.41	20.8^{b}	3.05	21.3^{b}	2.72	18.9^{b}	5.58	P<0.001***

Table 3.87 Mean values (± 1 standard error) of normally distributed plant variables (including '*no biomass*' zero values, and data back-transformed where necessary): aquatic bryophyte biomass per unit area (n = 79), aquatic bryophyte chlorophyll content per unit area (= 79), aquatic bryophyte cover (n = 79), bare area (n = 79), and periphyton cover (n = 79), between TWINSPAN sample-groups I (n = 8), II (n = 35), III (n = 15), and IV (n = 16) with the 'no bryophytes' sample-group V (n = 5) encompassing all other samples lacking aquatic bryophyte vegetation. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Refer to Chapter 4, section 4.5.13 for TWINSPAN output (Figure 4.26) and details of environmental habitat conditions (Table 4.86).

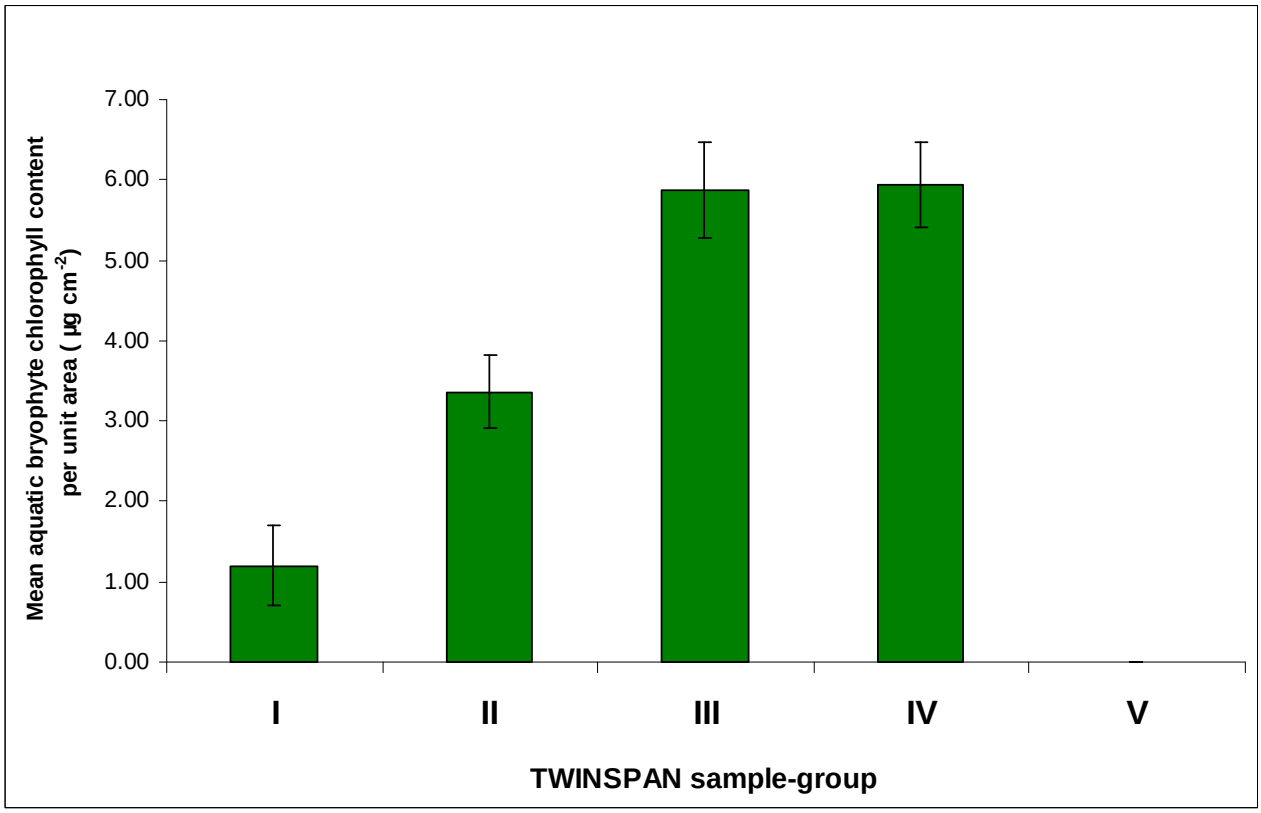


Figure 3.47 Comparison of mean values (± 1 standard error) of normally distributed plant variables (including '*no biomass*' zero values, and data back-transformed where necessary): aquatic bryophyte chlorophyll content per unit area (n = 79) between TWINSPAN sample-groups I (n = 8), II (n = 35), III (n = 15), and IV (n = 16) with the 'no bryophytes' sample-group V (n = 5) encompassing all other samples lacking aquatic bryophyte vegetation.

3.5.14 Relationships between aquatic bryophyte production and environmental habitat conditions

Aquatic bryophyte production (biomass and chlorophyll content) and abundance were strongly and significantly positively correlated with each other, and negatively correlated with increasing bare area (see Appendix 2e). In general, increased current velocity and proportion of boulders on the streambed were correlated with increased abundance and production of aquatic bryophytes. Increasing streamwater temperature, proportion of riparian shade experienced and presence of unstable particles in the streambed (e.g. small stones, gravel) tended to be negatively associated with aquatic bryophyte production and abundance. Water chemistry did not appear to exert much effect on aquatic bryophyte production or % cover, except for calcium and magnesium concentrations which were positively correlated with aquatic bryophyte biomass but had no significant relationship with either chlorophyll production or abundance. More resistant acidic geologies (e.g. Granite) tended to have higher aquatic bryophyte production and abundance, as did some softer calcareous geologies (e.g Durness limestone), whilst other rocks were identified as being particularly unfavourable for high aquatic bryophyte production.

3.5.15 Predicting freshwater aquatic bryophyte production

Several full models were developed for predicting aquatic bryophyte production ($\log_e$ chlorophyll content) of upland stream habitats using various combinations of environmental predictor variables (refer to Table 3.88). The selected model AqBRYOchlP1a was chosen because it produced the highest r^2 value (46.9%) and gave rise to variant minimal models with similar predictive power (see Table 3.89) which reasonably accurately predicted the response variable, mean aquatic bryophyte production of the third and final field surveys, for test data sets of the Water of Dye (Table 3.90, Figure 3.48), River Girnock (Table 3.91, Figure 3.49), and Knockan Burn (Table 3.92, Figure 3.50).

Full models	Regression equations	r^2-adj (%)	P_{value}
AqBRYOchlP1a: Aquatic Bryophyte Production (log_e Chl)	log_e Chl = 1.18 + 0.260 (BO) + 1.14 (log_e K) + 0.735 (log_e Zeu^3) - 0.524 (√ temp)	46.9	P<0.001***
AqBRYOchlP2a: Aquatic Bryophyte Production (log_e Chl)	log_e Chl = 2.27 + 0.163 (BO) - 0.454 (√ temp)	33.2	P<0.001***
AqBRYOchlP3a: Aquatic Bryophyte Production (log_e Chl)	log_e Chl = 0.11 + 0.193 (BO) + 0.694 (log_e) K + 0.659 (log_e Zeu^3)	28.2	P<0.01**
AqBRYOchlP4a: Aquatic Bryophyte Production (log_e Chl)	log_e Chl = 0.83 + 0.182 (BO) + 0.350 (log_e Zeu^3)	23.8	P<0.01**

Table 3.88 Statistically significant full models (n = 79) using combinations of environmental variable(s) for predicting freshwater aquatic bryophyte production (measured as log_e chlorophyll content in μg cm^{-2}) of upland stream habitats. Model codes: loge Chl: log_e chlorophyll production (μg cm^{-2}); BO: boulder cover (%); log_e Zeu^3: loge 3% euphotic depth (Zeu^3 m); √ temp: √ water temperature (°C).

Reduced (minimal) models	Regression equations	r^2-adj (%)	P_{value}
AqBRYOchlP1a: Aquatic Bryophyte Production (log_e Chl) excluding Water of Dye April 2006 test data set	log_e Chl = 1.21 + 0.295 (BO) + 1.22 (log_e K) + 0.732 (log_e Zeu^3) - 0.578 (√ temp)	43.6	P<0.001***
AqBRYOchlP1a: Aquatic Bryophyte Production (log_e Chl) excluding River Girnock April 2006 test data set	log_e Chl = 0.98 + 0.265 (BO) + 1.23 (log_e K) + 0.852 (log_e Zeu^3) - 0.490 (√ temp)	42.2	P<0.001***
AqBRYOchlP1a: Aquatic Bryophyte Production (log_e Chl) excluding Knockan Burn November 2006 test data set	log_e Chl = 1.69 + 0.172 (BO) + 1.02 (log_e K) + 0.582 (log_e Zeu^3) - 0.525 (√ temp)	44.5	P<0.001***

Table 3.89 Statistically significant minimal models (n = 70) of AqBRYOchlP1a for predicting freshwater aquatic bryophyte production (log_e chl) of the Water of Dye, River Girnock and Knockan Burn test data sets. For model codes refer to Table 3.88.

Mean test data	Observed log_e Chl: test data	Predicted log_e Chl: reduced model AqBRYOchlP1a	t-statistic	P-value
Brocky Burn (BB)	1.58	1.76	-0.69	NS
Charr Flume (CF)	1.32	1.38	-0.23	NS
Bogendreip (BD)	1.51	1.70	-1.22	NS
Water of Dye (WoD) April 2006	1.47	1.61	-1.08	NS

Table 3.90 Comparison of mean observed and predicted values of minimal model AqBRYOchlP1a for predicting freshwater aquatic bryophyte production (log_e chl) of the Water of Dye April 2006 test data set (see also Figure 3.48).

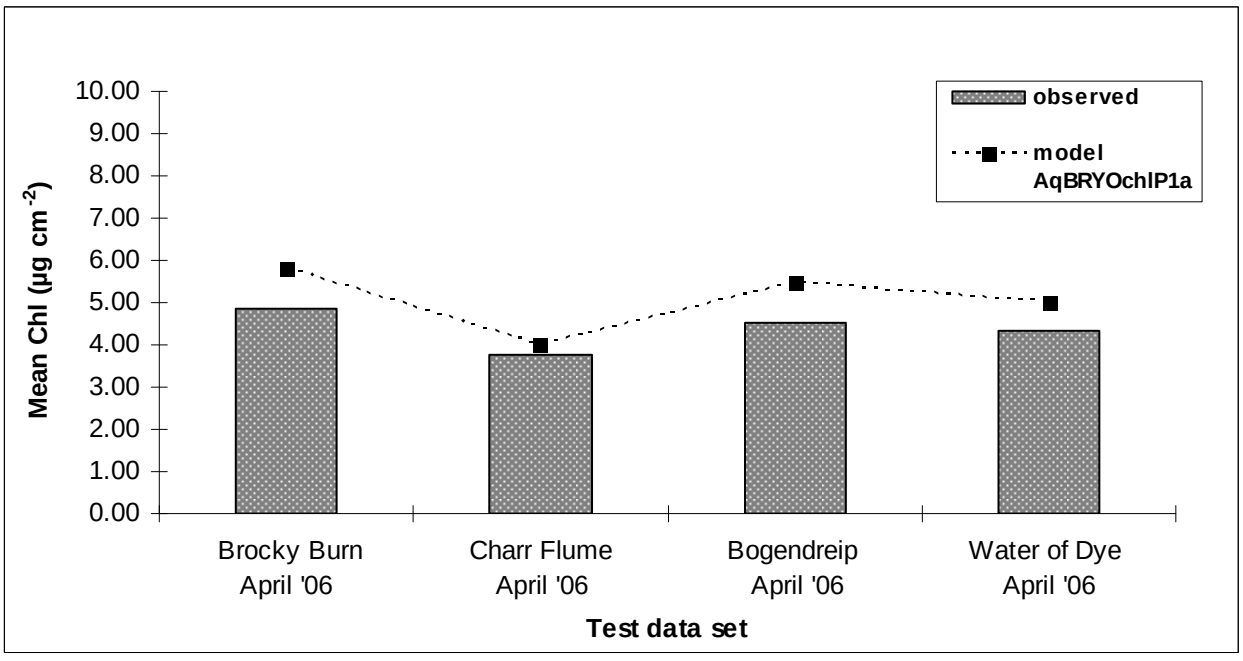


Figure 3.48 Comparison of mean observed and (back-transformed) predicted values of minimal model AqBRYOchlP1a for predicting freshwater aquatic bryophyte production (chl) of the Water of Dye April 2006 test data set.

Mean test data	Observed log_e Chl: test data	Predicted log_e Chl: reduced model AqBRYOchlP1a	t-statistic	P-value
Iron Bridge (IB)	1.35	1.30	0.25	NS
Hampshire's Bridge (HB)	0.80	1.12	-2.61	NS
Littlemill (LM)	0.63	0.42	1.93	NS
River Girnock April 2006	0.92	0.95	-0.15	NS

Table 3.91 Comparison of mean observed and predicted values of minimal model AqBRYOchlP1a for predicting freshwater aquatic bryophyte production (log_e chl) of the River Girnock April 2006 test data set (see also Figure 3.49).

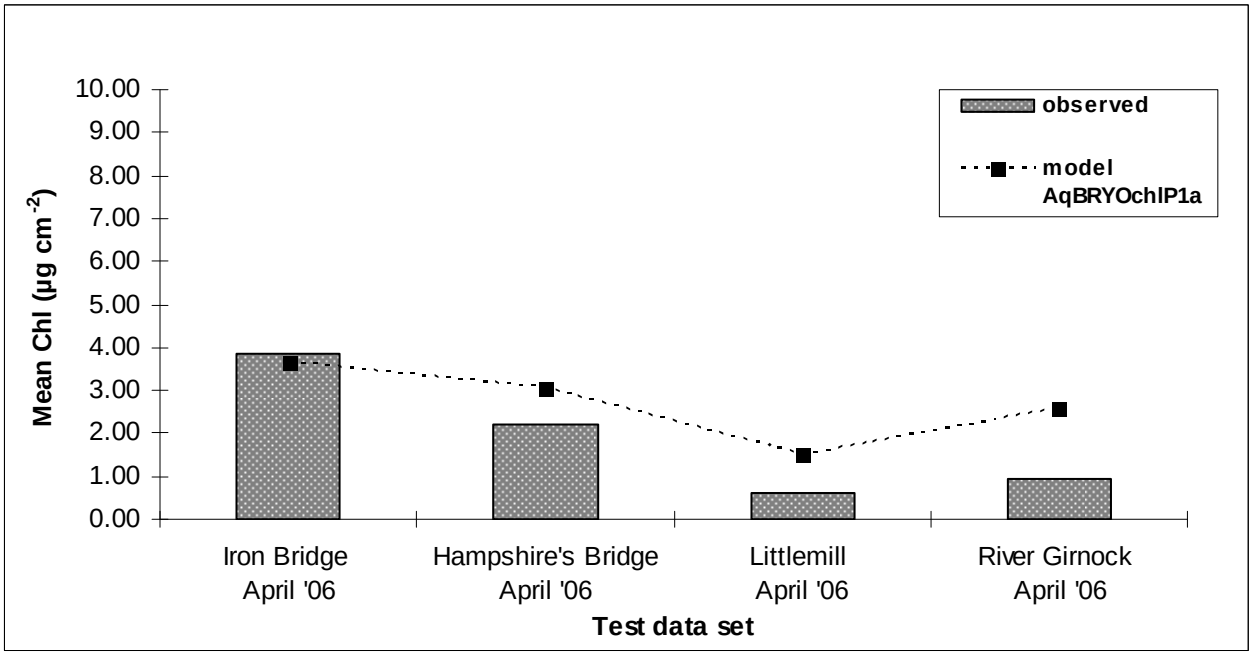


Figure 3.49 Comparison of mean observed and (back-transformed) predicted values of minimal model AqBRYOchlP1a for predicting freshwater aquatic bryophyte production (chl) of the River Girnock April 2006 test data set.

Mean test data	Observed log_e Chl: test data	Predicted log_e Chl: reduced model AqBRYOchlP1a	t-statistic	P-value
Upper Knockan (UK)	1.73	1.11	4.47	NS
Mid Knockan (KM)	0.88	0.98	-0.18	NS
Lower Knockan (LK)	2.31	1.41	0.77	NS
Knockan Burn November 2006	1.63	1.17	0.96	NS

Table 3.92 Comparison of mean observed and predicted values of minimal model AqBRYOchlP1a for predicting freshwater aquatic bryophyte production (log_e chl) of the Knockan Burn November 2006 test data set (see also Figure 3.50).

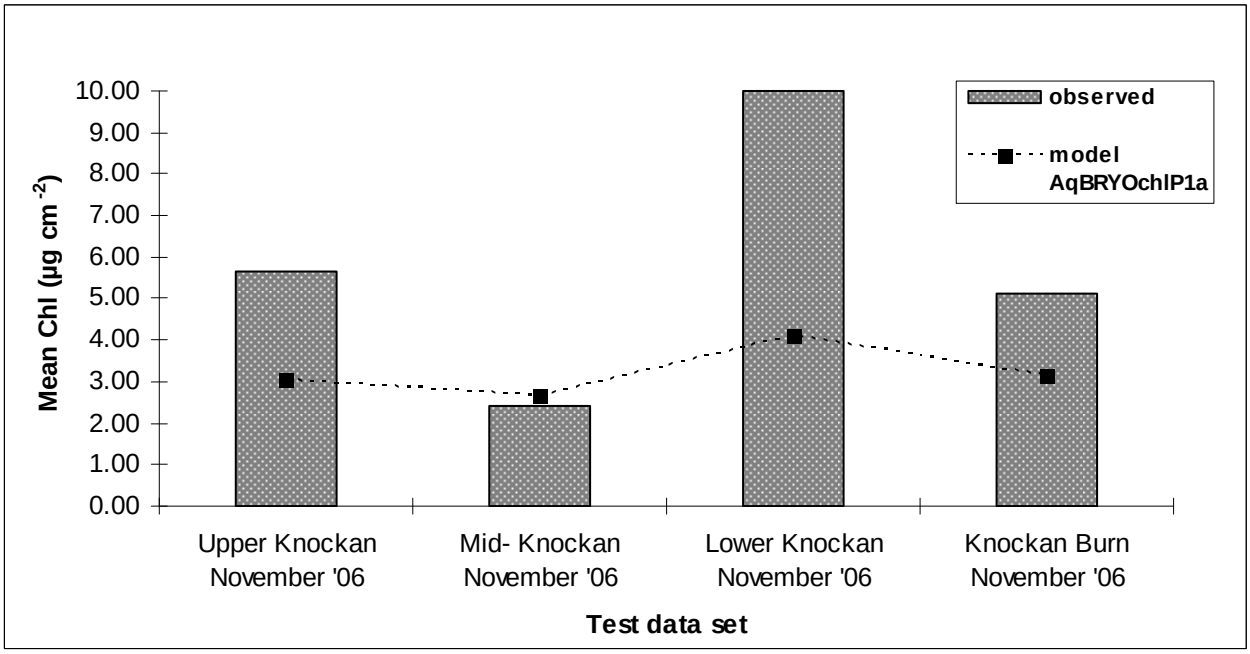


Figure 3.50 Comparison of mean observed and (back-transformed) predicted values of minimal model AqBRYOchlP1a for predicting freshwater aquatic bryophyte production (chl) of the Knockan Burn November 2006 test data set.

3.5.16 Variation in vascular submerged macrophyte production and environmental habitat conditions in the Knockan Burn sub-catchment and its sites

There were significant differences in vascular submerged macrophyte production (biomass and chlorophyll content) and plant cover between the three study sub-catchment streams (Table 3.93). Both the Water of Dye and River Girnock sub-catchment streams were significantly deficient in vascular submerged macrophytes, the occurrence of which was limited to the upper and lower parts of Knockan Burn (refer to Table 3.94 for details).

Variable	Water of Dye		River Girnock		Knockan Burn		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Vascular submerged macrophyte biomass (mg cm^{-2})	0.00		0.00		1.18		P<0.01**
Chlorophyll content of vascular submerged macrophytes (μg cm^{-2})	0.00		0.00		0.46		P<0.01**
Vascular submerged macrophyte cover (%)	0.0		0.0		7.0		P<0.01**
Bare area (%)	38.6^{a}	1.95	28.2^{b}	1.75	40.1^{a}	1.91	P<0.001***
D (m)	0.14	0.17	0.12	0.16	0.13	0.17	NS
K (m^{-1})	2.99^{a}	0.15	2.32^{b}	0.15	2.54^{b}	0.13	P<0.001***
$Z_{eu}^{3\%}$ (m)	0.84^{a}	0.15	0.89^{a}	0.19	1.26^{b}	0.14	P<0.001***
Z_{eu}:$D^{3\%}$	6.18^{a}	0.16	7.37^{a}	0.21	9.85^{b}	0.15	P<0.001***
pH	6.33^{a}	0.07	6.93^{b}	0.05	7.59^{c}	0.03	P<0.001***
Conductivity (μS cm^{-1})	45.8^{a}	0.11	51.8^{b}	0.13	142.1^{c}	0.11	P<0.001***
Water Temperature (^{o}C)	10.2^{ab}	0.05	11.2^{a}	0.07	9.1^{b}	0.04	P<0.01**
Flow (m s^{-1})	0.218^{a}	0.02	0.203^{a}	0.02	0.278^{b}	0.02	P<0.05*

Table 3.93 Mean values (± 1 standard error) of normally distributed plant variables (including '*no biomass*' zero values, and data back-transformed where necessary): vascular submerged macrophyte biomass per unit area (n = 429), vascular submerged macrophyte chlorophyll content per unit area (n = 429), vascular submerged macrophyte cover (n = 429), bare area (n = 429) and environmental habitat variables (n = 429) between study stream sub-catchments. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values are quoted for non-normal variables compared using Kruskal-Wallis test. For details of underlying geology, substrate morphology, alkalinity, nutrient status and heavy metal composition refer to Table 2.1 in Chapter 2.

	Water of Dye						River Girnock						Knockan Burn						
Variable	**BB**		**CF**		**BD**		**IB**		**HB**		**LM**		**UK**		**MK**		**LK**		**PANOVA**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Vascular submerged macrophyte biomass (mg cm^{-2})	0.00		0.00		0.00		0.00		0.00		0.00		2.07		0.00		1.48		P<0.01**
Chlorophyll content of vascular submerged macrophytes (μg cm^{-2})	0.00		0.00		0.00		0.00		0.00		0.00		0.89		0.00		0.60		P<0.01**
Vascular submerged macrophyte cover (%)	0.00		0.00		0.00		0.00		0.00		0.00		13.2		0.00		7.9		P<0.01**
Bare area (%)	33.3^{ab}	2.83	44.7^{a}	3.74	37.7^{ab}	3.34	25.5^{b}	3.02	25.0^{b}	2.95	33.9^{ab}	3.01	38.4^{a}	3.04	47.0^{a}	3.51	36.5^{a}	3.26	P<0.001***
D (m)	0.09^{a}	0.30	0.17^{b}	0.27	0.18^{b}	0.24	0.12^{ab}	0.25	0.11^{a}	0.28	0.13^{ab}	0.30	0.14^{ab}	0.38	0.11^{a}	0.29	0.14^{ab}	0.30	P<0.001***
K (m^{-1})	4.39^{a}	0.22	2.37^{b}	0.22	2.58^{b}	0.26	2.50^{b}	0.25	2.43^{b}	0.24	2.05^{b}	0.28	2.64^{b}	0.22	2.35^{b}	0.22	2.54^{b}	0.24	P<0.001***
$Z_{eu}{}^{3\%}$ (m)	0.56^{a}	0.26	1.20^{bd}	0.22	0.90^{d}	0.25	1.25^{b}	0.25	1.41^{b}	0.24	0.39^{c}	0.27	1.15^{bd}	0.24	1.29^{b}	0.22	1.36^{b}	0.24	P<0.001***
Z_{eu}:$D^{3\%}$	6.42^{a}	0.26	7.21^{ab}	0.30	5.09^{a}	0.27	10.06^{b}	0.25	12.80^{b}	0.31	3.11^{c}	0.31	8.50^{b}	0.26	11.67^{b}	0.27	9.90^{b}	0.27	P<0.001***
pH	5.89^{a}	0.13	6.80^{b}	0.07	6.29^{c}	0.12	6.51^{c}	0.08	7.15^{d}	0.06	7.13^{d}	0.06	7.31^{d}	0.01	7.43^{d}	0.02	7.94^{e}	0.03	P<0.001***
Conductivity (μS cm^{-1})	38.4^{a}	0.18	48.1^{b}	0.19	52.1^{bc}	0.19	39.0^{a}	0.22	58.6^{c}	0.22	60.9^{c}	0.21	115.3^{d}	0.17	110.9^{d}	0.18	206.5^{e}	0.16	P<0.001***
Water Temperature (^{o}C)	9.9^{ab}	0.10	10.7^{ab}	0.06	10.1^{ab}	0.10	10.9^{ab}	0.12	12.8^{a}	0.16	9.9^{ab}	0.05	8.7^{b}	0.08	8.2^{b}	0.03	10.1^{ab}	0.06	P<0.001***
Flow (m s^{-1})	0.228^{a}	0.06	0.216^{a}	0.06	0.209^{a}	0.06	0.224^{a}	0.07	0.198^{a}	0.07	0.188^{a}	0.06	0.169^{a}	0.05	0.475^{b}	0.08	0.260^{a}	0.04	P<0.001***

Table 3.94 Mean values (± 1 standard error) of normally distributed plant variables (including '*no biomass*' zero values, and data back-transformed where necessary): vascular submerged macrophyte biomass per unit area (n = 429), vascular submerged macrophyte chlorophyll content per unit area (n = 429), vascular submerged macrophyte cover (n = 429), bare area (n = 429) and environmental habitat variables (n = 429) between sampling sites. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values are quoted for non-normal variables compared using Kruskal-Wallis test. For details of underlying geology, substrate morphology, alkalinity, nutrient status and heavy metal composition refer to Table 2.2 in Chapter 2.

3.5.17 Seasonal variation in vascular submerged macrophyte production and environmental habitat conditions in Knockan Burn

Although overall there appeared to be a peak in vascular submerged macrophyte production and abundance in September 2006 compared to April and November 2006, these differences in the Knockan Burn sub-catchment between dates surveyed were not found to be significant (Table 3.95).

Variable	April 2006		September 2006		November 2006		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Vascular submerged macrophyte biomass (mg cm^{-2})	0.86		1.56		1.13		NS
Chlorophyll content of vascular submerged macrophytes (μg cm^{-2})	0.35		0.58		0.46		NS
Vascular submerged macrophyte cover (%)	5.0		9.0		7.0		NS
Bare area (%)	47.3^{a}	3.06	33.9^{b}	2.72	38.3^{ab}	4.29	P<0.01**
D (m)	0.21^{a}	0.21	0.08^{b}	0.26	0.12^{b}	0.39	P<0.001***
K (m^{-1})	1.77^{a}	0.18	3.48^{b}	0.21	2.80^{c}	0.26	P<0.001***
$Z_{eu}^{3\%}$ (m)	1.88^{a}	0.18	0.88^{b}	0.21	1.18^{c}	0.26	P<0.001***
Z_{eu}:$D^{3\%}$	8.96	0.22	10.82	0.26	9.96	0.36	NS
pH	7.47^{a}	0.03	7.73^{b}	0.05	7.53^{a}	0.04	P<0.001***
Conductivity (μS cm^{-1})	119.8^{a}	0.16	177.4^{b}	0.17	128.8^{a}	0.27	P<0.001***
Water Temperature (^{o}C)	6.6^{a}	0.03	12.5^{b}	0.02	8.3^{c}	0.01	P<0.001***
Flow (m s^{-1})	0.465^{a}	0.04	0.149^{b}	0.03	0.250^{b}	0.04	P<0.001***

Table 3.95 Mean values (± 1 standard error) of normally distributed plant variables (including '*no biomass*' zero values, and data back-transformed where necessary): vascular submerged macrophyte biomass per unit area (n = 159), vascular submerged macrophyte chlorophyll content per unit area (n = 159), vascular submerged macrophyte cover (n = 159), bare area (n = 159) and environmental habitat variables (n = 159) between sampling dates in the Knockan Burn sub-catchment. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values are quoted for non-normal variables compared using Kruskal-Wallis test. For details of substrate morphology, alkalinity, nutrient status and heavy metal composition refer to Table 2.6 in Chapter 2.

3.5.18 Response of vascular submerged macrophyte production and environmental habitat conditions in Knockan Burn to variation in flow regime: pool, glide and riffle zones

There was a significant response of vascular submerged macrophyte production and abundance to flow regime in the Knockan Burn sub-catchment (Table 3.96). Most notably vascular submerged macrophyte vegetation was found not to occur in fast-flowing, riffle habitats. Further, it may be possible to interpret that vascular submerged macrophyte production and abundance did not vary significantly between pools and glides, but was significantly higher in glide habitats than in riffle zones.

Variable	Pool		Glide		Riffle		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Vascular submerged macrophyte biomass (mg cm^{-2})	1.35		2.19		0.00		P<0.05*
Chlorophyll content of vascular submerged macrophytes (μg cm^{-2})	0.38		0.99		0.00		P<0.05*
Vascular submerged macrophyte cover (%)	7.5		13.5		0.00		P<0.05*
Bare area (%)	39.4	2.96	39.2	2.75	44.0	5.09	NS
D (m)	0.12	0.36	0.14	0.26	0.11	0.38	NS
K (m^{-1})	2.67	0.25	2.45	0.20	2.60	0.30	NS
$Z_{eu}^{3\%}$ (m)	1.14	0.25	1.34	0.20	1.26	0.30	NS
Z_{eu}:$D^{3\%}$	9.65	0.30	9.46	0.21	11.43	0.33	NS
pH	7.54	0.20	7.61	0.17	7.59	0.26	NS
Conductivity (μS cm^{-1})	148.6	0.20	139.4	0.16	133.6	0.26	NS
Water Temperature (^oC)	9.3	0.07	8.7	0.05	8.1	0.08	NS
Flow (m s^{-1})	0.040^a	0.02	0.360^b	0.03	0.747^c	0.03	P<0.001***

Table 3.96 Mean values (± 1 standard error) of normally distributed plant variables (including '*no biomass*' zero values, and data back-transformed where necessary): vascular submerged macrophyte biomass per unit area (n = 159), vascular submerged macrophyte chlorophyll content per unit area, vascular submerged macrophyte cover (n = 159), bare area (n = 159) and environmental habitat variables (n = 159) between flow regime (pool, glide, riffle habitats) in the Knockan Burn sub-catchment (n = 159). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values are quoted for non-normal variables compared using Kruskal-Wallis test. For details of environmental habitat conditions refer to Table 2.9 in Chapter 2.

3.5.19 Response of vascular submerged macrophyte production and environmental habitat conditions in Knockan Burn to variation in substrate morphology

Vascular submerged macrophyte production and abundance tended to be negatively correlated with streambeds characterised by a predominance of coarse substrate particles (e.g. high boulder cover), and generally increased as the proportion of fine substrate particles increased, particularly to an abundance of sandy loams (Table 3.97 - Table 3.101, inclusive).

Variable	0% BO		3% BO		15.5% BO		38% BO		63% BO		88% BO		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Vascular submerged macrophyte biomass per unit area (mg cm^{-2})	4.61		2.47		0.00		0.00		0.00		0.00		P<0.01**
Vascular submerged macrophyte chlorophyll content per unit area (μg cm^{-2})	1.65		1.13		0.00		0.00		0.00		0.00		P<0.05*
Vascular submerged macrophyte cover (%)	27.7		14.2		0.0		0.0		0.0		0.0		P<0.01**

Table 3.97 Mean values (± 1 standard error) of normally distributed plant variables (including '*no biomass*' zero values, and data back-transformed where necessary): response of vascular submerged macrophyte biomass, chlorophyll content and abundance to variation in the abundance (median % cover) of boulders (BO) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 429). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values are quoted for non-normal variables compared using Kruskal-Wallis test. For details of environmental habitat conditions refer to Table 2.11 in Chapter 2.

Variable	0% LS		3% LS		15.5% LS		38% LS		63% LS		88% LS		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Vascular submerged macrophyte biomass per unit area (mg cm^{-2})	5.00		2.08		0.00		0.00		0.00		0.00		P<0.01**
Vascular submerged macrophyte chlorophyll content per unit area (μg cm^{-2})	1.60		1.18		0.00		0.00		0.00		0.00		P<0.01**
Vascular submerged macrophyte cover (%)	29.6		12.5		0.0		0.0		0.0		0.0		P<0.01**

Table 3.98 Mean values (± 1 standard error) of normally distributed plant variables (including '*no biomass*' zero values, and data back-transformed where necessary): response of vascular submerged macrophyte biomass, chlorophyll content and abundance to variation in the abundance (median % cover) of large stones (LS) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 429). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values are quoted for non-normal variables compared using Kruskal-Wallis test. For details of environmental habitat conditions refer to Table 2.12 in Chapter 2.

Variable	0% SS		3% SS		15.5% SS		38% SS		63% SS		88% SS		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Vascular submerged macrophyte biomass per unit area (mg cm^{-2})	0.00		0.00		0.00		1.20		3.45		2.42		P<0.01**
Vascular submerged macrophyte chlorophyll content per unit area (μg cm^{-2})	0.00		0.00		0.00		0.85		1.02		0.90		P<0.01**
Vascular submerged macrophyte cover (%)	0.0		0.0		0.0		7.0		20.5		14.5		P<0.01**

Table 3.99 Mean values (± 1 standard error) of normally distributed plant variables (including '*no biomass*' zero values, and data back-transformed where necessary): response of vascular submerged macrophyte biomass, chlorophyll content and abundance to variation in the abundance (median % cover) of small stones (SS) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 429). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values are quoted for non-normal variables compared using Kruskal-Wallis test. For details of environmental habitat conditions refer to Table 2.13 in Chapter 2.

Variable	0% GR		3% GR		15.5% GR		38% GR		63% GR		88% GR		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Vascular submerged macrophyte biomass per unit area (mg cm^{-2})	0.00		0.00		0.00		2.30		3.75		1.00		P<0.05*
Vascular submerged macrophyte chlorophyll content per unit area (μg cm^{-2})	0.00		0.00		0.00		0.88		1.40		0.50		P<0.05*
Vascular submerged macrophyte cover (%)	0.0		0.0		0.0		13.5		18.5		10.0		P<0.05*

Table 3.100 Mean values (± 1 standard error) of normally distributed plant variables (including '*no biomass*' zero values, and data back-transformed where necessary): response of vascular submerged macrophyte biomass, chlorophyll content and abundance to variation in the abundance (median % cover) of gravel (GR) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 429). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values are quoted for non-normal variables compared using Kruskal-Wallis test. For details of environmental habitat conditions refer to Table 2.14 in Chapter 2.

Variable	0% SA		3% SA		15.5% SA		38% SA		63% SA		88% SA		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Vascular submerged macrophyte biomass per unit area (mg cm^{-2})	0.00		0.00		0.00		0.50		4.35		2.25		P<0.01**
Vascular submerged macrophyte chlorophyll content per unit area (μg cm^{-2})	0.00		0.00		0.00		0.38		1.30		1.10		P<0.01**
Vascular submerged macrophyte cover (%)	0.0		0.0		0.0		9.0		18.5		14.5		P<0.01**

Table 3.101 Mean values (± 1 standard error) of normally distributed plant variables (including '*no biomass*' zero values, and data back-transformed where necessary): response of vascular submerged macrophyte biomass, chlorophyll content and abundance to variation in the abundance (median % cover) of sand (SA) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 429). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values are quoted for non-normal variables compared using Kruskal-Wallis test. For details of environmental habitat conditions refer to Table 2.15 in Chapter 2.

3.5.20 Vascular submerged macrophyte production and environmental habitat conditions in Knockan Burn as determined by TWINSPAN classification

Plant communities I and II of the Knockan Burn sub-catchment stream were characterised by significant production and abundance of vascular submerged macrophytes, unlike sample-group I, which wholly lacked this sort of aquatic vegetation (Table 3.102, and also Figure 3.51). Consult Chapter 4, Figure 4.33 for details of the TWINSPAN classification here cited. See also Chapter 4, Table 4.102 and section 4.5.20 for details and discussion of variation in environmental habitat conditions between the TWINSPAN sample-groups.

	TWINSPAN sample-group						
Variable	**I**		**II**		**III**		**P_{ANOVA}**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Vascular submerged macrophyte biomass per unit area (mg cm^{-2})	2.07		1.48		0.00		P<0.01**
Vascular submerged macrophyte chlorophyll content per unit area (μg cm^{-2})	0.89		0.60		0.00		P<0.01**
Vascular submerged macrophyte cover (%)	13.2		7.9		0.0		P<0.01**
Bare area (%)	35.9	3.75	37.5	4.29	35.8	1.53	NS
Periphyton cover (%)	17.4	4.23	22.7	3.72	23.7	1.76	NS

Table 3.102 Mean values (± 1 standard error) of normally distributed plant variables (including '*no biomass*' zero values, and data back-transformed where necessary): vascular submerged macrophyte biomass per unit area (n = 79), vascular submerged macrophyte chlorophyll content per unit area (n = 79), vascular submerged macrophyte cover (n = 79), bare area (n = 79), and periphyton cover (n = 79), between TWINSPAN sample-groups I (n = 5) and II (n = 5), with the non-vascular submerged macrophyte sample-group III (n = 69) encompassing all other samples lacking aquatic macrophyte vegetation. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values are quoted for non-normal variables compared using Kruskal-Wallis test. Refer to Chapter 4, section 4.5.20 for TWINSPAN output (Figure 4.33) and details of environmental habitat conditions (Table 4.102).

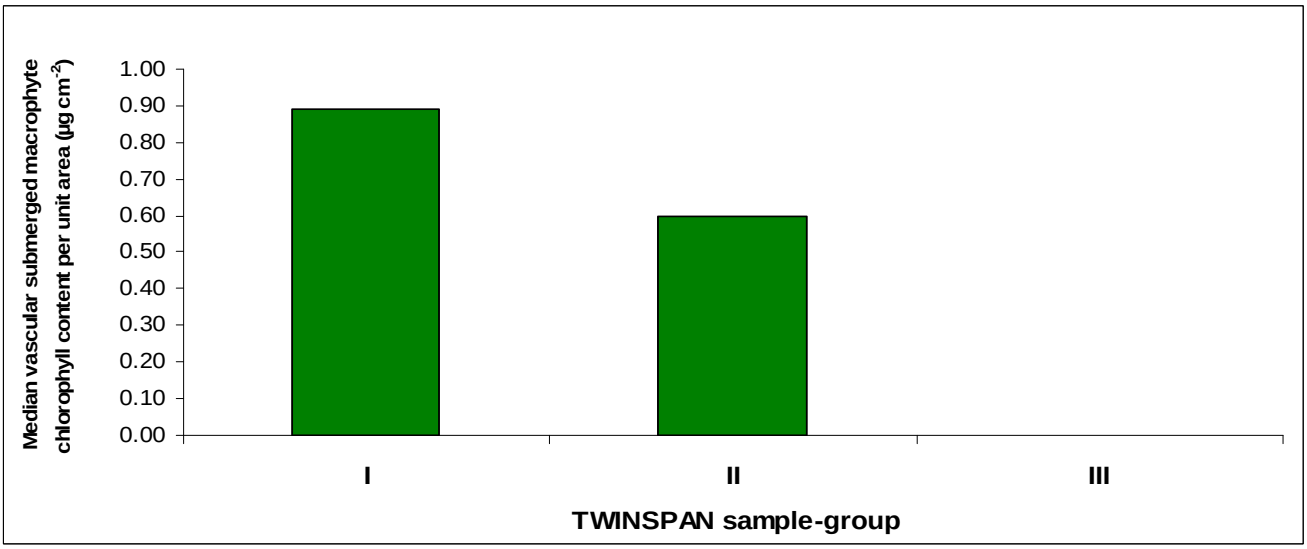


Figure 3.51 Comparison of mean values (± 1 standard error) of normally distributed plant variables (including '*no biomass*' zero values, and data back-transformed where necessary): vascular submerged macrophyte chlorophyll content per unit area (n = 79) between TWINSPAN sample-groups I (n = 5) and II (n = 5), with the non-vascular submerged macrophyte sample-group III (n = 69) encompassing all other samples lacking aquatic macrophyte vegetation.

3.5.21 Relationships between vascular submerged macrophyte production and environmental habitat conditions

Vascular submerged macrophyte production (biomass and chlorophyll content) and abundance were strongly and significantly positively correlated with each other, and negatively correlated with increasing bare area (see Appendix 2f). Overall, vascular submerged macrophyte production and abundance was positively correlated to increased underwater light availability, streamwater pH, conductivity and alkalinity, as well as to concentrations of calcium and magnesium cations. Generally, streambeds with diverse substrate morphology, particularly comprised of fine sized mineral substrate particles (e.g. gravel and sand) and of softer calcareous geology (e.g. Durness limestone) favoured the occurrence of vascular submerged macrophytes, unlike streambeds underlain with more resistant acid-sensitive geologies predominated by course substrates of impenetrable character wherein such aquatic vegetation did not tend to occur.

3.5.22 Predicting freshwater vascular submerged macrophyte production

Due to the limited size of the data set gathered on vascular submerged macrophytes in this research project, it was not appropriate to undertake multiple regression predictive modelling procedures.

Nevertheless, from other work that has been conducted herein, an indication is given that substrate morphology factors (e.g. predominance of fine sands) and base rich characteristics (e.g. increased streamwater pH, conductivity, concentrations of Ca^{2+} and Mg^{2+}) probably act as the principal environmental drivers in controlling vascular submerged macrophyte production and abundance, as shown from correlations (see Appendix 2f).

3.5.23 The three-tier approach to characterising upland stream habitat conditions by combining freshwater vegetation assemblages: periphyton, aquatic bryophyte and vascular submerged macrophyte production

To determine potential environmental drivers controlling differences in the functional response of freshwater vegetation assemblages, an integrated three-tier approach was utilised to characterise variation in primary production and abundance of freshwater vegetation in relation to stream habitat conditions by combining three groups of aquatic plants: periphyton, aquatic bryophytes and (where present) vascular submerged macrophytes.

3.5.23.1 Freshwater vegetation production and environmental habitat conditions as determined by TWINSPAN classification

Although TWINSPAN sample-group II was characterised by significantly lower biomass and chlorophyll content compared to the other two communities, there was no significant variation in chlorophyll production between freshwater vegetation assemblages I and III, despite the apparent significant difference in aquatic plant biomass (Table 3.103, and also Figure 3.52). Furthermore, although the overall abundance of plant cover did not vary significantly between the three assemblages, the composition of freshwater vegetation did (refer to Chapter 4, section 4.5.23.1). There was no significant difference in freshwater production or assemblage between vegetation sub-assemblages IIIa and IIIb (Table 3.104, and also Figure 3.53). Consult Chapter 4, Figure 4.35 for details of the TWINSPAN classification here cited. See also Chapter 4, Table 4.103 and section 4.5.23.1 for details and discussion of variation in environmental habitat conditions between the TWINSPAN sample-groups.

	TWINSPAN sample group						
Variable	**I**		**II**		**III**		**P**ANOVA
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Freshwater vegetation biomass per unit area (mg cm^{-2})	11.28^{a}	3.87	1.41^{b}	0.89	4.06^{c}	1.26	P<0.001***
Freshwater vegetation chlorophyll content per unit area (μg cm^{-2})	2.98^{a}	1.15	0.68^{b}	0.50	2.37^{a}	0.38	P<0.001***
Freshwater vegetation cover (%)	45.1	3.84	46.5	3.03	47.7	2.77	NS
Bare area (%)	38.6^{a}	2.27	33.1^{b}	2.30	37.8^{a}	1.89	P<0.01**

Table 3.103 Mean values (± 1 standard error) of normally distributed freshwater vegetation biomass per unit area, freshwater vegetation chlorophyll content per unit area, freshwater vegetation cover, and bare area (including '*no biomass*' zero values, and data back-transformed where necessary) between TWINSPAN sample-groups I (n = 25), II (n = 21) and III (n = 33): for combined periphyton, aquatic bryophyte and (where present) vascular submerged macrophyte assemblages (n = 79). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Refer to Chapter 4, section 4.5.23.1 for TWINSPAN output (Figure 4.35) and details of environmental habitat conditions (Table 4.103).

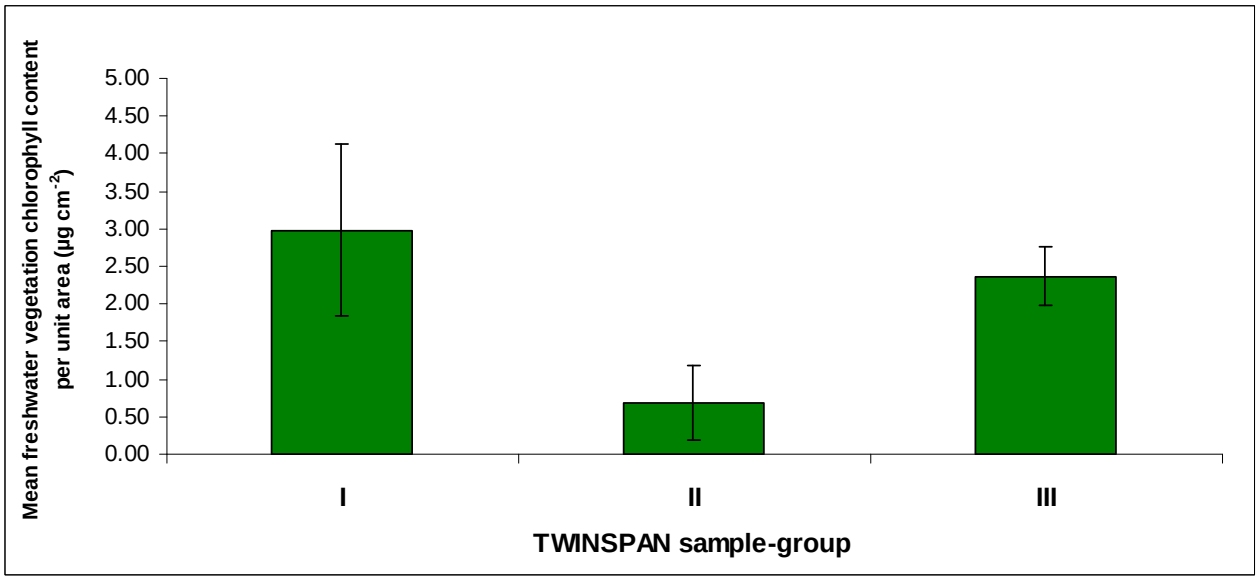


Figure 3.52 Comparison of mean values (± 1 standard error) of normally distributed (including '*no biomass*' zero values, and data back-transformed where necessary) freshwater vegetation chlorophyll content per unit area between TWINSPAN sample-groups I (n = 25), II (n = 21) and III (n = 33): for combined periphyton, aquatic bryophyte and (where present) vascular submerged macrophyte assemblages (n = 79).

Variable	TWINSPAN sub-assemblage IIIa		IIIb		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	
Freshwater vegetation biomass per unit area (mg cm^{-2})	4.34	2.83	3.78	3.29	NS
Freshwater vegetation chlorophyll content per unit area (µg cm^{-2})	2.56	0.58	2.18	0.45	NS
Freshwater vegetation cover (%)	50.5	3.41	44.9	3.53	NS
Bare area (%)	36.0	2.74	39.5	2.42	NS

Table 3.104 Mean values (± 1 standard error) of normally distributed freshwater vegetation biomass per unit area, freshwater vegetation chlorophyll content per unit area, freshwater vegetation cover, and bare area (including '*no biomass*' zero values, and data back-transformed where necessary) between TWINSPAN sample-group III sub-assemblages IIIa (n = 9) and IIIb (n = 24): for combined periphyton, aquatic bryophyte and (where present) vascular submerged macrophyte assemblages (n = 33). Significance testing: one-way ANOVA. Refer to Chapter 4, section 4.5.23.1 for TWINSPAN output (Figure 4.35) and details of environmental habitat conditions (Table 4.104).

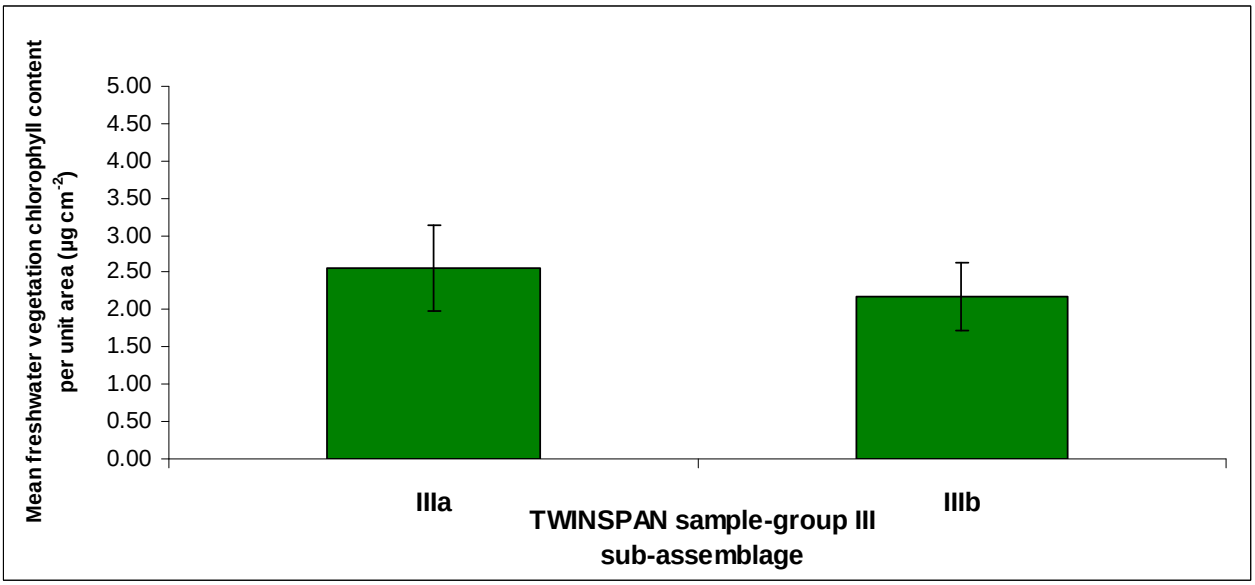


Figure 3.53 Comparison of mean values (± 1 standard error) of normally distributed (including '*no biomass*' zero values, and data back-transformed where necessary) freshwater vegetation chlorophyll content per unit area between TWINSPAN sample-group III sub-assemblages IIIa (n = 9) and IIIb (n = 24): for combined periphyton, aquatic bryophyte and (where present) vascular submerged macrophyte assemblages (n = 33).

3.5.23.2 Relationships between freshwater vegetation production and environmental habitat conditions

Freshwater biomass, chlorophyll content and abundance were positively correlated with each other (see Appendix 2g). Overall, freshwater production and abundance tended to be positively correlated to increased underwater light availability, and mostly negatively correlated to increased bare area, extent of riparian shade and certain geologies (e.g. Amphibolite, Serpentinite etc.).

3.5.23.3 Predicting freshwater vegetation production

Due to the small data set collected for vascular submerged macrophytes in this research project, it was not appropriate to integrate this information together with the more comprehensive data sets belonging to periphyton and aquatic bryophytes as tool for predicting freshwater vegetation vegetation production.

3.6 Discussion

3.6.1 Periphyton

3.6.1.1 Variation in periphyton production and environmental habitat conditions in the Water of Dye, River Girnock and Knockan Burn; their sub-catchments and sites

Mostly, periphyton production did not vary greatly between the three target streams and also on the whole, irrespective of the substrate type employed (save aquatic bryophytes). Notably substrata harvested from Knockan Burn and occasionally also the River Girnock, tended to accumulate higher quantities of periphyton biomass compared to the Water of Dye, yet chlorophyll production generally differed little between the three subcatchment streams. From field observations and laboratory notes, it was particularly obvious that this 'biomass' did not solely comprise live material and in fact, was a matrix of attached algae

and detritus (e.g. sand), typically of stream biofilms (Sládečková 1962, Biggs & Close 1989, Hill & Harvey 1990, Stevenson *et al*. 1996). In the lab, unlike other evident contaminants (e.g. plant fragments, macroinvertebrates, pieces of gravel) which could be easily spotted and removed using tweezers, it was impracticable to separate fine grained sediment from the periphytic algae present in the biofilm. Chlorophyll extractions are generally regarded as a more accurate measure of algal biomass (Stevenson *et al*. 1996), thus providing an indication of the proportion of photosynthetic and detrital material present in each of the harvested samples, in order to obtain a more reliable assessment of periphyton production. By and large, chlorophyll contents obtained for the attached algal communities from each of the three streams were similar, thus supporting prior discussion that differences in periphyton 'biomass' were mostly attributable to sediment contamination (though periphyton species composition varied markedly between the three streams: refer to Chapter 4, section 4.6.1.1).

The significant differences in the quantities of periphyton harvested from naturally-occurring aquatic bryophytes between the three target streams can easily be explained by variation in the morphology of species dominating the aquatic bryophyte vegetation at each sampling site. For example, parts of the River Girnock were mostly characterised by an abundance of turf mosses (e.g. *Schistidium*, *Blindia* and *Racomitrium*) which were small in form and surface area. By comparison, parts of Knockan Burn were dominated by canopy mosses (e.g. *Fontinalis antipyretica*), large in both form and surface area. The Water of Dye had a mixture of both cushion and trailing forms of mosses with variable surface areas. Typically, canopy mosses had larger, more complex surface areas which harboured greater quantities of periphyton, than smaller turfed forms in which periphyton colonisation was meagre. Therefore (apart perhaps from current velocity) it is principally the biomass and surface area available that controls the extent of epiphytic periphyton colonisation on stream bryophytes (Suren 1991, Muotka & Laasonen 2002), thus accounting for patterns of periphyton colonisation

(refer back to Table 3.16) which clearly reflect patterns of aquatic bryophyte production (see Table 3.73).

My findings suggest that there is a 'set-limit' to epilithic periphyton production in oligotrophic turbulent mountain headwaters in the Scottish Highlands, irrespective of pH (Winterbourn *et al*. 1992), which is governed by a combination of environmental factors, most probably flow disturbance, water temperature, light and nutrient (especially P) availability, a single or combined adjustment of which may act either to alleviate or further constrain the potential for algal production in these streams. Where appropriate and in turn, each shifting environmental constraining factor is addressed in subsequent sections of this chapter relating specifically to the ecology of attached algae in river habitats (see especially section 3.6.1.2). In contrast, the composition of periphyton species assemblages occurring in each of the three target streams was most strongly influenced by water chemistry (refer to Chapter 4, section 4.6.1.6), although the physical force of flow was also an important determining factor of algal microsuccession (refer to Chapter 4, section 4.6.1.2).

3.6.1.2 Temporal and seasonal variation in periphyton production and environmental habitat conditions in the Water of Dye, River Girnock and Knockan Burn

In general, both significant temporal and seasonal variation in periphyton production were observable in each of the three target streams. Throughout most of the sampling year, each stream was characterised by a background minimum or baseline of periphyton production (e.g. chlorophyll <0.05 μg cm^{-2}). However, distinct peaks or depressions in periphytic algal production usually occurred when flow contraints were alleviated (e.g. summer baseflows) or exacerbated (e.g. spring spates), respectively, and is subsequently discussed.

In my study, variation in periphyton production over time can largely be explained from changes in the community structure of the attached algae responding to alternations in environmental habitat conditions, which were especially apparent between sampling seasons (see Chapter 4, section 4.6.1.2). In frequently disturbed headwaters characterised by highly variable hydrological regimes, flow is probably the principal factor governing patterns of annual periphyton production in these low-order temperate streams. Current velocity is widely regarded one of the major environmental constraints of periphyton production in river systems (e.g. Weitzel 1979, Biggs & Close 1989, Peterson & Stevenson 1992, Biggs 1995, Biggs & Thomsen 1995, Biggs *et al*. 1998). The physical stress of shear and drag forces exerted by high-velocity flood events was particularly evident during marked spates in the early spring (refer back to Chapter 2, section 2.6.2). Major discharges such as these were capable of dramatically reducing periphyton production (Horner *et al*. 1990, Lohman *et al*. 1992, Biggs & Thomsen 1995) as a result of profound scouring effects on the community of attached algae (McIntire 1966b, 1968, Horner & Welch 1981, Peterson & Stevenson 1992, Biggs 1995, Biggs & Thomsen 1995, Biggs *et al*. 1998). Flow-pertubations interrupt community succession in periphytic algae by selecting for a predominance of pioneer growth forms adapted to stress or disturbance (SR-strategists: Grime 1979) such as tightly adhering prostrate morphologies (e.g. *Cocconeis placentula*, *Acthnanthidium minutissimum*), conveying the necessary resilience attributes for enduring high-velocity scour events (Peterson & Stevenson 1992, Blenkinsopp & Lock 1994, Biggs 1995, Biggs & Thomsen 1995, Stevenson *et al*. 1996, Passay 2007). Stalked, filamentous or other mature canopy-forming morphologies of loosely attached diatoms and green algae (e.g. *Gomphonema* sp., *Tabellaria* sp., *Mougeotia* sp., *Spirogyra* sp.) protrude into the water column, tending to be less hydraulically stable and more susceptible to becoming dislodged under high flows exceeding the threshold capacity (Hynes 1970, Steinman *et al*. 1991, Uehlinger 1991, Peterson & Stevenson 1992, Biggs 1995, Biggs & Thomsen 1995, Stevenson *et al*. 1996, Biggs *et al*. 1998).

Throughout most of the sampling year, each stream was characterised by a background minimum or baseline of periphyton production consisting of a thin layer of biofilm (usually, <2 mm thick) dominated by low-profile non-filamentous diatoms (usually, >90% of the total population sampled). This indicated that periphytic algal succession was stalled in the pioneer phase (or early colonisation stage) by frequent flow disturbances and high-velocity spates (Biggs 1995, Biggs & Thomsen 1995, Stevenson *et al*. 1996), thus constraining periphyton production to a minimum during the autumn-winter-spring period.

However, during summer baseflow conditions a notable peak in periphyton production characterised each stream, corresponding to variation in local climatic and hydrological patterns (refer back to Chapter 2, section 2.6.2). Generally, it is not coincidental that periphyton production reached its highest point during the summer when streamwaters were shallower and warmer, but essentially flow had fallen below a critical threshold lifting the major restraining factor governing the potential production and species composition (see Chapter 4, section 4.6.1.2) of the periphyton communities. Only during this period of more stable flow conditions did other interacting environmental factors (e.g. underwater light availability, water temperature) enter into play and become important secondary factors (by influencing rates of enzyme activity, metabolic processes, cell division, reproductive cycles) coupled to driving succession, stimulating photosynthetic activity and thereby biomass accrual of the attached algae (Hill 1996, Stevenson *et al*. 1996). This marked increase in periphyton production reflects an ecological shift in community composition attributable to an increase in the abundance of green filamentous algae (e.g. *Mougeotia* sp., *Ulothrix* sp., *Spirogyra* sp.). This indicated that a climax algal community had developed during the low flow summer months, contributing significantly to the biomass of the whole community of attached algae (Biggs 1995, Stevenson *et al*. 1996). Even so, diatoms remained a substantial component (usually >70%) of the algal population though the abundance and diversity of diatom species were usually higher during the summer than during peak flow periods (see Chapter 4, section 4.6.1.2). As

chlorophytes, green filamentous algae generally have high physiological requirements to sustain photosynthesis and growth (Rier *et al*. 2006). Therefore conditions of elevated light and warmer temperatures appeared to favour optimal production of green algal filaments. This is consistent with findings from manipulative laboratory stream experiments which also indicated that the growth of chlorophytes required high irradiance (e.g. Steinman & McIntire 1987, Lamberti *et al*. 1989, Steinman *et al*. 1989), and other field studies (e.g. Lowe *et al*. 1986, Duncan & Blinn 1989, Wellnitz *et al*. 1996, Mosisch *et al*. 2001, Kiffney *et al*. 2003). Under warmer, sufficiently lit conditions aggregations of loosely-attached green filamentous algae grew most profusely in the streams, with trailing floating mats often extending several centimetres, sometimes metres in length in the most slow-flowing waters or standing pools (see Figure 3.54). Similar findings have been described elsewhere (e.g. Biggs & Thomsen 1995). This also explains why green algal filaments occurred least abundantly at other times of the year (characterised by cooler, flashier streamwaters during shorter daylengths) and tended to disappear under conditions of heavy shade from riparian vegetation. Leafy canopies of riparian vegetation can potentially intercept >95% of the incoming photosynthetically active radiation, reducing both the quality and quantity of light reaching the streambed and capable of substantially suppressing periphyton production (DeNicola *et al*. 1992, Hill *et al*. 1995, 2001). Similar results have been found when heavy shade by dense forest canopies has been replicated artificially in manipulative stream experiments (e.g. Bourassa & Cattaneo 2000, Kiffney *et al*. 2004), or confirmed from measurable increases in periphyton production reponding to clear-cutting of riparian buffers (e.g. Noel *et al*. 1986, Boothroyd *et al*. 2004). Most forms of green filamentous algae are particularly sensitive to changes in light intensity from shade pressure because they lack the accessory pigments possessed by diatoms, which tend to be more tolerant of low irradiances (Lowe *et al*. 1986, DeNicola *et al*. 1992, Bourassa & Cattaneo 2000) and capable of photoacclimation (Rier *et al*. 2006). Confirming this speculation, Littlemill on the lower River Girnock was characterised by heavy shade from tall broadleaf trees, especially during the late spring and summer when incoming light was further

reduced by growth and expansion of the riparian canopy several metres above the streambed. Consequently, at Littlemill periphyton production tended to be lower (e.g. Table 3.15) compared with unshaded upstream sites in the River Girnock (e.g. Iron Bridge, Hampshire's Bridge). From microscopic analysis I concluded that in particular heavy shade suppressed the abundance of green algal filaments (e.g. *Mougeotia*) which were present in the periphyton population but usually considerably less abundant at Littlemill compared to occurrences at the unshaded sites further upstream. In contrast, diatom community composition appeared to be largely unaffected by changes in light intensity and appeared tolerant of low irradiances. Furthermore, an assortment of diatom morphologies (e.g. prostrate, stalked and filamentous) dominated the biofilm in heavily shaded low flow microhabitats from which green filaments were excluded. This suggested that in this particular instance current velocity was not the major restraining factor, and instead severe light limitation was responsible for preventing canopy growth of loosely-attached green filaments from becoming properly established. Supporting this deduction, similar findings have been reported in laboratory stream studies (e.g. McIntire & Phinney 1965, Steinman & McIntire 1987). Generally, temporal patterns in periphyton growth in each of the streams (consult: Table 3.10, Table 3.19, and Table 3.28) followed trends similar to variation in sunshine hours (refer to Appendix 3c, 3d) and air temperatures (see Appendix 3e, 3f) but on the whole, inversely related to precipitation inputs (see also Appendix 3a and 3b). Unlike the suggestions of the River Continuum Concept (Vannote *et al*. 1980), throughout my study I found that in low order streams of the Scottish Highlands shade from riparian vegetation often increased downstream, with sampling sites stationed nearest to the source characterised mostly by perennial shrubby vegetation (e.g. heather, gorse) or bracken, downstream towards the lowlands where tall woodland trees grew (e.g. Scots Pine, Alder, Birch, and Willow). Where streams were sufficiently wide, riparian shade was mostly restricted as an 'edge effect', akin to the RCC (Vannote *et al*. 1980), but capable of encroaching on the inner channel of particularly narrow streams (e.g. Brocky Burn) or those bordered by

thick forest (e.g. Littlemill), although the extent of shade cast varied seasonally (e.g. Hill *et al*. 2001, Hill & Dimick 2002).

In shaded streams it has been shown that additional pulses of nutrients are unlikely to exert a profound effect on periphyton growth because they cannot be utilised when photosynthesis is limited by an inadequate supply of light (e.g. Lowe *et al*. 1986, Hill & Knight 1988a, Hill *et al*. 1995, 2001, Larned & Santos 2000, Mosisch *et al*. 1999, 2001). However, in unshaded nutrient-poor streams periphyton production has been shown to respond appreciably to nutrient enrichment (Hill & Knight 1988a, Hill *et al*. 1992b, Rosemond *et al*. 2000, Mosisch *et al*. 2001). A rare finding by Hill & Fanta (2008) was that both light and nutrients (phosphorus) appeared to co-limit periphyton growth in flow-through laboratory streams at sub-saturating irradiances. However, the assemblages studied were dominated by diatoms because controlled conditions of low irradiance prevented formation of a climax community (expected to be characterised by green filamentous algae with higher nutrient demands). This underpins P as contributing a secondary role to light in restraining periphytic algal production in shaded, nutrient-poor streams (Hill & Fanta 2008). Together the results of the aforementioned studies support the theory that in oligotrophic streams, notwithstanding the effects of flow velocity, light controls the potential for periphyton production in the absence of shade from riparian vegetation and ascertains that the supply of nutrients is also of considerable importance.

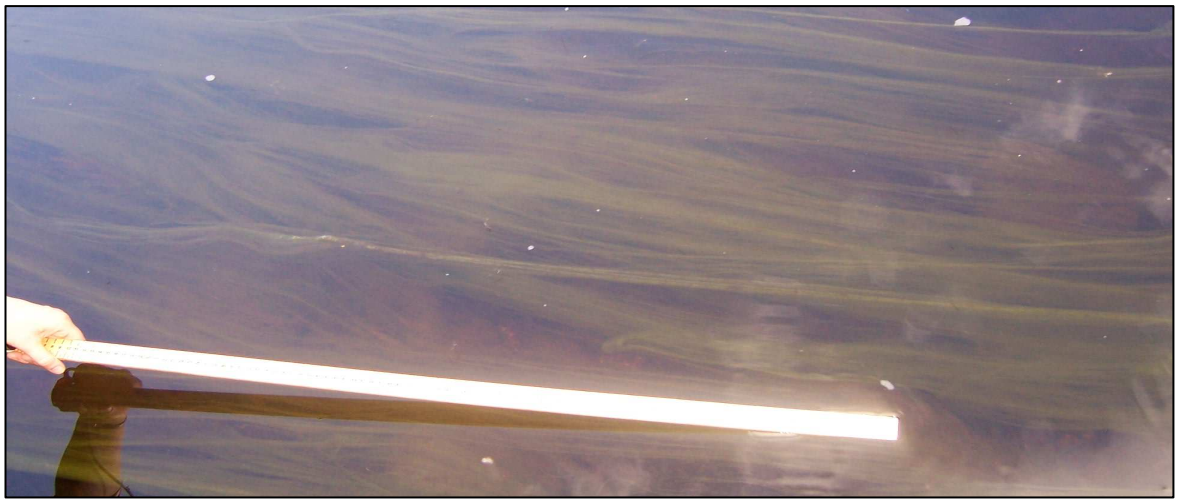


Figure 3.54 Extensive growth of green filamentous algae during summer baseflow conditions

Selective grazing by stream macroinvertebrates may become an important natural disturbance mechanism of stream periphyton communities particularly under low flows. Grazers can substantially reduce the density of species of attached algae in the loose overstorey, preferencing the occurrence of scour-resistant taxa and thereby capable of suppressing community biomass (e.g. Feminella *et al*. 1989). The low standing crops of intensely grazed periphyton assemblages can commonly resemble algal communities exposed to high flow disturbance. Thus similar to the effects of high velocity floods, herbivory interferes with natural successional progression in stream periphyton communities (e.g. Lamberti & Resh 1983, Lamberti *et al*. 1989, Steinman *et al.* 1989, Steinman 1992, Hill & Knight 1987, 1988b, Hill & Harvey 1990, Hill *et al*. 1992b, Marks *et al.* 2000, Jones *et al*. 2000a) and algal regrowth (e.g. Wellnitz & Poff 2006). A number of studies have employed manipulative experimental approaches to uncover grazer–periphyton interactions using in-situ exclosure channels or randomised block treatments to control the incidence and densities of herbivores (e.g. Hill & Knight 1987, 1988b, Hill & Harvey 1990, Hill *et al*. 1992b, Rosemond *et al*. 2000, Jones *et al*. 2000a). Yet, grazer-periphyton interactions are often found to be more complex than simple and straightforward especially in studies wherein levels of light (e.g. Lamberti *et al*. 1989, Steinman 1992, Wellnitz & Ward 1998) and/or nutrients (e.g. Mulholland *et al*. 1991, McCormick & Stevenson 1989, 1991) have been manipulated. Also interactions between different grazers can further complicate matters, though few papers (e.g. McAuliffe 1984) have examined resource competition in stream herbivores as it has been more common practice to manipulate abundance of a single grazer in experimental stream studies rather than attempt to control the whole macroinvertebrate community present. From my sample observations, the community composition of macroinvertebrates varied between each of the three target streams. Amongst other macroinvertebrates each stream often contained mayfly and stonefly nymphs as well as cased-caddisfly larvae (ecological indicators of good water quality). Had time not been limiting then it may have been useful to identify grazers to a least family level, quantify their relative abundance, categorise them into feeding guilds (e.g. shredder, filterer, etc.) and

examine gut contents to ascertain feeding preferences. However as the assemblage and abundance of macroinvertebrates was not incorporated into this particular study, it is unknown whether grazing pressure exerted significant impacts on the standing crop and community structure of stream periphyton in each of the three target streams. Therefore although I accept there will have been some unquantified losses of periphyton production liable to consumer-limited growth throughout the course of this study, other research has proven that biotic controls are overridden by the physical effects of flow and light disturbance (e.g. Kiffney *et al*. 2004), and I will therefore not discuss this topic further.

Subsequently the onset of heavy precipitation in the early autumn invoked variable high flows which terminated maximal periphyton production and the reign of canopy morphologies which had characterised the climax community of the summer months. At this time detached clumps of green algal filaments were frequently observed floating downstream and were usually the first indication of heightened flows. This emphasizes the overwhelming scour effects of fluctuations in current velocity on the abundance and species composition of attached algae (Peterson & Stevenson 1992, Biggs & Thomsen 1995, Biggs *et al*. 1998). In the approach to winter, most substrates had been scoured clean after flooding leaving behind remnants of a low growth form diatom-dominated community. This indicated succession had been reset to an early colonist phase (Biggs & Close 1989, Stevenson *et al*. 1996), which characterised minimal background periphyton production and persisted year round as a thin brownish coating upon the surfaces of submerged substrates in each of the streams.

Similar patterns of seasonal cycling in periphyton communities have been depicted for frequently disturbed streams elsewhere (e.g. Antoine & Benson-Evans 1985, Biggs & Close 1989, Uehlinger 1991, Lohman *et al*. 1992, reviewed in Stevenson *et al*. 1996). Overall, the disturbance regime, a function of catchment climate and hydrology, is a fundamental determinant of attached algae community biomass and there seems to be an interchangeable dominance in the

single or multiple combined environmental factors affecting periphyton production in near-pristine upland streams. Furthermore, the relative magnitude of environmental factors constraining stream periphyton production shifted seasonally (Rosemond *et al*. 2000). Principally, it is a shift in dominance from flow disturbance (predetermined by the prevailing climate and catchment hydrology) which is the major governing factor of stream periphyton production (Biggs *et al*. 1998, Elósegui & Pozo 1998) and determines whether other secondary interacting environmental factors become engaged in controlling the potential for these attached algal communities to accumulate large standing crops. Regulated rivers characterised by stable flow regimes lack distinct spate episodes and often harbour higher quantities of periphyton biomass compared to naturally frequently-disturbed streams (Uehlinger *et al.* 2003). However, notwithstanding 'top-down' disturbance (e.g. flow, grazing) to photosynthesis and succession, then it is probable that a nutrient deficiency would establish the upper limit of periphyton production in oligotrophic streams, and further that this control is more P-limited than N-limited. This consensus is based on the fact that all three of the target streams of this study were characteristically nutrient-poor and experienced a P-loading phenomenon during major spate events, particularly characteristic of UK upland-peat headwater regions during the early spring (e.g. Turner *et al*. 2003, Ellwood *et al*. 2008). In my study evidence of P-limitation exerting 'bottom-up' control on periphyton production was recorded during April 2006, from observations of biomass accrual in the form of a subsidiary algal bloom, or at least that periphyton production was slightly higher than expected for comparably high-flow episode wherein P-inputs were known to be near-negligible. Although, the extent of these P-inputs will likely vary between years, the general timing remains a phenomenon associated with the early spring melt. Periphyton growth was quite extensive in the River Girnock during April 2006 as indicated not only from laboratory investigation (refer back to Table 3.26) and notes taken by me in the field, but also which (unknowingly at the time) coincided with observations of "*pronounced filamentous algal growth*" made by colleagues as a common feature of this river during the spring period in recent years (C. Soulsby,

pers. comm.). This suggests that underlying these observations there is a climatic driver affecting the abundance of periphytic algae in upland stream habitats in the Scottish highlands. Most probably as our weather becomes milder yet wetter due to global climate change, increased precipitation is expected to enhance the release of phosphate from peaty upland soils draining into headwater streams (Whitton *et al*. 2009). In each of the three target streams, the filamentous chlorophyte *Stigeoclonium* tended to become more abundant during the early spring flushes, and production of *Stigeoclonium* has been shown elsewhere to increase response to nutrient (N, P) enrichment when light was in sufficient supply (e.g. Fairchild *et al*. 1985, Chessman *et al*. 1992, Marks *et al*. 2000). However with particular reference to the River Girnock, my theory is perhaps more strongly reinforced by the fact that a dominance of *Rivularia* characterised the periphytic algal community in this stream and was accountable for the notable bloom during the spring flush of 2006, when streamwaters were slightly enriched with phosphate. *Rivularia* is a filamentous cyanobacteria capable of utilising P when it becomes more readily available in the environment (Turner *et al*. 2003). Similarly, Mundie *et al*. (1991) found that treating experimental troughs with P additions stimulated an increase in chlorophyll production attributed to an ecological shift from diatoms (in control troughs) towards a community dominated by the cyanobacteria, *Oscillatoria* (in P-enriched troughs). Therefore although the genera of cyanobacteria differ between my study and that of Mundie *et al*. (1991), the underlying principle is essentially the same; that N-fixing cyanobacteria are unable to effectively compete with diatoms at low phosphate levels but will be expected to increase in abundance when this particular nutrient restraint is alleviated. Therefore there may be evidence that at certain times of the year nutrient enrichment via P-inputs can over-ride or at least, counteract the negative effects of high current-velocities on periphyton production (Lohman *et al*. 1992). Furthermore probably only when the supply of inorganic P has become saturated and other physiological requirements (light, temperature) are unlimited, will N become a crucial tertiary factor limiting to stream periphyton production, reflecting the highly oligotrophic character of the three target streams studied. However, having been the centre of long-

standing debate, the overriding importance of either P or N cycling to periphyton production is complicated given interactions with other factors (e.g. flow, shade, herbivory) and is generally not a well understood aspect of stream ecology. Some studies have attempted to unravel this but often unsuccessful in doing so, concluding that there is no clear-cut answer and raising more questions or identifying areas requiring more precise research. A handful of manipulative stream studies using nutrient-diffusing substrates have indicated that often P appeared to be secondarily limiting compared to N (e.g. Hill & Knight 1988a, Lohman *et al*. 1991, Chessman *et al*. 1992), whilst others pinpoint P as the overriding constraining nutrient in streams (e.g. McCormick & Stevenson 1989, Ghosh & Gaur 1994, Larned & Santos 2000). The answer to the contentious question "which macronutrient is most limiting to primary production in streams, N or P?" probably depends upon catchment land-use and other spatially variable catchment processes (e.g. Irvine & Jackson 2006). For example streams draining agricultural catchments will receive artificially-enhanced P-inputs and therefore may be more N-limited (e.g. Biggs 1995, Mosisch *et al*. 1999), whereas in relatively unimpacted catchments P-limitation is expected to be the major constraining factor over and above N-availability (e.g. Ghosh & Gaur 1994, Larned & Santos 2000). Collectively the evidence of resource limitation in stream periphyton is that as a rule of thumb, P is probably the overriding limiting nutrient except where in plentiful supply and then N-limitation will become more important in constraining the extent of further production.

Overall, profound variation in periphyton production reflected ecological shifts in the community composition of the attached algae, with green filamentous morphologies contributing to a substantial proportion of the biomass during the summer months and diatoms accounting for low standing crop throughout most of the sampling year (Welch *et al*. 1988, Lohman *et al*. 1992, Biggs 1995, Stevenson *et al*. 1996). Periphyton community structure and therefore standing crop responded to interchangeable environmental factors, the prevalence of which varied seasonally, but consistently across the three target streams (though species

composition differed in relation to water chemistry: Chapter 4, section 4.6.1.6). Physical flow disturbance was the dominant environmental factor responsible for structuring community composition and standing crop of attached algae throughout the sampling year. However, other environmental factors were of importance on certain sampling occasions, mostly when current velocity restraints were slackened (e.g. light intensity, temperature) although this was not consistently the case (e.g. P-enriched spring spates).

3.6.1.3 Response of periphyton production and environmental habitat conditions in the Water of Dye, River Girnock and Knockan Burn to variation in flow regime: pool, glide and riffle zones

It is not a novel finding that high current velocities scour periphyton and thereby can significantly reduce production (e.g. Biggs & Thomsen 1995, Biggs *et al*. 1998). My findings also show that to an extent periphyton communities were able to resist shear effects under moderate flow conditions (glides) and were mostly similar in terms of maintaining an overall community composition to assemblages congregating in pools despite at least a 35% loss of biomass. However, under extremely fast-flows periphyton community structure changed substantially, with high-velocity scours selecting for an abundance of firmly-attached adnate, prostrate and stalked morphologies adapted for surviving flood disturbance (e.g. *Achnanthidium minutissimum*, *Cocconeis placentula*) by plucking-out vunerable stalked or filamentous forms of algae, contributing to at least a further 35% loss in biomass compared to glides, and at least a 60% loss compared to pools. However despite notable changes in biomass and diversity (see Chapter 4, section 4.6.1.3) of periphytic algae, chlorophyll production did not seem to vary significantly between the three basic flow patterns. This suggested physiologically that either periphytic photosynthesis was unaffected by differences current velocity, or perhaps more feasibly that a contrasting 'trade-off' of environmental factors curtailed periphyton production in pools and riffles. In slow-flowing pools periphytic photosynthetic activity was probably hindered by lower rates of carbon

diffusion due to thickened boundary layer forming around biofilms under low-velocity conditions. Also lofty stalked or trailing filamentous forms of algae and detritus (forming part of the 'biomass') lodged in the overlying canopy layer of the periphyton matrix may attenuate light and cause diffusional resistance of nutrients to the understorey, thereby potentially limiting photosynthesis in particularly slow flowing waters (Boston & Hill 1991, Peterson & Stevenson 1992, Stevenson *et al*. 1996). Under such conditions competitor (C-strategist) traits (e.g. for exploiting or controlling access to light or nutrient sources in limited supply) may become more apparent (Stevenson *et al*. 1996). In highly turbulent riffles, C-supply would be expected to be sufficient for photosynthesis by thinning the boundary layer thus encouraging diffusion of gases and nutrients (Stevenson 1983, Stevenson *et al*. 1996, Biggs *et al*. 1998). For example, Kevern & Ball (1965) found that in artificially-constructed streams periphyton production tended to be higher in riffles than pools. However, with reference to the findings of my study (see again: section 3.5.3) it was more likely that fast-flows may have stimulated photosynthetic activity yet concurrently constrained production by sloughing-off algal material particularly more vulnerable forms (e.g. green filaments) in the canopy layer as well as older scenescing cells and detritus (Peterson & Stevenson 1992, Biggs & Stokseth 1996, Biggs *et al*. 1998, Ghosh & Gaur 1998). It is also quite possible that better adapted disturbance-resistant forms persevered and held their own in terms of growth when competitors had been more or less excluded from the niche under fast-moving flows (Biggs *et al*. 1998). For example, polysaccharide mucilage production enables some stalk-forming diatoms (e.g. *Cymbella*, *Gomphonema*) at high current velocities to manipulate their own microhabitat and protect against further scour by forming a hydraulic shield against the effects of surface friction and drag (Biggs & Hickey 1994, Dodds & Biggs 2002). Glides probably offered an intermediate microhabitat or 'half-way house' (amid pools and riffles) for attached algal communities with respect to flow conditions. Thus shear effects of increasing current velocity almost certainly accounts for the patchy growth of periphytic algae between flow patterns (Biggs *et al*. 1998).

3.6.1.4 Response of periphyton production and environmental habitat conditions in the Water of Dye, River Girnock and Knockan Burn to variation in substrate morphology

Overall, periphyton production appeared not to respond significantly to changes in the physical composition of substrate particles present in the streambed, and furthermore became established on all substrate samplers used in the study (see 3.6.1.5). Any changes in the unvegetated (bare) area were mostly attributed to the response of aquatic bryophytes which grew more abundantly on stable streambed structures such as large boulders and tended to leave unstable niches open to colonisation by other stream producers (see section 3.6.2.4). By comparison, at least a thin layer of periphyton biofilm coated the surface of every available submerged substrate irrespective of size or type (Stevenson *et al*. 1996), though species-assemblages formed were distinctive to prevailing water chemistry (Chapter 4, section 4.6.1.1) in each of the three streams sampled. On the whole, stream periphyton growth was transient, patchy and strongly constrained by localised flow patterns (refer back to 3.6.1.3).

3.6.1.5 Comparison of periphyton production and environmental habitat conditions between artificial and naturally-occurring substrata: do artificial substrates make good surrogates for naturally-occurring microhabitats?

Overall, artificial substrate samplers showed similar patterns of variation in periphyton production in respect to most aspects of the study in which they could be compared. However, the majority of naturally-occurring substrates accumulated significantly higher quantities of periphytic biomass and chlorophyll content than did their respective surrogate microhabitats. There may be several possible explanations for this, which are here discussed.

The first important point to emphasize is that differences in environmental habitat conditions cannot account for differences in periphyton production between the various types of artificial substrates and naturally-occurring microhabitat sampled

throughout the course of the study, as these were mostly similar (refer back to section 3.5.5). The notable exception was stands of vascular submerged macrophytes associated with higher streamwater pH and conductivity (compared to plastic aquarium plant samplers) in Knockan Burn. This is explained by the fact that dense beds of aquatic vegetation are capable of profoundly modifying the water chemistry of their surrounding environment whilst undertaking photosynthesis during daylight hours (Carpenter & Lodge 1986). Therefore the findings of this particular study mostly omit significant differences between substrate types as attributable to variation in environmental habitat conditions, meaning that other more feasible possibilities must be considered.

Secondly, I am not simply observing a contaminant 'sediment effect', which admittedly interfered with some of the periphyton biomass results in this study (particularly Knockan Burn samples – see again comments in section 3.6.1.1) but did not affect chlorophyll measures obtained. However, overall periphyton production (both biomass and chlorophyll content) harvested from naturally-occurring substrates was significantly higher compared to quantities obtained from artifical substrate samplers. A feasible explanation may be that although periphyton community composition of the surrogate samplers closely-resembled those harvested from their respective naturally-occurring microhabitats, the relative abundance of algae was usually greater on surfaces of the latter (data not shown) and thus could account for the higher levels of production obtained.

Thirdly, together the data suggest that naturally-occurring microhabitat surfaces are exposed to similar environmental habitat conditions yet have the propensity to gather a greater densities of algal cells than do artificial substrate samplers attempting to mimic them. One reason may be that naturally-occurring substrates were exposed to periphytic colonisation for an infinitely longer period and thus their surfaces are inclined to accumulate greater quantities of algal cells compared to artificial samplers. However, I am not inclined to argue this point because at initial inspection my findings suggest that length of colonisation period can

probably be ruled out as a factor controlling the abundance of algal cells as short-term and long-term studies yielded similar results – that yes, periphyton communities do show ecological succession but that this is radically perturbed by high-velocity scours, resetting community development to early successional stage on the majority of substrates sampled. I do however recognise that I did not 'prime' any of my selected artificial substrates prior to inset in each of the target streams, meaning that surrogates placed instream were essentially 'bare' and surfaces required time to develop a preliminary biofilm coating of which naturally-occurring substrata would already be conditioned with a polysaccharide matrix embedded with aufwuchs, so one might reason this is why naturally-occurring substrates possess higher densities of algal cells. I decided against priming my artificial samplers as although pre-conditioning substratum with agar can speed up colonisation rates (by mimicking polysaccharide biofilm produced by microbial bacteria), it can also inadvertently act to select against the abundance of specific taxa (Peterson & Stevenson 1989). Furthermore, an initial exposure period of at least 3-4 weeks is usually considered an ample exposure period to gather a periphyton flora representative of naturally-occurring microhabitat (Aloi 1990, Kelly *et al*. 1998, 2001), and was found to be sufficient in this study.

Fourthly, if one reasons that neither variation in environmental habitat conditions or exposure period is particularly helpful in explaining differences in periphytic algal production between the sampled substrata, then perhaps it is worth considering variation in the surface texture (e.g. roughness, porosity, micro-crevices, 3-D structure, refuge opportunities, boundary layer) and/or biotic interactions with chemical microgradients (e.g. mineral composition) of naturally-occurring substrata? The surface microtopography of naturally-occurring substrata is often more heterogenous than the surfaces of artifical samplers. Therefore the coarse physical characteristics of naturally-ocurring substrata (e.g. stones) may provide better-quality attachment sites for the sustainable production of periphytic algae in streams and rivers (Nielson *et al*. 1984). Another critical feature of substratum surface texture to contemplate is the role of micro-crevices

in protecting diatom-dominated assemblages from the effects of grazing. For example, often small-sized crevices exclude grazers and provide refuge for diatoms, whereas larger crevices tend to expose diatoms to intense grazing pressure, although the results obtained depend mostly upon grazer size, morphology and foraging behaviour (Bergey & Weaver 2004, Bergey 2005). Thus up to now I have established that variation in surface texture can profoundly influence the composition and abundance of periphyton. However, I also wish to explore the extent to which benthic algal communities may have been affected by differences in the geochemical composition of naturally-occurring mineral substrate particles. In my opinion, the recent work of Bergey (2008) provides a definitive answer to this question. She adopted an experimental approach which used diffusing substrates comprising various types of powdered rock to test the effects of chemical composition on periphyton production, but crucially at the same time, eliminated the effects of substrate texture from the study. The results were undisputed and in keeping with previous findings (e.g. Bergey 2005, 2006), that periphyton production was unaffected by the chemical composition of various rock types, underpinning the strong effect of surface roughness on algal biomass accrual. Therefore a fine-scale technique (e.g. Bergey & Getty 2006) which would have permitted the combed measurement and comparison of surface roughness of the various types of substrates utilised may have been more helpful in fully explaining biomass variation between naturally-occurring and artificial samplers in my study.

Fifthly, compared to naturally-occurring mineral substrata and surrogate linoleum samplers, both naturally-occurring aquatic bryophytes and surrogate Astroturf sampler bristles possessed higher quantities of periphyton production (biomass and chlorophyll content), respectively. Due to their inherently large and complex surface areas, canopy forming moss species (e.g. *Fontinalis antipyretica*, *Platyhypnidium riparioides*) tend to retain high quantities of periphyton and detritus, whereas small turfs (e.g. *Blindia*, *Schistidium*, *Racomitrium*) often trap the least (Suren 1991, Muotka & Laasonen 2002). Functionally similar to vascular

submerged macrophytes (see section 3.6.3.3), the high surface area (Wetzel 1983) and entangled matrices of aquatic bryophyte vegetation act as an effective sediment trap but additionally within their foliage create 'hydraulically quiescent' microhabitat for other lotic organisms such as periphyton and macroinvertebrates by altering near-bed flow regimes (Suren 1991, Lancaster & Hildrew 1993, Nikora *et al*. 1998). By the same principle, Astroturf segments were functionally similar to aquatic bryophytes due to their dense and complex bristle structure which provided a large surface area for trapping sediment particles and shelter from flow. This maintained laminar sub-layer flow (Smith 1975) can explain why higher quantities of periphyton and detritus were trapped in aquatic bryophyte foliage and Astroturf bristles compared to the surfaces of unvegetated substrata (naturally-occurring mineral particles and linoleum samplers), agreeing with similar findings reported elsewhere (e.g. Pentecost 1991, Suren 1991). Furthermore, this 'shielding effect' from flow may also explain why periphyton growth on aquatic bryophytes (common to boulder-riffle zones) harboured levels comparable to that harvested from plants occurring in slow-flowing pools (for example, see Table 3.46).

There may also be another interesting benefit to consider besides the effect of physical shelter regarding the interaction of epiphytic periphyton with aquatic macrophytes. That is the assimilation or exchange of simple sugars, metabolites and nutrients with plant foliage upon which epiphytes accrue (Wetzel 1983). This could possibly be tested experimentally by controlling flow regime and utilising either artifical nutrient-diffusing plastic aquarium plants or growing real aquatic macrophytes in pots of various nutrient treatments. However this may not yield straightforward results, if one anticipates complex interactions of sugars and nutrients with photosynthesis, biochemical signals and other metabolic processes occurring in live plants. In contrast, the neutral substrate theory (Cattaneo & Kalff 1979) upholds the view that aquatic plants are neutral attachment sites for epiphytes and do not offer other benefits than mainly refuge. Cattaneo & Kalff (1979) found that quantities of periphytic chlorophyll a sampled from the surfaces

of plastic plants and foliage of *Potamogeton richardsonii* was comparably similar. This concept infers that aquatic plants contribute negligibly to the production of epiphytes, which tends to agree with the findings of my study (specifically regarding periphyton harvested from vascular submerged macrophyte surfaces in Knockan Burn). However, perhaps too few samples were collected thus hiding any real significance in the Knockan Burn periphyton-macrophyte dataset, or that the switch between competitive or mutualistic interactions were masked by changes plant development (e.g. growth, scenescence) reponding to environmental cues. Some vascular submerged macrophytes (e.g. *Myriophyllum spicatum*) are capable of releasing allelopathic chemicals that may inhibit or suppress epiphytic growth upon their foliage (Hilt 2006). However, no evidence for or against this can be shown from my study.

A number of studies have reported findings similar to my own, that periphyton production was higher on naturally-occurring substrata than artificial substrate samplers, with most authors also speculating this was probably due to differences in surface texture (e.g. Tippett 1970, Herder-Brouwer 1975, Nielson *et al*. 1984, Antoine & Benson-Evans 1985, Coe *et al*. 2006). A possibility to consider is the effect substrate roughness exerts on surface flow, whether it is able to support laminar flow or keep water turbulent (Smith 1975). My finding that naturally-occurring substrata tended to support higher periphyton standing crops may be in part a consequence of the former supporting more extensive laminar flow.

In conclusion, each of the artificial substrate samplers utilised in this study did not appear to make good surrogates for capturing quantities of periphyton production comparable to growth on their respective naturally-occurring microhabitat. Thus artificial substrate samplers did not sufficiently mimic their naturally-occurring templates, which may have led to underestimation of periphyton production and therefore should be utilised with caution for this purpose, despite accomplishing similar algal assemblages overall (refer to Chapter 4, section 4.6.1.5). This was attributed to the greater abundance of periphyton growing on naturally-occurring

microhabitat, most probably as a result of biotic interactions with chemical microgradients and surface texture which may have encouraged the higher cell densities on mineral substrata and aquatic plants.

3.6.1.6 Periphyton production and environmental habitat conditions in the Water of Dye, River Girnock and Knockan Burn as determined by TWINSPAN classification

Generally there was no significant variation in periphyton production between the three TWINSPAN sample-groups. As prior discussed (section 3.6.1.1) there was an influential effect of sediment contamination in the periphyton harvested from Knockan Burn which affected biomass but overall chlorophyll was similar between the three TWINSPAN sample-groups. This tends to support the concept of a set upper limit of periphyton production in oligotrophic upland streams, irrespective of pH (Winterbourn *et al*. 1992) that is governed principally by flow disturbance and P-limitation, as earlier discussed (refer back to section 3.6.1.2).

Grime's (1979) theory predicts that often it is environments experiencing intermediate levels of stress or disturbance which are characterised by an intermediate standing crop, and support the highest species richness. Whereas fewer species are expected to occur in highly stressed or least disturbed environments, commonly corresponding to the lowest and most productive standing crops, respectively. Although a humpback trend was observed in my study between species richness and standing crop of stream periphyton communities (see Figure 3.55, Figure 3.56 and Figure 3.57), the r^2 value was low. This suggests that a physical disturbance gradient was not foremostly a key explanatory factor controlling the diversity of periphytic algal species in streams and rivers, regardless of a critical role in structuring aquatic bryophyte communities (see sections 3.6.2.5 and 4.6.2.5). Although flow was a dominant factor regulating ecological succession of periphytic algae (see Chapter 4, section 4.6.1.2), community composition was largely governed by mesoscale

environmental gradients of prevailing water chemistry and microscale factors (e.g. flow-substrate interactions) were of lesser importance (see Chapter 4, section 4.6.1.6). Unlike aquatic bryophytes which usually form relatively stable assemblages, the species composition, morphology and biomass of periphyton communities tend to exhibit a strong temporal response to an interchangeable dominance of environmental habitat conditions (see Chapter 4, section 4.6.1.2). This may help explain the weak relationship between the diversity and standing crop of stream periphyton to a gradient of physical disturbance. Perhaps a more prominent humpback relationship would have been observed for periphyton communities occurring within streams of widely ranging nutrient status. For example, in oligotrophic streams (such as those comprising this study) periphyton diversity and thus standing crop may be expected to be relatively low. Whereas by comparison, competitive dominants like *Cladophora glomerata* which often accumulate nuisance levels of biomass in nutrient-enriched rivers (Biggs 1995, Biggs *et al*. 1998), commonly displace other algal species from the niche. Therefore mesotrophic, moderately-enriched rivers may be expected to contain moderate standing crops of periphyton characterised by a more diverse flora.

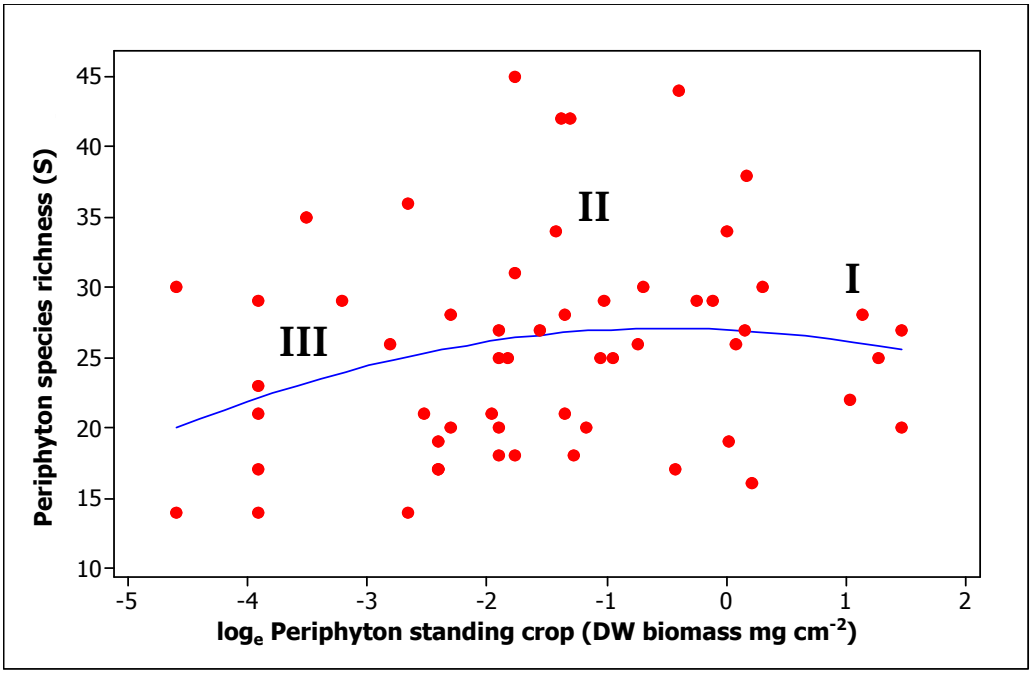


Figure 3.55 Scatterplot analysis of amalgamated periphyton data (harvested from short-term linoleum substrates; all routine sampling dates inclusive) showing a hump-back relationship between species richness and standing crop, with occurrence of TWINSPAN sample-groups I - III indicated. A quadratic regression produced the best line of fit: aquatic bryophyte species richness (S cm^{-2}) = 28.4 + 1.43 log$_e$ periphyton biomass mg cm^{-2}, r^2 (adj) = 6.7%, P<0.05*.

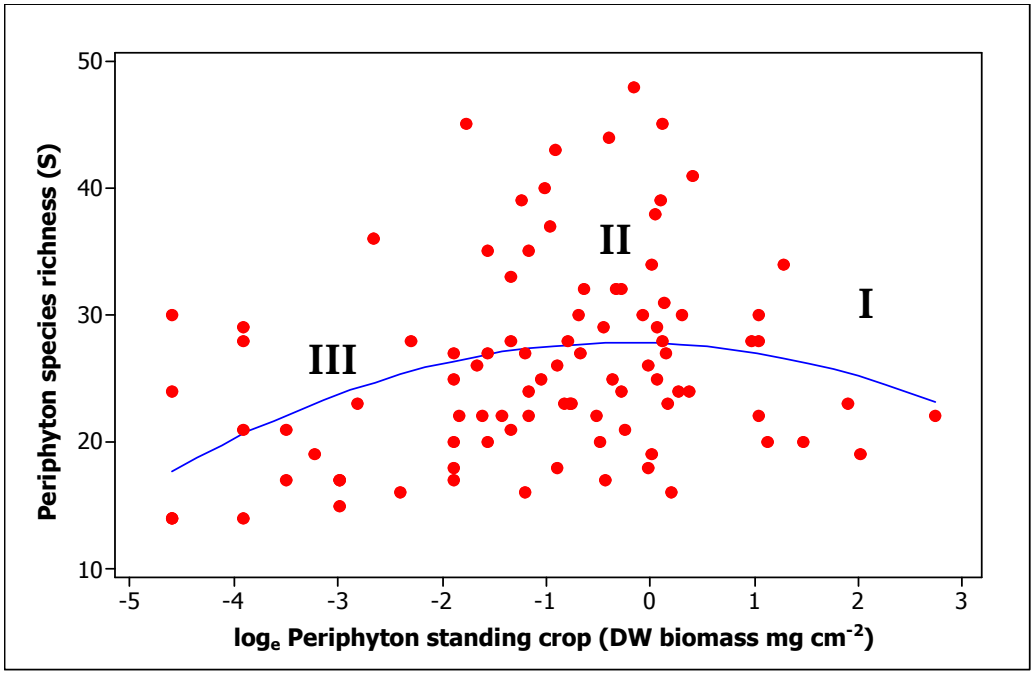


Figure 3.56 Scatterplot analysis of amalgamated periphyton data (harvested from all artificial substrates; survey dates only) showing a hump-back relationship between species richness and standing crop, with occurrence of TWINSPAN sample-groups I - III indicated. A quadratic regression produced the best line of fit: aquatic bryophyte species richness (S cm^{-2}) = 27.4 + 1.21 log$_e$ periphyton biomass mg cm^{-2}, r^2 (adj) = 5.3%, P<0.05*.

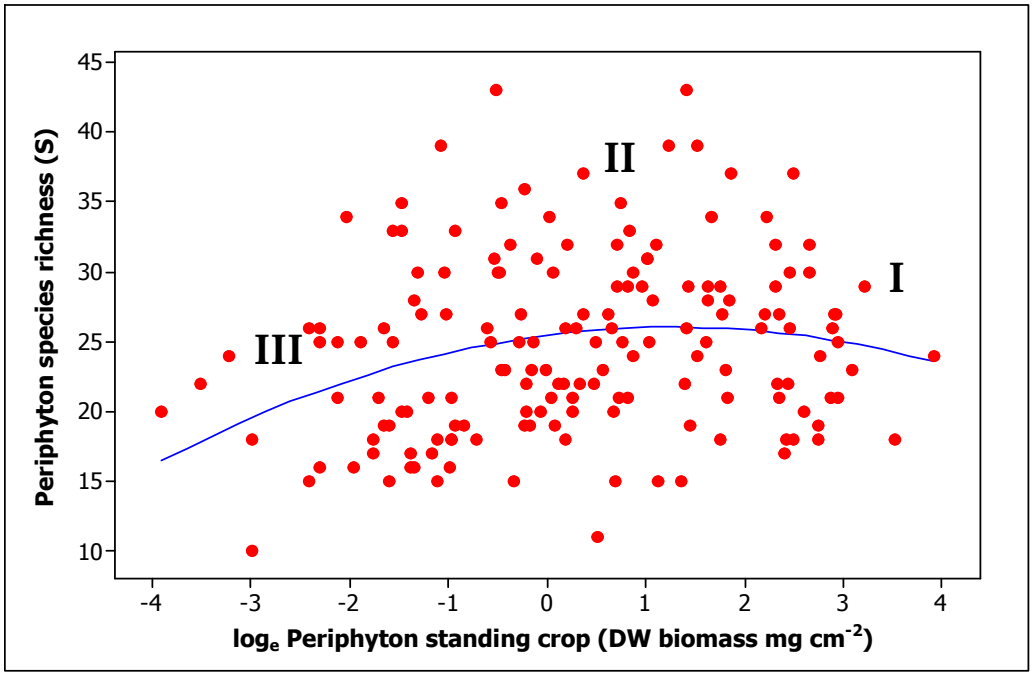


Figure 3.57 Scatterplot analysis of amalgamated periphyton data (harvested from all naturally-occurring substrata; survey dates only) showing a hump-back relationship between species richness and standing crop, with occurrence of TWINSPAN sample-groups I - III indicated. A quadratic regression produced the best line of fit: aquatic bryophyte species richness (S cm^{-2}) = 24.5 + 0.75 log$_e$ periphyton biomass mg cm^{-2}, r^2 (adj) = 4.9%, P<0.05*.

3.6.1.7 Predicting freshwater periphyton production

It was difficult to accurately predict freshwater periphyton production (measured as $\log_e$ chlorophyll content) from the single predictor variable (e.g. √ water temperature) utilised in construction of the PERIchlP1a model and its derivatives, which had low predictive power. Previous attempts were made to build a significant multiple regression model from various combinations of environmental parameters (e.g. underwater light availability, water temperature, flow) shown to be correlated with periphyton chlorophyll production (refer to Appendix 2b) but did not successfully help predict the response variable. Short-term substrate samplers were chosen for this purpose because they captured fluctuations in stream periphyton production and environmental habitat conditions over a defined period. However, I suspect that periphyton production relationships with other environmental variables were weakened because of the overriding effect of flow controlling the community composition, succession and morphology of the algal components and thus directly the biomass. On the occasions where water temperature particularly poorly predicted periphyton production (most notably in the spring months), spates had probably occurred before sampling and removed most of the biofilm that had been present. Hence explaining why at these times periphyton production was often observed to be lower than was predicted by the model. Yet flow could not be incorporated into the model as a significant predictor variable driving periphytic algal production. I suspect this concerns the fact that samples were rarely collected during the height of flow spates, and more commonly when current velocities had subsided. Thus the 'snap shot' data collected at the time of sampling did not accurately reflect preceding environmental conditions (e.g. intense current velocities) which had instigated the algal scour, thus disguising the governing effect of flow regime in the snap shot data. This may explain why production of periphyton was much less well predicted than for aquatic bryophytes, probably because the latter tend form relatively 'fixed' communities and respond positively to the effects of flow (boulder-riffle effect), in contrast to the transitory nature of periphyton communities constrained by current velocity. Therefore future research would

greatly benefit greatly from long-term logger data to pinpoint the frequency and intensity of spate events, as this would probably help construct more robust models than those presented herewith. It is also possible that because relationships of periphyton production with several environmental variables (e.g. flow, water temperature, underwater light and nutrient availability) were found to be seasonally interchangeable, that this may explain why it was difficult to build a single compatible model using these predictors in combination to strongly predicted the response variable. Also as previously discussed, the unquantified effects of grazing pressure may have weakened or uncoupled interactions with other environmental factors (e.g. light, nutrients) and thus may have affected the predictive power of the derived models in the first place.

3.6.2 Aquatic bryophytes

3.6.2.1 Variation in aquatic bryophyte production and environmental habitat conditions in the Water of Dye, River Girnock and Knockan Burn; their sub-catchments and sites

In general, assessments of aquatic bryophyte community biomass and % cover appeared to provide an accurate reflection of the abundance of aquatic bryophyte species with different growth forms and alternative life strategies within the target streams. For example canopy-forming bryophyte species (e.g. *Fontinalis antipyretica*, *Platyhypnidium riparioides*) exhibited inherently high biomass, compared to that of low-growing turf-forming species (e.g. *Blindia acuta*, *Racomitrium aciculare*, *Schistidium* spp.). This concurs with findings elsewhere (e.g. Muotka & Virtanen 1995, Virtanen *et al*. 2001). Besides their functional attributes, the habitat ecology of stream bryophytes is a critical aspect also to be considered. For example some species may exhibit a preferential occurrence in more calcareous stream habitats than in acid-sensitive conditions. Therefore the community composition of stream bryophytes may exhibit affiliations with streamwater chemistry (Thiebaut *et al*. 1998, Stream Bryophyte Group 1999), and it

is likely at least part of the observed variation in aquatic bryophyte production and abundance between the three sub-catchment streams reflects the variation in species composition between their communities. This is addressed in more detail in Chapter 4 (sections 4.6.2.1 and 4.6.2.5).

Aquatic bryophyte production and abundance might have been influenced by inherent differences in water nutrient status between the target streams. It is known that stream bryophytes can assimilate N and P at relatively low concentrations (Bowden *et al*. 1994, Finlay & Bowden 1994, Stream Bryophyte Group 1999) and that their morphology may play a critical role in nutrient retention (Stream Bryophyte Group 1999). However, in this study nutrient status is unlikely to have been a major factor influencing aquatic bryophyte production because the three streams were characterised by similarly low values of ammonia-nitrogen, nitrate-nitrogen and phosphate (refer back to Chapter 2, section 2.7.1). This indicated that the three target streams were of exceptionally high water quality, with oligotrophic status and in near-pristine condition. Other aspects of streamwater chemistry (e.g. pH, base cation and heavy metal composition) are more likely to be important here. For example, in the base-poor acid-sensitive stream habitats of this study, growth (e.g. shoot length) of *Fontinalis antipyretica* may have been impaired by sulphur toxicity, compared to occurrence of the moss in more calcareous, mineral-rich, well-buffered habitats of this study wherein the phyto-toxic effects of streamwater sulphur would have been alleviated (Davies 2007). Therefore in these oligotrophic streams of near-pristine reference condition inherent differences in streamwater pH (attributed to the predominant underlying geology) may have exerted an effect on stream bryophyte production. In this study, shade pressure had a weak negative effect on aquatic bryophyte production and abundance (refer to Appendix 2e). However, low light levels can probably be dismissed as an influential environmental factor affecting stream bryophyte production because aquatic bryophytes are shade-adapted (Stream Bryophyte Group 1999) and secondly, riparian vegetation was most abundant (and hence shade greatest) during the summer season at a time when aquatic bryophyte

vitality was probably more strongly affected by other abiotic forces (e.g. temperature): see next section (3.6.2.2).

3.6.2.2 Seasonal variation in aquatic bryophyte production and environmental habitat conditions in the Water of Dye, River Girnock and Knockan Burn

Pigment analysis is considered to be a useful indicator of aquatic bryophyte photosynthetic capacity (López & Carballeira 1989 and 1993b, Bruns *et al*. 1997, López *et al*. 1997, Davies 2007). It is also probably a more suitable metric for assessing the physiological condition and response of stream bryophytes to environmental variation, than perhaps DW biomass, the accuracy of which can be affected by presence of detritus and periphyton. Whereas chlorophyll a content is a useful proxy of plant health, with higher chlorophyll content generally reflecting higher photosynthetic activity (or adaptation to shade) and a reduction would indicate a physiologically stressed state (or acclimation to ambient light) induced by the environment. In stream bryophytes it has been shown previously that chlorophyll content can vary seasonally (e.g. Martínez-Ábaiger *et al*. 2004).

From the current study, it is apparent that the low base flow conditions of the temperate summer period (June – September), characterised a time when the stream bryophytes often became exposed to the air, potentially stressing the plants due to desiccation and photo-oxidation produced by the exposure to wind, high ambient temperatures and bright light. This desiccation is very likely to have exerted a negative effect on stream bryophyte photosynthesis as indicated by the significant reduction in production (biomass, abundance) and photosynthetic capacity (chlorophyll content) in all three sub-catchment stream bryophyte communities during the summer period. Aquatic bryophytes are poikilohydric and sensitive to the effects of desiccation (Richardson 1981, Seel *et al*. 1992, Suren 1996, Stream Bryophyte Group 1999). Furthermore, aquatic bryophytes use the C_3-photosynthetic pathway, meaning that under high temperatures (in the light),

the rubisco (ribulose-1,5-biphosphate carboxylase/oxygenase) enzyme shifts from photosynthetic (utilising CO_2 for carbon synthesis) to photo-respiration activity (consuming oxygen and releasing CO_2), resulting in a net carbon loss for the plants (Buchanan *et al*. 2000, Uno *et al*. 2001). Together with photo-oxidative stress experienced by plants under high ambient light conditions of summer (stream bryophytes are primarily shade-adapted and therefore sensitive to sun exposure: Stream Bryophyte Group 1999) this would also explain the reduction in chlorophyll content observed during the summer season (Hendry & Grime 1993). Alternatively, it may have been that the stream bryophytes adapted their light harvesting complexes to sunnier conditions of the summer period and simply that less chlorophyll was required to harvest ambient light available. However, the suggestion that stream bryophytes were enduring physiological stress during summer base flows accompanies the significant reduction in production and abundance, indicating that aquatic bryophyte production shrunk in response to desiccation and lost carbon to photorespiration during the summer season. *Fontinalis antipyretica* is documented as exhibiting optimal growth between 10 - 15^{o}C (Glime 1987) is usually limited by temperatures exceeding 20^{o}C and exposure to air during low base flow periods (Chemeris & Bobrov 2003). Bruns *et al*. (1997) reported that in the River Elbe, growth of *Fontinalis antipyretica* was most productive during the autumn and winter, and markedly suppressed during the summer months when shoots had visibly retracted. Significant variation in the pigment composition, most notably reductions in the chlorophyll and carotenoid content of *Fontinalis antipyretica* in response to increased temperatures and UV-exposure has been recorded in other studies (e.g. Nunez-Olivera *et al*. 2004, 2005). Overall, these findings tend to support my hypothesis that the stream bryophytes in this study were physiologically stressed, as indicated from a reduction in the photosynthetic apparatus during warmer summer base flows, compared to their augmented functioning during cooler deeper water conditions of the autumn-spring season when the plants were rehydrated.

The results presented here show that the aquatic bryophytes in the study streams resumed photosynthetic activity and regained carbon synthesis upon rehydration during the autumn-winter-spring period. Furthermore the light harvesting complexes of the stream bryophytes may even function more efficiently during the low light intensities of the autumn-winter-spring period, as suggested by increased chlorophyll content.

In the spring flush of 2006, I observed the appearance of distinguishable bright green foliage produced by the shoot tips on some species of stream bryophytes indicating recent growth (e.g. *Racomitrium aciculare*: see Figure 3.58). I have considered the possibility that such new growth may correspond with replenished phosphate availability in the spring flush of 2006 (refer to Chapter 2, section 2.7.2). However, no overall significant effect of streamwater phosphate on bryophyte community production was detected and so this will not be discussed further. Additional investigation would be required to determine which influential environmental parameters, in regards to nutrient status (N, P), stimulate (or are perhaps limiting to) the growth of stream bryophytes in near-pristine reference conditions, and whether a species-specific response can be detected as inferred from other research (e.g. Bowden *et al*. 1994, Finlay & Bowden 1994, Christmas & Whitton 1998ab).

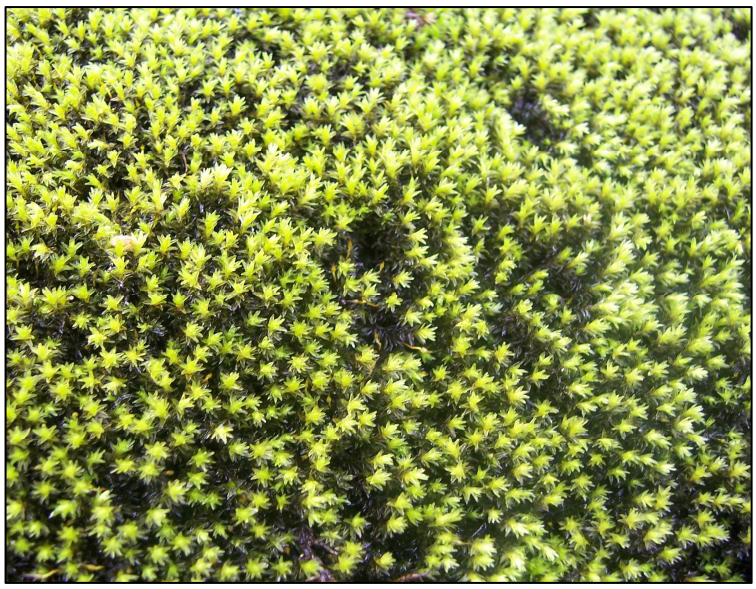


Figure 3.58 Shoot tips of *Racomitrium aciculare* showing fresh growth in the spring

3.6.2.3 Response of aquatic bryophyte production and environmental habitat conditions in the Water of Dye, River Girnock and Knockan Burn to variation in flow regime: pool, glide and riffle zones

The results of this study show that high current velocities favoured an increase in aquatic bryophyte production. Fast-flowing riffles were identified as the most productive microhabitats for stream bryophytes, and slow-flowing pools were the least productive zones wherein aquatic bryophyte abundance was usually nominal. Glides usually occupied an intermediate habitat, wherein stream bryophyte production attained moderate quantities. These findings applied in each of the sub-catchment streams studied and also when the data were amalgamated. The results were in line with numerous other observations that high-velocity riffle zones in streams are especially abundant in aquatic bryophyte vegetation (e.g. Glime 1987, Suren 1996, Linhart *et al*. 2002a, Chemeris & Bobrov 2003).

Whether acting independently or together, there are several practicable explanations as to why aquatic bryophyte production attained significantly higher levels in faster-flowing streamwaters than in slow-flowing pools. Aquatic bryophyte production mirrored an increase of species richness and diversity in response to increasing current velocity (see Chapter 4, sections 4.5.11 and 4.6.2.3). One logical reason for the high abundance of aquatic bryophytes in streambed zones characterised by high current velocities is the relationship that coexists between the occurrence of large, protruding stable substrates in the streambed and surface flow patterns; the boulder-riffle effect. Thorough discussion regarding flow interactions with predominant substrate morphologies is addressed in Chapter 2 (refer back to section 2.7.3). In this chapter, section 3.6.2.4 deals specifically with the effects of streambed substrate stability as an influential factor of aquatic bryophyte production and is therefore not further discussed here.

A further explanation could be that high current velocities are known to scour epiphytic periphyton from aquatic bryophyte surfaces thereby alleviating the

shade pressure imposed by attached epiphytes congregating (and trapping sediments in the process of "aufwuchs" formation) in low flow habitats (Finlay & Bowden 1994, Suren 1996). However, no evidence in the current study was found for this, as the production of periphyton harvested from naturally-occurring aquatic bryophytes did not vary significantly between flow regimes (refer back to sections 3.5.3). Furthermore, Suren (1991) actually found that despite their principal occurrence in stable, fast-flowing habitats, aquatic bryophytes harboured a higher abundance of periphyton and detritus compared to uncolonised (bare) mineral substrata. This reinforces the functional role of aquatic bryophytes in turbulent stream ecosystems as critical hydraulic refugia for other lotic biota (e.g. periphyton, macroinvertebrates, and juvenile fish) under high velocity conditions (Lancaster & Hildrew 1993, Nikora *et al*. 1998, Muotka & Syrjanen 2007).

A contributory factor may be that aquatic bryophytes utilise the C_3-photosynthetic pathway and therefore require a supply of free dissolved CO_2. Few aquatic bryophytes can utilise bicarbonate (HCO_3^-) as a carbon source, though *Fontinalis antipyretica* is a notable exception (Bain & Proctor 1980, Peñuelas 1985, Raven *et al*. 1985, Ballesteros *et al*. 1998). Boundary layer thickness may limit CO_2 diffusion in streamwaters, and therefore potentially constrain aquatic bryophyte production (Jenkins & Proctor 1985). Under turbulent high flows, atmospheric drawdown of CO_2 is encouraged (Hynes 1970, Bain & Proctor 1980) and perhaps more importantly, boundary layer thickness around stream plants is often reduced (Bain & Proctor 1980, Jenkins & Proctor 1985). A thinning of the boundary layer under high velocity conditions has been documented as a key factor in facilitating increased CO_2 diffusion and carbon acquisition by stream bryophytes thereby enhancing photosynthetic carbon-fixation of aquatic bryophytes (Jenkins & Proctor 1985), particularly for canopy-forming species (e.g. *Fontinalis antipyretica*) characterised by a high surface area. The hydraulic force of high flows can exert a detrimental effect on stream bryophyte production either by directly shearing plant material from stream bryophytes, or indirectly by abrading foliage due to the action of fine-grained particles suspended in the currents (Jenkins & Proctor

1985). *Fontinalis antipyretica* is known to be susceptible to such physical forces (Glime 1987). Further evidence of mechanical stress of flow on aquatic bryophyte foliage comes from the existence of (at least) two contrasting ecotypes of *Fontinalis antipyretica* responding to different flow regimes, sampled from a Black Forest stream in south-west Germany (Biehle *et al*. 1998). Therefore at high flow regimes, shear-stress may have limited the potential of stream bryophyte production in fast-flowing riffles, whereas in low current velocity pools the boundary layer resistance of CO_2 diffusion was probably the factor most limiting to aquatic bryophyte growth.

3.6.2.4 Response of aquatic bryophyte production and environmental habitat conditions in the Water of Dye, River Girnock and Knockan Burn to variation in substrate morphology

It has been commonly reported in the literature that aquatic bryophyte growth thrives on stable substrates: the well-known saying is that '*rolling stones never gather mosses*' (Slack & Glime 1985, Englund 1991, Suren 1991, Suren & Winterbourn 1992, Muotka & Virtanen 1995, Suren 1996, Suren & Ormerod 1998, Duncan *et al*. 1999, Suren & Duncan 1999).

The results of this study support findings elsewhere regarding discussion of stable substrates as common establishment zones for aquatic bryophytes (e.g. Slack & Glime 1985, Englund 1991, Steinman & Boston 1993, Muotka & Virtanen 1995, Duncan *et al.* 1999, Suren & Duncan 1999, Suren 1996, Suren *et al*. 2000). Stable substrates resist flow-induced streambed movements and offer persistent microhabitat for aquatic bryophytes. On the contrary, unstable substrates (e.g. cobbles) are susceptible to motion and becoming dislodged under heightened flows. The multiplier effect of innate streambed instability is that substratum motion can lead to destruction of plant material during high-velocity spates. Therefore unstable substrates provide poor foundations for aquatic bryophyte colonisation and are typically unavailable to most species (Suren 1996, Duncan *et*

al. 1999, Suren & Duncan 1999). The exception is a few species adapted to such frequently-disturbed habitats which can rapidly colonise an open niche or possesses a growth form for withstanding scour events (e.g. *Blindia*-type: Muotka & Virtanen 1995). In this study, aquatic bryophyte growth was most abundant on stable streambeds and least abundant in unstable habitats or on small substrate particles, thus agreeing with other work (e.g. Slack & Glime 1985, Englund 1991, Muotka & Virtanen 1995, Suren 1996, Suren & Ormerod 1998, Stream Bryophyte Group 1999).

Strong vertical zonation patterns, a gradient consisting of aquatic bryophyte species occurring in mostly submerged to mostly exposed microhabitats, are often observed on substratum with tall profiles extending beyond the water surface (e.g. Muotka & Virtanen 1995, Virtanen *et al*. 2001). My study also found that the largest substrates (e.g. boulders) tended to gather the greatest number of species (refer to Chapter 4, sections 4.5.12 and 4.6.2.4) and were therefore often characterised by the highest production and abundance of aquatic bryophytes.

3.6.2.5 Aquatic bryophyte production and environmental habitat conditions in the Water of Dye, River Girnock and Knockan Burn as determined by TWINSPAN classification

Variation in aquatic bryophyte production between the TWINSPAN sample-groups (see Chapter 4, section 4.6.2.5) reflected life-form variation in sets of aquatic bryophyte species comprising each of the assemblages, and my results correspond with the findings of other works (e.g. Muotka & Virtanen 1995, Suren & Ormerod 1998, Virtanen *et al*. 2001). Canopy formers are often obligatory aquatic species (e.g. *Fontinalis*-type: Muotka & Virtanen 1995) with characteristically long shoots, large complex surface areas, often growing in dense clumps with a tendency to overhang the substratum (Siebert *et al*. 1996, Biehle *et al*. 1998, Davies 2007). Such morphologies are often perceived as good competitors (C-Strategists: Grime 1979), reproducing principally by vegetation reproduction

(fragmentation) rendering them capable of monopolising the space available and thereby excluding other bryophyte species from the niche under certain habitat conditions (Muotka & Virtanen 1995, Virtanen *et al*. 2001). On the other hand, cushion-formers (e.g. *Blindia*-type) exhibit a short-stature and often form mats adhering to the surface of the substratum (Muotka & Virtanen 1995). Typically, short-turfed forms are adapted to enduring scours and movement in the streambed, making them good stress-disturbance tolerators (SR-strategists: Grime 1979).

In this study, Group I was characterised by a low diversity aquatic bryophyte community with correspondingly low biomass and cover, owing to the meagre turf morphologies of the indicator species comprising this sample-group (e.g. *Blindia acuta*, *Schistidium agassizii*).

Group II encompassed an aquatic bryophyte community of mixed morphologies including some canopy-formers (e.g. *Fontinalis antipyretica* and *Hygrohypnum ochraceum*) and an abundance of short mat formers (e.g. *Scapania undulata*, *Racomitrium aciculare*).

Groups III and IV supported an abundance of high biomass aquatic bryophyte vegetation characterised by weft-carpet formers (e.g. *Fontinalis antipyretica*, *Platyhypnidium riparioides*). Although common to both groups, the greater water moss, *Fontinalis antipyretica*, existed in Group III as a near-monoculture standing crop, but occurred to a lesser extent in Group IV with other bryophyte morphologies (e.g. carpet-turfs such as *Palustriella falcata*, *Hygrohypnum luridum*, and short-statured species like the liverwort *Chiloscyphus polyanthos* and *Fissidens adianthoides*) to form a high diversity assemblage.

The hump-back model relating diversity to environmental stress or disturbance, derived from C-S-R theory: Grime (1979), predicts that few species, termed SR-strategists, are likely to occur in environments experiencing a combination of moderate to high stress and disturbance pressures, and that the corresponding

standing crop is expected to be low. Yet the most productive or least disturbed environments are often home to competitive dominants (C-strategists), and will also not necessarily support the highest number of species. Often, it is environments experiencing intermediate levels of stress or disturbance which are characterised by an intermediate standing crop, and support the highest species richness, with a coexisting array of intermediate C-S-R species.

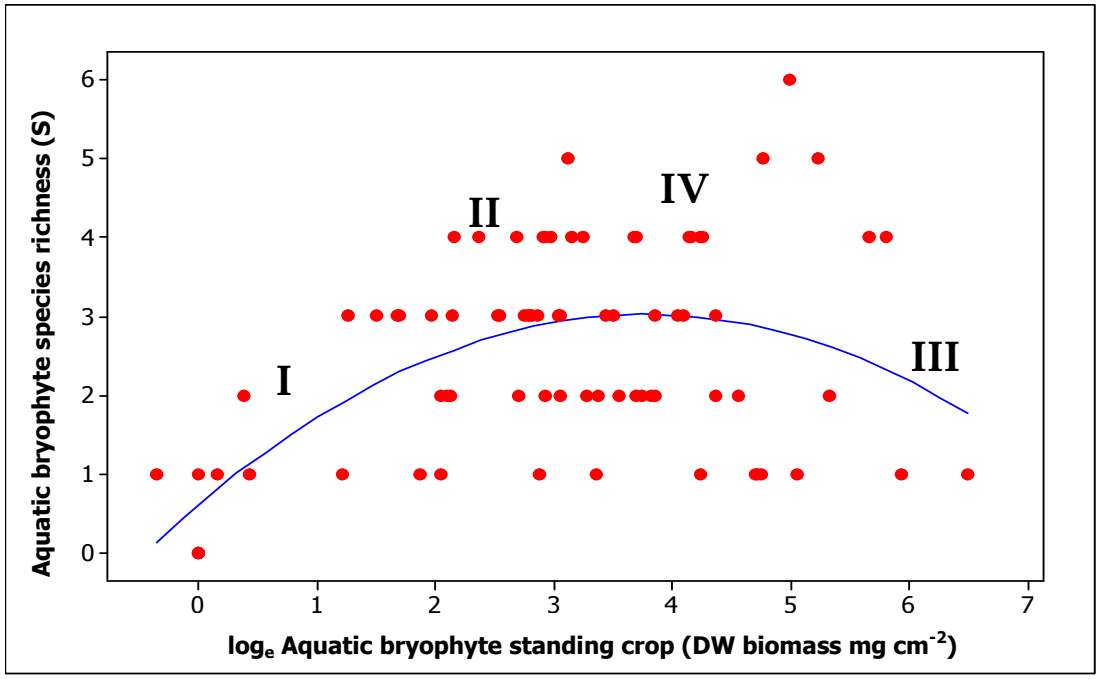


Figure 3.59 Scatterplot analysis of amalgamated aquatic bryophyte data showing a hump-back relationship between species richness and standing crop, with occurrence of TWINSPAN sample-groups I-IV indicated. A quadratic regression produced the best line of fit: aquatic bryophyte species richness (S cm^{-2}) = 0.6082 + 1.279 log$_e$ bryo biomass mg cm^{-2} - 0.1694 log$_e$ bryo biomass mg cm^{-2} **2, r^2 (adj) = 29.4%, P<0.001*.**

Therefore the results of this study (Figure 3.59) generally agree with the predictions of the hump-back model of Grime (1979), which has also been applied in a small number of other studies (e.g. Muotka & Virtanen 1995, Suren & Ormerod 1998, Virtanen *et al*. 2001) to describe the relationship between species richness (or diversity) and standing crop of stream bryophytes. In Figure 3.59, the x-axis (standing crop) probably represents a stream disturbance-stability gradient (e.g. flow frequency and intensity, propensity for substratum movement), in which the most unstable disturbance-prone habitats are characterised by a small

number of turfed SR-strategists (Group I community-type) and a low standing crop, whereas the most stable habitats are often predominated by a single C-strategist (with few co-occurring species) and a naturally high standing crop (Group III community-type). The continuum existing between either extreme on the disturbance gradient is characterised by moderate standing crop owed to the rich assemblage of species morphologies occurring therein (Group II and IV community-types), in fitting with the framework of the C-S-R strategist theory of Grime (1979) and Intermediate Disturbance Hypothesis (IDH) described by Connell (1978). For further detailed discussion of aquatic bryophyte morphology in relation to life strategy refer to Chapter 4 (section 4.6.2.5).

3.6.2.6 Predicting freshwater aquatic bryophyte production

Together streamwater physico-chemical variables (e.g. underwater light availability, temperature) and substrate morphology factors (e.g. predominance of boulders) acted as the most effective environmental drivers for reasonably predicting the production of aquatic bryophyte vegetation in the upland stream habitats of the study using model AqBRYOchlP1a. As previously discussed, aquatic bryophyte production is strongly determined by the growth morphology of individual species occurring therein. Therefore the incorporation of vegetation state variables (e.g. species richness: S) may have further improved the predictive power of the model.

3.6.3 Vascular submerged macrophytes

3.6.3.1 Variation in vascular submerged macrophyte production and environmental habitat conditions in the Knockan Burn sub-catchment and its sites

It is not surprising that there were significant differences in aquatic macrophyte production and abundance between the three subcatchment streams in this

particular study, as vascular submerged macrophytes were completely absent from seven of the nine sampling sites investigated. The Water of Dye and River Girnock lacked this type of vegetation entirely whereas stands of vascular submerged macrophyte vegetation occurred in the upper (mixed macrophyte composition of *Potamogeton polygonifolius*, *Chara globularis* var. *globularis*, *Eleogiton fluitans* and *Ranunculus flammula*) and lower (mostly only *Myriophyllum alterniflorum*) parts of Knockan Burn but river plants were absent from the middle section of this stream (for further discussion refer to Chapter 4, section 4.6.3).

Although possibly not significant, the observed differences in production and abundance between upper and lower Knockan can probably be attributed more to variation in species morphology and diversity of vascular submerged macrophyte vegetation present (Riis *et al*. 2003), rather than due to the effects of water quality. Although, nutrient enrichment has been shown in other studies to exert profound effects on the growth of macrophyte vegetation, particularly in rivers affected by inputs from sewage disposal (e.g. Carr & Chambers 1998), neither N nor P status varied greatly enough in my study to be considered factors stimulating (or otherwise) to vascular plant production. Nevertheless, it is certain that the environment played a key role in shaping the community composition and distribution of river plants, particularly the substrate morphology and water chemistry (e.g. pH, conductivity, alkalinity, Ca^{2+}) of upland stream habitats in this study (see Chapter 4, section 4.6.3.5).

It should be taken into consideration that the data available for vascular submerged macrophytes is much smaller by comparison to the other plant groups (e.g. periphyton, aquatic bryophytes) comprising this study. This is simply because river plants were sparsely distributed compared to periphyton and aquatic bryophytes which occurred in almost all stream habitats sampled as part of this study. In hindsight this meant that the dataset collected for vascular submerged macrophytes was limited in size, not normally distributed and further, could not be transformed. Therefore the data presented for this particular plant

group in the chapters of this thesis, represents the median value (note the absence of standard errors), following statistical analysis using the Kruskal Wallis test (as noted in table or figure captions where relevant). This probably explains why in some instances the median value may be larger than expected than if it were the mean value presented. For example in dense, productive stands of vascular macrophyte vegetation dominated by floating-leaved plants (e.g. *Potamogeton polygonifolius*), assessments of plant cover could quite easily equate near to 100%. Further to this, I decided to include the zero values (e.g. where this plant-group simply did not occur) in the dataset for vascular submerged macrophyte, enabling me to avoid overestimating production, and explore reasons why these plant-groups were absent from certain environmental habitat conditions across the range of upland streams assessed for this study.

3.6.3.2 Seasonal variation in vascular submerged macrophyte production and environmental habitat conditions in Knockan Burn

It may be expected that in streams and rivers, aquatic macrophytes will accrue more biomass during summer by responding to increasing irradiance, photoperiod and water temperature, than at other times of the year, particularly the autumn-winter period when plants usually grow less and undergo scenescence (e.g. Sand-Jensen 1998, Riis *et al*. 2003). However, no significant difference in the production or abundance of vascular submerged macrophytes was detected between sampling seasons (e.g. April, September and November) in Knockan Burn during 2006, and from personal observations the communities did not appear to change visibly during this time period. Perhaps had the aquatic macrophyte vegetation been sampled earlier in the summer season (e.g. June or July 2006) then a significant peak in growth may have been detected. It is also possible that aquatic plant production was physiologically limited by water temperatures exceeding optimum growth requirements, photorespiration, shading (self or epiphytic), low nutrient (N, P) conditions, or capped by grazers (Carr *et al*. 1997). It is also quite possible that usable dissolved inorganic carbon sources may

have become limiting for aquatic plants in Knockan Burn during the summer as streamwater pH increased significantly (refer back to Chapter 2, section 2.6.2.3), as this may have encouraged a compositional shift towards an abundance of either bicarbonate or carbonate ions (Drever 1982), which are unusable forms to most vascular submerged macrophytes (Carr *et al*. 1997, Riis *et al*. 2000). Some aquatic plants possess adaptations which allow them to use HCO_3^- (e.g. *Myriophyllum alterniflorum*, *Potamogeton pectinatus*) thus giving them a 'competitive edge' under certain environmental conditions (wherein the carbon dioxide supply is inadequate), whilst many other species have a much lower affinity for bicarbonate or rely exclusively on sequestering free-CO_2 from the atmosphere (Carr *et al*. 1997, Riis *et al*. 2000). An abundance of unusable forms of dissolved inorganic carbon, coupled to low velocity flows experienced during the summer period may have led to thicker boundary layers forming around aquatic plants and thereby further limited production by potentially reducing rates of diffusion and uptake of atmospheric CO_2. However, quantities of dissolved inorganic carbon constituents (e.g. free-CO_2, HCO_3^-, CO_3^{2-}) were not measured directly (although alkalinity is often considered a reliable indicator of their abundance: Feijoo & Lombardo 2007) so to a certain extent previous mention remains assumption, and therefore cannot be discussed any further. In the natural environment under near-pristine reference conditions, it is unlikely that any one of aforementioned factors over-rides another, and more plausible that they interact together to attenuate or promote plant production. These could probably only be teased apart by following a more specific line of research required to collate models to be able to truly understand and predict changes of aquatic macrophyte production in streams and rivers (e.g. Carr *et al*. 1997). Another important point perhaps worth considering is that some of the aquatic macrophyte species could have been diverting valuable carbon resources away from photosynthetic assimilation and investing in their sexual or vegetative reproductive effort (e.g. seeds or other propagules) to secure the niche and prepare for over-wintering. Therefore the construction costs associated with resource allocation to propagation may have come at the expense of an overall gain plant growth (Spencer *et al*. 1997). The

dominant macrophyte species will determine the overall pattern of plant growth in the vegetation communities (Riis *et al*. 2003). Other species belonging to the watermilfoil family includes the Eurasian species *Myriophyllum spicatum*, often described as an invasive nusiance aquatic 'weed' in many countries around the world extending beyond its native range (e.g. Madsen *et al*. 1991, Ali & Soltan 2006) and a strong competitor strategist (Murphy *et al*. 1990), relying principally on mechanisms of vegetative reproduction (e.g. fragmentation, stolons) to attain the niche. It is also likely that the relatively small sample size and non-normality of this particular dataset may have made it difficult to obtain a significant result. It may also be possible that the morphological (e.g. shoot elongation, leaf expansion) or photosynthetic (e.g. pigment composition) plasticity of individual aquatic macrophyte species in my study was obscured because I chose to examine the amalgamated response of the whole plant community to seasonal variation.

3.6.3.3 Response of vascular submerged macrophyte production and environmental habitat conditions in Knockan Burn to variation in flow regime: pool, glide and riffle zones

Exposure to high current velocities can scour and uproot vascular submerged macrophytes susceptible to the effects of fast-flowing waters (Biggs 1996, Riis & Biggs 2003, Sand-Jensen 2003, Schutten *et al*. 2005, Riis *et al*. 2008), and explains their distinct absence from riffle habitats in Knockan Burn. Further, it was suspected that in slow-flowing pools, the production and abundance of aquatic plants may have been light-limited by aufwuchs accumulating on their surface (causing shade pressure: Carr *et al*. 1997; and/or, impeding carbon supply: Jones *et al*. 2000b) and thick boundary layer (resisting diffusion of inorganic carbon and other nutrients: Smith & Walker 1980, Black *et al*. 1981, Sand-Jensen *et al*. 1992, Carr *et al*. 1997, Riis & Biggs 2003).

My study not only showed that vascular submerged macrophytes were largely restricted to low-moderate flows and excluded from highly disturbed high-

velocity riffle zones, but also that the abundance of aquatic plants appeared to 'peak' in swiftly (though not turbulent) flowing waters. I suspect that microhabitat characterised by glides offered a compromise to aquatic macrophytes in that flows were probably sufficiently fast enough to scour epiphytes and reduce the boundary layer resistance to nutrient or gas exchange, but critically did not attain velocities high enough to destroy or dislodge plants from the streambed (Riis & Biggs 2003, Riis *et al*. 2008). This may explain why river plants appeared to perform better photosynthetically in glides compared to slow-flowing pools or highly-disturbed riffles, and therefore is also in fitting with the IDH theory of Connell (1978). A study of large prarie rivers in western Canada subject to nutrient enrichment pinpointed that localised increases in flow velocity dramatically reduced the biomass of aquatic macrophytes (Chambers *et al*. 1991). However more comparable with the results of my study, Riis & Biggs (2003) showed that aquatic macrophyte vegetation in New Zealand streams exhibited a similar humpback relationship to increasing current velocity, with a higher abundance occurring at moderate flows (0.3 - 0.5 m s^{-1}) than under slow (<0.2 m s^{-1}) or extremely high velocities (>0.8 m s^{-1}).

It is well known that dense patches of submerged vegetation can also modify their surrounding environment by reducing near-bed current velocities (Sand-Jensen 1998, Dodds & Biggs 2002). Therefore considering this point, it may not be surprising that unvegetated zones of streambed were characterised by higher current velocities compared to other habitats in which plants grew (Riis & Biggs 2003). This underpins complex interaction existing between plant morphology and flow regime patterns in streams and rivers wherein vascular macrophytes are a notable feature.

Aquatic macrophytes can also influence streambed substrate composition by reducing within-bed flows which encourages sediment deposition (Sand-Jensen 1998). Furthermore, there are the dual effects of substrate-flow interactions to consider: slow-flowing habitats were more abundant in fine sediment particles

and faster-flows were characterised by coarser substrates (refer back to Chapter 2, section 2.6.3 and 2.7.3). Ultimately the size, composition and packing of substrate particles along with their cohesion properties and liability to erode, determine whether aquatic plants can establish themselves by affecting the extent to which their roots successfully become implanted and anchored to the streambed (Biggs 1996, Schutten *et al*. 2005). Consequently, this will also govern the vulnerability of vascular macrophyte vegetation to increased current velocities and their propensity to breakage or becoming dislodged during major spates (Riis & Biggs 2003), although this is also dependent on the flow-resistance mechanisms of individual plant species and their morphological trade-offs between strong stems or roots (Schutten *et al*. 2005). For further discussion of the distribution of aquatic macrophytes in respect to substrate morphology then see next section (3.6.3.4).

3.6.3.4 Response of vascular submerged macrophyte production and environmental habitat conditions in Knockan Burn to variation in substrate morphology

In this study, vascular submerged macrophytes were most productive in habitats characterised by fine substrates, least abundant wherein coarse substrates were a dominant feature of the streambed, and moreover were generally absent from streams characterised by hard, impenetrable substrate morphologies. The results of this study indicate that the distribution and abundance of river plants was strongly influenced by substrate morphology. In particular and in contrast to aquatic bryophytes, the presence of fine sediment particles (e.g. sand) favoured the occurrence of vascular submerged macrophytes, agreeing with the ecology of river plants noted elsewhere (e.g. Haslam 1978, 2006, Biggs 1996). Furthermore the species composition of the river plant communities appeared to be influenced by variation in substrate morphology and water chemistry (see Chapter 4, section 4.6.3.5).

3.6.3.5 Vascular submerged macrophyte production and environmental habitat conditions in Knockan Burn as determined by TWINSPAN classification

Variation in vascular submerged macrophyte production and abundance was linked mostly to the presence or absence of river plants in this study, and probably also to inherent differences in species-composition and diversity of life forms present in each of the TWINPSAN sample-groups (refer to Chapter 4, section 4.6.3.5). However the small size and non-normality of the dataset limited further analysis and interpretation.

In this study, Group I was characterised by a mixed assemblage of aquatic macrophytes from large floating-leaved morphologies (e.g. *Potamogeton polygonifolius*) often growing in fusion with *Chara globularis*, which usually occupied the majority of plant community biomass and cover, together with smaller, less conspicuous life forms (e.g. *Eleogiton fluitans*, *Ranunculus flammula*).

Group II community production and abundance was dominated by monospecific stands of *Myriophyllum alterniflorum*, which possesses a slender fine-leaved yet complex surface area comprised of a highly-divided 'feathered' morphology (well-adapted for CO_2 sequestration: Madsen & Sand-Jensen 1994), and tended not to co-occur with any other aquatic macrophyte species.

For further detailed discussion of vascular submerged macrophyte species in relation to environmental habitat conditions refer to Chapter 4 (section 4.6.3.5).

3.6.3.6 Predicting freshwater vascular submerged macrophyte production

I propose that it would be feasible to build a model using combinations of environmental variables (probably alkalinity, Ca^{2+} and substrate particle size) to predict the production of vascular submerged macrophytes occurring in upland stream habitats in the UK by collecting a much larger dataset for this purpose. However the dataset presented here is too small to conduct further analysis than

has been presented and discussed elsewhere in this thesis regarding true river plants.

Neither water level nor underwater light availability was found to be a major environmental factor constraining the production and abundance of aquatic macrophytes. This is probably because the streams sampled in my study were characteristically narrow (1 - 8 m), shallow (usually < 0.5 m) and the waters though slightly peat-stained, remained relatively clear year round. Therefore I feel it is unlikely that either streamwater depth or light penetration were limiting to vascular submerged plant production in Knockan Burn but more plausibly, that any differences in production or plant cover reflect variation in the functional attributes of the dominant morphologies present in the vegetation community, as in this study species-assemblages themselves were governed by interacting physical and chemical factors of the environment (see Chapter 4, section 4.6.3.5). This is of course expected be a very different case for the biomass of submerged macrophyte vegetation occurring in deep, major river systems especially those experiencing eutrophication or flow intervention such as the sub-tropical drainage basin of the Rio Paraná in Brazil (e.g. Murphy *et al*. 2003), and tropical streams in in Zambia in southern Africa (e.g. Lang *et al*. 2008), but certainly not for shallow, low order mountain streams of near-pristine water quality in the Scottish highlands.

Besides recording plant community biomass, chlorophyll content and cover, I recommend that similar future work should examine the morphological traits of individual aquatic plant species, as field-measured sets of functional attributes (e.g. leaf area index, length of stems, internodes, and roots, nodal spacing, shoot density, propagule production, propensity to reproduce vegetatively, etc.) of submerged vegetation, which often respond predictably to environmental variables (e.g. Ali *et al*. 1999, Garbey *et al*. 2004). Also for example, Asaeda *et al*. (2007) found that two charophyte species in a shallow lake ecosystem in Austrailia showed differing morphological and reproductive adaptations at contrasting

depths. Furthermore, Milne *et al*. (2006) described significant variation in morphological traits of free-floating *Eichhornia azurea* and *Eichhornia crassipes* in response to a number of environmental parameters (including water depth, underwater light regime, sediment Ca^{2+}) in the floodplain of the Rio Paraná, Brazil. In UK navigation canals, Willby *et al*. (2001) found that trait attributes were useful in assessing the functional response of aquatic vegetation to environmental disturbances (e.g. boat-trafficking and waterway management). In France, Chatenet *et al*. (2006) found evidence of morphological ecotypes in populations of *Myriophyllum alterniflorum* responding to habitats of varying nutrient status in Limounsin river systems. Similarly, Harris *et al*. (1992) described marked genetic variation in populations of *Myriophyllum alterniflorum* sampled mostly from parts of north-west Scotland, which may have diverged by responding plastically to differing water chemistries.

Therefore I am generally in agreement with the opinion of Daniel *et al*. (2006) that current biomonitoring protocols would benefit from an improved understanding of the environmental processes driving potential differences in the morphological characteristics of aquatic macrophytes, particularly valuable indicator species, in UK and other European rivers exposed to varying human disturbances.

3.6.4 The three-tier approach to characterising upland stream habitat conditions by combining freshwater vegetation assemblages: periphyton, aquatic bryophyte and vascular submerged macrophyte production

3.6.4.1 Freshwater vegetation production and environmental habitat conditions as determined by TWINSPAN classification

The three-tier integrated approach made it possible to evaluate the role of underlying geology as a major determining factor of water chemistry and

substrate morphology, and further how the whole vegetation community responded to these environmental gradients.

The calcareous stream, Knockan Burn, supported a high biomass of aquatic vegetation because of competitive dominance by *Fontinalis antipyretica* and soft bottom sediments coupled to a relatively base-rich chemistry which accommodated an additional plant community: vascular submerged macrophytes. Rarely, aquatic bryophytes were able to coexist in the same niche as vascular submerged macrophytes, as the distribution of each plant group traded-off to contrasting specific substrate requirements. In the Water of Dye, chlorophyll production appeared to be similar compared to that of Knockan Burn. However, vegetation in the former was dominated by a diverse flora of aquatic bryophytes growing on boulders and hard bedrock, meaning that there was no available niche to support the growth of rooted aquatic macrophytes. The cobbled streambed morphology characterising the mid- and lower River Girnock was unable to functionally sustain an abundance of aquatic plants, instead supporting the occurrence of low growing, disturbance-tolerant turf mosses.

3.6.4.2 Predicting freshwater vegetation production

The lack of sufficient aquatic macrophyte data made it impracticable to incorporate this information together with that of periphyton and aquatic bryophytes to build a sensible model. It may have been feasible to construct a model based on a much larger data set and use combinations of probable environmental drivers (e.g. light availability, water temperature, flow) to predict the production of freshwater vegetation occurring in upland stream habitats in the UK.

However, I suspect one might run into problems attempting to model the response variable of whole vegetation communities in this way, opposed to perhaps more sensibly trying to model each component plant group individually

(e.g. periphyton: sections 3.5.8, 3.6.1.7; aquatic bryophytes: sections 3.5.15, 3.6.2.6). My reasoning for this is that although light availability (indicated in section 3.5.23.2 as a potential predictor variable) is certainly a key driver of photosynthesis and collectively as photosynthetic organisms all were responsive to variation in underwater light regime, other environmental factors (e.g. water temperature, flow velocity) are often also of importance in determining freshwater plant production in upland streams. Part of the underlying problem is that each of the three plant groups (periphyton, aquatic bryophytes and vascular submerged submerged macrophytes) tended to respond differentially to some of the environmental pressures (e.g. flow velocity, water temperature) one might expect to affect production. Thus potentially it could be difficult to build a model sufficiently compatible for the purpose of predicting the combined response of assemblages of freshwater vegetation. Similarly, water chemistry (e.g. alkalinity, Ca^{2+}) and (where applicable) substrate morphology were important environmental factors determining plant species distribution, growth form and therefore production. Thus in this study, the calcareous stream (Knockan Burn) tended to be more productive than the more acid streams (Water of Dye, River Girnock) because it could support an additional plant group: vascular submerged macrophytes, due to fine sediment particles and base-rich water chemistry, thus not necessarily because the waters were perhaps clearer and received more light.

3.7 Conclusions

- There appears to be a 'set-limit' to periphyton production in oligotrophic turbulent mountain headwater streams in the Scottish Highlands, which is governed by flow disturbance, water temperature, underwater light availability and nutrient (especially P) limitation. There is a seasonally interchangeable dominance of these environmental factors, a single or combined adjustment of which acts either to alleviate or further constrain the potential for periphytic algal

production in these streams through regulation of community succession and predominant growth morphologies of the species present.

- Aquatic bryophyte production was generally a function of predominant growth morphology and life strategy of the species assemblages present. For example canopy-forming bryophyte species (e.g. *Fontinalis antipyretica*, *Platyhypnidium riparioides*) exhibited inherently high biomass, compared to that of low-growing turf-forming species (e.g. *Blindia acuta*, *Racomitrium aciculare*, *Schistidium* spp.). Aquatic bryophytes became physiologically stressed during low baseflow conditions of the summer, probably experiencing desiccation problems in response to exposure to air, warm ambient temperatures and bright light; but resumed photosynthetic functionality following rehydration when streams re-flooded. Stream bryophytes grew best mostly in fast-flowing riffles characterised by a predominance of stable substrates, particularly on boulders.

- As Knockan Burn was characterised, at least in part, by fine sediment substrates and (in total) by moderately calcareous water chemistry it was additionally able to support river macrophytes, unlike the other two streams of the study which were dominated by coarse substrates, impenetrable to macrophyte roots. Aquatic macrophytes appeared to perform better photosynthetically in glides compared to slow-flowing pools or highly-disturbed riffles, probably because flows were sufficiently fast enough to scour epiphytes and reduce the boundary layer resistance to nutrient or gas exchange, but critically did not attain velocities high enough to destroy or dislodge plants from the streambed.

- The minimal models proposed in this study predicted the response variables quite well, but freshwater plant production was much less well predicted than diversity (see Chapter 4, section 4.6.1.7 and 4.6.2.6). Other ecological studies (e.g. Murphy *et al*. 2003) have also found plant biomass to be less well predicted than plant diversity from multiple regression models using combinations of environmental variables. Biological systems are inherently noisy and it can be

difficult to accurately predict response variables because they do not necessarily respond unimodally to environmental pressures, thus modelling using linear regressions may not be wholly appropriate (Murphy & Hootsmans 2002). Upland headwater streams are frequently disturbed ecosystems subjected to highly variable intense spates, a phenomenon which is particularly characteristic of the spring period. This may have introduced difficulties in accurately predicting the response variable as often sampling occurred when flows had subsided and therefore current velocity measurements did not capture a precise representation of those endured during peak spates. Therefore some of the constructed models, presented here, may have been sensitive to the highly dynamic physical nature of upland streams, which may also help explain why periphyton production was particularly poorly predicted. Additionally, plant morphology had a considerable bearing on levels of production therefore future work should try to take account of plant traits (e.g. leaf area index, length of stems) to improve our understanding of how the functional attributes of aquatic vegetation respond predictably to the environment.

Chapter 4. Upland Stream Freshwater Plant Community Composition and Diversity

4.1 Objectives

- To quantify natural variation in freshwater community composition (assemblage structure), richness and diversity of species present between the three target streams over one full growing season, for each of three aquatic plant groups: periphytic algae, bryophytes and (where present) vascular submerged macrophytes.

- To characterise habitat conditions associated with the assemblages identified and biodiversity of aquatic vegetation.

- To determine the effectiveness of various types of artificial substrate samplers in acting as surrogate microhabitat for periphyton communities compared to naturally-occurring substrata.

- To determine potential environmental factors driving differences in freshwater community composition and diversity in response to variation in habitat characteristics and seasonality, for each of periphyton, aquatic bryophytes and (where present) vascular submerged macrophytes.

- To determine the nature, strength and significance of any associations between freshwater assemblages, species diversity and these habitat conditions, for each of periphyton, aquatic bryophytes and (where present) vascular submerged macrophytes.

- Establish the characteristics of near-pristine reference communities associated with environmental habitat conditions for each of periphyton, aquatic bryophytes and (where present) vascular submerged macrophytes.

- To demonstrate the potential of the project outcomes in helping implement the biomonitoring of upland stream water quality in Scotland by using multiple

regression modelling procedures to determine the relative predictive strength of combinations of environmental factors in acting as drivers of functional attributes (aquatic plant assemblage and species diversity) of stream vegetation.

4.2 Introduction

Central to the primary objective of the Water Framework Directive (WFD; EC 2000) in achieving at least 'good ecological status', by 2015, is the pending requirement to define ecological benchmark communities. Further, the aim is to identify indicator species, representative of near-pristine reference (or minimally-impacted) conditions as a baseline tool for evaluating the water quality status of inland waters in the UK (Boon & Howell 1997).

Upland headwater streams in Scotland are considered to be predominantly of near-pristine reference condition (SEPA 2007) and thereby, environmental habitat markers of high water quality status. Species-assemblages of aquatic plants can provide integrated projections of environmental habitat characteristics, and thus an overall indication of ecological integrity as a comparable benchmark for impacted rivers. There is a scarcity of knowledge surrounding the ecology of freshwater vegetation inhabiting small mountain streams, and potential bioindicator capacity of periphyton, aquatic bryophytes and (where present) vascular macrophytes for purposes of assessing environmental water quality remains largely unexplored compared to the disturbed habitats of lowland rivers. Consequently, new contributions are fundamental to the Scottish Environment Protection Agency (SEPA) to ensure effective implementation of the WFD. This is to be achieved through development of baseline monitoring tools for the improved assessment of ecological status and sustainable management of inland water quality in Scotland.

4.3 Methods

4.3.1 Periphyton

4.3.1.1 Periphyton sub-sampling and specimen preservation

Refer back to Chapter 3 (section 3.3.1) for details of field sampling of periphyton. The pre-collected 2 ml sub-samples of periphyton were dispensed into pre-labelled sterile glass vials, preserved with Lugol's Iodine solution (Kelly 2000) and kept refrigerated until initial microscopic analysis of algal composition could be undertaken (refer to 4.3.1.2).

Diatom specimens (originally preserved with Lugol's Iodine) were chemically digested using 30% Hydrogen Peroxide on a hotplate for 2-3 h and mounted permanently onto slides using Naphrax™ in accordance with standard procedures outlined in the revised TDI Manual (Kelly *et al*. 2001).

Chemically 'cleaning' diatoms in this way involves the use of oxidizing agents (e.g. hot hydrogen peroxide method or strong acids) to digest intracellular components (e.g. nucleus, chloroplast, cytoplasm etc.) and any contaminant organic material that may be present in the sample (Kelly 2000, Kelly *et al*. 2001). Known as the 'frustule', diatom cell walls are composed of silica and two overlapping valves. Usually sufficient chemical digestion separates the frustule valves to facilitate microscopic identification but due to their siliceous properties, diatoms are chemically resistant to digestion and the morphology of cleaned frustule valves (e.g. shape, dimensions, raphe, striation orientation and density, fibulae, stigmata, etc.) is used to distinguish individual specimens to species level (refer to 4.3.1.2). Mounting specimens with the resin Naphrax™ provided a permanent library of slides for microscopic analysis and future reference.

4.3.1.2 Periphyton identification, quantification and community composition

Initial observations of periphyton community composition (suspended in Lugol's Iodine solution) were undertaken using a compound light microscope, usually within one month of sample collection. 1 - 2 droplets of each periphyton sub-sample was dispensed onto a clean glass microscope slide using a sterile pipette and sealed with a glass coverslip. Slides were analysed using a Leitz S.M. Lux brightfield (phase contrast) microscope and periphyton genera (other than diatoms) were identified from Belcher & Swale (1976ab), and John *et al.* (2002), and were mostly classified according to John *et al*. (2002), then scored in terms of relative abundance (see below).

Periphyton community composition was quantified using a Sedgwick-Rafter Counting Chamber S50 to measure relative abundance (or 'success': Sládečková 1962, NRC 1969, Cunningham & Purewall 1983) of each algal genera. The Sedgwick-Rafter Counting Chamber is a grid-like graticule comprised of a thousand 1 mm^2 grids (referred to as 'whipple fields') etched onto the base, and has a 1 ml volume capacity. Relative abundance was calculated as mean % frequency of occurrence (by scoring the presence or no. of 'hits') of each algal genera occurring in randomly selected whipple squares, divided by the total no. of Sedgwick-Rafter whipple grid units examined from a thoroughly mixed 1 ml sub-sample (preserved in Lugol's): APHA 1971. Analysing sub-samples for a period of 15-30 minutes (or at least two minutes per whipple grid unit) is considered adequate effort for algal quantification (Woelkerling *et al.* 1976). The mean proportion of the main algal groups present: diatoms, desmids, green filamentous algae and cyanobacteria were recorded similarly, based on the % frequency of their associated genera. The main advantage of the Sedgwick-Rafter Cell is that it facilitates a quick and easy method for quantifying algal composition and abundance (NRC 1969, Woelkerling *et al.* 1976). A drawback is that samples could only be analysed at low (x10) magnification (due to the thickened coverslip) which made it problematic to identify particularly complex groups of algae such as the

Bacillariophyceae (diatoms). Therefore it was necessary to examine cleaned diatom specimens at higher magnification (see next).

Naphrax-mounted diatom frustules were usually inspected under x1000 magnification with immersion oil using a Leica Polyvar 2 photographic light microscope attached to a digital camera (courtesy of Prof. D. G. Mann, RBGE), in combination with the Microsoft™ Photoshop 6 package. Slides were consistently analysed in horizontal traverses and only intact valves counted (Kelly *et al*. 2001). Diatom community composition was recorded as the relative abundance of each individual species: mean % frequency of occurrence (no. of fields of view in which each individual species valve occurred, divided by the total no. of fields of view examined per slide). Species abundance was categorised on a five-point scale: scarce, 0-20%; occasional, 21-40%; frequent, 41-60%; highly abundant, 61-80%; and dominant, 81-100% (as adapted from Sládečková 1962). Although a range of keys and references were used for identification purposes, for consistency, diatom species were mostly classified according to Krammer & Lange-Bertalot (1986-1991). Exceptions were *Brachysira procera* (Lange-Bertalot & Moser 1994), *Navicula aquaedurae* (Lange-Bertalot 1993), and *Craticula acidoclinata* (Lange-Bertalot & Metzeltin 1996): identified from publications post Krammer & Lange-Bertalot (1986-1991) as the aformentioned series did not contain evidence of any suitable species matches for these particular specimens. Other official diatom identification keys also referred to for assistance were: Barber & Hayworth (1981), Prygiel & Coste (2000), Kelly (2000), Kelly *et al*. (2005), Taylor *et al*. (2007a) and the Automatic Diatom Identification and Classification (ADIAC) website (http://rbg-web2.rbge.org.uk/ADIAC/db/adiacdb.htm). For quality assurance purposes, Prof. David Mann (RBGE) and Dr. Jan Krokowski (SEPA) verified the identification of diatom specimens included in this study. A photographic library of diatom specimens is presented in Appendix 1.

4.3.2 Aquatic Bryophytes

A dissecting microscope was used to separate and prepare aquatic bryophyte specimens for identification, from sub-samples collected in the field (refer back to Chapter 3, section 3.3.3). Bryophyte specimens were moistened and individual leaves carefully detached from sample tissue using forceps, according to standard methodology (Watson 1981, Smith 2004). A razor blade was used occasionally to examine features of the leaves or stems that help clarify species identification. Specimens were inspected using a light compound microscope (as utilised for periphyton). Aquatic bryophyte identification followed the most recent nomenclature available: moss species were identified according to Smith (2004), and liverwort taxonomy followed that of Paton (1999).

After completing preliminary identification of bryophyte specimens at Glasgow University, the specialist expertise of Dr. Liz Kungu at the Royal Botanic Gardens Edinburgh was utilised to confirm the identity of these specimens to ensure accuracy and precision of the data. Furthermore, Mr. S.D.S. Bosanquet of the Countryside Council for Wales confirmed identification of *Schistidium agassizii*, which has a particularly rare distribution in Scotland. Following this work, there is now an official record of *S. agassizzii* in the NBN Gateway, and a specimen from Hampshire's Bridge (River Girnock) resides in the herbarium at RBGE.

The relative abundance of each aquatic bryophyte species was determined as % frequency of occurrence: by scoring the presence of each species in each sample (species composition), divided by the total no. of samples collected. Aquatic bryophyte abundance was categorized on the five-point scale, as previously described for periphyton (refer to section 4.3.1.2).

4.3.3 Vascular Submerged Macrophytes

Specimens of aquatic vascular submerged macrophytes collected in the field (refer back to Chapter 3, section 3.3.5) were identified to species level using Haslam (1975) and *Chara globularis* var. *globularis* was identified with the aid of Moore (1986). Dr. Kevin Murphy confirmed the identity of these specimens. Relative abundance of each macrophyte species was calculated as % frequency, as described for periphyton and aquatic bryophytes (refer back to 4.3.1.2 and 4.3.2, respectively).

4.4 Data Analysis

Refer to Chapter 3 (section 3.4) for details of statistical analyses conducted such as one-way ANOVA, Tukey's and Kruskal-Wallis tests, correlations, non-parametric methods (where applicable), and multiple regression analysis (minimal modelling). All statistical analyses were conducted using Minitab version 15.1.0 except where otherwise stated. Multivariate analyses were performed with TWINSPAN (Hill 1979) and CANOCO software packages (ter Braak & Šmilauer 1998), and Microsoft Excel 2003 was used to plot graphs, as well as the observed and predicted values of each response variable for the test data.

Species diversity (H) is a measure of the variety of (or number of different) species present according to their relative abundance within a given unit area sampled. Species diversity was calculated as the Shannon Wiener Index using the *Species Diversity and Richness* software package version 4 (Seaby & Henderson 2006). Species richness (S) is a measure of the mean number of species present. The Berger-Parker Dominance Index assessed species dominance, with values closer to 1 indicating that the community is dominated by one particular species, and values closer to 0 representing a more diverse assemblage. These indices were determined using the same software program.

4.4.1 Multivariate ordination of species data and environmental habitat conditions

Canonical Correspondence Analysis (CCA: ter Braak & Šmilauer 1998) is an ordination technique which analyses vegetational (samples-by-species) and environmental (samples-by-environmental variables) data matrices simultaneously (Gauch 1982). CCA is a unimodal response model used in this chapter to explore and characterise the sample-groups in terms of their species assemblage and distribution in relation to potential underlying environmental gradients to reveal predominant patterns in the data that help explain the occurrence of aquatic vegetation communities (ter Braak 1986). Similar to other ordination techniques (e.g. Principal Components Analysis: PCA: Goodall 1954; Detrended Correspondence Analysis: DCA: Hill & Gauch 1980), CCA reduces multivariate (or multidimensional) data onto two or more axes and arranges data based on their intrinsic similarity or dissimilarity to one another, ordinating this information usually in a two-dimensional space according to their eigenvalues (proportion of the variance explained) on the two major ordination axes (Gauch 1982). However, unlike PCA and DCA, CCA conducts a direct gradient analysis of the data, firstly ordinating the samples based on their variation in species composition and then reanalysing the data, to constrain sample ordination in relation to environmental variation. Those samples positioned closer together are expected to be more similar in species composition and thus share overlapping environmental habitat conditions, than those points distributed further away in the ordination space. The first few algorithm steps in CCA reiterate that of its predecessor: DCA, but the procedure supersedes indirect gradient analysis, with the incorporation of a multiple regression step which is performed thereafter. Arbitrary values are assigned to the sites (or samples) to represent scores on an artificial gradient. These are referred to as site (or sample) scores, which are then used to determine the species scores by means of weighted averaging. The mean of the species scores weighted by the abundance of each species, is then used to calculate a new set of site (or sample) scores. CCA then conducts a multiple

regression of the new site (or sample) scores with the environmental variables and the fitted values of the regression are used to produce new site (or sample) scores, and in turn new species scores. With CCA, the distribution of species scores on the artificial gradient are thereby constrained by the new site (or sample) scores. This facilitates direct gradient analysis, in which the samples or species are orientated in a CCA diagram to present the optimal and most probable solution that explains their distribution in relation to environmental variation. On a CCA diagram, points (or dots) symbolize individual samples or species, and arrows represent the measured environmental variables (ter Braak 1986). The direction of the arrows on the CCA diagram indicates the direction of each of the environmental gradients. The length of the arrow depicts the importance of the underlying environmental variable in driving species ordination that has emerged from patterns in the vegetation. In general, the longer the arrow the more influential and closely correlated the environmental variable is likely to be to the ordination. The vicinity of the dots around the arrows provides an indication of which environmental factors are likely to be principally correlated with individual samples or species and their associated assemblages. Overall, CCA diagrams help reveal the ecological response and niche preferences of individual species, to determine the potential environmental factors driving the occurrence and community composition of freshwater vegetation. Furthermore, the 'arch effect' encountered in earlier ordination techniques (e.g. PCA) is suppressed in subsequent versions like CCA by detrending (as in DCA).

The effectiveness of CCA in explaining natural variation in species composition and distribution in relation to environmental drivers can be measured by examining the output from eigenvalues of the axes and Monte Carlo permutation tests. Eigenvalues can range between 0 and +1 with values close to zero indicating that little of the variation has been accounted for by the ordination and a substantial proportion of the variance remains unexplained. Values nearer to 1 infer the converse. The Monte Carlo permutation test is a statistical test that can be conducted to test the validity of the CCA ordination. It tests the null

hypothesis that the 'species-environment data are unrelated' against a new sample data set (permutated at random from the species data whilst holding the environmental variables constant) from which each scenario would be equally likely according to the null hypothesis. The null hypothesis could be rejected at $P<0.05$ (499 permutations), indicating that species data respond significantly to the measured environmental variation (ter Braak & Smilauer 1998).

4.4.2 TWINSPAN cluster analysis, sample-group characterisation and community classification

Two-Way Indicator Species Analysis (TWINSPAN: Hill 1979) is a hierarchical classification method used to partition data into sample-groups which are most similar in terms of species composition and separate these from other dissimilar data (Gauch 1982). Similar to Cluster Analysis (applied in Chapter 2), TWINSPAN is a polythetic divisive technique used for sample classification. However, unlike Cluster Analysis, TWINSPAN classifies samples based on their species composition and relative abundances. Furthermore, TWINSPAN is considered an advanced clustering technique because it does not solely rely on presence or absence data, through application of the 'pseudospecies' concept by converting % frequency data into pre-determined scores of abundance, for example set at 5 cut levels: 1, <3%; 2, ≤15.5%; 3, ≤38%; 4, ≤63%; 5, ≥88%. Initially, TWINSPAN ordinates the samples by reciprocal averaging and is then adjusted using indicator scores. The data set is partitioned into two primary groups, one positive (+ve) and the other negative (-ve), by identifying the indicator species that tend to occur in either of the two clusters (Gauch 1982). The process continues to successively divide the data into smaller sub-groups supporting assemblages that are more similar within- than between-clusters, until a minimum size criterion is attained and groups become too small to be considered ecologically significant. TWINSPAN output is presented in a two-way ordered table showing sample-group divisions. Eigenvalues are used as a measure of how well separated these

communities (sample-groups) are from each other. Generally, eigenvalues ≥0.4 are considered an acceptable marker. TWINSPAN is a useful approach for subjectively identifying ecological boundaries that may exist between vegetation communities (species assemblages) characterised by sample-groups also deemed similar by multivariate ordination (e.g. CCA).

CANOCO (for Windows) version 4.12 was used to perform the CCA analyses. TWINSPAN was conducted using VESPAN.

Analysis of variance (ANOVA), with subsequent separation of sample-group means using Tukey's mean comparison test, for ANOVA outcomes significant at $P<0.05$, was applied to test the significance of differences in mean values of environmental variables between sample-groups produced by TWINSPAN.

4.5 Results

4.5.1 Variation in periphyton community composition and diversity in the Water of Dye, River Girnock and Knockan Burn; their sub-catchments and sites

A total of twenty-five diatom genera (c. 85 individual species), two desmids, seven green filamentous algae, a single type of cyanobacterium, and two rhodophytes were identified from a mean total of 256 samples including artificial and naturally-occurring substrates combined (refer to Table 4.1 for listed periphyton flora).

Diatoms were the dominant assemblage of periphyton at mostly all sites, and samples, often comprising >70% of community composition. Usually, the second most abundant in the periphyton community were the green filamentous algae, and to a lesser extent cyanobacteria (e.g. filamentous *Rivularia* sp., distinct to the River Girnock). Desmids (e.g. *Closterium* sp., *Cosmarium* sp.) and rhodophytes (e.g. *Lemanea fluviatilis* occurring in the Water of Dye and River Girnock, and

Batrachospermum sp. distribution confined to Knockan Burn) occupied the lowest proportions of the periphyton populations.

There was significant variation in periphyton community composition between the three sub-catchment streams, however the most notable shift in assemblage structure was attributed to the diatoms.

Periphyton species	**Synonym(s)**	**Order**	**Class / Family**
[1]*Achnanthes lanceolata* (Brébisson) Grunow in Cleve & Grunow	= *Planothidium lanceolatum*	Achnanthales	Bacillariophyceae / Acthnanthaceae
[1]*Achnanthidium minutissima* Kützing	= *Achnanthes minutissima*, *Acthnanthidium minutissimum*	Achnanthales	Bacillariophyceae / Acthnanthaceae
[1]*Cocconeis placentula* Ehrenberg	-	Achnanthales	Bacillariophyceae / Acthnanthaceae
[1]*Denticula tenuis* Kützing	= *Denticula frigida*, *Denticula crassula*, *Denticula inflata*	Bacillariales	Bacillariophyceae / Epithemiaceae
[1]*Nitzschia gracilis* Hantzsch	= *Nitzschia graciloides*	Bacillariales	Bacillariophyceae / Bacillariaceae
[1]*Nitzschia hantzschiana* Rabenhorst	= *Nitzschia perpusilla*, *Nitzschia frustulum* var. *glacialis*	Bacillariales	Bacillariophyceae / Bacillariaceae
[1]*Nitzschia perminuta* agg. (Grunow) M. Peragallo	= *Nitzschia palea* var. *perminuta*	Bacillariales	Bacillariophyceae / Bacillariaceae
[1]*Nitzschia intermedia* agg. Hantzsch ex Cleve & Grunow	-	Bacillariales	Bacillariophyceae / Bacillariaceae
[1]*Nitzschia* cf. *acula* Hantzsch ex Cleve & Grunow	-	Bacillariales	Bacillariophyceae / Bacillariaceae
[1]*Nitzschia palea* agg. (Kützing) W. Smith	-	Bacillariales	Bacillariophyceae / Bacillariaceae
[1]*Nitzschia sublinearis* Hustedt	-	Bacillariales	Bacillariophyceae / Bacillariaceae
[1]*Nitzschia angustata* (W. Smith) Grunow in Cleve & Grunow	= *Tryblionella angustata*	Bacillariales	Bacillariophyceae / Bacillariaceae
[1]*Nitzschia* undefined sp. [no corresponding species	-	Bacillariales	Bacillariophyceae / Bacillariaceae

identifed to date]			
[1]*Cymbella silesiaca* Bleisch in Rabenhorst	= *Cymbella ventricosa*, *Cymbella minuta* var. *silesiaca*, *Encyonema silesiacum*	Cymbellales	Bacillariophyceae / Naviculaceae
[1]*Cymbella gracilis* (Ehrenberg) Kützing	= *Cocconema gracile*, *Cymbella lunata*, *Encyonema gracile*, *Encyonema neogracile*	Cymbellales	Bacillariophyceae / Naviculaceae
[1]*Cymbella cistula* (Ehrenberg) Kirchner	= *Bacillaria cistula*, *Cymbella maculata*	Cymbellales	Bacillariophyceae / Naviculaceae
[1]*Cymbella cymbiformis* Agardh	-	Cymbellales	Bacillariophyceae / Naviculaceae
[1]*Cymbella helvetica* Kützing	= *Cymbella compacta*	Cymbellales	Bacillariophyceae / Naviculaceae
[1]*Cymbella affinis* Kützing	= *Cymbella excisa*, *Cocconema parvum*	Cymbellales	Bacillariophyceae / Naviculaceae
[1]*Cymbella lanceolata* (Ehrenberg) Kirchner	= *Cocconema lanceolatum*	Cymbellales	Bacillariophyceae / Naviculaceae
[1]*Cymbella caespitosa* (Kützing) Brun	= *Encyonema caespitosum*	Cymbellales	Bacillariophyceae / Naviculaceae
[1]*Cymbella naviculiformis* (Auerswald) Cleve	= *Cymbella cuspidata* var. *naviculiformis*	Cymbellales	Bacillariophyceae / Naviculaceae
[1]*Cymbella microcephala* Grunow in Van Heurck	= *Encyonopsis microcephala*	Cymbellales	Bacillariophyceae / Naviculaceae
[1]*Didymosphenia geminata* (Lyngbye) M. Schmidt	= *Echinella geminata*, *Gomphonema geminatum*	Cymbellales	Bacillariophyceae / Naviculaceae
[1]*Gomphonema* cf. *parvulum* var. *exilissimum* (Kützing) Kützing	-	Cymbellales	Bacillariophyceae / Naviculaceae
[1]*Gomphonema clavatum* Ehrenberg	= *Gomphonema subclavatum*, *Gomphonema montanum*	Cymbellales	Bacillariophyceae / Naviculaceae
[1]*Gomphonema truncatum*	= *Gomphonema*	Cymbellales	Bacillariophyceae /

Ehrenberg	*constrictum*, *Gomphonema capitatum*, *Gomphonema turgidum*		Naviculaceae
[1]*Gomphonema acuminatum* Ehrenberg	= *Gomphonema brebissonii*	Cymbellales	Bacillariophyceae / Naviculaceae
[1]*Gomphonema olivaceum* (Hornemann) Brébisson	-	Cymbellales	Bacillariophyceae / Naviculaceae
[1]*Gomphonema olivaceum* (Hornemann) Brébisson var. *olivaceoides* (Hornemann) Brébisson	= *Gomphonema olivaceoides*	Cymbellales	Bacillariophyceae / Naviculaceae
[1]*Gomphonema gracile* Ehrenberg	-	Cymbellales	Bacillariophyceae / Naviculaceae
[1]*Gomphonema ventricosum* Gregory	-	Cymbellales	Bacillariophyceae / Naviculaceae
[1]*Eunotia arcus* sensu Ehrenberg	= *Himantidium arcus*	Eunotiales	Bacillariophyceae / Eunotiaceae
[1]*Eunotia exigua* (Brébisson ex Kützing) Rabenhorst	= *Himantidium exiguum*	Eunotiales	Bacillariophyceae / Eunotiaceae
[1]*Eunotia muscicola* Krasske var. *tridentula* Nörpel & Lange-Bertalot	= *Eunotia tridentula*, *Eunotia quaternaria*, *Eunotia quinaria*, *Eunotia polydentula*, *Eunotia ehrenbergii*, *Eunotia perpusilla*	Eunotiales	Bacillariophyceae / Eunotiaceae
[1]*Eunotia* cf. *incisa* Gregory	= *Himantidium veneris*	Eunotiales	Bacillariophyceae / Eunotiaceae
[1]*Eunotia meisteri* Hustedt	-	Eunotiales	Bacillariophyceae / Eunotiaceae
[1]*Eunotia bilunaris* (Ehrenberg) Mills var. *linearis* (Okuno) Lange-Bertalot & Nörpel	= *Eunotia flexuosa* var. *linearis*, *Eunotia okavangoi*, *Eunotia curvata* var. *linearis*	Eunotiales	Bacillariophyceae / Eunotiaceae
[1]*Eunotia bilunaris* (Ehrenberg) Mills var.	= *Eunotia lunaris* var. *subarcuata*	Eunotiales	Bacillariophyceae / Eunotiaceae

mucophila Lange-Bertalot & Nörpel			
[1]*Eunotia serra* Ehrenberg	-	Eunotiales	Bacillariophyceae / Eunotiaceae
[1]*Eunotia implicata* Nörpel, Lange-Bertalot & Alles	-	Eunotiales	Bacillariophyceae / Eunotiaceae
[1]*Brachysira vitrea* (Grunow) Ross	= *Anomoeoneis exilis*, *Navicula variabilis*, *Anomoeoneis variabilis*, *Navicula exilis*, *Anomoeoneis vitrea*	Naviculales	Bacillariophyceae / Naviculaceae
[1]*Brachysira procera* Lange-Bertalot & Moser	-	Naviculales	Bacillariophyceae / Naviculaceae
[1]*Craticula acidoclinata* Lange-Bertalot & Metzeltin	-	Naviculales	Bacillariophyceae / Naviculaceae
[1]*Diploneis* cf. *elliptica* (Kutzing) Cleve	= *Navicula elliptica*	Naviculales	Bacillariophyceae / Naviculaceae
[1]*Diploneis marginestriata* Hustedt	-	Naviculales	Bacillariophyceae / Naviculaceae
[1]*Diploneis oblongella* (Naegeli) Cleve-Euler	= *Navicula oblongella*, *Diploneis ovalis* var. *oblongella*	Naviculales	Bacillariophyceae / Naviculaceae
[1]*Frustulia rhomboides* (Ehrenberg) De Toni var. *rhomboides*	= *Navicula rhomboides*	Naviculales	Bacillariophyceae / Naviculaceae
[1]*Frustulia rhomboides* (Ehrenberg) De Toni var. *crassinervia* (Brébisson ex Smith) Ross	= *Navicula crassinervia*, *Frustulia crassinervia*	Naviculales	Bacillariophyceae / Naviculaceae
[1]*Frustulia vulgaris* (Thwaites) De Toni	= *Schizonema vulgare*	Naviculales	Bacillariophyceae / Naviculaceae
[1]*Navicula rhynchocephala* Kützing	-	Naviculales	Bacillariophyceae / Naviculaceae
[1]*Navicula lanceolata*	= *Frustulia lanceolata*,	Naviculales	Bacillariophyceae /

(Agardh) Ehrenberg	*Schizonema thwaitesii*		Naviculaceae
[1]*Navicula* cf. *aquaedurae* Lange-Bertalot	-	Naviculales	Bacillariophyceae / Naviculaceae
[1]*Navicula angusta* Grunow	= *Navicula cari* var. *angusta, Navicula cincta* var. *angusta, Navicula cincta* var. *linearis, Navicula pseudocari, Navicula lobeliae*	Naviculales	Bacillariophyceae / Naviculaceae
[1]*Navicula radiosa* Kützing	-	Naviculales	Bacillariophyceae / Naviculaceae
[1]*Navicula tripunctata* (O. F. Müller) Bory	= *Vibrio tripunctatus*, *Navicula gracilis*, *Schizonema neglectum*	Naviculales	Bacillariophyceae / Naviculaceae
[1]*Navicula capitatoradiata* Germain	= *Navicula cryptocephala* var. *intermedia*, *Navicula salinarum* var. *intermedia*	Naviculales	Bacillariophyceae / Naviculaceae
[1]*Navicula* cf. *gregaria* Donkin	= *Navicula cryptocephala*, *Navicula gregalis*, *Navicula gotlandica*, *Navicula phyllepta*	Naviculales	Bacillariophyceae / Naviculaceae
[1]*Navicula* cf. *pygmaea* agg. Kützing	= *Navicula minutula*, *Navicula rotundata*, *Navicula hudsonis*, *Diploneis hudsonis*	Naviculales	Bacillariophyceae / Naviculaceae
[1]*Navicula jaernefeltii* Hustedt	= *Cavinula jaernefeltii*	Naviculales	Bacillariophyceae / Naviculaceae
[1]*Navicula minima* Grunow in Van Heurck	= *Navicula minutissima, Navicula atomoides, Navicula minima* var. *atomoides, Navicula tantula*	Naviculales	Bacillariophyceae / Naviculaceae
[1]*Nedium bisulcatum* (Lagerstedt) Cleve	= *Navicula bisulcata*	Naviculales	Bacillariophyceae / Naviculaceae
[1]*Pinnularia subcapitata* Gregory	= *Pinnularia hilseana*, *Navicula hilseana*	Naviculales	Bacillariophyceae / Naviculaceae

[1]*Pinnularia* cf. *sudetica* (Hilse) M. Peragallo	= *Navicula sudetica*	Naviculales	Bacillariophyceae / Naviculaceae
[1]*Pinnularia* cf. *divergens* W. Smith	-	Naviculales	Bacillariophyceae / Naviculaceae
[1]*Epithemia adnata* (Kützing) Brébisson	= *Epithemia zebra*, *Frustulia adnata*, *Eunotia zebra*, *Epithemia kurzeana*	Rhopalodiales	Bacillariophyceae / Epithemiaceae
[1]*Epithemia sorex* Kützing	-	Rhopalodiales	Bacillariophyceae / Epithemiaceae
[1]*Rhopalodia gibba* (Ehrenberg) O. Müller	-	Rhopalodiales	Bacillariophyceae / Epithemiaceae
[1]*Surirella roba* Leclercq	-	Surirellales	Bacillariophyceae / Surirellaceae
[1]*Surirella brebissonii* Krammer & Lange-Bertalot	-	Surirellales	Bacillariophyceae / Surirellaceae
[1]*Diatoma mesodon* (Ehrenberg) Kützing	= *Fragilaria mesodon*, *Diatoma hiemalis* var. *mesodon*	Fragilariales	Fragilariophyceae / Fragilariaceae
[1]*Diatoma moniliformis* Kützing	= *Diatoma tenuis* var. *moniliformis*	Fragilariales	Fragilariophyceae / Fragilariaceae
[1]*Diatoma tenuis* Agardh	= *Diatoma tenuis* var. *elongatum*, *Diatoma elongatum*, *Diatoma mesoleptum*	Fragilariales	Fragilariophyceae / Fragilariaceae
[1]*Fragilaria capucina* var. *vaucheriae* (Kützing) Lange-Bertalot	= *Exilaria vaucheriae*, *Fragilaria intermedia*, *Synedra rumpens* var. *meneghiniana*, *Fragilaria vaucheriae*	Fragilariales	Fragilariophyceae / Fragilariaceae
[1]*Fragilaria capucina* var. *gracilis* (Oestrup) Hustedt	= *Synedra rumpens*, *Fragilaria gracilis*, *Synedra familiaris*, *Synedra famelica*	Fragilariales	Fragilariophyceae / Fragilariaceae
[1]*Fragilaria virescens* Ralfs	= *Fragilaria aequalis*	Fragilariales	Fragilariophyceae / Fragilariaceae

[1]*Fragilaria arcus* (Ehrenberg) Cleve	= *Navicula arcus*, *Hannaea arcus*, *Ceratoneis arcus*	Fragilariales	Fragilariophyceae / Fragilariaceae
[1]*Fragilaria pulchella* (Ralfs ex Kützing) Lange-Bertalot	= *Exilaria pulchella*, *Synedra pulchella*, *Synedra familiaris*, *Ctenophora pulchella*	Fragilariales	Fragilariophyceae / Fragilariaceae
[1]*Meridion circulare* (Greville) C. A. Agardh var. *constrictum* (Ralfs) Van Heurck	= *Meridion constrictum*	Fragilariales	Fragilariophyceae / Fragilariaceae
[1]*Meridion circulare* (Greville) C. A. Agardh	= *Echinella circularis*, *Meridion zinckenii*	Fragilariales	Fragilariophyceae / Fragilariaceae
[1]*Synedra ulna* (Nitzsch) Ehrenberg	= *Bacillaria ulna, Fragilaria ulna*	Fragilariales	Fragilariophyceae / Fragilariaceae
[1]*Tabellaria flocculosa* (Roth) Kützing	= *Conferva flocculosa*	Tabellariales	Fragilariophyceae / Fragilariaceae
[1]*Tetracyclus glans* (Ehrenberg) Mills	= *Navicula glans*, *Tetracyclus lacustris*	Tabellariales	Fragilariophyceae / Fragilariaceae
[2]*Closterium* sp.	-	Zygnematales	Chlorophyceae
[2]*Cosmarium* sp.	-	Zygnematales	Chlorophyceae
[3]*Mougeotia* sp.	-	Zygnematales	Chlorophyceae
[3]*Spirogyra* sp.	-	Zygnematales	Chlorophyceae
[3]*Zygnema* sp.	-	Zygnematales	Chlorophyceae
[3]*Microspora* sp.	-	Microsporales	Chlorophyceae
[3]*Ulothrix zonata*	-	Ulotrichales	Chlorophyceae
[4]*Stigeoclonium tenue*	-	Chaetophorales	Chlorophyceae
[4]*Bulbochaete* sp.	-	Oedogoniales	Chlorophyceae
[5]*Rivularia* sp.	-	Nostocales	Cyanophyceae
[6]*Batrachospermum* sp.	-	Batrachospermales	Rhodophyceae
[6]*Lemanea fluviatilis*	-	Batrachospermales	Rhodophyceae

Table 4.1 Periphyton species list: [1]Diatoms (mostly Krammer & Lange-Bertalot, 1986-1991); [2]Desmids (John *et al.*, 2002); [3]Unbranched green filamentous algae (John *et al.*, 2002); [4]Branched green filamentous algae (John *et al.*, 2002); [5]Cyanobacteria (John *et al.*, 2002) and [6]Rhodophytes (John *et al.*, 2002).

4.5.1.1 Periphyton community composition and diversity of artificial substrata

Data from the use of short-term linoleum substrate samplers indicated that the River Girnock had a richer assemblage of periphyton species than the Water of Dye and Knockan Burn (Table 4.2). Further to this, the River Girnock and Knockan Burn were characterised by significantly high-diversity periphyton assemblages compared to the Water of Dye which possessed a low diversity periphyton community and tended to be dominated by fewer species.

Long-term periphyton samplers showed similar community composition to that of their respective short-term linoleum samplers, and although abundances may have varied between the various types of substrates these differences were mostly insignificant (data therefore not presented). Both long-term linoleum (Table 4.4) and Astroturf samplers (Table 4.6) showed similar variation in periphyton species richness, diversity and dominance between the three streams as described for the aforementioned short-term linoleum substrates.

4.5.1.2 Periphyton community composition and diversity of naturally-occurring substrata

Overall, despite often being more abundant, periphyton communities harvested from natural-occurring substrata were similar in composition to assemblages observed on artificial substrates. Also natural variation in periphyton species richness, diversity and dominance on naturally-occurring mineral particles (Table 4.12) and aquatic bryophytes (Table 4.14) reflected the trends observed from artificial samplers.

Mean Variable	Water of Dye		River Girnock		Knockan Burn		PANOVA
	Mean	*S.E*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	22.6[a]	1.11	33.6[b]	2.17	26.1[a]	1.87	P<0.001***
Periphyton species diversity: **H**	2.53[a]	0.06	2.89[b]	0.09	2.77[b]	0.07	P<0.01**
Periphyton species dominance	0.19[a]	0.03	0.15[b]	0.02	0.16[ab]	0.02	P<0.05*

Table 4.2 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 400 cm^2), periphyton species diversity (per 400 cm^2), and periphyton species dominance (per 400 cm^2) of short-term linoleum substrates between study stream sub-catchments (n = 56 samples). For details of environmental habitat conditions refer to Chapter 3, Table 3.4. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

	Water of Dye						River Girnock								Knockan Burn				
Mean Variable	**BB**		**CF**		**BD**		**IB**		**HB**		**LM**		**UK**		**MK**		**LK**		**P_{ANOVA}**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton richness: **S**	21.0[a]	2.01	24.0[a]	2.07	22.7[a]	1.74	34.0[b]	3.16	34.4[b]	3.49	32.4[b]	4.93	24.8[a]	2.96	23.6[a]	2.23	29.8[ab]	4.19	P<0.05*
Periphyton species diversity: **H**	2.39[a]	0.11	2.63[a]	0.10	2.56[a]	0.08	3.04[b]	0.12	2.87[b]	0.16	2.77[b]	0.17	2.72[b]	0.14	2.70[b]	0.09	2.89[b]	0.15	P<0.01**
Periphyton species dominance	0.22[a]	0.03	0.18[a]	0.02	0.18[a]	0.01	0.13[b]	0.03	0.14[b]	0.03	0.17[ab]	0.02	0.17[ab]	0.02	0.17[ab]	0.03	0.13[b]	0.01	P<0.05*

Table 4.3 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 400 cm^2), periphyton species diversity (per 400 cm^2), and periphyton species dominance (per 400 cm^2) of short-term linoleum substrates between sampling sites (n = 56 samples). For details of environmental habitat conditions refer to Chapter 3, Table 3.5. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Mean Variable	Water of Dye		River Girnock		Knockan Burn		P_{ANOVA}
	Mean	*S.E*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	22.1^{a}	2.08	32.7^{b}	3.01	26.7^{a}	2.07	P<0.001***
Periphyton species diversity: **H**	2.45^{a}	0.07	2.72^{b}	0.12	2.73^{b}	0.08	P<0.01**
Periphyton species dominance	0.24^{a}	0.02	0.15^{b}	0.02	0.18^{ab}	0.01	P<0.05*

Table 4.4 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 100 cm^2), periphyton species diversity (per 100 cm^2), and periphyton species dominance (per 100 cm^2) of long-term linoleum substrates between study stream sub-catchments (n = 27 samples). For details of environmental habitat conditions refer to Chapter 3, Table 3.6. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

	Water of Dye								River Girnock						Knockan Burn				
Mean Variable	**BB**		**CF**		**BD**		**IB**		**HB**		**LM**		**UK**		**MK**		**LK**		**P_{ANOVA}**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton richness: **S**	19.0^{a}	2.65	22.7^{a}	4.67	24.7^{a}	3.84	33.3^{b}	4.09	35.0^{b}	6.36	29.8^{ab}	6.44	23.9^{a}	1.33	27.7^{ab}	3.84	28.5^{ab}	4.33	P<0.05*
Periphyton species diversity: **H**	2.37^{a}	0.14	2.45^{a}	0.13	2.52^{a}	0.10	2.76^{b}	0.14	2.76^{b}	0.24	2.64^{b}	0.29	2.60^{b}	0.14	2.74^{b}	0.14	2.84^{b}	0.13	P<0.01**
Periphyton species dominance	0.22^{a}	0.04	0.25^{a}	0.06	0.25^{a}	0.04	0.14^{b}	0.04	0.14^{b}	0.02	0.17^{ab}	0.02	0.20^{a}	0.03	0.18^{a}	0.04	0.17^{ab}	0.03	P<0.05*

Table 4.5 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 100 cm^2), periphyton species diversity (per 100 cm^2), and periphyton species dominance (per 100 cm^2) of long-term linoleum substrates between sampling sites (n = 27 samples). For details of environmental habitat conditions refer to Chapter 3, Table 3.7. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Mean Variable	Water of Dye		River Girnock		Knockan Burn		PANOVA
	Mean	*S.E*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	23.3[a]	1.35	35.9[b]	1.91	25.3[a]	2.05	P<0.001***
Periphyton species diversity: **H**	2.46[a]	0.04	2.74[b]	0.08	2.76[b]	0.06	P<0.01**
Periphyton species dominance	0.22[a]	0.02	0.17[b]	0.02	0.18[ab]	0.02	P<0.05*

Table 4.6 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 1440 cm^2), periphyton species diversity (per 1440 cm^2), and periphyton species dominance (per 1440 cm^2) of long-term Astroturf substrates between study stream sub-catchments (n = 27 samples). For details of environmental habitat conditions refer to Chapter 3, Table 3.8. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

	Water of Dye						River Girnock								Knockan Burn				
Mean Variable	**BB**		**CF**		**BD**		**IB**		**HB**		**LM**		**UK**		**MK**		**LK**		**P**ANOVA
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton richness: **S**	22.3[a]	1.20	25.0[a]	3.61	22.7[a]	2.33	36.0[b]	2.65	36.3[b]	4.37	35.3[b]	4.18	21.3[a]	1.20	23.0[a]	2.89	31.7[ab]	3.18	P<0.05*
Periphyton species diversity: **H**	2.43[a]	0.05	2.44[a]	0.11	2.51[a]	0.02	2.82[b]	0.06	2.76[b]	0.14	2.64[ab]	0.21	2.68[ab]	0.12	2.71[ab]	0.11	2.89[b]	0.10	P<0.05*
Periphyton species dominance	0.23[a]	0.02	0.20[a]	0.05	0.23[a]	0.02	0.15[b]	0.02	0.15[b]	0.03	0.20[ab]	0.03	0.20[a]	0.03	0.18[a]	0.02	0.15[ab]	0.01	P<0.05*

Table 4.7 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 1440 cm^2), periphyton species diversity (per 1440 cm^2), and periphyton species dominance (per 1440 cm^2) of long-term Astroturf substrates between sampling sites (n = 27 samples). For details of environmental habitat conditions refer to Chapter 3, Table 3.9. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Mean Variable	Water of Dye		River Girnock		Knockan Burn		P_{ANOVA}
	Mean	*S.E*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	N/A		N/A		20.3	1.48	N/A
Periphyton species diversity: **H**	N/A		N/A		2.60	0.09	N/A
Periphyton species dominance	N/A		N/A		0.18	0.02	N/A

Table 4.8 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 87.7 cm^2), periphyton species diversity (per 87.7 cm^2), and periphyton species dominance (per 87.7 cm^2) of long-term plastic aquarium *Potamogeton*-like substrates between study stream sub-catchments (n = 6 samples). For details of environmental habitat conditions refer to Chapter 3, Table 3.10. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

	Water of Dye								River Girnock						Knockan Burn				
Mean Variable	**BB**		**CF**		**BD**		**IB**		**HB**		**LM**		**UK**		**MK**		**LK**		**P_{ANOVA}**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton richness: **S**	N/A		N/A		N/A		N/A		N/A		N/A		18.0^{a}	1.53	N/A		22.6^{b}	1.67	P<0.01**
Periphyton species diversity: **H**	N/A		N/A		N/A		N/A		N/A		N/A		2.46^{a}	0.12	N/A		2.74^{b}	0.08	P<0.01**
Periphyton species dominance	N/A		N/A		N/A		N/A		N/A		N/A		0.21^{a}	0.04	N/A		0.14^{b}	0.02	P<0.01**

Table 4.9 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 87.7 cm^2), periphyton species diversity (per 87.7 cm^2), and periphyton species dominance (per 87.7 cm^2) of long-term plastic aquarium *Potamogeton*-like substrates between sampling sites (n = 6 samples). For details of environmental habitat conditions refer to Chapter 3, Table 3.11. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Mean Variable	Water of Dye		River Girnock		Knockan Burn		P_{ANOVA}
	Mean	*S.E*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	N/A		N/A		19.8	1.62	N/A
Periphyton species diversity: **H**	N/A		N/A		2.59	0.09	N/A
Periphyton species dominance	N/A		N/A		0.18	0.03	N/A

Table 4.10 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 26.1 cm^2), periphyton species diversity (per 26.1 cm^2), and periphyton species dominance (per 26.1 cm^2) of long-term plastic aquarium *Myriophyllum*-like substrates between study stream sub-catchments (n = 6 samples). For details of environmental habitat conditions refer to Chapter 3, Table 3.12. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

	Water of Dye								River Girnock								Knockan Burn				
Mean Variable	**BB**		**CF**		**BD**		**IB**		**HB**		**LM**		**UK**				**MK**		**LK**		**P**ANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*			*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	N/A		N/A		N/A		N/A		N/A		N/A		16.5^{a}	1.33			N/A		23.0^{b}	1.72	P<0.01**
Periphyton species diversity: **H**	N/A		N/A		N/A		N/A		N/A		N/A		2.42^{a}	0.14			N/A		2.75^{b}	0.10	P<0.01**
Periphyton species dominance	N/A		N/A		N/A		N/A		N/A		N/A		0.21^{a}	0.05			N/A		0.15^{b}	0.02	P<0.01**

Table 4.11 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 26.1 cm^2), periphyton species diversity (per 26.1 cm^2), and periphyton species dominance (per 26.1 cm^2) of long-term plastic aquarium *Myriophyllum*-like substrates between sampling sites (n = 6 samples). For details of environmental habitat conditions refer to Chapter 3, Table 3.13. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Mean Variable	Water of Dye		River Girnock		Knockan Burn		PANOVA
	Mean	*S.E*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	20.4[a]	1.02	31.3[b]	1.10	22.8[a]	0.94	P<0.001***
Periphyton species diversity: **H**	2.34[a]	0.04	2.84[b]	0.04	2.72[b]	0.04	P<0.001***
Periphyton species dominance	0.24[a]	0.01	0.16[b]	0.01	0.14[b]	0.01	P<0.001***

Table 4.12 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 4.91 cm^2), periphyton species diversity (per 4.91 cm^2), and periphyton species dominance (per 4.91 cm^2) of naturally-occurring mineral substrata between study stream sub-catchments (n = 79 samples). For details of environmental habitat conditions refer to Chapter 3, Table 3.14. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

	Water of Dye						River Girnock						Knockan Burn						
Mean Variable	**BB**		**CF**		**BD**		**IB**		**HB**		**LM**		**UK**		**MK**		**LK**		**P_{ANOVA}**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton richness: **S**	19.2^{a}	1.10	19.9^{a}	1.72	22.0^{a}	2.34	33.1^{b}	1.16	32.0^{b}	2.46	28.9^{b}	1.92	21.5^{a}	0.97	22.0^{a}	1.39	25.0^{a}	1.60	P<0.001***
Periphyton species diversity: **H**	2.28^{a}	0.06	2.30^{a}	0.07	2.44^{a}	0.08	2.94^{b}	0.04	2.81^{b}	0.08	2.78^{b}	0.09	2.62^{b}	0.06	2.70^{b}	0.06	2.83^{b}	0.07	P<0.001***
Periphyton species dominance	0.22^{a}	0.02	0.24^{a}	0.02	0.25^{a}	0.02	0.16^{b}	0.01	0.16^{b}	0.01	0.17^{b}	0.01	0.16^{b}	0.01	0.14^{b}	0.01	0.13^{b}	0.01	P<0.001***

Table 4.13 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 4.91 cm^2), periphyton species diversity (per 4.91 cm^2), and periphyton species dominance (per 4.91 cm^2) of naturally-occurring mineral substrata between sampling sites (n = 79 samples). For details of environmental habitat conditions refer to Chapter 3, Table 3.15. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Mean Variable	Water of Dye		River Girnock		Knockan Burn		P_{ANOVA}
	Mean	*S.E*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	21.8^{a}	0.97	29.3^{b}	1.18	23.5^{a}	0.91	P<0.001***
Periphyton species diversity: **H**	2.42^{a}	0.05	2.75^{b}	0.05	2.76^{b}	0.04	P<0.001***
Periphyton species dominance	0.23^{a}	0.01	0.16^{b}	0.01	0.18^{b}	0.01	P<0.01**

Table 4.14 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 19.64 cm^2), periphyton species diversity (per 19.64 cm^2), and periphyton species dominance (per 19.64 cm^2) of naturally-occurring aquatic bryophytes between study stream sub-catchments (n = 74 samples). For details of environmental habitat conditions refer to Chapter 3, Table 3.16. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

	Water of Dye								River Girnock						Knockan Burn				
Mean Variable	**BB**		**CF**		**BD**		**IB**		**HB**		**LM**		**UK**		**MK**		**LK**		**P_{ANOVA}**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton richness: **S**	20.7^{a}	1.31	21.8^{a}	1.97	23.0^{a}	1.76	31.2^{b}	1.18	31.4^{b}	2.17	25.3^{a}	1.77	21.4^{a}	1.71	23.2^{a}	1.42	26.0^{a}	1.16	P<0.001***
Periphyton species diversity: **H**	2.31^{a}	0.07	2.47^{a}	0.10	2.50^{a}	0.08	2.90^{b}	0.06	2.85^{b}	0.07	2.50^{a}	0.08	2.63^{a}	0.06	2.82^{b}	0.04	2.82^{b}	0.05	P<0.001***
Periphyton species dominance	0.25^{a}	0.03	0.22^{a}	0.02	0.21^{a}	0.02	0.15^{b}	0.01	0.15^{b}	0.01	0.19^{a}	0.01	0.21^{a}	0.02	0.16^{b}	0.01	0.16^{b}	0.01	P<0.001***

Table 4.15 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 19.64 cm^2), periphyton species diversity (per 19.64 cm^2), and periphyton species dominance (per 19.64 cm^2) of naturally-occurring aquatic bryophytes between sampling sites (n = 74 samples). For details of environmental habitat conditions refer to Chapter 3, Table 3.17. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

Mean Variable	Water of Dye		River Girnock		Knockan Burn		P_{ANOVA}
	Mean	*S.E*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	N/A		N/A		23.3	1.60	N/A
Periphyton species diversity: **H**	N/A		N/A		2.66	0.05	N/A
Periphyton species dominance	N/A		N/A		0.16	0.02	N/A

Table 4.16 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 400 cm^2), periphyton species diversity (per 400 cm^2), and periphyton species dominance (per 400 cm^2) of naturally-occurring vascular submerged macrophytes between study stream sub-catchments (n = 10 samples). For details of environmental habitat conditions refer to Chapter 3, Table 3.18. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

	Water of Dye								River Girnock								Knockan Burn				
Mean Variable	**BB**		**CF**		**BD**		**IB**		**HB**		**LM**		**UK**		**MK**		**LK**		**P_{ANOVA}**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton richness: **S**	N/A		N/A		N/A		N/A		N/A		N/A		20.4	1.72	N/A		26.2	2.05	P<0.05*
Periphyton species diversity: **H**	N/A		N/A		N/A		N/A		N/A		N/A		2.54	0.03	N/A		2.78	0.06	P<0.05*
Periphyton species dominance	N/A		N/A		N/A		N/A		N/A		N/A		0.19	0.03	N/A		0.13	0.02	P<0.05*

Table 4.17 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 400 cm^2), periphyton species diversity (per 400 cm^2), and periphyton species dominance (per 400 cm^2) of naturally-occurring vascular submerged macrophytes between sampling sites (n = 10 samples). For details of environmental habitat conditions refer to Chapter 3, Table 3.19. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

4.5.2 Temporal and seasonal variation in periphyton community composition and diversity in the Water of Dye, River Girnock and Knockan Burn

4.5.2.1 Periphyton community composition and diversity of artificial substrata in the Water of Dye

In the Water of Dye, periphyton species richness, diversity and dominance showed significant temporal variation on short-term linoleum substrates ranging between 15.3 – 31.7, 2.15 – 2.89, and 0.12 - 0.30, respectively (Table 4.18). During October 2004 through to April 2005 periphyton species richness, diversity and dominance exhibited minor fluctuations but did not vary significantly between these sampling dates. However, in May 2005 there was a significant reduction in periphyton species richness and diversity, coupled to an increase in dominance when a limited number of species occurred in the samples. In July and August 2005, periphyton species richness and diversity peaked significantly, whilst species dominance was significantly lower compared to previous samplings due to the array of periphyton species present in the community during the summer months. Periphyton species richness, diversity and dominance had returned to background levels (similar to pre-May 2005) when short-term linoleum substrates were sampled in April 2006. In general, trends in periphyton species diversity mirrored temporal variation observed for periphyton species richness on short-term linoleum substrates (Figure 4.1), but unsurprisingly periphyton species dominance showed an inverse relationship with each of these community attributes (Figure 4.2 and Figure 4.3, respectively).

Periphyton species richness, diversity and dominance harvested from short-term linoleum substrates exhibited significant seasonal variation between survey dates in the Water of Dye (Table 4.19). Periphyton species richness was significantly lower in May 2005 compared to both August 2005 and April 2006 (which also differed significantly from each other). Periphyton species diversity was

significantly higher, and species dominance lower in August 2005 and April 2006 compared to May 2005.

Long-term linoleum (Table 4.20) and Astroturf (Table 4.21) substrates showed similar trends in periphyton species richness, diversity and dominance between survey dates, as described for short-term linoleum samplers.

4.5.2.2 Periphyton community composition and diversity of naturally-occurring substrata in the Water of Dye

Periphyton species richness, diversity and dominance harvested from naturally-occurring mineral particles (Table 4.22) and aquatic bryophytes (Table 4.23) in the Water of Dye, varied similarly between sampling dates as described previously for artificial substrates.

4.5.2.3 Periphyton community composition and diversity of artificial substrata in the River Girnock

In the River Girnock, periphyton species richness, diversity and dominance also showed significant temporal variation, ranging between 23.3 – 41.7, 2.54 – 3.23, and 0.10 – 0.19, respectively (Table 4.24). Minimal periphyton species richness and diversity characterised the April and May 2005 harvests, which peaked significantly in July and August 2005 and fell to low background levels in April 2006. Overall, periphyton species richness and diversity showed parallel patterns of temporal variation on short-term linoleum substrates (Figure 4.4) but species dominance exhibited the opposite response compared to these community attributes (Figure 4.5 and Figure 4.6, respectively).

Periphyton species richness and diversity were significantly higher, and species dominance lower, on short-term linoleum substrates harvested in August 2005 compared to May 2005 and April 2006 in the River Girnock (Table 4.25).

In the River Girnock, long-term linoleum (Table 4.26) and Astroturf (Table 4.27) samplers showed similar trends in periphyton species richness, diversity and dominance between survey dates, as described for short-term linoleum substrates.

4.5.2.4 Periphyton community composition and diversity of naturally-occurring substrata in the River Girnock

Periphyton species richness, diversity and dominance harvested from naturally-occurring mineral particles (Table 4.28) and aquatic bryophytes (Table 4.29) in the River Girnock, varied similarly between sampling dates as described previously for artificial substrates.

4.5.2.5 Periphyton community composition and diversity of artificial substrata in Knockan Burn

In Knockan Burn, significant temporal variation was observed for periphyton species richness, diversity and dominance harvested from short-term linoleum substrates, ranging between 18.7 – 34.7, 2.47 – 3.14, and 0.11 – 0.19, respectively (Table 4.30). Periphyton species richness and diversity were significantly lower and species dominance higher in December 2005 and November 2006 compared to all other dates sampled. Periphyton species richness and diversity peaked in July 2006 but did not vary significantly between the April and September 2006 harvests. Patterns of temporal variation in periphyton species richness and diversity on short-term samplers reflected each other (Figure 4.7), but species dominance showed inverse trends with these community attributes (Figure 4.8 and Figure 4.9, respectively).

In Knockan Burn, periphyton species richness and diversity were significantly higher and species dominance lower on short-term linoleum substrates in both April and September 2006, than in November 2006 (Table 4.31).

Long-term linoleum (Table 4.32), Astroturf (Table 4.33) and plastic plant samplers (Table 4.34 and Table 4.35) exhibited similar trends in periphyton species richness, diversity and dominance in Knockan Burn as described for short-term linoleum substrates between survey dates sampled.

4.5.2.6 Periphyton community composition and diversity of naturally-occurring substrata in Knockan Burn

Periphyton species richness, diversity and dominance harvested from naturally-occurring mineral particles (Table 4.36), aquatic bryophytes (Table 4.37) and (where present) vascular submerged macrophytes (Table 4.38) in Knockan Burn, varied similarly between sampling dates as described previously for artificial substrates.

Mean Variable	October 2004		November 2004		January 2005		March 2005		April 2005		May 2005		July 2005		August 2005		April 2006		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	22.0^{a}	4.36	18.3^{a}	0.88	18.7^{a}	1.21	22.0^{a}	2.08	25.7^{ab}	0.33	15.3^{c}	1.33	31.7^{b}	1.33	29.0^{b}	1.02	20.3^{a}	0.67	P<0.01**
Periphyton species diversity: **H**	2.51^{a}	0.35	2.46^{a}	0.13	2.43^{a}	0.09	2.44^{a}	0.13	2.68^{ab}	0.06	2.15^{c}	0.18	2.89^{b}	0.08	2.59^{ab}	0.10	2.51^{a}	0.03	P<0.05*
Periphyton species dominance	0.22^{a}	0.03	0.20^{a}	0.04	0.19^{a}	0.02	0.18^{a}	0.02	0.15^{ab}	0.02	0.30^{c}	0.06	0.12^{b}	0.02	0.16^{ab}	0.02	0.23^{a}	0.03	P<0.05*

Table 4.18 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 400 cm^2), periphyton species diversity (per 400 cm^2), and periphyton species dominance (per 400 cm^2) of short-term linoleum substrates between sampling dates (October 2005 – April 2006) in the Water of Dye sub-catchment (n = 27 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.20.

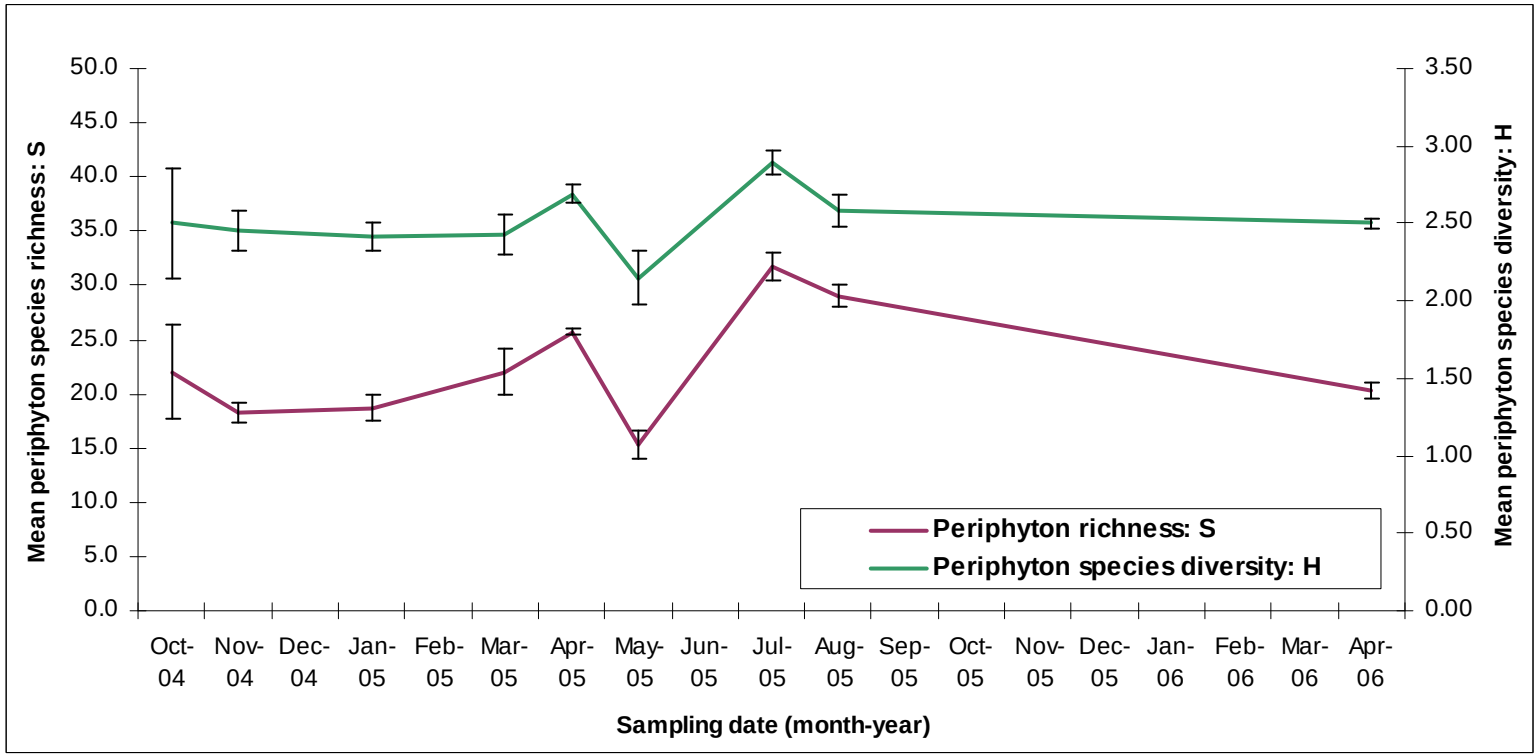


Figure 4.1 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) periphyton species richness (per 400 cm^2), and periphyton species diversity (per 400 cm^2) harvested from short-term linoleum substrates in the Water of Dye sub-catchment between October 2004 and April 2006 (n = 27 samples).

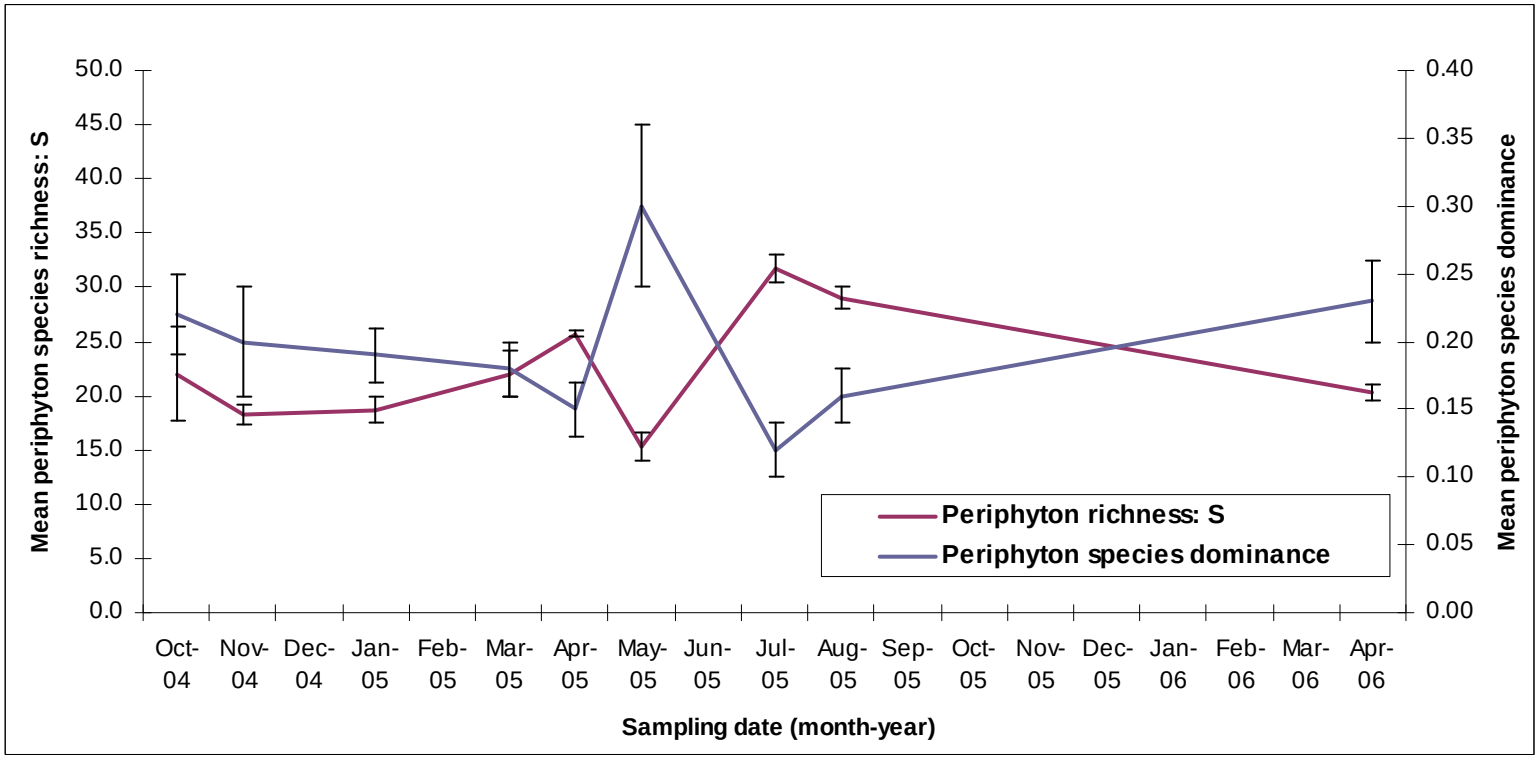


Figure 4.2 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) periphyton species richness (per 400 cm^2), and periphyton species dominance (per 400 cm^2) harvested from short-term linoleum substrates in the Water of Dye sub-catchment between October 2004 and April 2006 (n = 27 samples).

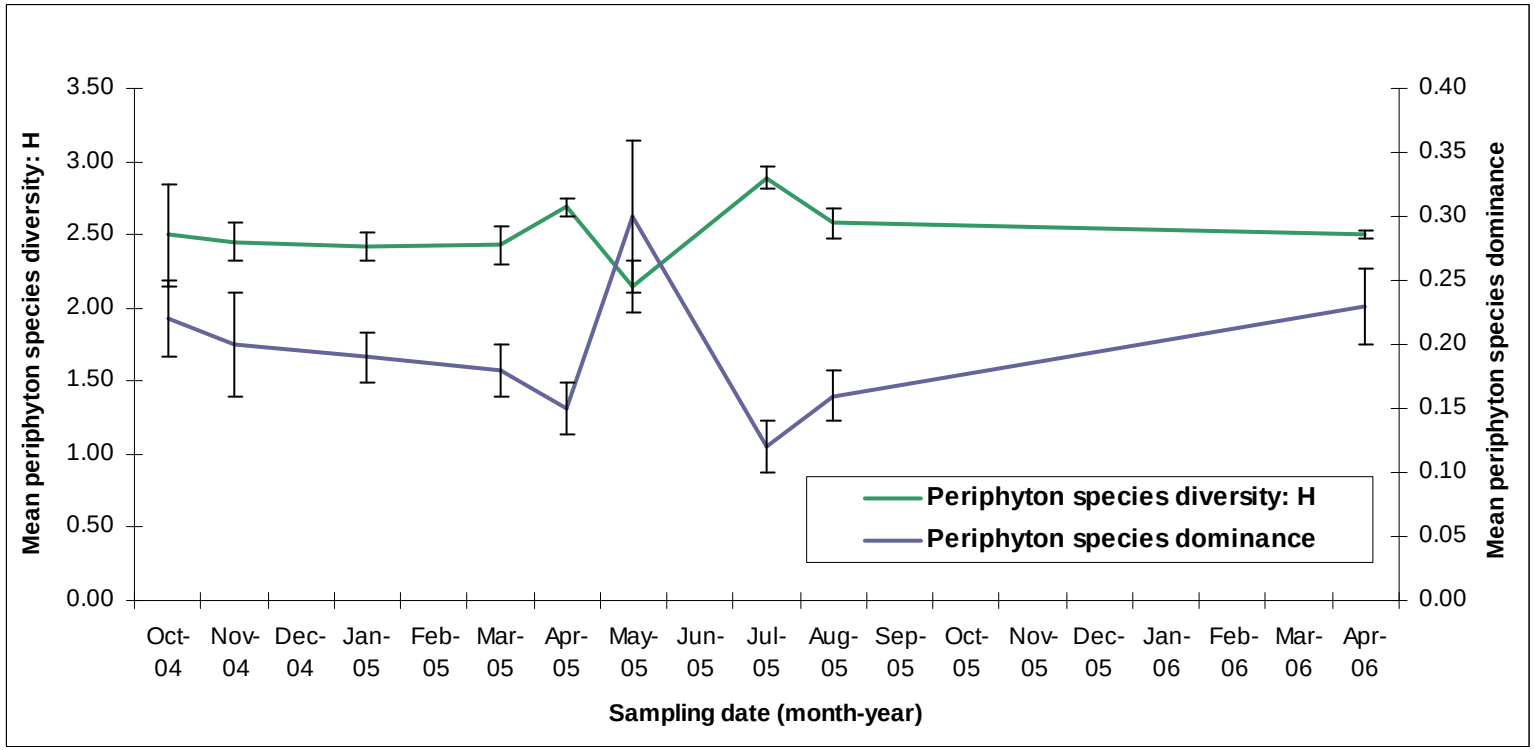


Figure 4.3 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) periphyton species diversity (per 400 cm^2), and periphyton species dominance (per 400 cm^2) harvested from short-term linoleum substrates in the Water of Dye sub-catchment between October 2004 and April 2006 (n = 27 samples).

Mean Variable	May 2005		August 2005		April 2006		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	15.3[a]	1.33	29.0[b]	1.02	20.3[c]	0.67	P<0.001***
Periphyton species diversity: **H**	2.15[a]	0.18	2.59[b]	0.10	2.51[b]	0.03	P<0.01**
Periphyton species dominance	0.30[a]	0.06	0.16[b]	0.02	0.23[b]	0.03	P<0.01**

Table 4.19 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 400 cm^2), periphyton species diversity (per 400 cm^2), and periphyton species dominance (per 400 cm^2) of short-term linoleum substrates between sampling dates in the Water of Dye sub-catchment (n = 9 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.21.

Mean Variable	May 2005		August 2005		April 2006		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	15.3[a]	0.88	28.5[b]	1.76	22.5[c]	2.18	P<0.001***
Periphyton species diversity: **H**	2.22[a]	0.09	2.59[b]	0.06	2.53[b]	0.10	P<0.01**
Periphyton species dominance	0.29[a]	0.02	0.19[b]	0.02	0.23[b]	0.03	P<0.01**

Table 4.20 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 100 cm^2), periphyton species diversity (per 100 cm^2), and periphyton species dominance (per 100 cm^2) of long-term linoleum substrates between sampling dates in the Water of Dye sub-catchment (n = 9 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.22.

Mean Variable	May 2005		August 2005		April 2006		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	18.7[a]	0.37	28.3[b]	2.59	23.0[c]	0.58	P<0.001***
Periphyton species diversity: **H**	2.34[a]	0.05	2.56[b]	0.04	2.49[b]	0.07	P<0.01**
Periphyton species dominance	0.28[a]	0.02	0.19[b]	0.02	0.20[b]	0.02	P<0.01**

Table 4.21 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 1440 cm^2), periphyton species diversity (per 1440 cm^2), and periphyton species dominance (per 1440 cm^2) of long-term Astroturf substrates between sampling dates in the Water of Dye sub-catchment (n = 9 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.23.

Table 4.22 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per

Mean Variable	May 2005		August 2005		April 2006		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	16.1[a]	0.70	24.7[b]	2.15	20.3[c]	1.94	P<0.001***
Periphyton species diversity: **H**	2.23[a]	0.03	2.44[b]	0.07	2.35[b]	0.05	P<0.01**
Periphyton species dominance	0.28[a]	0.01	0.21[b]	0.02	0.22[b]	0.02	P<0.01**

4.91 cm^2), periphyton species diversity (per 4.91 cm^2), and periphyton species dominance (per 4.91 cm^2) of naturally-occurring mineral substrata between sampling dates in the Water of Dye sub-catchment (n = 27 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.24.

	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	17.8[a]	0.76	26.2[b]	1.53	21.3[c]	1.48	P<0.001***
Periphyton species diversity: **H**	2.22[a]	0.05	2.57[b]	0.06	2.49[b]	0.08	P<0.01**
Periphyton species dominance	0.29[a]	0.01	0.19[b]	0.02	0.20[b]	0.02	P<0.01**

Table 4.23 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 19.64 cm^2), periphyton species diversity (per 19.64 cm^2), and periphyton species dominance (per 19.64 cm^2) of naturally-occurring aquatic bryophytes between sampling dates in the Water of Dye sub-catchment (n = 27 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.25.

Mean Variable	April 2005		May 2005		July 2005		August 2005		April 2006		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	33.1^{ab}	2.00	29.0^{a}	0.59	40.7^{b}	1.33	41.7^{b}	2.85	23.3^{a}	3.28	P<0.05*
Periphyton species diversity: **H**	2.86^{ab}	0.01	2.62^{a}	0.13	3.23^{b}	0.03	3.19^{b}	0.03	2.54^{a}	0.14	P<0.05*
Periphyton species dominance	0.15^{ab}	0.01	0.19^{a}	0.03	0.10^{b}	0.01	0.11^{b}	0.01	0.18^{a}	0.02	P<0.05*

Table 4.24 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 400 cm^2), periphyton species diversity (per 400 cm^2), and periphyton species dominance (per 400 cm^2) of short-term linoleum substrates between sampling dates (April 2005 – April 2006) in the River Girnock sub-catchment (n = 14 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.26.

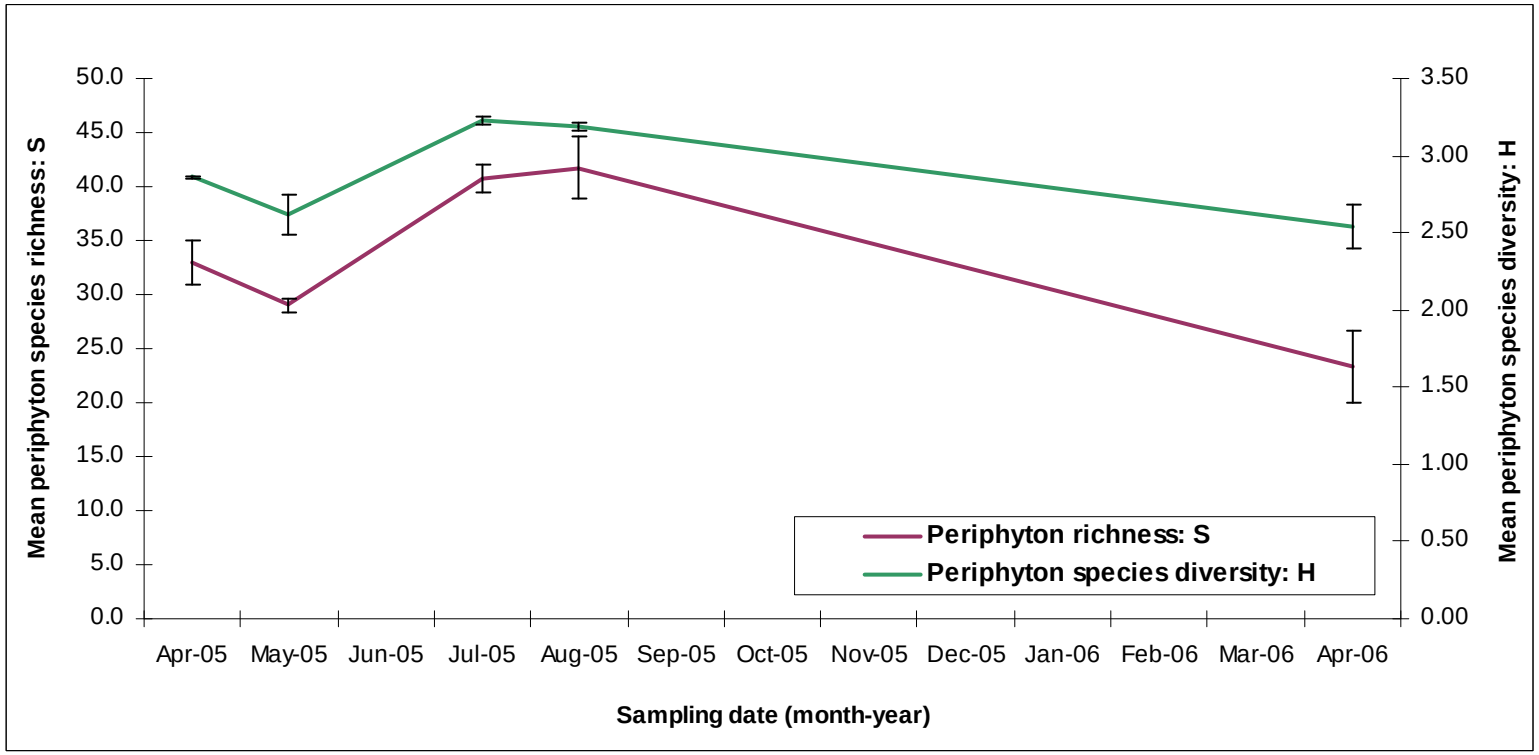


Figure 4.4 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) periphyton species richness (per 400 cm^2), and periphyton species diversity (per 400 cm^2) harvested from short-term linoleum substrates in the River Girnock sub-catchment between April 2005 and April 2006 (n = 14 samples).

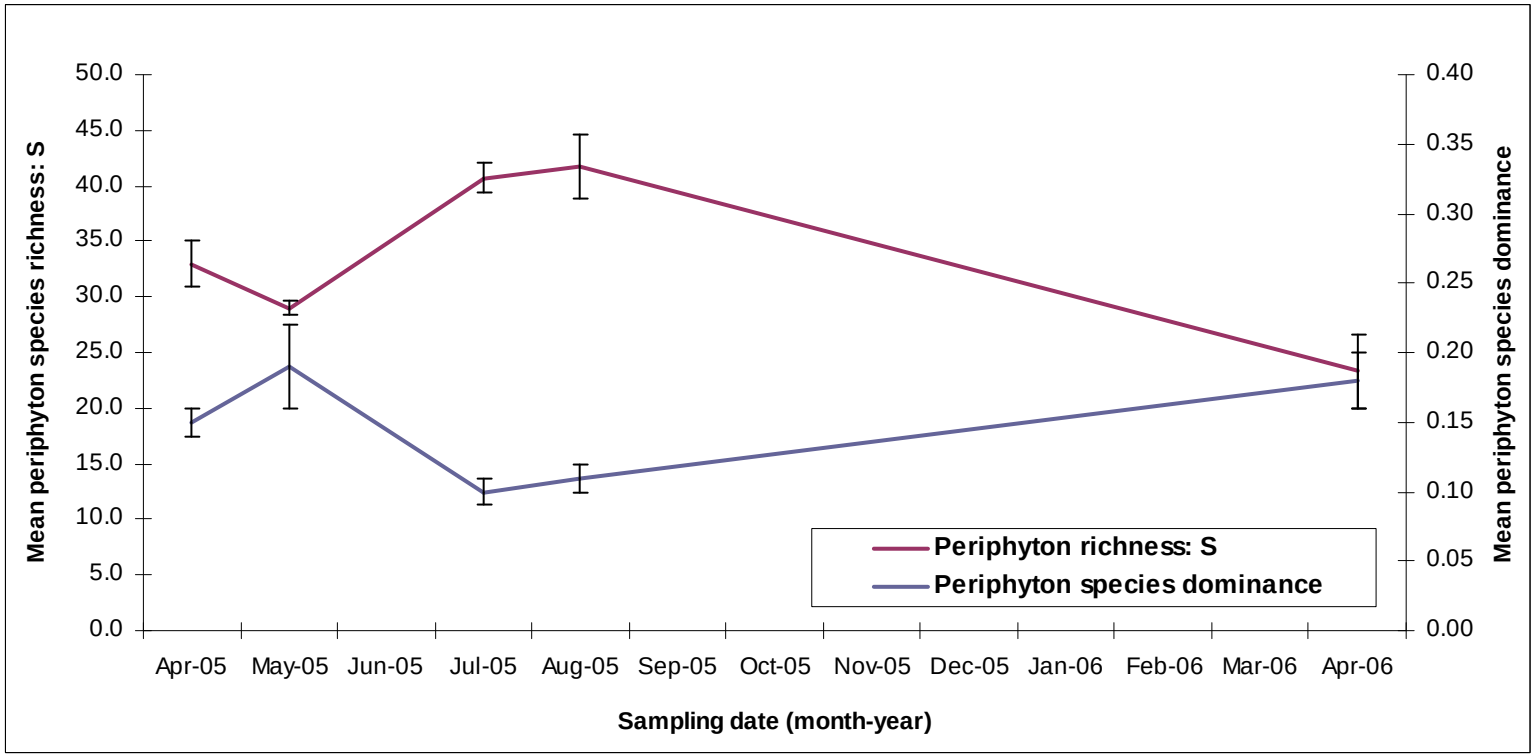


Figure 4.5 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) periphyton species richness (per 400 cm^2), and periphyton species dominance (per 400 cm^2) harvested from short-term linoleum substrates in the River Girnock sub-catchment between April 2005 and April 2006 (n = 14 samples).

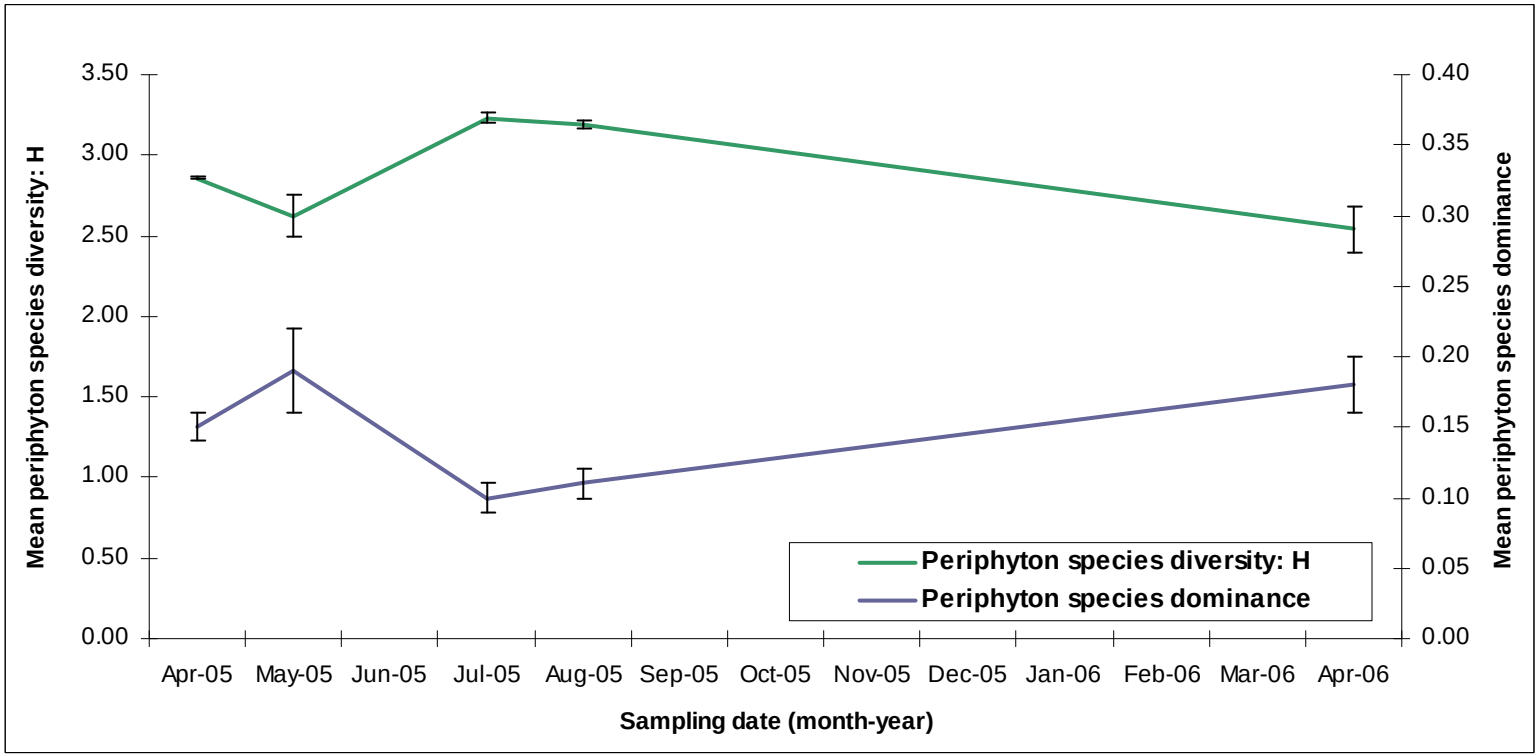


Figure 4.6 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) periphyton species diversity (per 400 cm^2), and periphyton species dominance (per 400 cm^2) harvested from short-term linoleum substrates in the River Girnock sub-catchment between April 2005 and April 2006 (n = 14 samples).

Mean Variable	May 2005		August 2005		April 2006		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	29.0[a]	0.59	41.7[b]	2.85	23.3[a]	3.28	P<0.01**
Periphyton species diversity: **H**	2.62[a]	0.13	3.19[b]	0.03	2.54[a]	0.14	P<0.01**
Periphyton species dominance	0.19[a]	0.03	0.11[b]	0.01	0.18[a]	0.02	P<0.01**

Table 4.25 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 400 cm^2), periphyton species diversity (per 400 cm^2), and periphyton species dominance (per 400 cm^2) of short-term linoleum substrates between sampling dates in the River Girnock sub-catchment (n = 8 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.27.

Mean Variable	May 2005		August 2005		April 2006		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	25.3[a]	1.86	42.7[b]	3.18	30.0[a]	1.15	P<0.01**
Periphyton species diversity: **H**	2.41[a]	0.09	3.15[b]	0.06	2.60[a]	0.08	P<0.01**
Periphyton species dominance	0.20[a]	0.02	0.10[b]	0.02	0.16[a]	0.02	P<0.01**

Table 4.26 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 100 cm^2), periphyton species diversity (per 100 cm^2), and periphyton species dominance (per 100 cm^2) of long-term linoleum substrates between sampling dates in the River Girnock sub-catchment (n = 9 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.28.

Mean Variable	May		August		April		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	33.7[a]	1.76	42.0[b]	1.53	32.0[a]	1.62	P<0.01**
Periphyton species diversity: **H**	2.48[a]	0.06	3.10[b]	0.04	2.65[a]	0.08	P<0.01**
Periphyton species dominance	0.19[a]	0.02	0.13[b]	0.02	0.19[a]	0.02	P<0.01**

Table 4.27 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 1440 cm^2), periphyton species diversity (per 1440 cm^2), and periphyton species dominance (per 1440 cm^2) of long-term Astroturf substrates between sampling dates in the River Girnock sub-catchment (n = 9 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.29.

Table 4.28 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness per

Mean Variable	May 2005		August 2005		April 2006		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	28.2[a]	1.91	36.5[b]	1.23	29.3[a]	1.94	P<0.001***
Periphyton species diversity: **H**	2.61[a]	0.08	3.16[b]	0.03	2.75[a]	0.05	P<0.001***
Periphyton species dominance	0.19[a]	0.01	0.12[b]	0.01	0.17[a]	0.02	P<0.01**

(per 4.91 cm^2), periphyton species diversity (per 4.91 cm^2), and periphyton species dominance (per 4.91 cm^2) of naturally-occurring mineral substrata between sampling dates in the River Girnock sub-catchment (n = 27 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.30.

	2005		2005		2006		
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	25.8[a]	1.87	36.0[b]	1.70	26.2[a]	0.78	P<0.001***
Periphyton species diversity: **H**	2.50[a]	0.09	3.11[b]	0.04	2.65[a]	0.04	P<0.001***
Periphyton species dominance	0.20[a]	0.02	0.12[b]	0.01	0.17[a]	0.01	P<0.01**

Table 4.29 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 19.64 cm^2), periphyton species diversity (per 19.64 cm^2), and periphyton species dominance (per 19.64 cm^2) of naturally-occurring aquatic bryophytes between sampling dates in the River Girnock sub-catchment (n = 25 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.31.

Mean Variable	December 2005		April 2006		July 2006		September 2006		November 2006		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	20.7^{a}	2.33	28.7^{b}	2.73	34.7^{b}	3.48	27.7^{b}	3.48	18.7^{a}	1.33	P<0.01**
Periphyton species diversity: **H**	2.59^{a}	0.05	2.85^{b}	0.12	3.11^{b}	0.13	2.89^{b}	0.12	2.47^{a}	0.10	P<0.01**
Periphyton species dominance	0.20^{a}	0.03	0.15^{b}	0.02	0.11^{b}	0.02	0.13^{b}	0.01	0.19^{a}	0.02	P<0.01**

Table 4.30 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 400 cm^2), periphyton species diversity (per 400 cm^2), and periphyton species dominance (per 400 cm^2) of short-term linoleum substrates between sampling dates in the Knockan Burn sub-catchment (n = 15 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.32.

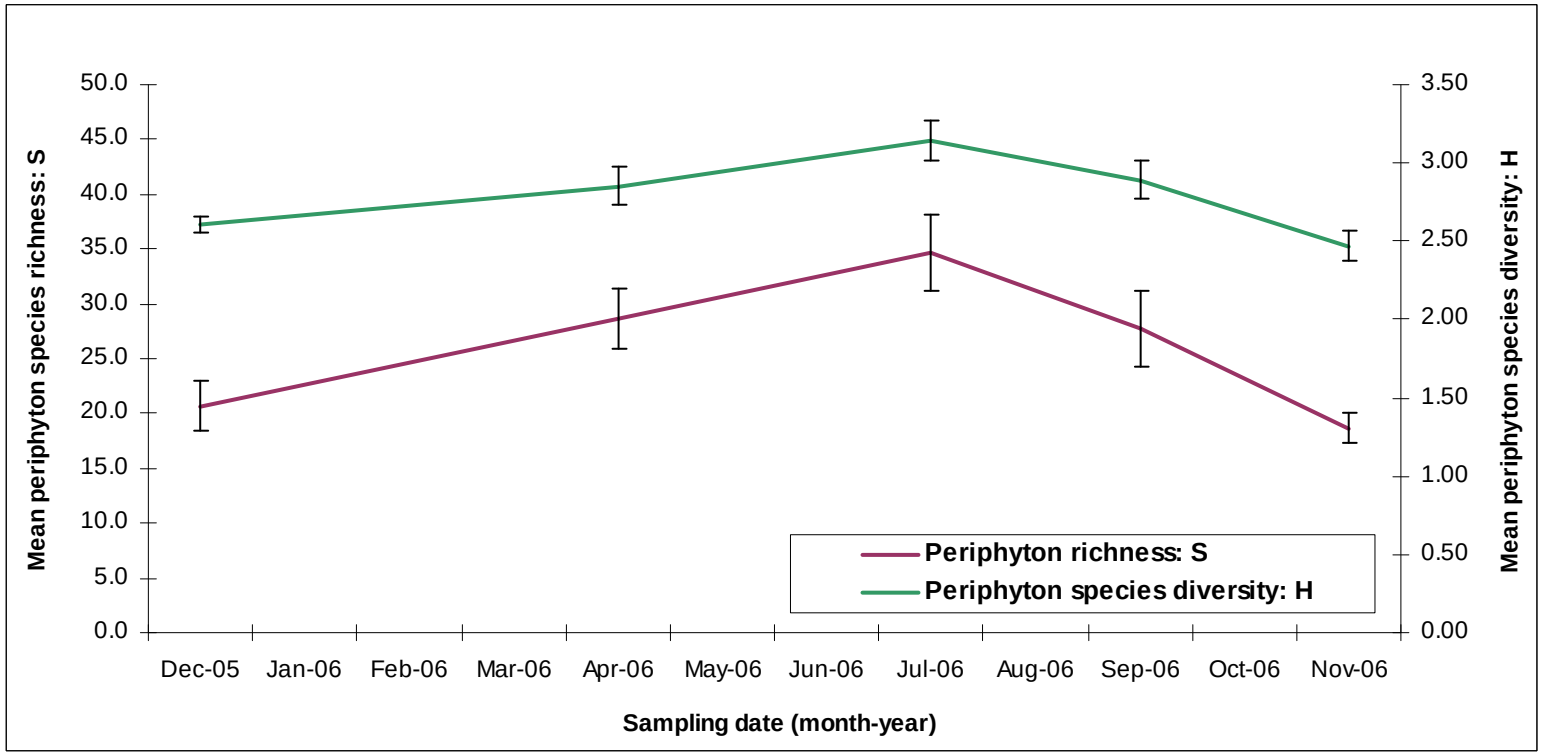


Figure 4.7 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) periphyton species richness (per 400 cm^2), and periphyton species diversity (per 400 cm^2) harvested from short-term linoleum substrates in the Knockan Burn sub-catchment between December 2005 and November 2006 (n = 15 samples).

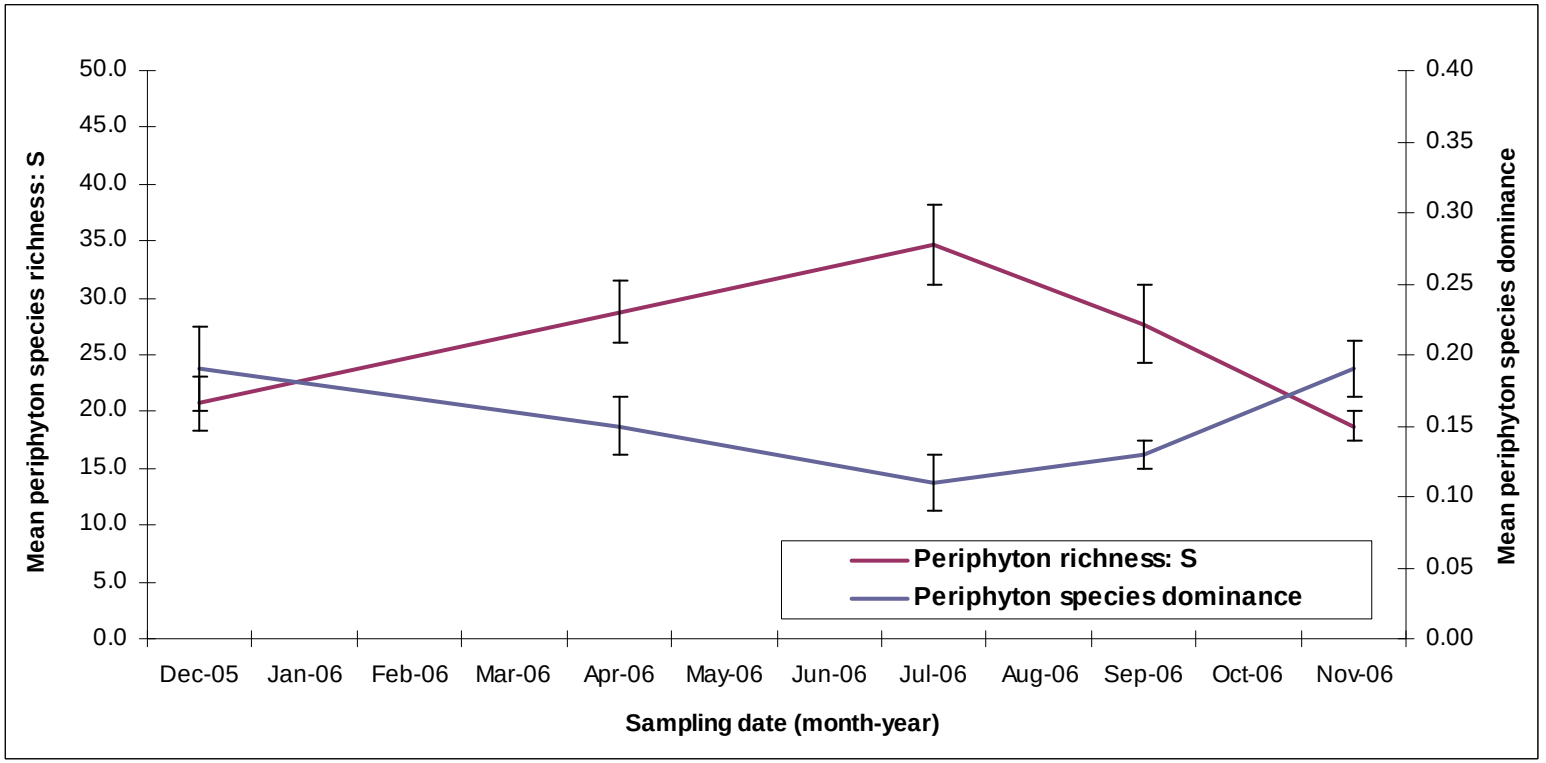


Figure 4.8 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) periphyton species richness (per 400 cm^2), and periphyton species dominance (per 400 cm^2) harvested from short-term linoleum substrates in the Knockan Burn sub-catchment between December 2005 and November 2006 (n = 15 samples).

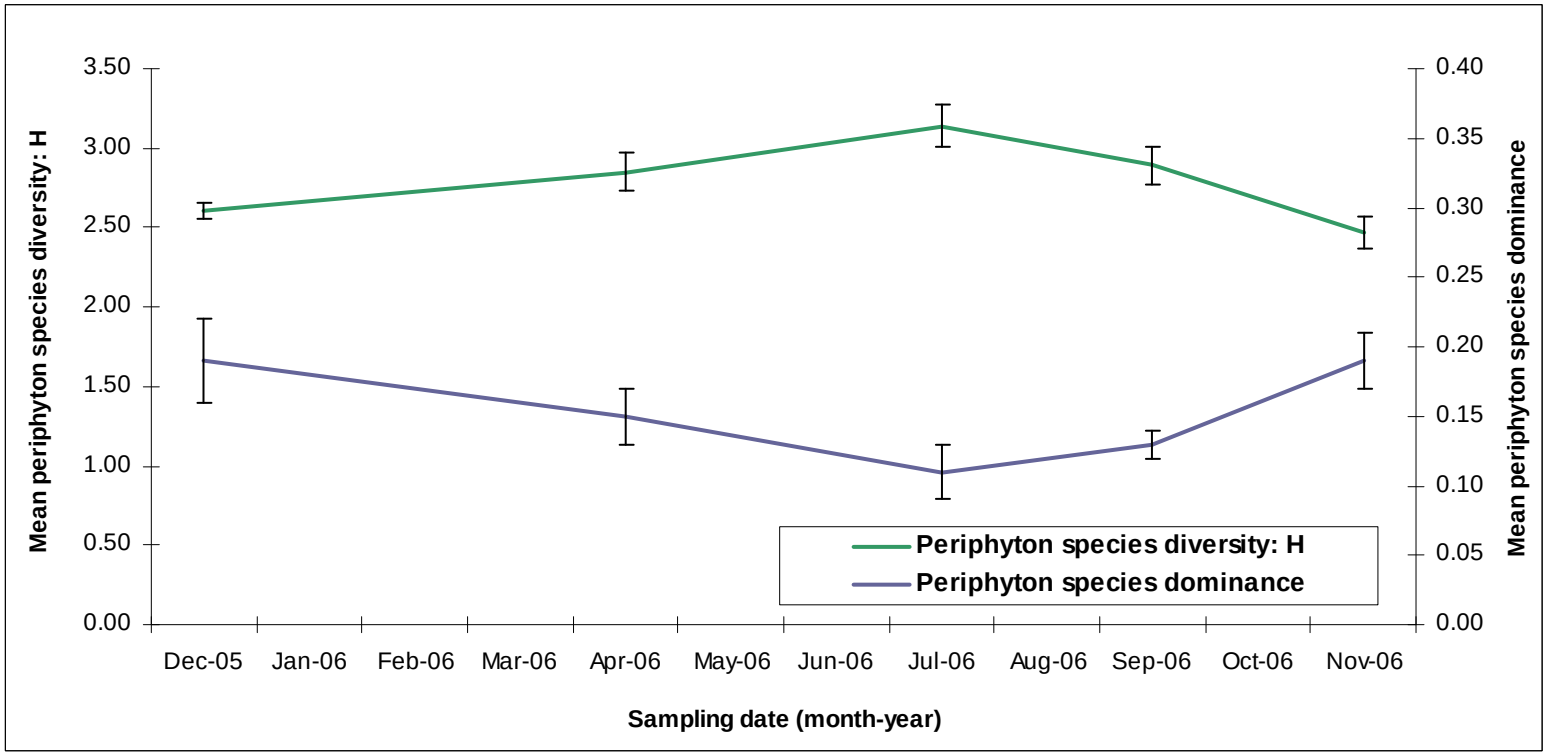


Figure 4.9 Variation in the mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) periphyton species diversity (per 400 cm^2), and periphyton species dominance (per 400 cm^2) harvested from short-term linoleum substrates in the Knockan Burn sub-catchment between December 2005 and November 2006 (n = 15 samples).

Mean Variable	April 2006		September 2006		November 2006		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	28.7[a]	2.73	27.7[a]	3.48	18.7[b]	1.33	P<0.01**
Periphyton species diversity: **H**	2.85[a]	0.12	2.89[a]	0.12	2.47[b]	0.10	P<0.01**
Periphyton species dominance	0.15[a]	0.02	0.13[a]	0.01	0.19[b]	0.02	P<0.01**

Table 4.31 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 400 cm^2), periphyton species diversity (per 400 cm^2), and periphyton species dominance (per 400 cm^2) of short-term linoleum substrates between sampling dates in the Knockan Burn sub-catchment (n = 9 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.33.

Mean Variable	April 2006		September 2006		November 2006		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	26.7[a]	2.19	32.0[a]	4.16	21.3[b]	0.88	P<0.01**
Periphyton species diversity: **H**	2.76[a]	0.08	2.94[a]	0.06	2.48[b]	0.07	P<0.01**
Periphyton species dominance	0.16[a]	0.02	0.14[a]	0.01	0.22[b]	0.02	P<0.01**

Table 4.32 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 100 cm^2), periphyton species diversity (per 100 cm^2), and periphyton species dominance (per 100 cm^2) of long-term linoleum substrates between sampling dates in the Knockan Burn sub-catchment (n = 9 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.34.

Mean Variable	April *Mean*	*S.E.*	September *Mean*	*S.E.*	November *Mean*	*S.E.*	PANOVA
Periphyton richness: **S**	24.7[a]	1.67	29.3[a]	4.67	22.0[b]	3.51	P<0.01**
Periphyton species diversity: **H**	2.64[a]	0.04	2.87[a]	0.05	2.43[b]	0.10	P<0.01**
Periphyton species dominance	0.17[a]	0.02	0.15[a]	0.01	0.21[b]	0.02	P<0.01**

Table 4.33 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 1440 cm^2), periphyton species diversity (per 1440 cm^2), and periphyton species dominance (per 1440 cm^2) of long-term Astroturf substrates between sampling dates in the Knockan Burn sub-catchment (n = 9 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.35.

Table 4.34 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 87.7 cm^2), periphyton species diversity (per 87.7 cm^2), and periphyton species dominance

Mean Variable	April 2006		September 2006		November 2006		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	20.9[ab]	1.52	22.0[a]	2.50	18.0[b]	1.96	P<0.05*
Periphyton species diversity: **H**	2.58[ab]	0.13	2.72[a]	0.04	2.50[b]	0.14	P<0.05*
Periphyton species dominance	0.18[ab]	0.06	0.14[a]	0.01	0.21[b]	0.03	P<0.05*

(per 87.7 cm^2) of long-term plastic aquarium *Potamogeton*-like substrates between sampling dates in the Knockan Burn sub-catchment (n = 6 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.36.

Mean Variable	April 2006		September 2006		November 2006		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	19.9[ab]	3.52	22.0[a]	2.98	17.5[b]	2.00	P<0.05*
Periphyton species diversity: **H**	2.55[ab]	0.10	2.73[a]	0.03	2.48[b]	0.08	P<0.05*
Periphyton species dominance	0.19[ab]	0.07	0.14[a]	0.02	0.22[b]	0.04	P<0.05*

Table 4.35 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 26.1 cm^2), periphyton species diversity (per 26.1 cm^2), and periphyton species dominance (per 26.1 cm^2) of long-term plastic aquarium *Myriophyllum*-like substrates between sampling dates in the Knockan Burn sub-catchment (n = 6 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.37.

Table 4.36 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per

Mean Variable	April 2006		September 2006		November 2006		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	23.2[a]	1.52	26.5[a]	1.49	18.8[b]	1.13	P<0.01**
Periphyton species diversity: **H**	2.76[a]	0.06	2.91[a]	0.04	2.50[b]	0.06	P<0.01**
Periphyton species dominance	0.13[a]	0.01	0.12[a]	0.01	0.20[b]	0.02	P<0.01**

4.91 cm^2), periphyton species diversity (per 4.91 cm^2), and periphyton species dominance (per 4.91 cm^2) of naturally-occurring mineral substrata between sampling dates in the Knockan Burn sub-catchment (n = 25 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.38.

	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	23.8[a]	1.33	26.5[a]	1.20	20.1[b]	1.06	P<0.01**
Periphyton species diversity: **H**	2.79[a]	0.05	2.84[a]	0.04	2.65[b]	0.05	P<0.01**
Periphyton species dominance	0.16[a]	0.02	0.15[a]	0.01	0.22[b]	0.02	P<0.01**

Table 4.37 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 19.64 cm^2), periphyton species diversity (per 19.64 cm^2), and periphyton species dominance (per 19.64 cm^2) of naturally-occurring aquatic bryophytes between sampling dates in the Knockan Burn sub-catchment (n = 22 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.39.

Table 4.38 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 400 cm^2), periphyton species diversity (per 400 cm^2), and periphyton species dominance (per

Mean Variable	**April 2006**		**September 2006**		**November 2006**		**P_{ANOVA}**
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	24.7[a]	1.59	25.8[a]	2.26	19.3[b]	1.73	P<0.01**
Periphyton species diversity: **H**	2.69[a]	0.07	2.75[a]	0.09	2.55[b]	0.03	P<0.01**
Periphyton species dominance	0.14[a]	0.02	0.13[a]	0.03	0.22[b]	0.02	P<0.01**

400 cm^2) of naturally-occurring vascular submerged macrophytes between sampling dates in the Knockan Burn sub-catchment (n = 10 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.40.

4.5.3 Response of periphyton community composition and diversity in the Water of Dye, River Girnock and Knockan Burn to variation in flow regime: pool, glide and riffle zones

4.5.3.1 Periphyton community composition and diversity of naturally-occurring substrata

Overall, the amalgamated data set indicated that periphyton assemblages harvested from naturally-occurring mineral substrata displayed a significant response to variation in flow regime (Table 4.42). In general, species richness and diversity decreased with increasing current velocity, and unsurprisingly species dominance showed the inverse trend. Therefore, riffle habitats tended to be dominated by fewer species and were characterised by significantly lower species richness and diversity compared to pools and glides. These observed trends were consistent with findings in each individual sub-catchment stream: Water of Dye (Table 4.39), River Girnock (Table 4.40) and Knockan Burn (Table 4.41).

Overall, the amalgamated data set of periphyton assemblages harvested from the surfaces of naturally-occurring aquatic bryophytes exhibited a similar trend as for that obtained for mineral substrata (Table 4.46). This was reflected in each of the sub-catchment streams: Water of Dye (Table 4.43), River Girnock (Table 4.44) and Knockan Burn (Table 4.45).

There was no significant difference in periphyton species richness, diversity or dominance on the surfaces of vascular submerged macrophytes occurring in pools or glides (note: vascular submerged macrophytes were completely absent from riffle zones) in Knockan Burn (Table 4.47).

Variable	Pool		Glide		Riffle		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	23.6[a]	1.60	21.7[a]	1.46	15.9[b]	1.23	P<0.01**
Periphyton species diversity: **H**	2.46[a]	0.06	2.38[a]	0.06	2.18[b]	0.06	P<0.01**
Periphyton species dominance	0.22[a]	0.01	0.23[a]	0.01	0.27[b]	0.02	P<0.01**

Table 4.39 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 4.91 cm^2), periphyton species diversity (per 4.91 cm^2), and periphyton species dominance (per 4.91 cm^2) of naturally-occurring mineral substrata between flow regime (pool, glide, riffle habitats) in the Water of Dye sub-catchment (n = 27 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.41.

Variable	Pool		Glide		Riffle		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	35.4[a]	1.38	32.3[a]	1.90	26.3[b]	2.20	P<0.01**
Periphyton species diversity: **H**	2.96[a]	0.06	2.88[a]	0.06	2.68[b]	0.07	P<0.01**
Periphyton species dominance	0.15[a]	0.02	0.15[a]	0.02	0.19[b]	0.01	P<0.01**

Table 4.40 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 4.91 cm^2), periphyton species diversity (per 4.91 cm^2), and periphyton species dominance (per 4.91 cm^2) of naturally-occurring mineral substrata between flow regime (pool, glide, riffle habitats) in the River Girnock sub-catchment (n = 27 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.42.

Variable	Pool		Glide		Riffle		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	25.7[a]	1.70	23.6[a]	1.62	19.1[b]	1.55	P<0.01**
Periphyton species diversity: **H**	2.80[a]	0.05	2.75[a]	0.05	2.60[b]	0.06	P<0.01**
Periphyton species dominance	0.12[a]	0.01	0.13[a]	0.02	0.18[b]	0.02	P<0.01**

Table 4.41 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 4.91 cm^2), periphyton species diversity (per 4.91 cm^2), and periphyton species dominance (per 4.91 cm^2) of naturally-occurring mineral substrata between flow regime (pool, glide, riffle habitats) in the Knockan Burn sub-catchment (n = 25 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.43.

Variable	Pool		Glide		Riffle		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	28.2[a]	1.70	25.9[a]	1.62	20.4[b]	1.55	P<0.01**
Periphyton species diversity: **H**	2.74[a]	0.05	2.67[a]	0.05	2.49[b]	0.06	P<0.01**
Periphyton species dominance	0.16[a]	0.01	0.16[a]	0.02	0.22[b]	0.02	P<0.01**

Table 4.42 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 4.91 cm^2), periphyton species diversity (per 4.91 cm^2), and periphyton species dominance (per 4.91 cm^2) of naturally-occurring mineral substrata between flow regime (pool, glide, riffle habitats) for amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 79 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.44.

Variable	Pool		Glide		Riffle		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	24.5[a]	1.51	23.4[a]	1.72	17.6[b]	1.39	P<0.01**
Periphyton species diversity: **H**	2.60[a]	0.07	2.51[a]	0.09	2.19[b]	0.08	P<0.01**
Periphyton species dominance	0.20[a]	0.02	0.21[a]	0.02	0.28[b]	0.02	P<0.01**

Table 4.43 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 19.64 cm^2), periphyton species diversity (per 19.64 cm^2), and periphyton species dominance (per 19.64 cm^2) of naturally-occurring aquatic bryophytes between flow regime (pool, glide, riffle habitats) in the Water of Dye sub-catchment (n = 27 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.45.

Variable	Pool		Glide		Riffle		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	30.0[a]	2.04	32.7[a]	2.73	25.2[b]	1.52	P<0.01**
Periphyton species diversity: **H**	2.82[a]	0.08	2.90[a]	0.08	2.54[b]	0.08	P<0.01**
Periphyton species dominance	0.15[a]	0.01	0.15[a]	0.01	0.19[b]	0.01	P<0.01**

Table 4.44 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 19.64 cm^2), periphyton species diversity (per 19.64 cm^2), and periphyton species dominance (per 19.64 cm^2) of naturally-occurring aquatic bryophytes between flow regime (pool, glide, riffle habitats) in the River Girnock sub-catchment (n = 25 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.46.

Variable	Pool		Glide		Riffle		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	24.5[a]	1.20	25.2[a]	1.67	20.7[b]	1.55	P<0.01**
Periphyton species diversity: **H**	2.86[a]	0.06	2.90[a]	0.07	2.51[b]	0.06	P<0.01**
Periphyton species dominance	0.16[a]	0.01	0.15[a]	0.02	0.22[b]	0.02	P<0.05*

Table 4.45 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 19.64 cm^2), periphyton species diversity (per 19.64 cm^2), and periphyton species dominance (per 19.64 cm^2) of naturally-occurring aquatic bryophytes between flow regime (pool, glide, riffle habitats) in the Knockan Burn sub-catchment (n = 22 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.47.

Variable	Pool		Glide		Riffle		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	26.3[a]	1.07	27.1[a]	1.29	21.2[b]	1.24	P<0.01**
Periphyton species diversity: **H**	2.75[a]	0.05	2.78[a]	0.05	2.41[b]	0.06	P<0.01**
Periphyton species dominance	0.17[a]	0.01	0.17[a]	0.01	0.23[b]	0.02	P<0.01**

Table 4.46 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 19.64 cm^2), periphyton species diversity (per 19.64 cm^2), and periphyton species dominance (per 19.64 cm^2) of naturally-occurring aquatic bryophytes between flow regime (pool, glide, riffle habitats) for amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 74 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.48.

Variable	Pool		Glide		Riffle		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	22.1	1.47	24.5	2.52	N/A		NS
Periphyton species diversity: **H**	2.61	0.06	2.70	0.07	N/A		NS
Periphyton species dominance	0.17	0.04	0.15	0.02	N/A		NS

Table 4.47 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): periphyton species richness (per 400 cm^2), periphyton species diversity (per 400 cm^2), and periphyton species dominance (per 400 cm^2) of naturally-occurring vascular submerged macrophytes between flow regime (pool, glide, riffle habitats) in the Knockan Burn sub-catchment (n = 10 samples). Significance testing: one-way ANOVA. For details of environmental habitat conditions refer to Chapter 3, Table 3.49.

4.5.4 Response of periphyton community composition and diversity in the Water of Dye, River Girnock and Knockan Burn to variation in substrate morphology

4.5.4.1 Periphyton community composition and diversity of naturally-occurring substrata

In general, periphyton species richness and diversity tended to show a 'humpback response' to variation in substrate morphology, peaking at intermediate proportions of substrate particles, or more diverse streambed morphologies containing a mixed assortment of size categories, whereas periphyton species dominance tended to be most pronounced at proportional extremes wherein substrate diversity was low and streambed substrate composition tended to be predominated by a single particle mode (refer to Table 4.48 – Table 4.52, inclusive).

Variable	0% BO		3% BO		15.5% BO		38% BO		63% BO		88% BO		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	21.6^{a}	0.91	28.7^{b}	1.87	27.6^{b}	1.35	25.5^{ab}	2.33	23.0^{a}	1.95	22.4^{a}	1.10	P<0.05*
Periphyton species diversity: **H**	2.45^{a}	0.04	2.84^{b}	0.06	2.80^{b}	0.06	2.69^{ab}	0.08	2.55^{a}	0.07	2.50^{a}	0.05	P<0.05*
Periphyton species dominance	0.16^{a}	0.01	0.16^{a}	0.01	0.17^{a}	0.01	0.20^{ab}	0.03	0.23^{b}	0.02	0.23^{b}	0.02	P<0.05*

Table 4.48 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): response of periphyton species richness (per 4.91 cm^2), periphyton species diversity (per 4.91 cm^2), and periphyton species dominance (per 4.91 cm^2) of naturally-occurring mineral substrata to variation in the abundance (median % cover) of boulders (BO) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 79 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.50.

Variable	0% LS		3% LS		15.5% LS		38% LS		63% LS		88% LS		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	23.6	1.91	24.1	2.25	27.2	1.58	26.4	1.81	24.6	2.79	23.0	1.88	NS
Periphyton species diversity: **H**	2.60	0.09	2.65	0.13	2.72	0.06	2.70	0.05	2.65	0.11	2.54	0.08	NS
Periphyton species dominance	0.20	0.03	0.19	0.03	0.16	0.02	0.17	0.02	0.19	0.02	0.20	0.03	NS

Table 4.49 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): response of periphyton species richness (per 4.91 cm^2), periphyton species diversity (per 4.91 cm^2), and periphyton species dominance (per 4.91 cm^2) of naturally-occurring mineral substrata to variation in the abundance (median % cover) of large stones (LS) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 79 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.51.

Variable	0% SS		3% SS		15.5% SS		38% SS		63% SS		88% SS		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	22.3[a]	1.45	23.8[a]	1.75	27.6[b]	1.10	28.7[b]	1.33	24.2[a]	2.00	21.9[a]	1.16	P<0.01**
Periphyton species diversity: **H**	2.53[a]	0.09	2.58[a]	0.05	2.76[b]	0.05	2.84[b]	0.06	2.60[a]	0.07	2.48[a]	0.04	P<0.01**
Periphyton species dominance	0.21[a]	0.03	0.21[a]	0.03	0.15[b]	0.02	0.14[b]	0.01	0.20[a]	0.02	0.22[a]	0.03	P<0.01**

Table 4.50 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): response of periphyton species richness (per 4.91 cm^2), periphyton species diversity (per 4.91 cm^2), and periphyton species dominance (per 4.91 cm^2) of naturally-occurring mineral substrata to variation in the abundance (median % cover) of small stones (SS) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 79 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.52.

Variable	0% GR		3% GR		15.5% GR		38% GR		63% GR		88% GR		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	23.3^{a}	1.63	25.0^{ab}	1.53	26.7^{b}	1.15	27.5^{b}	2.21	24.1^{ab}	1.75	22.7^{a}	1.77	P<0.05*
Periphyton species diversity: **H**	2.56^{a}	0.04	2.67^{ab}	0.05	2.70^{b}	0.05	2.73^{b}	0.06	2.66^{ab}	0.06	2.51^{a}	0.05	P<0.05*
Periphyton species dominance	0.20^{a}	0.02	0.19^{ab}	0.02	0.16^{b}	0.02	0.15^{b}	0.01	0.18^{ab}	0.02	0.21^{b}	0.02	P<0.05*

Table 4.51 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): response of periphyton species richness (per 4.91 cm^2), periphyton species diversity (per 4.91 cm^2), and periphyton species dominance (per 4.91 cm^2) of naturally-occurring mineral substrata to variation in the abundance (median % cover) of gravel (GR) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 79 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.53.

Variable	0% SA		3% SA		15.5% SA		38% SA		63% SA		88% SA		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	23.4	2.38	24.7	2.11	26.4	2.88	23.9	1.77	25.6	2.45	24.5	2.32	NS
Periphyton species diversity: **H**	2.59	0.05	2.66	0.08	2.70	0.07	2.61	0.06	2.68	0.06	2.64	0.05	NS
Periphyton species dominance	0.22	0.02	0.20	0.02	0.16	0.02	0.19	0.02	0.17	0.02	0.18	0.02	NS

Table 4.52 Mean values (± 1 standard error) of normally distributed normally distributed plant variables (data back-transformed where necessary): response of periphyton species richness (per 4.91 cm^2), periphyton species diversity (per 4.91 cm^2), and periphyton species dominance (per 4.91 cm^2) of naturally-occurring mineral substrata to variation in the abundance (median % cover) of sand (SA) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 79 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.54.

4.5.5 Comparison of periphyton community composition and diversity between artificial and naturally-occurring substrata: do artificial substrates make good surrogates for naturally-occurring microhabitats?

4.5.5.1 Water of Dye

In the Water of Dye there was no significant difference in periphyton community composition and structural attributes (species richness, diversity and dominance) between short-term linoleum, long-term linoleum and naturally-occurring mineral substrata (Table 4.53).

Similarly, there was no significant difference in periphyton community structure between long-term Astroturf substrates and that harvested from aquatic bryophytes in the Water of Dye (Table 4.54).

There was no significant difference in any of the environmental habitat variables measured between the linoleum and mineral substrates, or between the Astroturf and aquatic bryophyte substrates (refer to Chapter 3, section 3.5.5.1).

4.5.5.2 River Girnock

Also shown from periphyton communities harvested from the River Girnock was that species richness, diversity and dominance did not vary significantly between artificial substrates and their respective naturally-occurring microhabitat (refer to Table 4.55 and Table 4.56 for details).

There was no significant difference in any of the environmental habitat variables measured between the artificial and naturally-occurring substrates (refer to Chapter 3, section 3.5.5.2).

4.5.5.3 Knockan Burn

In Knockan Burn, periphyton communities harvested from artificial samplers did not differ significantly in terms of composition and structural attributes (species richness, diversity and dominance) compared to naturally-occurring substrates (refer to Table 4.57, Table 4.58 and Table 4.59).

Mostly there were no significant difference in environmental habitat conditions except for streamwater pH and conductivity, which tended to be higher in naturally-occurring stands of vascular submerged macrophytes than for plastic plant samplers (refer to Chapter 3, section 3.5.5.3).

4.5.5.4 Amalgamated data

On the whole, although relative abundances may have varied the assemblage of periphyton species and their structural attributes (species richness, diversity and dominance) found on artificial substrata reflected those communities harvested from naturally-occurring substrata (Table 4.60). Periphyton species richness, diversity and dominance did not vary significantly between the various types of substrates used in the study (see Table 4.60, and also Figure 4.10).

Mostly, there was no significant variation in any of the environmental habitat conditions between the artificial substrates and their naturally-occurring counterparts, with the exception of detected differences in streamwater pH and conductivity between the naturally-occurring vascular submerged macrophyte zones and plastic plant samplers in the Knockan Burn sub-catchment (refer to Chapter 3, section 3.5.5.4).

Mean Variable	Short-term linoleum		Long-term linoleum		Mineral substrata		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	21.6	2.06	22.1	2.08	20.4	1.02	NS
Periphyton species diversity: **H**	2.41	0.09	2.44	0.07	2.34	0.04	NS
Periphyton species dominance	0.23	0.03	0.24	0.02	0.24	0.01	NS

Table 4.53 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton species richness, periphyton species diversity, and periphyton species dominance per area sampled (i.e. 400 cm^2, 100 cm^2, and 4.91 cm^2, respectively) between short-term linoleum (n = 9), long-term linoleum (n = 9), and naturally-occurring mineral substrata (n = 27) in the Water of Dye sub-catchment (n = 45 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.55.

Mean Variable	Long-term Astroturf		Aquatic Bryophytes		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	23.3	1.35	21.8	0.97	NS
Periphyton species diversity: **H**	2.46	0.04	2.42	0.05	NS
Periphyton species dominance	0.22	0.02	0.23	0.01	NS

Table 4.54 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton species richness, periphyton species diversity, and periphyton species dominance per area sampled (i.e. 1440 cm^2, 19.64 cm^2, respectively) between long-term Astroturf (n = 9), and naturally-occurring aquatic bryophytes (n = 27) in the Water of Dye sub-catchment (n = 36 samples). Significance testing: one-way ANOVA. For details of environmental habitat conditions refer to Chapter 3, Table 3.56.

Mean Variable	Short-term linoleum		Long-term linoleum		Mineral substrata		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	31.3	2.98	31.7	3.01	31.1	1.10	NS
Periphyton species diversity: **H**	2.79	0.13	2.72	0.11	2.84	0.04	NS
Periphyton species dominance	0.16	0.02	0.19	0.02	0.16	0.01	NS

Table 4.55 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton species richness, periphyton species diversity, and periphyton species dominance per area sampled (i.e. 400 cm^2, 100 cm^2, and 4.91 cm^2, respectively) between short-term linoleum (n = 8), long-term linoleum (n = 9), and naturally-occurring mineral substrata (n = 27) in the River Girnock sub-catchment (n = 44 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.57.

Mean Variable	Long-term Astroturf		Aquatic Bryophytes		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	35.9	1.92	29.3	1.18	NS
Periphyton species diversity: **H**	2.84	0.08	2.75	0.05	NS
Periphyton species dominance	0.18	0.02	0.18	0.01	NS

Table 4.56 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton species richness, periphyton species diversity, and periphyton species dominance per area sampled (i.e. 1440 cm^2, and 19.64 cm^2, respectively) between long-term Astroturf (n = 9), and naturally-occurring aquatic bryophytes (n = 25) in the River Girnock sub-catchment (n = 34 samples). Significance testing: one-way ANOVA. For details of environmental habitat conditions refer to Chapter 3, Table 3.58.

Mean Variable	Short-term linoleum		Long-term linoleum		Mineral substrata		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	25.0	2.07	27.7	2.05	22.8	0.95	NS
Periphyton species diversity: **H**	2.74	0.11	2.73	0.10	2.72	0.04	NS
Periphyton species dominance	0.16	0.02	0.15	0.02	0.14	0.01	NS

Table 4.57 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton species richness, periphyton species diversity, and periphyton species dominance per area sampled (i.e. 400 cm^2, 100 cm^2, and 4.91 cm^2, respectively) between short-term linoleum (n = 9), long-term linoleum (n = 9), and naturally-occurring mineral substrata (n = 25) in the Knockan Burn sub-catchment (n = 43 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.59.

Mean Variable	Long-term Astroturf		Aquatic Bryophytes		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	25.3	2.05	23.5	0.95	NS
Periphyton species diversity: **H**	2.66	0.07	2.76	0.04	NS
Periphyton species dominance	0.18	0.02	0.15	0.01	NS

Table 4.58 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton species richness, periphyton species diversity, and periphyton species dominance per area sampled (i.e. 1440 cm^2, and 19.64 cm^2, respectively) between long-term Astroturf (n = 9), and naturally-occurring aquatic bryophytes (n = 22 samples) in the Knockan Burn sub-catchment (n = 31 samples). Significance testing: one-way ANOVA. For details of environmental habitat conditions refer to Chapter 3, Table 3.60.

Mean Variable	**Long-term plastic *Potamogeton***		**Long-term plastic *Myriophyllum***		**Vascular submerged macrophytes**		**PANOVA**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	NS
Periphyton richness: **S**	20.3	1.38	19.8	1.62	23.3	1.59	
Periphyton species diversity: **H**	2.60	0.08	2.59	0.09	2.66	0.05	NS
Periphyton species dominance	0.18	0.03	0.18	0.03	0.16	0.02	NS

Table 4.59 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton species richness, periphyton species diversity, and periphyton species dominance per area sampled (i.e. 87.7 cm^2, 26.1 cm^2, and 400 cm^2, respectively) between long-term plastic *Potamogeton*-like (n = 6), long-term plastic *Myriophyllum*-like (n = 6), and naturally-occurring vascular submerged macrophytes (n = 10) in the Knockan Burn sub-catchment (n = 22 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.61.

Mean Variable	Short-term linoleum		Long-term linoleum		Mineral substrata		Long-term Astroturf		Aquatic bryophytes		Long-term plastic *Potamogeton*		Long-term plastic *Myriophyllum*		Vascular submerged macrophytes		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Periphyton richness: **S**	26.0	1.56	27.2	1.55	24.8	0.80	28.2	1.47	25.0	0.70	20.3	1.38	19.8	1.62	23.3	1.59	NS
Periphyton species diversity: **H**	2.64	0.06	2.63	0.06	2.63	0.04	2.65	0.05	2.64	0.04	2.60	0.08	2.59	0.09	2.66	0.05	NS
Periphyton species dominance	0.18	0.01	0.19	0.02	0.18	0.01	0.19	0.01	0.19	0.01	0.18	0.03	0.18	0.03	0.16	0.03	NS

Table 4.60 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): periphyton species richness, periphyton species diversity, and periphyton species dominance per area sampled (i.e. 400 cm^2, 100 cm^2, 4.91 cm^2, 1440 cm^2, 19.64 cm^2, 87.7 cm^2, 26.1 cm^2, and 400 cm^2, respectively) between the various types of substrates utilised: short-term linoleum (n = 27), long-term linoleum (n = 27), naturally-occurring mineral substrata (n = 79), long-term Astroturf (n = 27), aquatic bryophytes (n = 74), long-term plastic *Potamogeton*-like (n = 6), long term plastic *Myriophyllum*-like (n = 6) and vascular submerged macrophytes (n = 10) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 256). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.62.

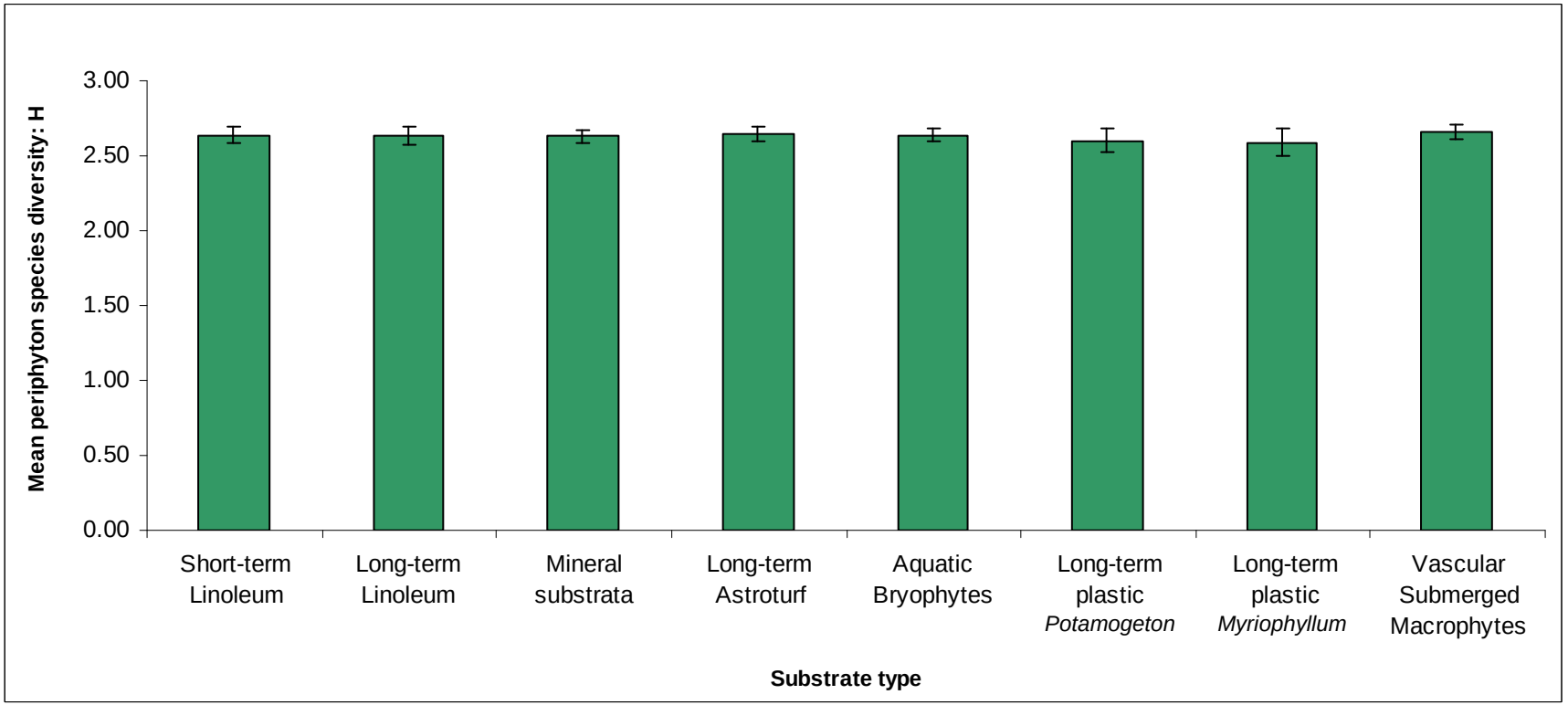


Figure 4.10 Comparison of mean values (± 1 standard error) of normally distributed (data back-transformed where necessary) periphyton species diversity per area sampled (i.e. 400 cm^2, 100 cm^2, 4.91 cm^2, 1440 cm^2, 19.64 cm^2, 87.7 cm^2, 26.1 cm^2, and 400 cm^2, respectively) between the various types of substrates utilised: short-term linoleum (n = 27), long-term linoleum (n = 27), naturally-occurring mineral substrata (n = 79), long-term Astroturf (n = 27), aquatic bryophytes (n = 74), long-term plastic *Potamogeton*-like (n = 6), long term plastic *Myriophyllum*-like (n = 6) and vascular submerged macrophytes (n = 10) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 256).

4.5.6 Periphyton community composition, diversity and environmental habitat conditions in the Water of Dye, River Girnock and Knockan Burn as determined by multivariate ordination and TWINSPAN classification

4.5.6.1 Periphyton community composition, diversity and environmental habitat conditions of short-term linoleum substrates only

Three primary periphyton species assemblages emerged from analysis of the short-term linoleum substrate data (n = 56) using TWINSPAN: Figure 4.11. These were indicated by the presence of *Fragilaria pulchella* (Group I), *Gomphonema acuminatum* (Group II), and an abundance of *Frustulia rhomboides* var. *rhomboides* (Group III). The three groups overlapped with each other due to the presence of a background periphyton community composed of several common ubiquitous species that characterised almost all samples (e.g. *Achnanthidium minutissima*, Amin; *Fragilaria capucina* var. *gracilis*, Fcgr; *Fragilaria capucina* var. *vaucheriae*, Fcva; *Synedra ulna*, Suln; *Tabellaria flocculosa*, Tfloc; *Mougeotia* sp., Moug; and *Ulothrix* sp., Ulox) and were mostly centrally ordinated in the CCA diagram (Figure 4.12).

Community Type I (n = 15 samples: UKDEC05SL, MKDEC05SL, LKDEC05SL, UKAP06SL, MKAP06SL, LKAP06SL, UKJY06SL, MKJY06SL, LKJY06SL, UKSM06SL, MKSM06SL, LKSM06SL, UKNV06SL, MKNV06SL, LKNV06SL: for key to sample codes see caption to Figure 4.12). Distribution of this moderately-diverse periphyton assemblage was restricted to Knockan Burn. This community was indicated primarily by the presence of *Fragilaria pulchella*, usually with several co-occurring diatom species exclusive to this particular stream: *Cocconeis placentula, Didymosphenia geminata, Cymbella lanceolata, Gomphonema olivaceum* and *Gomphonema olivaceum* var. *olivaceoides*. This group was parted from the other two periphyton communities with an eigenvalue of 0.363 (at level 1 of the classification).

Community Type II (n = 13 samples: HBAP05SL, LMAP05SL, IBMY05SL, HBMY05SL, LMMY05SL, IBJY05SL, HBJY05SL, LMJY05SL, IBAU05SL,

HBAU05SL, LMAU05SL, HBAP06SL, LMAP06SL). The Group II periphyton community corresponded to the mid- and lower basin regions of the River Girnock throughout the sampling year, but also occurred at Iron Bridge in the Upper Girnock during summer baseflow conditions (which contained elements of both the Group III during other times and made the transition). Group II encompassed a high diversity periphyton community indicated by *Gomphonema acuminatum* and was characterised by many species exclusive to that sample-group (e.g. *Epithemia* spp., *Navicula angusta*, *Nitzschia* cf. *acula*, *Nitzschia intermedia* agg., *Tetracyclus glans*). However, the Group II community also harboured some species that occurred in TWINSPAN sample-groups I and III, thus acting as a bridge or mediatory transition between the two other communities. This community type was separated from Group III by an eigenvalue 0.334 (at level 2 of the TWINSPAN classification). Periphyton community composition was predominated by *Synedra ulna*, *Tabellaria flocculosa*, *Fragilaria arcus* and several *Cymbella* spp.

Community Type III (n = 28 samples: BBOC04SL, CFOC04SL, BDOC04SL, BBNV04SL, CFNV04SL, BDNV04SL, BBJA05SL, CFJA05SL, BDJA05SL, BBMR05SL, CFMR05SL, BDMR05SL, BBAP05SL, CFAP05SL, BDAP05SL, BBMY05SL, CFMY05SL, BDMY05SL, BBJY05SL, CFJY05SL, BDJY05SL, BBAU05SL, CFAU05SL, BDAU05SL, BBAP06SL, CFAP06SL, BDAP06SL, IBAP06SL). This community type characterised the Water of Dye and upper River Girnock. Low in terms of richness and diversity, this periphyton assemblage was predominated by few species and characterised by an abundance of *Frustulia rhomboides* var. *rhomboides*. *Tabellaria flocculosa* was often a co-dominant member of this periphyton community, along with other commonly occurring species such as *Gomphonema parvulum* var. *exilissimum*, *Meridion circulare* var. *constrictum* and *Pinnularia subcapitata*, as well as less frequent *Eunotia* spp.

Performing one-way ANOVA on the environmental characteristics of TWINSPAN groups I – III (Table 4.61), enabled me to address the following questions: "*What*

environmental variables drive the distribution of periphyton assemblages in upland stream habitats?" Further evidence of periphyton species affiliations with environmental variables could also be determined from CCA analysis (Figure 4.13).

CCA ordination of the 97 periphyton species (85 diatom species and 12 other algal genera inclusive) constrained by the nine environmental variables used in the analysis suggested that water physico-chemical factors, mainly variation in streamwater pH and conductivity were the primary drivers of periphyton community composition between the three target streams (Figure 4.13). Evidence from the outcome of the multivariate analyses, together with ANOVA and Tukey's pairwise comparisons of mean environmental data for the sample-units comprising each TWINSPAN sample-group (Table 4.61) indicated that Zeu:D[1], pH, conductivity, water temperature, and extent of riparian shade showed significant inter-group differences between the samples comprising each community type, as did periphyton species diversity (see Table 4.61, and also Figure 4.14).

The Group I periphyton community appeared to exhibit a distinct ecological preference for stream environmental habitat conditions characterised by higher values of mean pH (>7) and conductivity, than the other two periphyton assemblages. The Group II community occurred in near-circumneutral streamwaters, and the Group III assemblage was most pronounced under acid-sensitive conditions (pH <7).

4.5.6.2 Periphyton community composition, diversity and environmental habitat conditions of all artificial substrata

Three comparable periphyton communities emerged from TWINSPAN analysis of the artificial sampler data set (n = 93) involving both short-term and long-term substrates (linoleum, Astroturf and where applicable plastic aquarium plants) harvested during the field survey campaigns: Figure 4.15. These were also

indicated by the presence of *Fragilaria pulchella* (Group I), *Gomphonema acuminatum* (Group II), and a high abundance of *Frustulia rhomboides* var. *rhomboides* (Group III). Several common ubiquitous species positioned centrally on the CCA diagram caused the three groups to slightly overlap (refer to Figure 4.16).

Community Type I (n = 39 samples: UKAP06SL, MKAP06SL, LKAP06SL, UKSM06SL, MKSM06SL, LKSM06SL, UKNV06SL, MKNV06SL, LKNV06SL, UKAP06LL, MKAP06LL, LKAP06LL, UKSM06LL, MKSM06LL, LKSM06LL, UKNV06LL, MKNV06LL, LKNV06LL, UKAP06AS, MKAP06AS, LKAP06AS, UKSM06AS, MKSM06AS, LKSM06AS, UKNV06AS, MKNV06AS, LKNV06AS, UKAP06PM, LKAP06PM, UKSM06PM, LKSM06PM, UKNV06PM, LKNV06PM, UKAP06PP, LKAP06PP, UKSM06PP, LKSM06PP, UKNV06PP, LKNV06PP: for key to sample codes see caption to Figure 4.16). This moderately-diverse periphyton community characterised Knockan Burn. It was moderately-well delineated from the other two groups by an eigenvalue of 0.452 (at level 1 of the TWINSPAN classification), and was indicated by *Fragilaria pulchella* (refer back to previous description of this community type if necessary).

Community Type II (n = 21 samples: HBMY05SL, LMMY05SL, IBAU05SL, HBAU05SL, LMAU05SL, HBAP06SL, LMAP06SL, HBMY05LL, LMMY05LL, IBAU05LL, HBAU05LL, LMAU05LL, HBAP06LL, LMAP06LL, HBMY05AS, LMMY05AS, IBAU05AS, HBAU05AS, LMAU05AS, HBAP06AS, LMAP06AS). This highly diversity community type depicted the periphyton assemblage found in the mid- and lower portions of the R. Girnock, and also occasionally (e.g. during the summer) in the upper part of the sub-catchment stream. The presence of *Gomphonema acuminatum* parted this community from Group III with an eigenvalue of 0.336 (at level 2 of the classification). If required, refer back to the overview of this community as earlier outlined.

Community Type III (n = 33 samples: BBMY05SL, CFMY05SL, BDMY05SL, BBAU05SL, CFAU05SL, BDAU05SL, BBAP06SL, CFAP06SL, BDAP06SL, IBMY05SL, IBAP06SL, BBMY05LL, CFMY05LL, BDMY05LL, BBAU05LL,

CFAU05LL, BDAU05LL, BBAP06LL, CFAP06LL, BDAP06LL, IBMY05LL, IBAP06LL, BBMY05AS, CFMY05AS, BDMY05AS, BBAU05AS, CFAU05AS, BDAU05AS, BBAP06AS, CFAP06AS, BDAP06AS, IBMY05AS, IBAP06AS). This periphyton assemblage characterised by few co-occurring dominant species, mainly *Frustulia rhomboides* var. *rhomboides* (again refer back to previous description of this community) and typified the Water of Dye throughout the sampling year and upper Girnock (except during summer baseflows).

CCA ordination of the 97 periphyton species constrained by the twenty-nine environmental variables used in the analysis supported prior findings (refer back to section 4.5.6.1) that variation in streamwater pH and conductivity were key drivers in determining periphyton species assemblage composition (Figure 4.17). Furthermore, from CCA output coupled to ANOVA and Tukey's pairwise comparisons of mean environmental data for the sample-units comprising each TWINSPAN sample-group (Table 4.62) it could be gathered that alkalinity and other water chemistry parameters (particularly heavy metals) were also important constraints of periphyton community structure and showed significant inter-group differences between the samples comprising each community type, as did periphyton species diversity (see Table 4.62, and also Figure 4.18).

The Group I periphyton community appeared to favour base-rich environmental habitat conditions, occurring in a stream characterised by high pH (>7), conductivity, alkalinity, concentrations of calcium and magnesium. Conversely, the Group III assemblage was mostly restricted to base-poor, acid-sensitive environmental habitat conditions experiencing elevated sulphate and heavy metal (especially lead, zinc and aluminium) availability. The Group II community occurred in near-circumneutral streamwaters, and had moderate streamwater levels of sulphate, heavy metals and base cations.

4.5.6.3 Periphyton community composition, diversity and environmental habitat conditions of all naturally-occurring substrata

Three periphyton assemblages similar in species composition to those uncovered previously for the artificial samplers were revealed from TWINSPAN classification of the naturally-occurring data set (n = 163) utilising mineral particles, aquatic bryophytes and (where present) vascular submerged macrophytes harvested during the field survey campaigns: Figure 4.19. These were also indicated by the presence of *Fragilaria pulchella* (Group I), *Gomphonema acuminatum* (Group II), and a high abundance of *Frustulia rhomboides* var. *rhomboides* (Group III). The three sample-groups shared a ubiquitous community comprised of several commonly occurring periphyton species ordinated centrally in the CCA diagram (refer to Figure 4.20).

Community Type I (n = 57 samples: UKAPP06MIN, UKAPG06MIN, UKAPR06MIN, UKSMP06MIN, UKSMG06MIN, UKNVP06MIN, UKNVG06MIN, MKAPP06MIN, MKAPG06MIN, MKAPR06MIN, MKSMP06MIN, MKSMG06MIN, MKSMR06MIN, MKNVP06MIN, MKNVG06MIN, MKNVR06MIN, LKAPP06MIN, LKAPG06MIN, LKAPR06MIN, LKSMP06MIN, LKSMG06MIN, LKSMR06MIN, LKNVP06MIN, LKNVG06MIN, LKNVR06MIN, UKAPP06BRY, UKAPG06BRY, UKAPR06BRY, UKSMP06BRY, UKNVP06BRY, UKNVG06BRY, MKAPP06BRY, MKAPG06BRY, MKAPR06BRY, MKSMP06BRY, MKSMG06BRY, MKSMR06BRY, MKNVG06BRY, MKNVR06BRY, LKAPP06BRY, LKAPG06BRY, LKAPR06BRY, LKSMP06BRY, LKSMG06BRY, LKSMR06BRY, LKNVP06BRY, LKNVG06BRY, UKAPP06VSM, UKAPG06VSM, UKSMP06VSM, UKSMG06VSM, UKNVG06VSM, LKAPP06VSM, LKAPG06VSM, LKSMG06VSM, LKNVP06VSM, LKNVG06VSM: for key to sample codes see caption to Figure 4.20). The Group I periphyton community type indicated by *Fragilaria pulchella* characterised Knockan Burn and was demarcated from the other two groups (at level 1 of the TWINSPAN classification) by an eigenvalue of 0.480. If necessary, refer back to previous descriptions of this community type (see sections 4.5.6.1 and 4.5.6.2).

Community Type II (n = 52 samples: IBMYP05MIN, IBMYG05MIN, IBMYR05MIN, IBAUP05MIN, IBAUG05MIN, IBAUR05MIN, IBAPP06MIN, IBAPG06MIN, IBAPR06MIN, HBMYP05MIN, HBMYG05MIN, HBMYR05MIN, HBAUP05MIN, HBAUG05MIN, HBAUR05MIN, HBAPP06MIN, HBAPG06MIN, HBAPR06MIN, LMMYP05MIN, LMMYG05MIN, LMMYR05MIN, LMAUP05MIN, LMAUG05MIN, LMAUR05MIN, LMAPP06MIN, LMAPG06MIN, LMAPR06MIN, IBMYP05BRY, IBMYG05BRY, IBMYR05BRY, IBAUP05BRY, IBAUG05BRY, IBAUR05BRY, IBAPP06BRY, IBAPG06BRY, IBAPR06BRY, HBMYP05BRY, HBMYG05BRY, HBMYR05BRY, HBAUP05BRY, HBAUG05BRY, HBAUR05BRY, HBAPP06BRY, HBAPG06BRY, HBAPR06BRY, LMMYP05BRY, LMMYG05BRY, LMMYR05BRY, LMAUR05BRY, LMAPP06BRY, LMAPG06BRY, LMAPR06BRY). This community type depicted the periphyton community found in the River Girnock. The occurrence of *Gomphonema acuminatum* separated this community from Group III with an eigenvalue of 0.304 (at level 2 of the classification). If required, refer back to an overview of this community type (see sections 4.5.6.1 and 4.5.6.2).

Community Type III (n = 54 samples: BBMYP05MIN, BBMYG05MIN, BBMYR05MIN, BBAUP05MIN, BBAUG05MIN, BBAUR05MIN, BBAPP06MIN, BBAPG06MIN, BBAPR06MIN, CFMYP05MIN, CFMYG05MIN, CFMYR05MIN, CFAUP05MIN, CFAUG05MIN, CFAUR05MIN, CFAPP06MIN, CFAPG06MIN, CFAPR06MIN, BDMYP05MIN, BDMYG05MIN, BDMYR05MIN, BDAUP05MIN, BDAUG05MIN, BDAUR05MIN, BDAPP06MIN, BDAPG06MIN, BDAPR06MIN, BBMYP05BRY, BBMYG05BRY, BBMYR05BRY, BBAUP05BRY, BBAUG05BRY, BBAUR05BRY, BBAPP06BRY, BBAPG06BRY, BBAPR06BRY, CFMYP05BRY, CFMYG05BRY, CFMYR05BRY, CFAUP05BRY, CFAUG05BRY, CFAUR05BRY, CFAPP06BRY, CFAPG06BRY, CFAPR06BRY, BDMYP05BRY, BDMYG05BRY, BDMYR05BRY, BDAUP05BRY, BDAUG05BRY, BDAUR05BRY, BDAPP06BRY, BDAPG06BRY, BDAPR06BRY). Group III portrayed the periphyton community occurring mainly in the Water of Dye and was indicated by a predominance of

Frustulia rhomboides var. *rhomboides* (again refer back to previous descriptions of this community type: 4.5.6.1 and 4.5.6.2).

CCA ordination of the 97 periphyton species constrained by the fifty-two environmental variables used in the analysis concurred with previous results for artificial samplers (refer back to sections 4.5.6.1 and 4.5.6.2) that variation in streamwater pH, conductivity, alkalinity and water chemistry (mainly heavy metal and base cation availability) were the principal drivers structuring periphyton community composition and occurrence (Figure 4.21). In addition to CCA, ANOVA and Tukey's pairwise comparisons of mean environmental data for the sample-units comprising each TWINSPAN sample-group (Table 4.63) indicated that under near-pristine reference conditions, water physico- (pH, conductivity, alkalinity) and chemistry (heavy metals, base cations) properties inherent to the underlying geology were the principal drivers of periphyton community structure and showed significant inter-group differences between the samples comprising each community type, as did periphyton species diversity (Table 4.63, and also Figure 4.22).

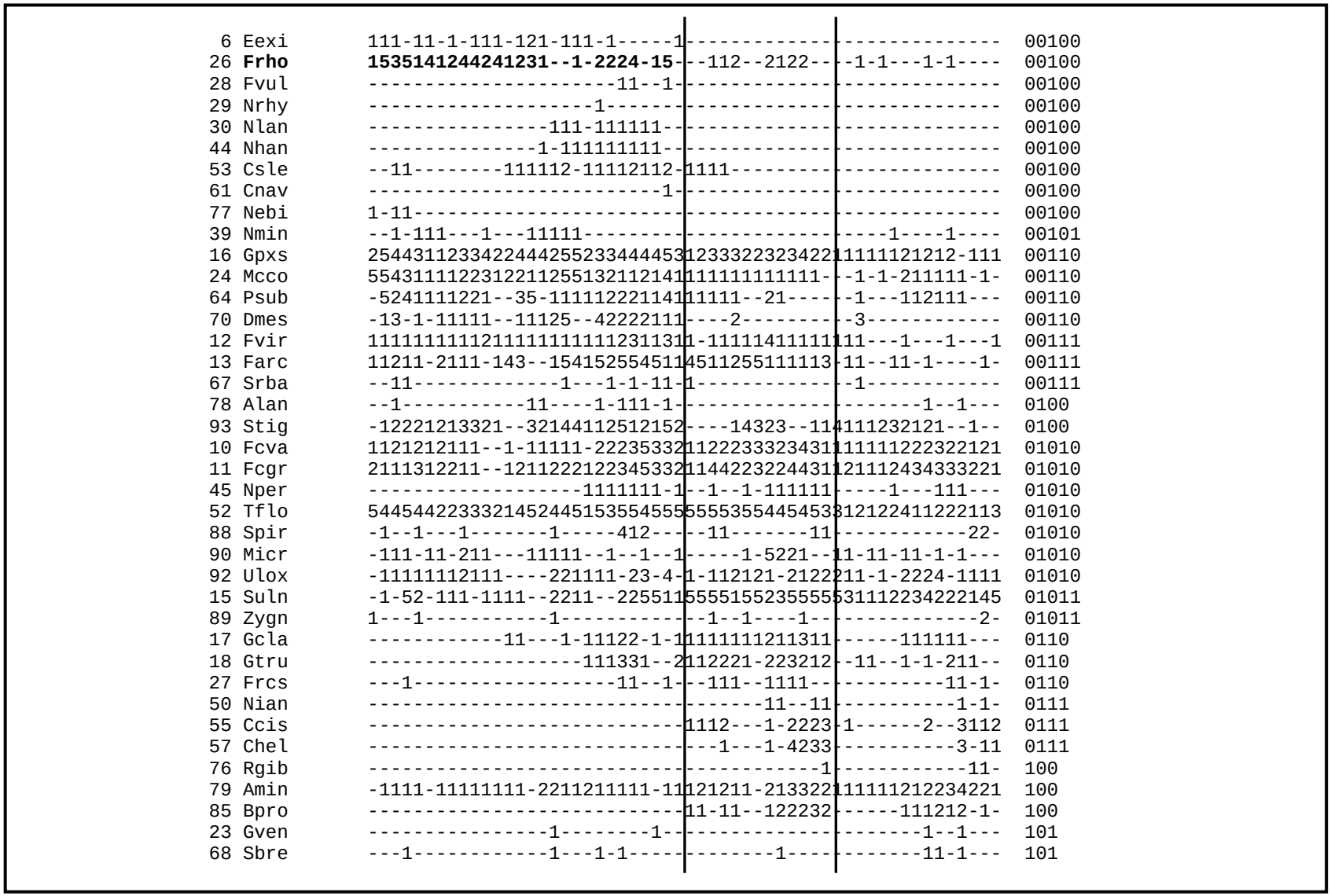
The Group I periphyton community occurred in base-rich streamwaters characterised by high pH (>7), conductivity, alkalinity, base cation concentrations and buffering capacity, resulting from the highly calcareous rock types situated beneath. The Group II community occurred in near-circumneutral streamwaters, and was moderately-well buffered due to the mixed composition of underlying base-poor and ultra-basic strata. The Group III assemblage was mostly restricted to streamwaters draining base-poor, acid-sensitive geologies with streamwaters characterised by low pH (<7) that contained accentuated levels of sulphate and heavy metal cations (especially lead, zinc and aluminium).

Samples are columns, species are rows.
Entries in the table are the pseudospecies levels not quantitative values.

Species Samples, relative numbers.

Rel. True

		III	II	I	
		12 111122 1121122221333223443333335544454444445555			
		1732345689067857212645013499128900136457845234156798023 6			
19	**Gacu**	----------**1122111212222**----------			00000
31	Naqu	----------1--------11----------			00000
32	Naan	----------11---122222----------			00000
40	Crac	----------1----------			00000
42	Nsin	----------1111----------			00000
46	Nint	----------12142----------			00000
47	Nacu	----------212121----------			00000
51	Nspp	----------111----------			00000
56	Ccym	----------1-2211----------			00000
65	Psud	----------1111211-111111----------			00000
69	Tgla	----------1111111-111-11----------			00000
74	Eadn	----------111----------			00000
75	Esor	----------1-1----------			00000
82	Dmar	----------1----------			00000
86	Clos	---1----------1----------11-------11----------			00000
94	Bulb	----------3-----4-425252----------			00000
95	Rivu	----------2-----1-441141----------			00000
5	Ebil	---1----------1------11111111111--111111----------			00001
43	Ngra	---1----------1---11111-------111111----------			00001
54	Cgra	---1----------1-11112111111111213111----------			00001
66	Pdiv	----------1----1--1-----1----------			00001
84	Bvit	----------11111--11--1--1111--1----------			00001
87	Cosm	1--11-11--1-----1--------1--111----1-1121----------			00001
96	Lema	----------1---1-------1-2----1------1----------			00001
3	Einc	-11--11---1---1-----1---------11---1-----1----------			0001
4	Emei	----1----------1-----11111111---1-11----1----------			0001
9	Eimp	111-11-1111111111111--11--1111-11--11--11----------			0001
81	Dell	--11----------11----------1--------1-1----------			0001
2	Emus	-111-11---111111------1-111----1--------1----------			00100


6 Eexi 111-11-1-111-121-111-1-----1 00100
26 Frho 1535141244241231--1-2224-15---112--2122---1-1---1-1---- 00100
28 Fvul ----------11--1 00100
29 Nrhy ----------1 00100
30 Nlan ----------111-111111 00100
44 Nhan ----------1-111111111 00100
53 Csle --11--------111112-11112112-1111 00100
61 Cnav ----------1 00100
77 Nebi 1-11 00100
39 Nmin --1-111---1---11111----------1----1----1---- 00101
16 Gpxs 254431123342244425523344445312333223234221111121212-111 00110
24 Mcco 554311112231221125513211214111111111111--1-1-211111-1- 00110
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70 Dmes -13-1-11111--11125--42222111----2---------3---------- 00110
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13 Farc 11211-2111-143--154152554511451125511111 3-11--11-1----1- 00111
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11 Fcgr 2111312211--12112221223453321144223224431121112434333221 01010
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52 Tflo 5445442233321452445153554555555535544545381212241122211 3 01010
88 Spir -1--1---1-------1-----412------11-------11----------22- 01010
90 Micr -111-11-211---11111--1--1--1-----1-5221--11-11-11-1-1--- 01010
92 Ulox -11111112111----221111-23-4-1-112121-2122211-1-2224-1111 01010
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89 Zygn 1---1----------1----------1--1----1----------2- 01011
17 Gcla ----------11---1-11122-1-111111111211311-----111111--- 0110
18 Gtru ----------111331--2112221-223212--11--1-1-211-- 0110
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79 Amin -1111-11111111-2211211111-11121211-213322111111212234221 100
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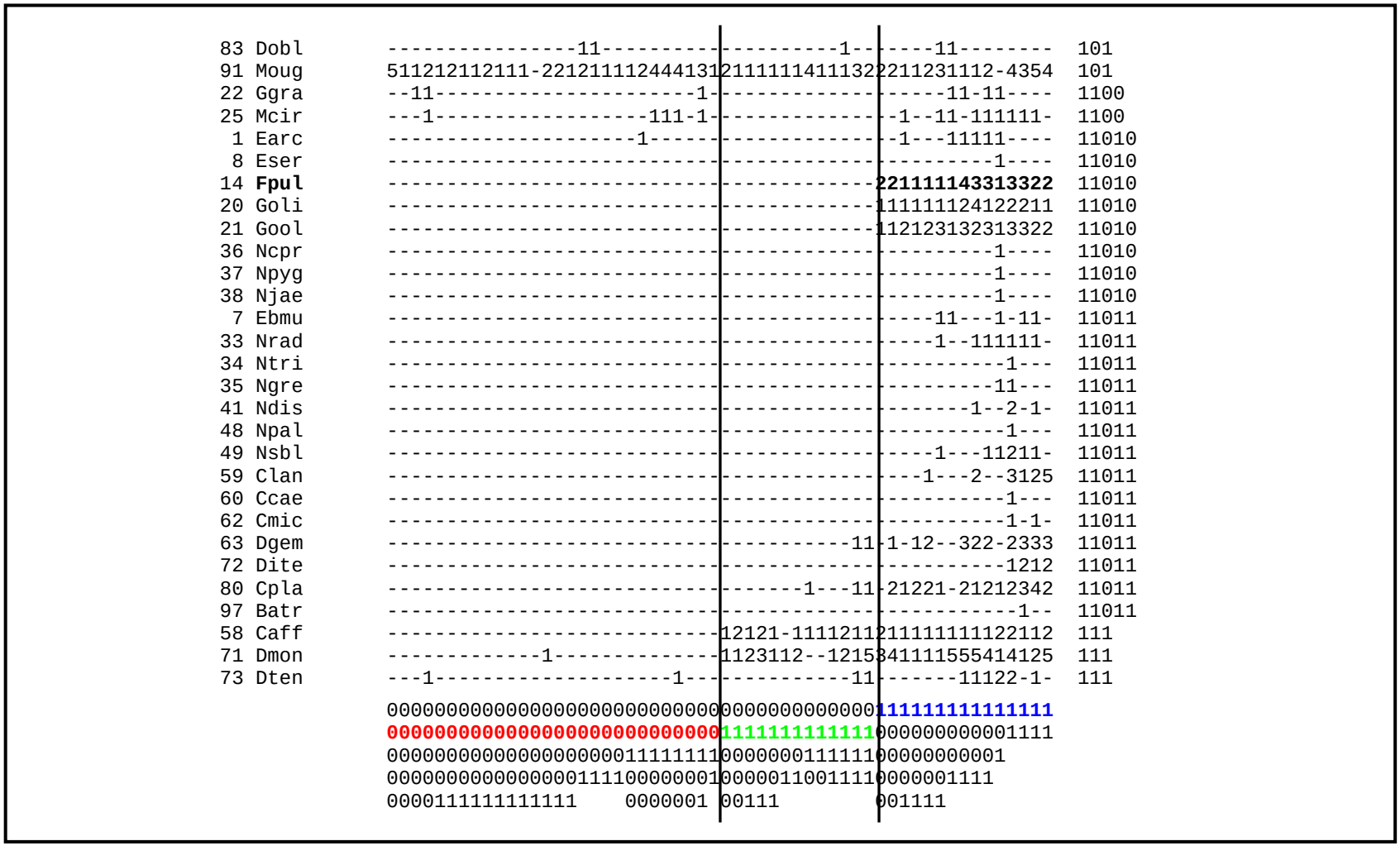


Figure 4.11 TWINSPAN output depicting 56 samples and 3 periphyton species assemblages, with indicator species highlighted in bold font and colour-coding as appropriate for TWINSPAN sample-groups I (blue), II (green), and III (red). For periphyton species codes refer to Figure 4.13.

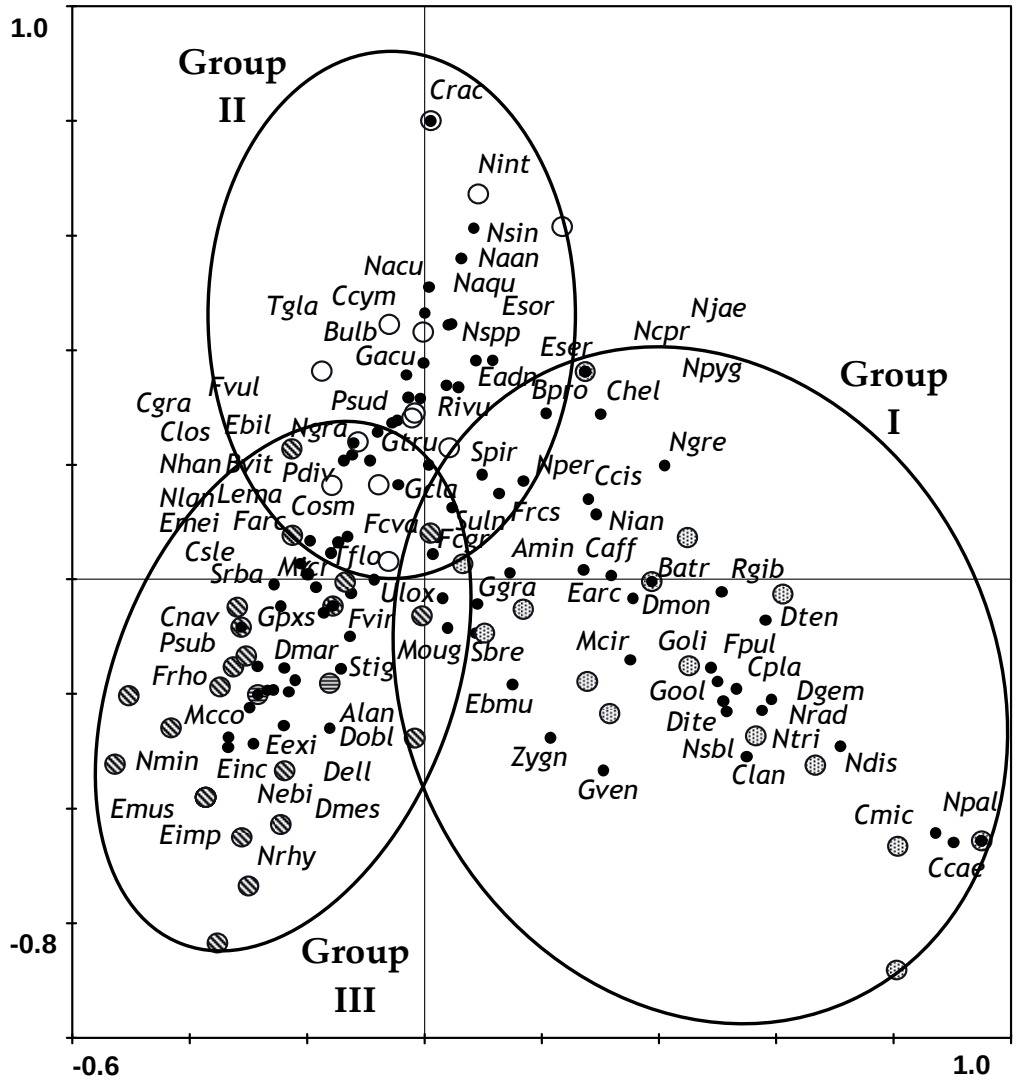


Figure 4.12 CCA ordination of 97 periphyton species and 56 samples, with TWINSPAN sample-group boundaries overlaid. TWINSPAN sample-group identifiers as follows: Group I (n=15: UKDEC05SL, MKDEC05SL, LKDEC05SL, UKAP06SL, MKAP06SL, LKAP06SL, UKJY06SL, MKJY06SL, LKJY06SL, UKSM06SL, MKSM06SL, LKSM06SL, UKNV06SL, MKNV06SL, LKNV06SL): dotted circles ; Group II (n=13: HBAP05SL, LMAP05SL, IBMY05SL, HBMY05SL, LMMY05SL, IBJY05SL, HBJY05SL, LMJY05SL, IBAU05SL, HBAU05SL, LMAU05SL, HBAP06SL, LMAP06SL): open circles ; Group III (n=28: BBOC04SL, CFOC04SL, BDOC04SL, BBNV04SL, CFNV04SL, BDNV04SL, BBJA05SL, CFJA05SL, BDJA05SL, BBMR05SL, CFMR05SL, BDMR05SL, BBAP05SL, CFAP05SL, BDAP05SL, BBMY05SL, CFMY05SL, BDMY05SL, BBJY05SL, CFJY05SL, BDJY05SL, BBAU05SL, CFAU05SL, BDAU05SL, BBAP06SL, CFAP06SL, BDAP06SL, IBAP06SL): diagonally striped circles . For sample site-codes: Water of Dye sites: Brocky Burn (BB), Charr Flume (CF) and Bogendreip (BD); River Girnock sites: Iron Bridge (IB), Hampshire's Bridge (HB) and Littlemill (LM); Knockan Burn sites: Upper Knockan (UK), Mid-Knockan (MK) and Lower Knockan (LK). Each site code is completed using code letters for survey date (AP: April; MY: May; AU: August; SM: September; NV: November), year sampled (05: 2005; 06: 2006) and substrate type (SL: short-term linoleum artificial sampler). Example: BBMY05SL = Brocky Burn May 2005 using short-term linoleum artificial samplers. For periphyton species codes and ordination statistics refer to Figure 4.13.

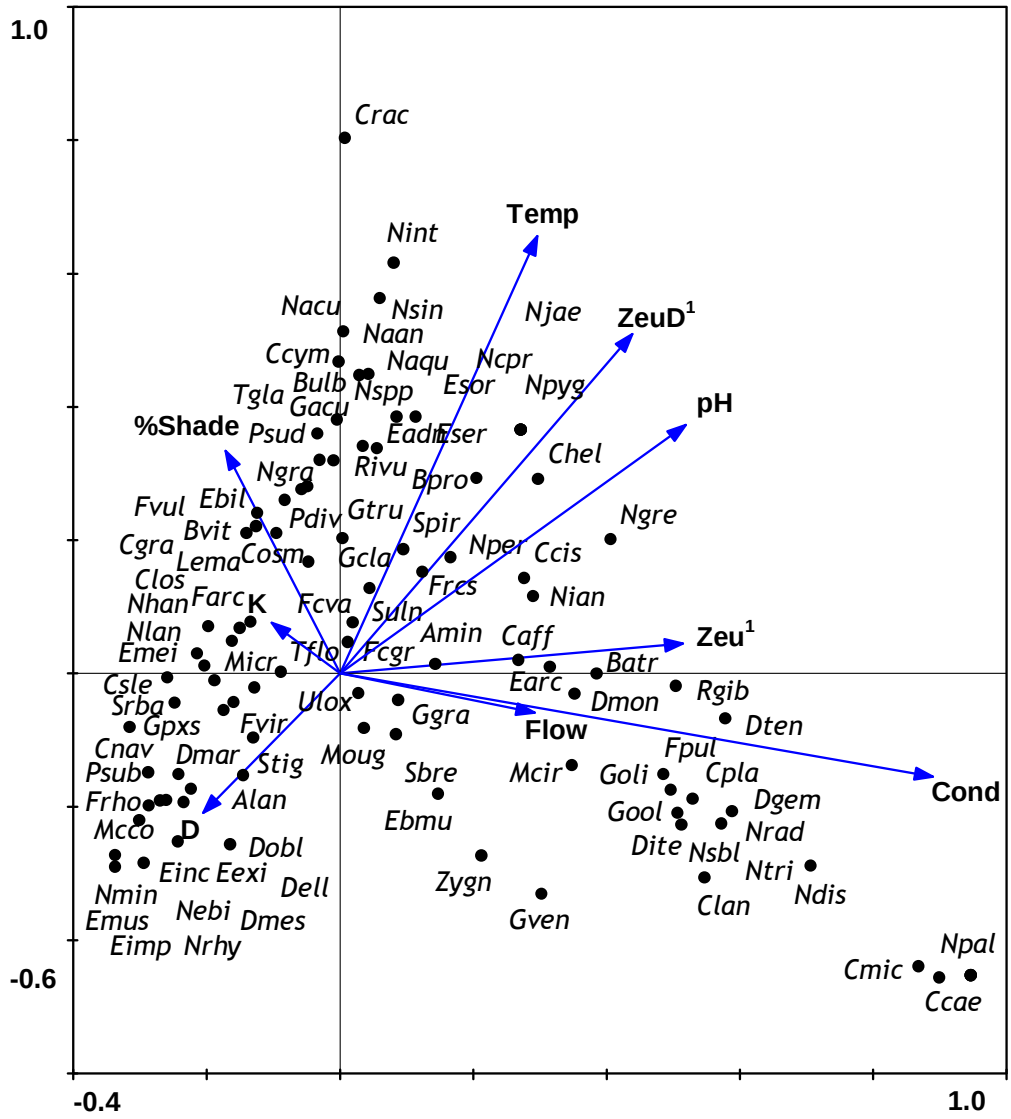


Figure 4.13 CCA ordination of periphyton species and environmental variables. Periphyton species codes: *Eunotia arcus* sensu (Earc), *Eunotia muscicola* var. *tridentula* (Emus), *Eunotia* cf. *incisa* (Einc), *Eunotia meisteri* (Emei), *Eunotia bilunaris* var. *linearis* (Ebil), *Eunotia exigua* (Eexi), *Eunotia bilunaris* var. *mucophila* (Ebmu), *Eunotia serra* (Eser), *Eunotia implicata* (Eimp), *Fragilaria capucina* var. *vaucheriae* (Fcva), *Fragilaria capucina* var. *gracilis* (Fcgr), *Fragilaria virescens* (Fvir), *Fragilaria arcus* (Farc), *Fragilaria pulchella* (Fpul), *Synedra ulna* (Suln),*Gomphonema* cf. *parvulum* var. *exilissimum* (Gpxs), *Gomphonema clavatum* (Gcla), *Gomphonema truncatum* (Gtru), *Gomphonema acuminatum* (Gacu), *Gomphonema olivaceum* (Goli), *Gomphonema olivaceum* var. *olivaceoides* (Gool), *Gomphonema gracile* (Ggra), *Gomphonema ventricosum* (Gven), *Meridion circulare* var. *constrictum* (Mcco), *Meridion circulare* (Mcir), *Frustulia rhomboides* var. *rhomboides* (Frho), *Frustulia rhomboides* var. *crassinervia* (Frcs), *Frustulia vulgaris* (Fvul), *Navicula rhynchocephala* (Nrhy), *Navicula lanceolata* (Nlan), *Navicula* cf. *aquaedurae* (Naqu), *Navicula angusta* (Naan), *Navicula radiosa* (Nrad), *Navicula tripunctata* (Ntri), *Navicula* cf. *gregaria* (Ngre), *Navicula capitatoradiata* (Ncpr), *Navicula* cf. *pygmaea* agg. (Npyg), *Navicula jaernefeltii* (Njae), *Navicula minima* (Nmin), *Craticula acidoclinata* (Crac), *Nitzschia dissipata* (Ndis), *Nitzschia sinuata* (Nsin), *Nitzschia gracilis* (*Ngra*), *Nitzschia*

hantzschiana **(Nhan),** ***Nitzschia perminuta*** **agg. (Nper),** ***Nitzschia intermedia*** **agg. (Nint),** ***Nitzschia*** **cf.** ***acula*** **(Nacu),** ***Nitzschia palea*** **agg. (Npal),** ***Nitzschia sublinearis*** **(Nsbl),** ***Nitzschia angustata*** **(Nian),** ***Nitzschia*** **undefined sp. (Nspp),** ***Tabellaria flocculosa*** **(Tflo),** ***Cymbella silesiaca*** **(Csle),** ***Cymbella gracilis*** **(Cgra),** ***Cymbella cistula*** **(Ccis),** ***Cymbella cymbiformis*** **(Ccym),** ***Cymbella helvetica*** **(Chel),** ***Cymbella affinis*** **(Caff),** ***Cymbella lanceolata*** **(Clan),** ***Cymbella caespitosa*** **(Ccae),** ***Cymbella naviculiformis*** **(Cnav),** ***Cymbella microcephala*** **(Cmic),** ***Didymosphenia geminata*** **(Dgem),** ***Pinnularia subcapitata*** **(Psub),** ***Pinnularia*** **cf.** ***sudetica*** **(Psud),** ***Pinnularia*** **cf.** ***divergens*** **(Pdiv),** ***Surirella roba*** **(Srba),** ***Surirella brebissonii*** **(Sbre),** ***Tetracyclus glans*** **(Tgla),** ***Diatoma mesodon*** **(Dmes),** ***Diatoma moniliformis*** **(Dmon),** ***Diatoma tenuis*** **(Dite),** ***Denticula tenuis*** **(Dten),** ***Epithemia adnata*** **(Eadn),** ***Epithemia sorex*** **(Esor),** ***Rhopalodia gibba*** **(Rgib),** ***Nedium bisulcatum*** **(Nebi),** ***Achnanthes lanceolata*** **(Alan),** ***Achnanthidium minutissima*** **(Amin),** ***Cocconeis placentula*** **(Cpla),** ***Diploneis*** **cf.** ***elliptica*** **(Dell),** ***Diploneis marginestriata*** **(Dmar),** ***Diploneis oblongella*** **(Dobl),** ***Brachysira vitrea*** **(Bvit),** ***Brachysira procera*** **(Bpro),** ***Closterium*** **sp. (Clos),** ***Cosmarium*** **sp. (Cosm),** ***Spirogyra*** **sp. (Spir),** ***Zygnema*** **sp. (Zygn),** ***Microspora*** **sp. (Micr),** ***Mougeotia*** **sp. (Moug),** ***Ulothrix*** **sp. (Ulox),** ***Stigeoclonium*** **sp. (Stig),** ***Bulbochaete*** **sp. (Bulb),** ***Rivularia*** **sp. (Rivu),** ***Lemanea fluviatilis*** **(Lema),** ***Batrachospermum*** **sp. (Batr). Environmental variables: Water physico-chemistry: benthic depth (D: m), light attenuation coefficient (K: m^{-1}), euphotic depth 1% (Zeu^1: m^{-1}), Zeu:D^1 ratio, pH, conductivity (Cond: µS cm^{-1}), water temperature (Temp: ^{O}C), current velocity (Flow: m s^{-1}) and % Shade. Monte Carlo significance test: Axis 1: P<0.005; all canonical axes: P<0.005. Eigenvalues: Axis 1: 0.372; Axis 2: 0.205.**

	TWINSPAN sample-group						
Variable	**I**		**II**		**III**		**PANOVA**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton species richness: **S**	26.1[a]	1.87	33.0[b]	2.30	23.2[a]	1.23	P<0.01**
Periphyton species diversity: **H**	2.77[a]	0.07	2.90[a]	0.10	2.53[b]	0.06	P<0.01**
Periphyton species dominance	0.16[ab]	0.02	0.15[a]	0.02	0.19[b]	0.02	P<0.05*
D (m)	0.10	0.14	0.09	0.22	0.17	0.15	NS
K (m^{-1})	2.30	0.39	2.53	0.39	2.94	0.32	NS
$Z_{eu}^{1\%}$ (m)	0.43	0.31	0.28	0.33	0.28	0.24	NS
Z_{eu}:$D^{1\%}$	4.07[a]	0.48	2.38[ab]	0.46	1.54[b]	0.32	P<0.05*
pH	7.50[a]	0.09	6.91[b]	0.10	6.31[c]	0.16	P<0.001***
Conductivity (μS cm^{-1})	146.8[a]	0.12	54.4[b]	0.10	43.0[c]	0.05	P<0.001***
Water Temperature (^oC)	8.7[a]	0.14	11.2[b]	0.14	7.9[a]	0.16	P<0.01**
Flow (m s^{-1})	0.249	0.05	0.156	0.06	0.168	0.05	NS
% Shade	8.4[a]	2.09	32.7[b]	9.10	22.2[ab]	3.28	P<0.05

Table 4.61 Mean values (± 1 standard error) of normally distributed periphyton species richness (per 400 cm^2), periphyton species diversity (per 400 cm^2), periphyton species dominance (per 400 cm^2), and environmental habitat variables (data back-transformed where necessary) between TWINSPAN sample-groups I (n = 15), II (n = 13), and III (n = 28): for short-term linoleum substrates (n = 56). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different.

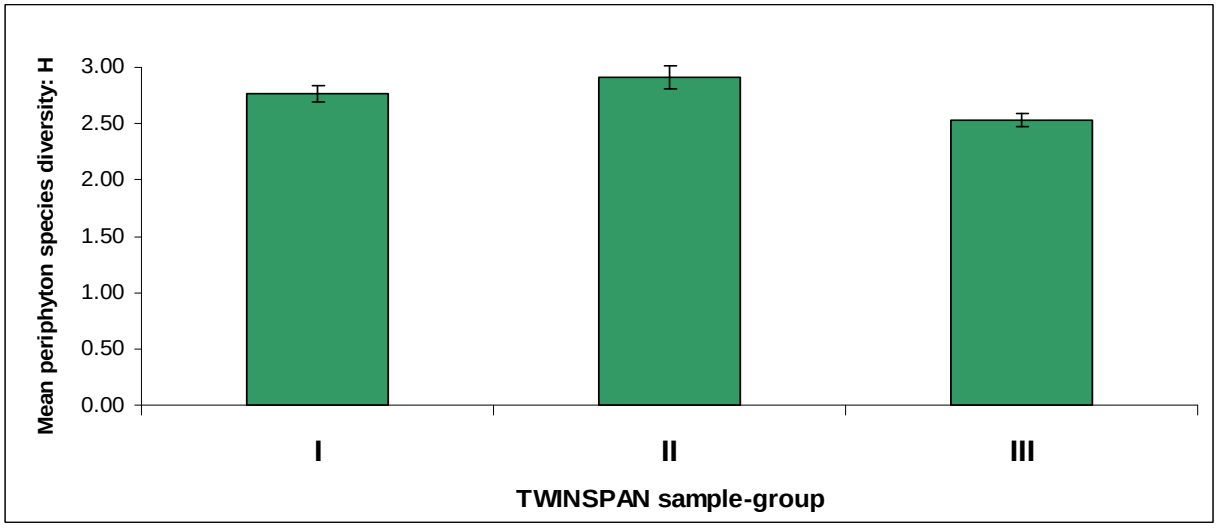
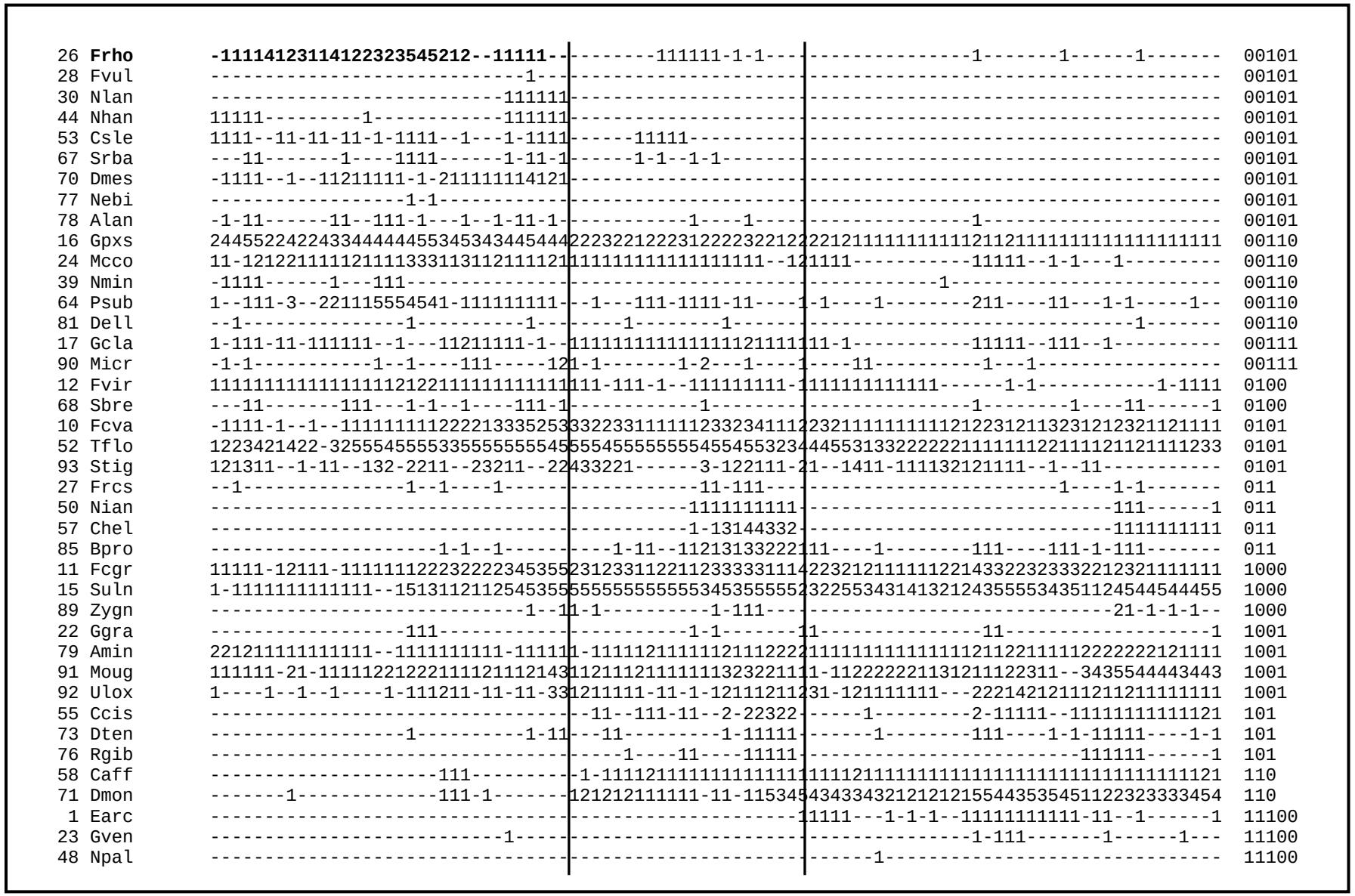


Figure 4.14 Comparison of mean values (± 1 standard error) of normally distributed periphyton species diversity per 400 cm^2 (data back-transformed where necessary) between TWINSPAN sample-groups I (n = 15), II (n = 13), and III (n = 28) of short-term linoleum substrates (n = 56).

Samples are columns, species are rows.
Entries in the table are the pseudospecies levels not quantitative values.

Species Samples, relative numbers.
Rel. True

		III	II	I	
		336 2235556 36 35136147 356 3	1144771133661614461461	47882257889924722488584772572578899528	
		8956212389056737414180746306390527	8451212895637401852996	3285629460229610839307453074185713471	
19	**Gacu**	----------111---------	**1111111111111122222**	----------	00000
31	Naqu	----------	----------1---1-11111	----------	00001
32	Naan	----------1--1----------	----11--1-11222223232	----------	00001
42	Nsin	----------	----------1-111---	----------	00001
46	Nint	----------	----------114145233	----------	00001
47	Nacu	----------1---------	----------11112123111	----------	00001
51	Nspp	----------	-----1--------1-11111	----------	00001
56	Ccym	----------	---1-------1--1-11111	----------	00001
65	Psud	----------1-----1---1-11-1------	1-1-11111-11111111111	----------	00001
74	Eadn	----------	---111----11--1-11111	----------	00001
75	Esor	----------	--11-1-----1----11111	----------	00001
86	Clos	----------1----------	-------1------1----1-	----------	00001
87	Cosm	----------1----1---11-----	--1---11--1--1211-11-	----------	00001
94	Bulb	----------322------	4-1-1-------215253231	----------	00001
95	Rivu	----------221------	1-2-1-------444525121	----------	00001
4	Emei	----------111111111	1-111-11111111--------	----------	00010
5	Ebil	-1-1----------1111-1111111111	--1111111111111111111	----------	00010
13	Farc	1-1111431321521--1111211113555443	555555454543111111322111---11-----1111-11-----1---11--------		00010
18	Gtru	1-11----------224221--11111	1-1-1111113231211211111-1-----------1-11------1---1--------		00010
45	Nper	1-1-1----------111111111	1-111-------111111111------------1-1---------1-1----------		00010
54	Cgra	1-11----------1--11111111-11111111	1111111111111111111111----------		00010
69	Tgla	----------114111------	1-111-111111111111111----------		00010
43	Ngra	----------111------111111	----------111111111----------		00011
66	Pdiv	----------1-11--------	----------1---1------	----------	00011
84	Bvit	----------111--1111111	----1-11-11111-1------	----------	00011
9	Eimp	111111111113231122---111111------	----11111-11111--1111----------		00100
96	Lema	1111----------11--------	-1-1---2----------	----------	00100
2	Emus	-1-1-1111111111121111111---111111	----------	----------	00101
3	Einc	----------1111----------	----------	----------	00101
6	Eexi	112221-11-12-22222------111------	----------	----------	00101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28 Fvul
30 Nlan
44 Nhan
53 Csle
67 Srba
70 Dmes
77 Nebi
78 Alan
16 Gpxs
24 Mcco
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81 Dell
17 Gcla
90 Micr
12 Fvir
68 Sbre
10 Fcva
52 Tflo
93 Stig
27 Frcs
50 Nian
57 Chel
85 Bpro
11 Fcgr
15 Suln
89 Zygn
22 Ggra
79 Amin
91 Moug
92 Ulox
55 Ccis
73 Dten
76 Rgib
58 Caff
71 Dmon
1 Earc
23 Gven
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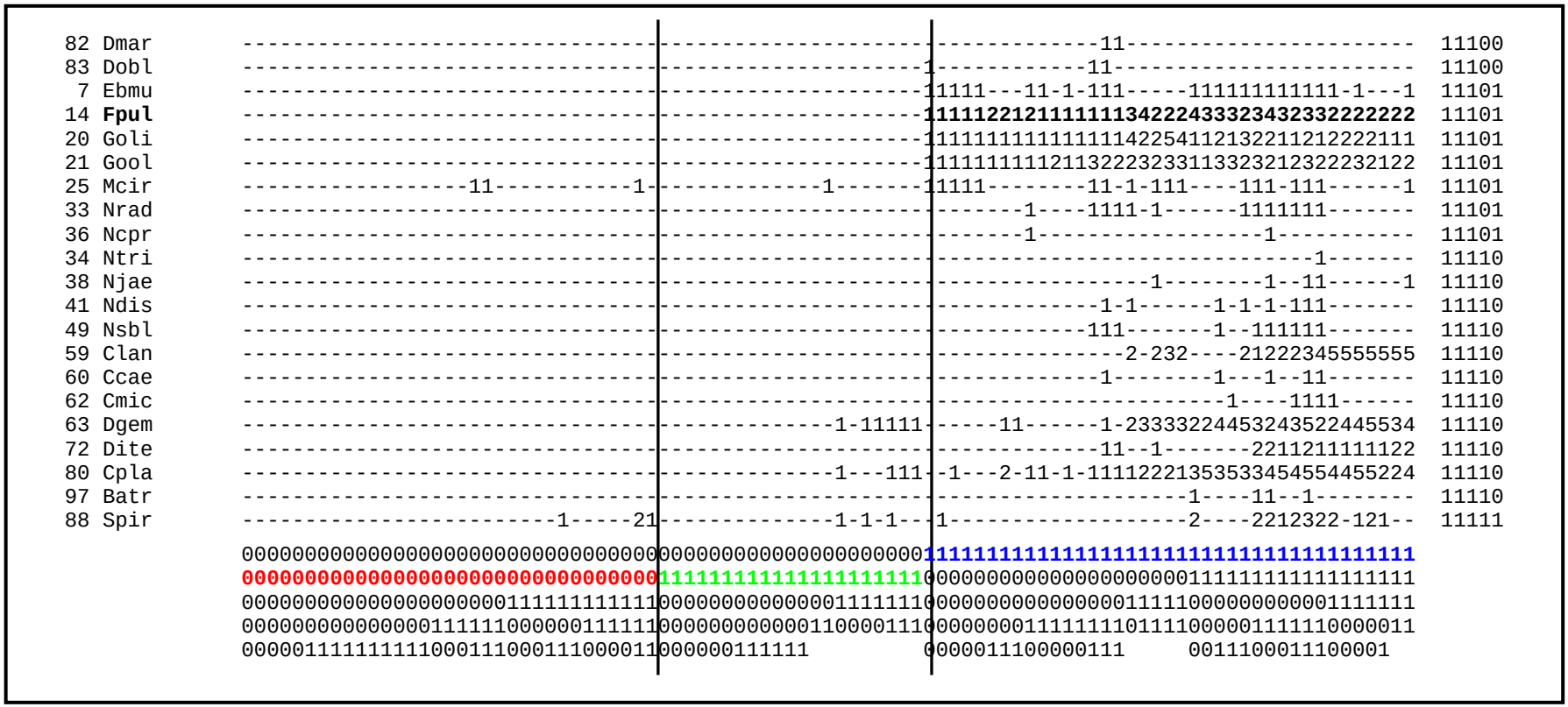


Figure 4.15 TWINSPAN output depicting 93 samples and 3 periphyton species assemblages, with indicator species highlighted in bold font and colour-coding as appropriate for TWINSPAN sample-groups I (blue), II (green), and III (red). For periphyton species codes refer to Figure 4.17.

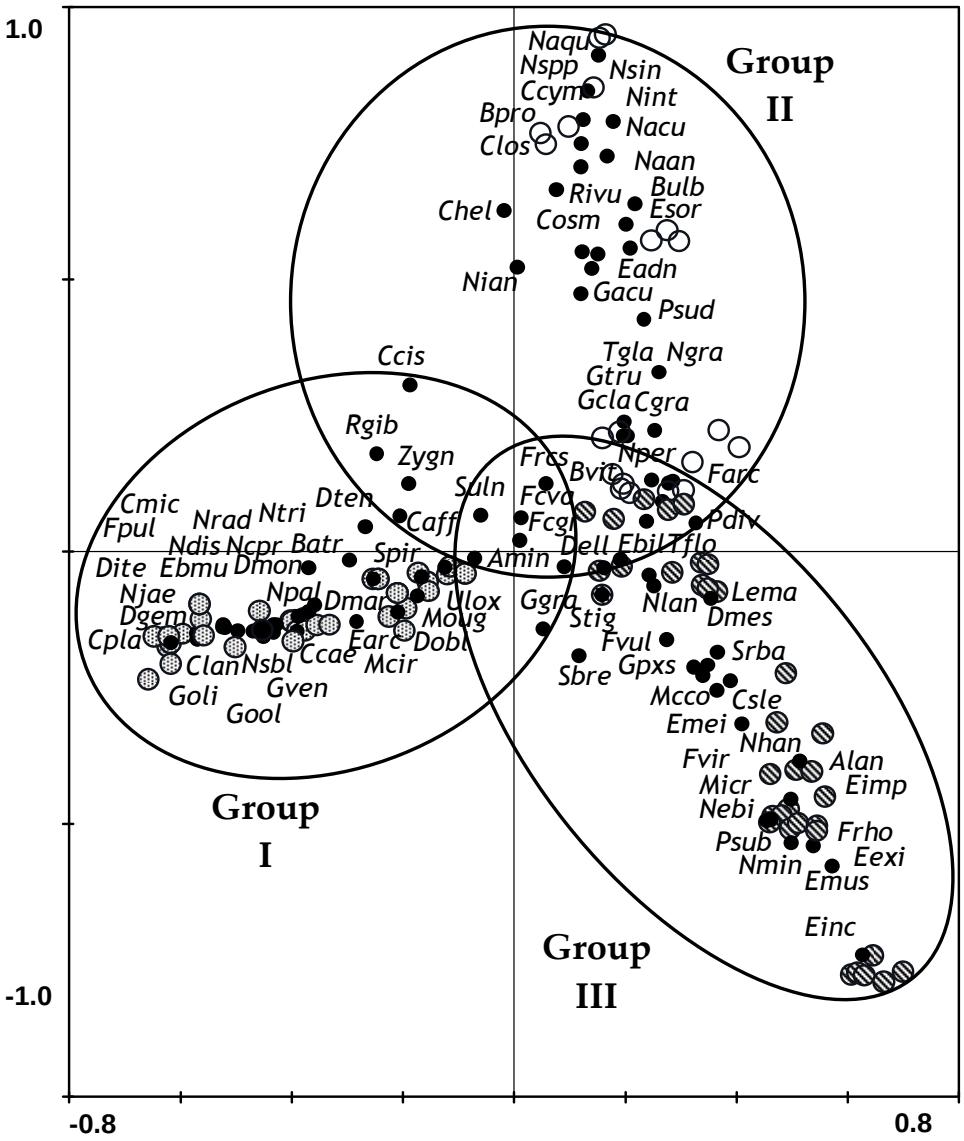


Figure 4.16 CCA ordination of 97 periphyton species and 93 samples, with TWINSPAN sample-group boundaries overlaid. TWINSPAN sample-group identifiers as follows: Group I (n=39: UKAP06SL, MKAP06SL, LKAP06SL, UKSM06SL, MKSM06SL, LKSM06SL, UKNV06SL, MKNV06SL, LKNV06SL, UKAP06LL, MKAP06LL, LKAP06LL, UKSM06LL, MKSM06LL, LKSM06LL, UKNV06LL, MKNV06LL, LKNV06LL, UKAP06AS, MKAP06AS, LKAP06AS, UKSM06AS, MKSM06AS, LKSM06AS, UKNV06AS, MKNV06AS, LKNV06AS, UKAP06PM, LKAP06PM, UKSM06PM, LKSM06PM, UKNV06PM, LKNV06PM, UKAP06PP, LKAP06PP, UKSM06PP, LKSM06PP, UKNV06PP, LKNV06PP): dotted circles ; Group II (n=21: HBMY05SL, LMMY05SL, IBAU05SL, HBAU05SL, LMAU05SL, HBAP06SL, LMAP06SL, HBMY05LL, LMMY05LL, IBAU05LL, HBAU05LL, LMAU05LL, HBAP06LL, LMAP06LL, HBMY05AS, LMMY05AS, IBAU05AS, HBAU05AS, LMAU05AS, HBAP06AS, LMAP06AS): open circles ; Group III (n=33: BBMY05SL, CFMY05SL, BDMY05SL, BBAU05SL, CFAU05SL, BDAU05SL, BBAP06SL, CFAP06SL, BDAP06SL, IBMY05SL, IBAP06SL, BBMY05LL, CFMY05LL, BDMY05LL, BBAU05LL, CFAU05LL, BDAU05LL, BBAP06LL, CFAP06LL, BDAP06LL, IBMY05LL, IBAP06LL, BBMY05AS, CFMY05AS, BDMY05AS, BBAU05AS, CFAU05AS, BDAU05AS, BBAP06AS, CFAP06AS, BDAP06AS, IBMY05AS, IBAP06AS): diagonally striped circles . For sample site-codes: Water of Dye sites: Brocky Burn (BB), Charr Flume (CF) and Bogendreip

(BD); River Girnock sites: Iron Bridge (IB), Hampshire's Bridge (HB) and Littlemill (LM); Knockan Burn sites: Upper Knockan (UK), Mid-Knockan (MK) and Lower Knockan (LK). Each site code is completed using code letters for survey date (AP: April; MY: May; AU: August; SM: September; NV: November), year sampled (05: 2005; 06: 2006) and substrate type (SL: short-term linoleum artificial sampler; LL: long-term linoleum artificial sampler; AS: long-term Astroturf artificial sampler; PM: long-term plastic aquarium *Myriophyllum*-like artificial sampler, PP: long-term plastic aquarium *Potamogeton*-like artificial sampler). Example: BBMY05SL = Brocky Burn May 2005 using short-term linoleum artificial samplers. For periphyton species codes and ordination statistics refer to Figure 4.17.

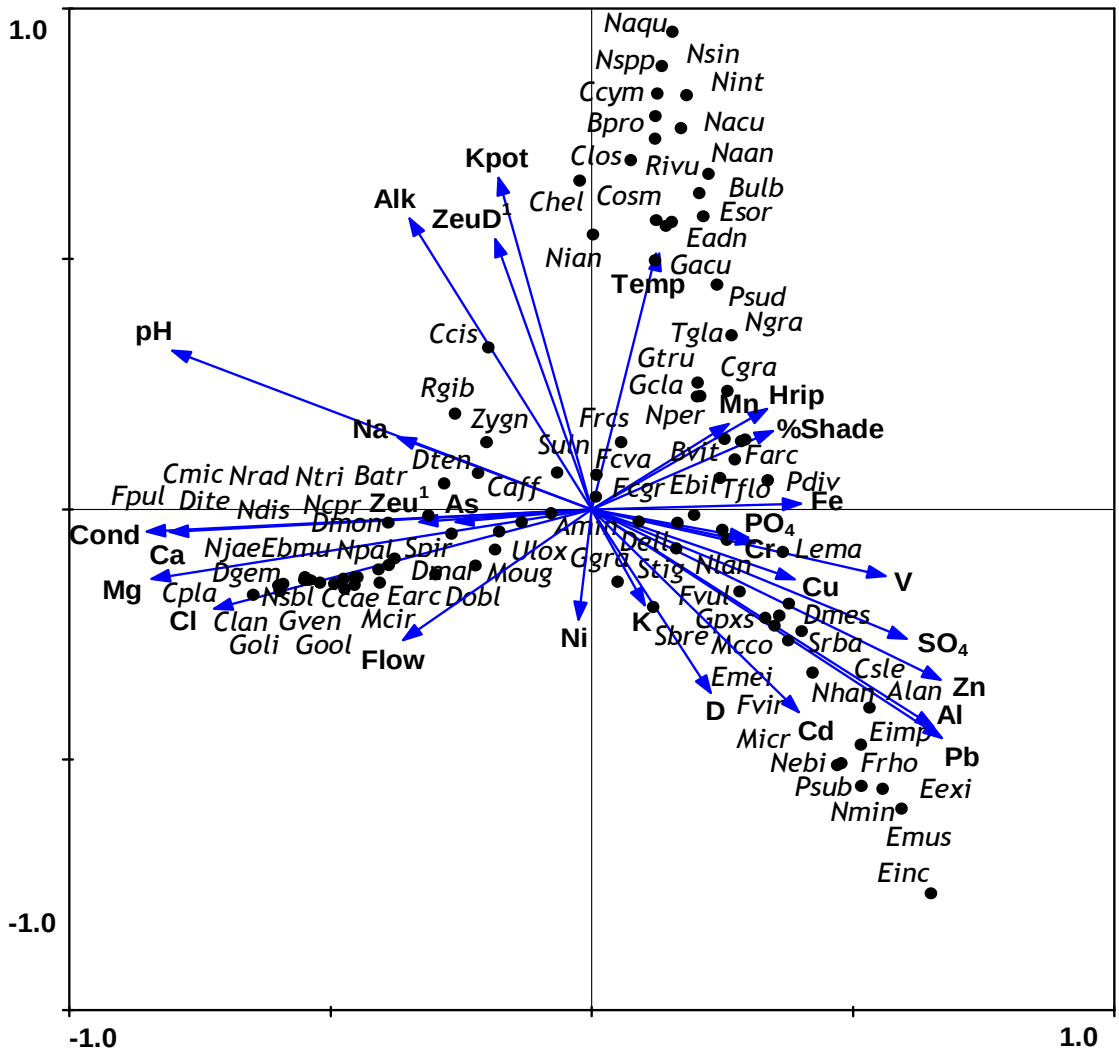


Figure 4.17 CCA ordination of periphyton species and environmental variables. Periphyton species codes: *Eunotia arcus* sensu (Earc), *Eunotia muscicola* var. *tridentula* (Emus), *Eunotia* cf. *incisa* (Einc), *Eunotia meisteri* (Emei), *Eunotia bilunaris* var. *linearis* (Ebil), *Eunotia exigua* (Eexi), *Eunotia bilunaris* var. *mucophila* (Ebmu), *Eunotia serra* (Eser), *Eunotia implicata* (Eimp), *Fragilaria capucina* var. *vaucheriae* (Fcva), *Fragilaria capucina* var. *gracilis* (Fcgr), *Fragilaria virescens* (Fvir), *Fragilaria arcus* (Farc), *Fragilaria pulchella* (Fpul), *Synedra ulna* (Suln),*Gomphonema* cf. *parvulum* var. *exilissimum* (Gpxs), *Gomphonema clavatum* (Gcla), *Gomphonema truncatum* (Gtru), *Gomphonema acuminatum* (Gacu), *Gomphonema olivaceum* (Goli), *Gomphonema olivaceum* var. *olivaceoides* (Gool), *Gomphonema gracile* (Ggra), *Gomphonema ventricosum* (Gven), *Meridion circulare* var. *constrictum* (Mcco), *Meridion circulare* (Mcir), *Frustulia rhomboides* var. *rhomboides* (Frho), *Frustulia rhomboides* var. *crassinervia* (Frcs), *Frustulia vulgaris* (Fvul), *Navicula rhynchocephala* (Nrhy), *Navicula lanceolata* (Nlan), *Navicula* cf. *aquaedurae* (Naqu), *Navicula angusta* (Naan), *Navicula radiosa* (Nrad), *Navicula tripunctata* (Ntri), *Navicula* cf. *gregaria* (Ngre), *Navicula capitatoradiata* (Ncpr), *Navicula* cf. *pygmaea* agg. (Npyg), *Navicula jaernefeltii* (Njae), *Navicula minima* (Nmin), *Nitzschia dissipata* (Ndis), *Nitzschia sinuata* (Nsin), *Nitzschia gracilis* (*Ngra*), *Nitzschia hantzschiana* (Nhan), *Nitzschia*

***perminuta* agg. (Nper), *Nitzschia intermedia* agg. (Nint), *Nitzschia* cf. *acula* (Nacu), *Nitzschia palea* agg. (Npal), *Nitzschia sublinearis* (Nsbl), *Nitzschia angustata* (Nian), *Nitzschia* undefined sp. (Nspp), *Tabellaria flocculosa* (Tflo), *Cymbella silesiaca* (Csle), *Cymbella gracilis* (Cgra), *Cymbella cistula* (Ccis), *Cymbella cymbiformis* (Ccym), *Cymbella helvetica* (Chel), *Cymbella affinis* (Caff), *Cymbella lanceolata* (Clan), *Cymbella caespitosa* (Ccae), *Cymbella naviculiformis* (Cnav), *Cymbella microcephala* (Cmic), *Didymosphenia geminata* (Dgem), *Pinnularia subcapitata* (Psub), *Pinnularia* cf. *sudetica* (Psud), *Pinnularia* cf. *divergens* (Pdiv), *Surirella roba* (Srba), *Surirella brebissonii* (Sbre), *Tetracyclus glans* (Tgla), *Diatoma mesodon* (Dmes), *Diatoma moniliformis* (Dmon), *Diatoma tenuis* (Dite), *Denticula tenuis* (Dten), *Epithemia adnata* (Eadn), *Epithemia sorex* (Esor), *Rhopalodia gibba* (Rgib), *Nedium bisulcatum* (Nebi), *Achnanthes lanceolata* (Alan), *Achnanthidium minutissima* (Amin), *Cocconeis placentula* (Cpla), *Diploneis* cf. *elliptica* (Dell), *Diploneis marginestriata* (Dmar), *Diploneis oblongella* (Dobl), *Brachysira vitrea* (Bvit), *Brachysira procera* (Bpro), *Closterium* sp. (Clos), *Cosmarium* sp. (Cosm), *Spirogyra* sp. (Spir), *Zygnema* sp. (Zygn), *Microspora* sp. (Micr), *Mougeotia* sp. (Moug), *Ulothrix* sp. (Ulox), *Stigeoclonium* sp. (Stig), *Bulbochaete* sp. (Bulb), *Rivularia* sp. (Rivu), *Lemanea fluviatilis* (Lema), *Batrachospermum* sp. (Batr). Water physico-chemistry: benthic depth (D: m), light attenuation coefficient (K: m^{-1}), euphotic depth 1% (Zeu^1: m^{-1}), $Zeu:D^1$ ratio, pH, alkalinity (Alk: mg/l), conductivity (Cond: µS cm^{-1}), water temperature (Temp: ^oC), current velocity (Flow: m s^{-1}), %Shade and height of riparian vegetation (Hrip). Water Chemistry: Phosphate (PO_4-P), Chloride (Cl), Sulphate (SO_4), Cadmium (Cd), Chromium (Cr), Copper (Cu), Lead (Pb), Nickel (Ni), Zinc (Zn), Aluminium (Al), Vanadium (V), Arsenic (As), Sodium (Na), Potassium (Kpot), Calcium (Ca), Magnesium (Mg), Iron (Fe), and Manganese (Mn). Monte Carlo significance test: Axis 1: P<0.005; all canonical axes: P<0.005. Eigenvalues: Axis 1: 0.509; Axis 2: 0.344.**

	TWINSPAN sample-group						
Variable	**I**		**II**		**III**		**P_{ANOVA}**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton species richness: **S**	24.2[a]	0.97	34.0[b]	1.88	23.6[a]	1.02	P<0.01**
Periphyton species diversity: **H**	2.67[a]	0.04	2.79[a]	0.08	2.50[b]	0.04	P<0.01**
Periphyton species dominance	0.17[a]	0.01	0.17[a]	0.01	0.22[b]	0.02	P<0.01**
D (m)	0.12	0.10	0.10	0.18	0.17	0.12	NS
K (m^{-1})	2.58	0.24	2.23	0.34	2.74	0.26	NS
$Z_{eu}^{1\%}$ (m)	0.35	0.25	0.25	0.38	0.28	0.27	NS
Z_{eu}:$D^{1\%}$	3.07[a]	0.23	2.47[ab]	0.41	1.76[b]	0.29	P<0.05*
pH	7.47[a]	0.06	6.87[b]	0.09	6.24[c]	0.12	P<0.001***
Conductivity (μS cm^{-1})	139.7[a]	0.07	53.8[b]	0.08	41.7[c]	0.05	P<0.001***
Alkalinity (mg l^{-1})	55.96[a]	5.49	25.48[b]	8.89	5.58[c]	6.65	P<0.001***
Water Temperature (^oC)	8.5[a]	0.06	11.0[b]	0.12	9.2[a]	0.11	P<0.01**
Flow (m s^{-1})	0.281	0.08	0.196	0.06	0.233	0.05	NS
% Shade	7.6[a]	1.27	34.9[b]	7.98	21.8[b]	2.68	P<0.001***
Height of Riparian Vegetation (m)	<0.04	0.00	<0.04	0.00	<0.04	0.00	NS
NH_3-N (mg l^{-1})	<0.01		<0.01		<0.01		NS
NO_3-N (mg l^{-1})	<0.04		<0.04		<0.04		NS
PO_4-P (mg l^{-1})	<0.003		<0.003		<0.003		NS
Cl (mg l^{-1})	12.86[a]	0.25	8.38[b]	0.30	8.60[b]	0.37	P<0.01**
SO_4 (mg l^{-1})	0.17[a]	0.03	1.18[b]	0.17	2.19[c]	0.28	P<0.001***
Cd (μg l^{-1})	0.02	0.00	0.02	0.09	0.03	0.10	NS
Cr (μg l^{-1})	0.13[a]	0.02	0.19[ab]	0.23	0.23[b]	0.16	P<0.05*
Cu (μg l^{-1})	0.17[a]	0.04	0.25[ab]	0.03	0.30[b]	0.02	P<0.05*
Pb (μg l^{-1})	0.06[a]	0.09	0.15[b]	0.17	0.48[c]	0.12	P<0.001***
Ni (μg l^{-1})	0.25	0.12	0.30	0.14	0.27	0.10	NS
Zn (μg l^{-1})	1.00[a]	0.10	1.79[b]	0.10	2.96[c]	0.09	P<0.001***
Al (μg l^{-1})	45.6[a]	5.65	73.7[b]	8.54	117.1[c]	8.76	P<0.001***
V (μg l^{-1})	0.15[a]	0.08	0.25[ab]	0.13	0.33[b]	0.09	P<0.05*
As (μg l^{-1})	0.59	0.00	0.47	0.06	0.50	0.05	NS
Na (mg l^{-1})	5.73[a]	0.15	4.94[b]	0.21	4.94[b]	0.20	P<0.01**

K (mg l^{-1})	0.53	0.11	0.64	0.13	0.43	0.05	NS
Ca (mg l^{-1})	11.19^{a}	0.08	3.87^{b}	0.09	2.02^{c}	0.08	P<0.001***
Mg (mg l^{-1})	6.87^{a}	0.09	1.46^{b}	0.08	0.78^{c}	0.06	P<0.001***
Fe (mg l^{-1})	0.16^{a}	0.08	0.24^{ab}	0.15	0.29^{b}	0.08	P<0.05*
Mn (mg l^{-1})	0.006^{a}	0.07	0.012^{ab}	0.14	0.016^{b}	0.10	P<0.05*

Table 4.62 Mean values (± 1 standard error) of normally distributed periphyton species richness (per 410.76 cm^2), periphyton species diversity (per 410.76 cm^2), periphyton species dominance (per 410.76 cm^2), and environmental habitat variables (data back-transformed where necessary) between TWINSPAN sample-groups I (n = 39), II (n = 21), and III (n = 33) of all artificial substrates sampled on survey dates only (n = 93): short-term linoleum, long-term linoleum, long-term Astroturf and plastic aquarium plants. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values are quoted for non-normal variables compared using Kruskal-Wallis test.

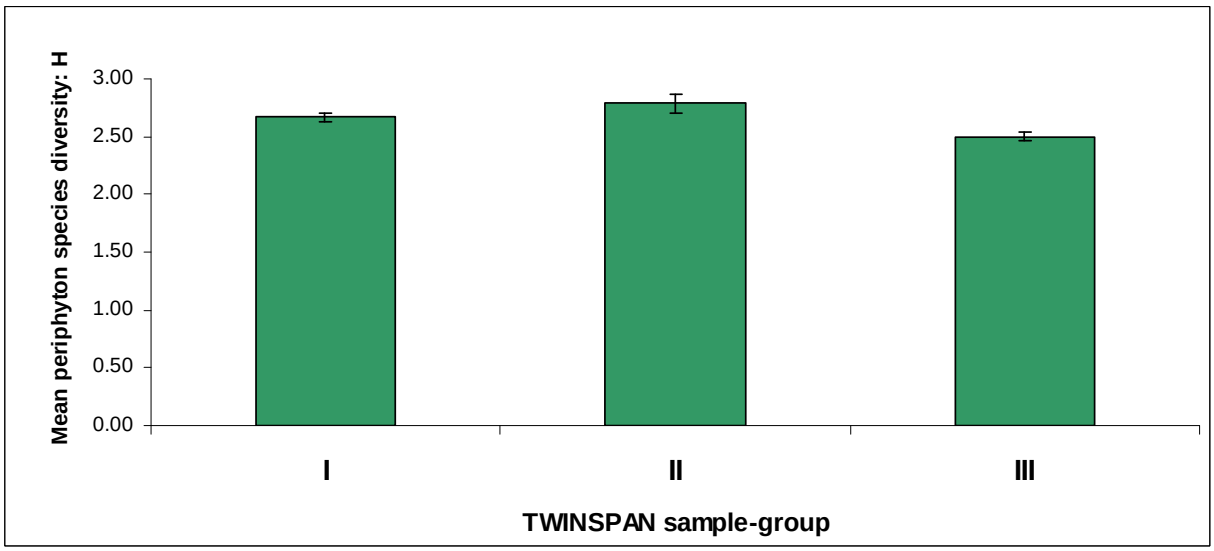
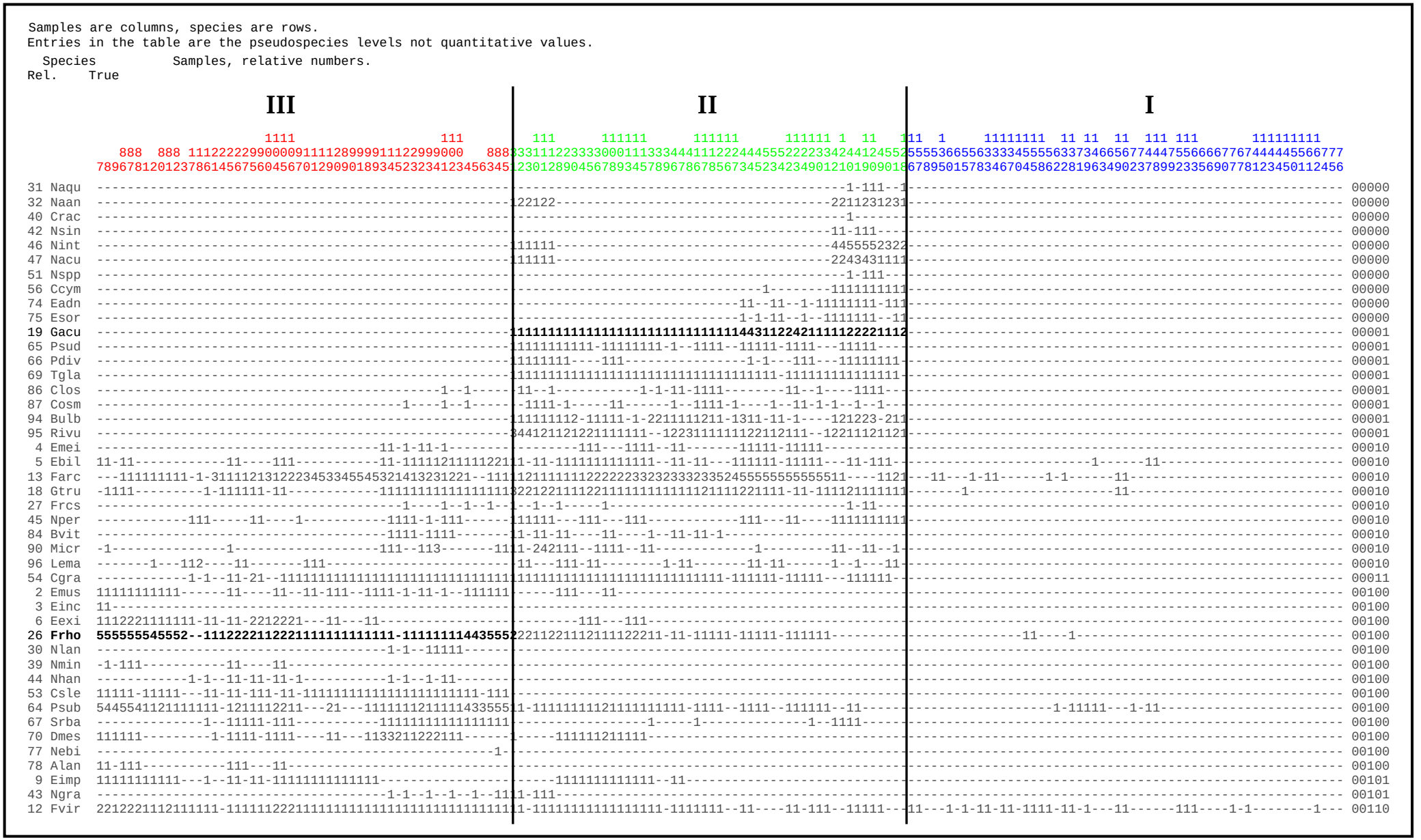


Figure 4.18 Comparison of mean values (± 1 standard error) of normally distributed periphyton species diversity per 410.76 cm^2 (data back-transformed where necessary) between TWINSPAN sample-groups I (n = 39), II (n = 21), and III (n = 33) of all artificial substrates sampled on survey dates only (n = 93): short-term linoleum, long-term linoleum, long-term Astroturf and plastic aquarium plants.


Samples are columns, species are rows.
Entries in the table are the pseudospecies levels not quantitative values.
Species Samples, relative numbers.
Rel. True
III
II
I
31 Naqu
32 Naan
40 Crac
42 Nsin
46 Nint
47 Nacu
51 Nspp
56 Ccym
74 Eadn
75 Esor
19 Gacu
65 Psud
66 Pdiv
69 Tgla
86 Clos
87 Cosm
94 Bulb
95 Rivu
4 Emei
5 Ebil
13 Farc
18 Gtru
27 Frcs
45 Nper
84 Bvit
90 Micr
96 Lema
54 Cgra
2 Emus
3 Einc
6 Eexi
26 Frho
30 Nlan
39 Nmin
44 Nhan
53 Csle
64 Psub
67 Srba
70 Dmes
77 Nebi
78 Alan
9 Eimp
43 Ngra
12 Fvir
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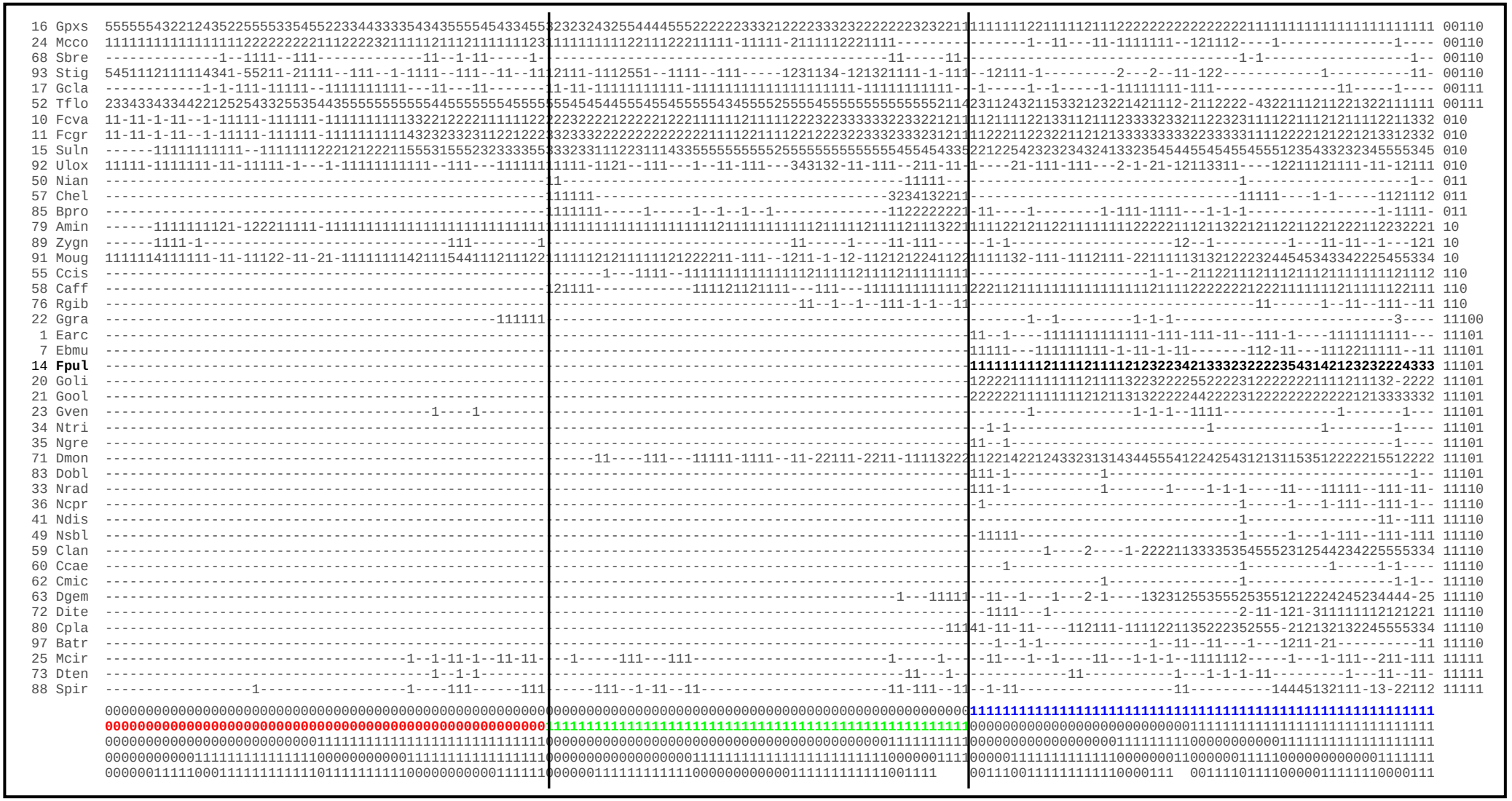


Figure 4.19 TWINSPAN output depicting 163 samples and 3 periphyton species assemblages, with indicator species highlighted in bold font and colour-coding as appropriate for TWINSPAN sample-groups I (blue), II (green), and III (red). For periphyton species codes refer to Figure 4.21.

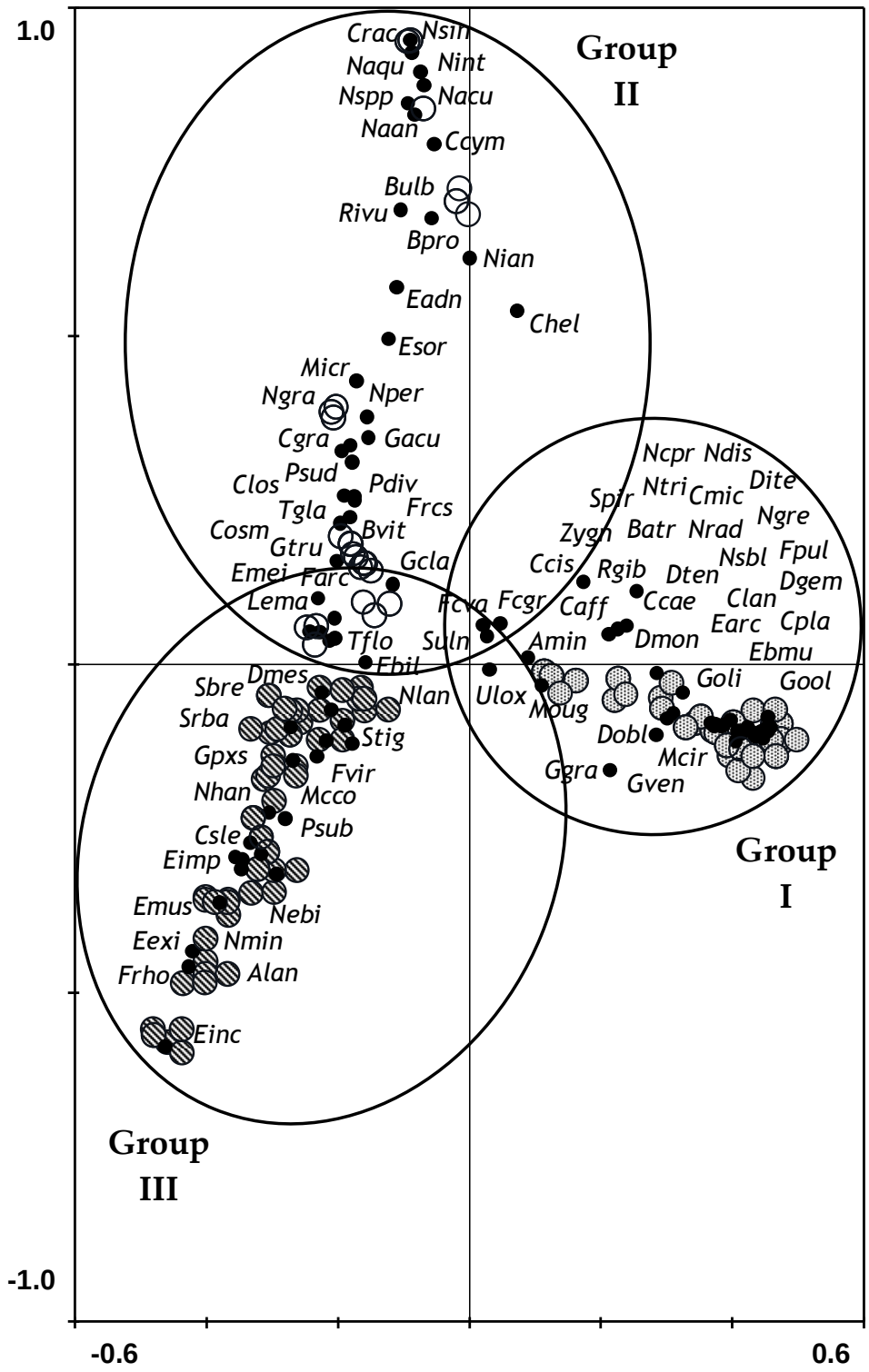


Figure 4.20 CCA ordination of 97 periphyton species and 163 samples, with TWINSPAN sample-group boundaries overlaid. TWINSPAN sample-group identifiers as follows: Group I (n=57: UKAPP06MIN, UKAPG06MIN, UKAPR06MIN, UKSMP06MIN, UKSMG06MIN, UKNVP06MIN, UKNVG06MIN, MKAPP06MIN, MKAPG06MIN, MKAPR06MIN, MKSMP06MIN, MKSMG06MIN, MKSMR06MIN, MKNVP06MIN, MKNVG06MIN, MKNVR06MIN, LKAPP06MIN, LKAPG06MIN, LKAPR06MIN, LKSMP06MIN, LKSMG06MIN, LKSMR06MIN, LKNVP06MIN, LKNVG06MIN, LKNVR06MIN, UKAPP06BRY, UKAPG06BRY, UKAPR06BRY, UKSMP06BRY, UKNVP06BRY,

UKNVG06BRY, MKAPP06BRY, MKAPG06BRY, MKAPR06BRY, MKSMP06BRY, MKSMG06BRY, MKSMR06BRY, MKNVG06BRY, MKNVR06BRY, LKAPP06BRY, LKAPG06BRY, LKAPR06BRY, LKSMP06BRY, LKSMG06BRY, LKSMR06BRY, LKNVP06BRY, LKNVG06BRY, UKAPP06VSM, UKAPG06VSM, UKSMP06VSM, UKSMG06VSM, UKNVG06VSM, LKAPP06VSM, LKAPG06VSM, LKSMG06VSM, LKNVP06VSM, LKNVG06VSM): dotted circles ; Group II (n=52: IBMYP05MIN, IBMYG05MIN, IBMYR05MIN, IBAUP05MIN, IBAUG05MIN, IBAUR05MIN, IBAPP06MIN, IBAPG06MIN, IBAPR06MIN, HBMYP05MIN, HBMYG05MIN, HBMYR05MIN, HBAUP05MIN, HBAUG05MIN, HBAUR05MIN, HBAPP06MIN, HBAPG06MIN, HBAPR06MIN, LMMYP05MIN, LMMYG05MIN, LMMYR05MIN, LMAUP05MIN, LMAUG05MIN, LMAUR05MIN, LMAPP06MIN, LMAPG06MIN, LMAPR06MIN, IBMYP05BRY, IBMYG05BRY, IBMYR05BRY, IBAUP05BRY, IBAUG05BRY, IBAUR05BRY, IBAPP06BRY, IBAPG06BRY, IBAPR06BRY, HBMYP05BRY, HBMYG05BRY, HBMYR05BRY, HBAUP05BRY, HBAUG05BRY, HBAUR05BRY, HBAPP06BRY, HBAPG06BRY, HBAPR06BRY, LMMYP05BRY, LMMYG05BRY, LMMYR05BRY, LMAUR05BRY, LMAPP06BRY, LMAPG06BRY, LMAPR06BRY): open circles ; Group III (n=54: BBMYP05MIN, BBMYG05MIN, BBMYR05MIN, BBAUP05MIN, BBAUG05MIN, BBAUR05MIN, BBAPP06MIN, BBAPG06MIN, BBAPR06MIN, CFMYP05MIN, CFMYG05MIN, CFMYR05MIN, CFAUP05MIN, CFAUG05MIN, CFAUR05MIN, CFAPP06MIN, CFAPG06MIN, CFAPR06MIN, BDMYP05MIN, BDMYG05MIN, BDMYR05MIN, BDAUP05MIN, BDAUG05MIN, BDAUR05MIN, BDAPP06MIN, BDAPG06MIN, BDAPR06MIN, BBMYP05BRY, BBMYG05BRY, BBMYR05BRY, BBAUP05BRY, BBAUG05BRY, BBAUR05BRY, BBAPP06BRY, BBAPG06BRY, BBAPR06BRY, CFMYP05BRY, CFMYG05BRY, CFMYR05BRY, CFAUP05BRY, CFAUG05BRY, CFAUR05BRY, CFAPP06BRY, CFAPG06BRY, CFAPR06BRY, BDMYP05BRY, BDMYG05BRY, BDMYR05BRY, BDAUP05BRY, BDAUG05BRY, BDAUR05BRY, BDAPP06BRY, BDAPG06BRY, BDAPR06BRY): diagonally striped circles . For sample site-codes: Water of Dye sites: Brocky Burn (BB), Charr Flume (CF) and Bogendreip (BD); River Girnock sites: Iron Bridge (IB), Hampshire's Bridge (HB) and Littlemill (LM); Knockan Burn sites: Upper Knockan (UK), Mid-Knockan (MK) and Lower Knockan (LK). Each site code is completed using code letters for survey date (AP: April; MY: May; AU: August; SM: September; NV: November), flow regime (P: Pool; G: Glide; R: Riffle), year sampled (05: 2005; 06: 2006) and substrate type (MIN: naturally-occurring mineral substrata; BRY: naturally-occurring aquatic bryophytes; VSM: naturally-occurring vascular submerged macrophytes). Example: BBMYR05MIN = Brocky Burn May Riffle 2005 harvested from naturally-occurring mineral substrata. For periphyton species codes and ordination statistics refer to Figure 4.21.

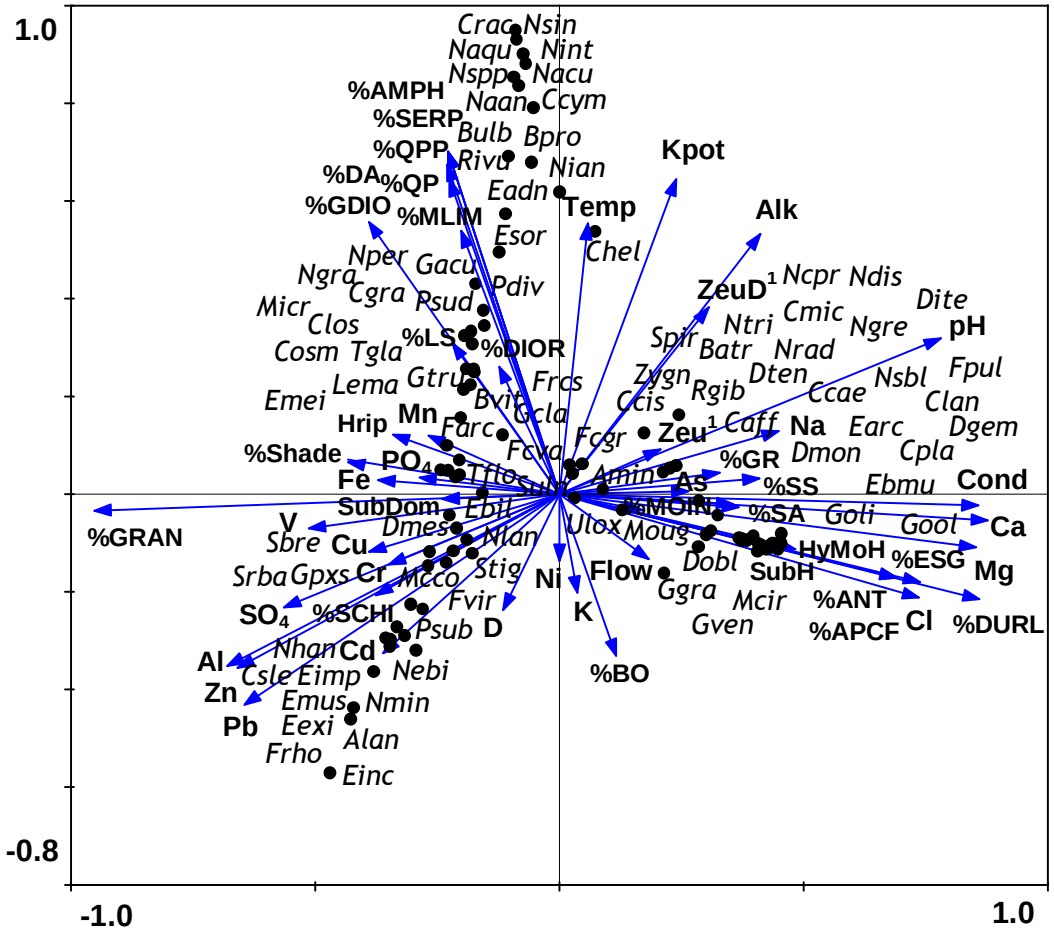


Figure 4.21 CCA ordination of periphyton species and environmental variables. Periphyton species codes: *Eunotia arcus* sensu (Earc), *Eunotia muscicola* var. *tridentula* (Emus), *Eunotia* cf. *incisa* (Einc), *Eunotia meisteri* (Emei), *Eunotia bilunaris* var. *linearis* (Ebil), *Eunotia exigua* (Eexi), *Eunotia bilunaris* var. *mucophila* (Ebmu), *Eunotia serra* (Eser), *Eunotia implicata* (Eimp), *Fragilaria capucina* var. *vaucheriae* (Fcva), *Fragilaria capucina* var. *gracilis* (Fcgr), *Fragilaria virescens* (Fvir), *Fragilaria arcus* (Farc), *Fragilaria pulchella* (Fpul), *Synedra ulna* (Suln),*Gomphonema* cf. *parvulum* var. *exilissimum* (Gpxs), *Gomphonema clavatum* (Gcla), *Gomphonema truncatum* (Gtru), *Gomphonema acuminatum* (Gacu), *Gomphonema olivaceum* (Goli), *Gomphonema olivaceum* var. *olivaceoides* (Gool), *Gomphonema gracile* (Ggra), *Gomphonema ventricosum* (Gven), *Meridion circulare* var. *constrictum* (Mcco), *Meridion circulare* (Mcir), *Frustulia rhomboides* var. *rhomboides* (Frho), *Frustulia rhomboides* var. *crassinervia* (Frcs), *Frustulia vulgaris* (Fvul), *Navicula rhynchocephala* (Nrhy), *Navicula lanceolata* (Nlan), *Navicula* cf. *aquaedurae* (Naqu), *Navicula angusta* (Naan), *Navicula radiosa* (Nrad), *Navicula tripunctata* (Ntri), *Navicula* cf. *gregaria* (Ngre), *Navicula capitatoradiata* (Ncpr), *Navicula* cf. *pygmaea* agg. (Npyg), *Navicula jaernefeltii* (Njae), *Navicula minima* (Nmin), *Craticula acidoclinata* (Crac), *Nitzschia dissipata* (Ndis), *Nitzschia sinuata* (Nsin), *Nitzschia gracilis* (*Ngra*), *Nitzschia hantzschiana* (Nhan), *Nitzschia perminuta* agg. (Nper), *Nitzschia intermedia* agg. (Nint), *Nitzschia* cf. *acula* (Nacu), *Nitzschia palea* agg. (Npal), *Nitzschia sublinearis* (Nsbl), *Nitzschia angustata* (Nian), *Nitzschia* undefined sp. (Nspp), *Tabellaria flocculosa* (Tflo),

Cymbella silesiaca **(Csle),** ***Cymbella gracilis*** **(Cgra),** ***Cymbella cistula*** **(Ccis),** ***Cymbella cymbiformis*** **(Ccym),** ***Cymbella helvetica*** **(Chel),** ***Cymbella affinis*** **(Caff),** ***Cymbella lanceolata*** **(Clan),** ***Cymbella caespitosa*** **(Ccae),** ***Cymbella naviculiformis*** **(Cnav),** ***Cymbella microcephala*** **(Cmic),** ***Didymosphenia geminata*** **(Dgem),** ***Pinnularia subcapitata*** **(Psub),** ***Pinnularia*** **cf.** ***sudetica*** **(Psud),** ***Pinnularia*** **cf.** ***divergens*** **(Pdiv),** ***Surirella roba*** **(Srba),** ***Surirella brebissonii*** **(Sbre),** ***Tetracyclus glans*** **(Tgla),** ***Diatoma mesodon*** **(Dmes),** ***Diatoma moniliformis*** **(Dmon),** ***Diatoma tenuis*** **(Dite),** ***Denticula tenuis*** **(Dten),** ***Epithemia adnata*** **(Eadn),** ***Epithemia sorex*** **(Esor),** ***Rhopalodia gibba*** **(Rgib),** ***Nedium bisulcatum*** **(Nebi),** ***Achnanthes lanceolata*** **(Alan),** ***Achnanthidium minutissima*** **(Amin),** ***Cocconeis placentula*** **(Cpla),** ***Diploneis*** **cf.** ***elliptica*** **(Dell),** ***Diploneis marginestriata*** **(Dmar),** ***Diploneis oblongella*** **(Dobl),** ***Brachysira vitrea*** **(Bvit),** ***Brachysira procera*** **(Bpro),** ***Closterium*** **sp. (Clos),** ***Cosmarium*** **sp. (Cosm),** ***Spirogyra*** **sp. (Spir),** ***Zygnema*** **sp. (Zygn),** ***Microspora*** **sp. (Micr),** ***Mougeotia*** **sp. (Moug),** ***Ulothrix*** **sp. (Ulox),** ***Stigeoclonium*** **sp. (Stig),** ***Bulbochaete*** **sp. (Bulb),** ***Rivularia*** **sp. (Rivu),** ***Lemanea fluviatilis*** **(Lema),** ***Batrachospermum*** **sp. (Batr). Environmental variables: Underlying geology: Granite (%GRAN), Mica Schist (%SCHI), Granodiorite (%GDIO), Diorite (%DIOR), Quartz/Psammite (%QP), Quartz/Psammite/Pelite (%QPP), Diorite/Amphibolite (%DA), Amphibolite (%AMPH), Serpentinite (%SERP), Metamorphic Limestone (%MLIM), Durness Limestone (%DURL), Moine Schist (%MOIN), Eriboll Sandstone Group (%ESG), Applecross Formation (%APCF) and An-T'sron (%ANT). Substrate morphology: substrate particle diversity (SubH), substrate particle dominance (SubDom), hydromorphological diversity (HyMoH), streambed cover of Boulders (%BO), Large Stones (%LS), Small Stones (%SS), Gravel (%GR), and Sand (%SA). Water physico-chemistry: benthic depth (D: m), light attenuation coefficient (K: m^{-1}), euphotic depth 1% (Zeu^{1}: m^{-1}), Zeu:D^{1} ratio, pH, alkalinity (Alk: mg/l), conductivity (Cond: µS cm^{-1}), water temperature (Temp: ^{o}C), current velocity (Flow: m s^{-1}), % Shade and height of riparian vegetation (Hrip). Water Chemistry: Phosphate (PO_4-P), Chloride (Cl), Sulphate (SO_4), Cadmium (Cd), Chromium (Cr), Copper (Cu), Lead (Pb), Nickel (Ni), Zinc (Zn), Aluminium (Al), Vanadium (V), Arsenic (As), Sodium (Na), Potassium (Kpot), Calcium (Ca), Magnesium (Mg), Iron (Fe), and Manganese (Mn). Monte Carlo significance test: Axis 1: P<0.005; all canonical axes: P<0.005. Eigenvalues: Axis 1: 0.535; Axis 2: 0.319.**

	TWINSPAN sample-group						
Variable	**I**		**II**		**III**		**PANOVA**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Periphyton species richness: **S**	23.2[a]	0.60	30.2[b]	0.81	21.1[a]	0.70	P<0.001***
Periphyton species diversity: **H**	2.73[a]	0.02	2.80[a]	0.03	2.38[b]	0.03	P<0.001***
Periphyton species dominance	0.15[a]	0.01	0.16[a]	0.01	0.24[b]	0.01	P<0.001***
D (m)	0.15	0.09	0.14	0.07	0.16	0.08	NS
K (m^{-1})	2.68[a]	0.14	2.43[a]	0.15	2.97[b]	0.15	P<0.01**
$Z_{eu}^{1\%}$ (m)	0.36[a]	0.14	0.27[b]	0.16	0.26[b]	0.15	P<0.01**
Z_{eu}:$D^{1\%}$	3.12[a]	0.15	2.36[b]	0.18	1.95[c]	0.16	P<0.001***
pH	7.58[a]	0.04	6.91[b]	0.07	6.34[c]	0.11	P<0.001***
Conductivity (µS cm^{-1})	139.8[a]	0.06	50.6[b]	0.05	47.0[c]	0.04	P<0.001***
Alkalinity (mg l^{-1})	56.14[a]	4.48	25.51[b]	8.72	5.63[c]	5.52	P<0.001***
Water Temperature (^oC)	8.6[a]	0.05	10.8[b]	0.10	8.9[a]	0.08	P<0.01**
Flow (m s^{-1})	0.270	0.03	0.213	0.02	0.228	0.02	NS
% Shade	7.1[a]	0.92	28.6[b]	2.47	24.9[b]	1.17	P<0.001***
Height of Riparian Vegetation (m)	0.15[a]	0.05	3.39[b]	0.47	2.69[b]	0.38	P<0.001***
Substrate diversity (Simpson's D)	3.62[a]	0.11	3.11[b]	0.07	3.00[b]	0.09	P<0.01**
Substrate dominance (Berger-Parker)	0.39[a]	0.02	0.45[b]	0.02	0.45[b]	0.02	P<0.01**
Hydromorphological diversity (D)	3.71[a]	0.11	3.22[b]	0.07	3.11[b]	0.09	P<0.01**
% Granite	0.0[a]	0.00	62.2[b]	1.95	84.9[c]	1.29	P<0.001***
% Granodiorite	0.0[a]	0.00	9.0[b]	0.42	0.0[a]	0.00	P<0.001***
% Diorite	0.0[a]	0.00	0.3[b]	0.05	0.0[a]	0.00	P<0.001***
% Mica Schist	0.0[a]	0.00	0.0[a]	0.00	11.5[b]	0.98	P<0.001***
% Amphibolite	0.0[a]	0.00	7.7[b]	0.62	0.0[a]	0.00	P<0.001***
% Serpentinite	0.0[a]	0.00	1.0[b]	0.09	0.0[a]	0.00	P<0.001***
% QP	0.0[a]	0.00	0.7[b]	0.06	0.0[a]	0.00	P<0.001***
% DA	0.0[a]	0.00	2.3[b]	0.20	0.0[a]	0.00	P<0.001***
% QPP	0.0[a]	0.00	9.5[b]	0.78	0.0[a]	0.00	P<0.001***
% Limestone	0.0[a]	0.00	7.4[b]	0.72	0.0[a]	0.00	P<0.001***
% Durness Limestone	73.3[a]	2.62	0.0[b]	0.00	0.0[b]	0.00	P<0.001***
% Eriboll Sandstone	10.0[a]	1.29	0.0[b]	0.00	0.0[b]	0.00	P<0.001***
% Moine Schist	6.7[a]	1.24	0.0[b]	0.00	0.0[b]	0.00	P<0.001***
% Applecross Formation	6.7[a]	1.05	0.0[b]	0.00	0.0[b]	0.00	P<0.001***

% An-t'Sron	3.3[a]	0.53	0.0[b]	0.00	0.0[b]	0.00	P<0.001***
% Boulders	13.9[a]	2.34	21.8[a]	2.17	40.9[b]	2.82	P<0.001***
% Large Stones	26.8[a]	2.23	41.5[b]	2.22	25.3[a]	2.19	P<0.001***
% Small Stones	30.0[a]	2.39	21.1[b]	1.92	14.4[c]	1.63	P<0.001***
% Gravel	23.9[a]	2.41	10.9[b]	1.53	14.9[b]	2.00	P<0.01**
% Sand	6.1[a]	1.42	0.0[b]	0.00	0.0[b]	0.00	P<0.01**
NH_3-N (mg l^{-1})	<0.04		<0.04		<0.04		NS
NO_3-N (mg l^{-1})	<0.01		<0.01		<0.01		NS
PO_4-P (mg l^{-1})	<0.003		<0.003		<0.003		NS
Cl (mg l^{-1})	12.84[a]	0.53	8.03[b]	0.51	9.00[b]	0.71	P<0.01**
SO_4 (mg l^{-1})	0.15[a]	0.06	1.11[b]	0.27	2.49[c]	0.54	P<0.001***
Cd (µg l^{-1})	0.02	0.01	0.02	0.02	0.03	0.02	NS
Cr (µg l^{-1})	0.13[a]	0.05	0.19[ab]	0.21	0.23[b]	0.15	P<0.05*
Cu (µg l^{-1})	0.17[a]	0.08	0.25[ab]	0.05	0.31[b]	0.05	P<0.05*
Pb (µg l^{-1})	0.06[a]	0.19	0.17[b]	0.26	0.53[c]	0.23	P<0.001***
Ni (µg l^{-1})	0.24	0.26	0.27	0.23	0.28	0.18	NS
Zn (µg l^{-1})	1.00[a]	0.20	1.84[b]	0.15	3.38[c]	0.17	P<0.001***
Al (µg l^{-1})	48.6[a]	11.63	82.5[b]	12.39	129.7[c]	18.24	P<0.001***
V (µg l^{-1})	0.15[a]	0.17	0.24[ab]	0.23	0.36[b]	0.13	P<0.05*
As (µg l^{-1})	0.59	0.00	0.37	0.23	0.49	0.14	NS
Na (mg l^{-1})	5.71[a]	0.33	4.85[b]	0.36	5.04[b]	0.37	P<0.01**
K (mg l^{-1})	0.53	0.14	0.58	0.13	0.42	0.07	NS
Ca (mg l^{-1})	10.74[a]	0.56	3.28[b]	0.55	2.06[c]	0.57	P<0.001***
Mg (mg l^{-1})	6.56[a]	0.18	1.28[b]	0.17	0.82[c]	0.13	P<0.001***
Fe (mg l^{-1})	0.16[a]	0.17	0.24[ab]	0.22	0.29[b]	0.18	P<0.05*
Mn (mg l^{-1})	0.006[a]	0.003	0.012[ab]	0.002	0.016[b]	0.001	P<0.05*

Table 4.63 Mean values (± 1 standard error) of normally distributed periphyton species richness (per 141.52 cm^2), periphyton species diversity (per 141.52 cm^2), periphyton species dominance (per 141.52 cm^2), and environmental habitat variables (data back-transformed where necessary) between TWINSPAN sample-groups I (n = 57), II (n = 52), and III (n = 54) of all naturally-occurring substrata sampled on survey dates only (n = 163): mineral substrata, aquatic bryophytes and vascular submerged macrophytes. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values are quoted for non-normal variables compared using Kruskal-Wallis test.

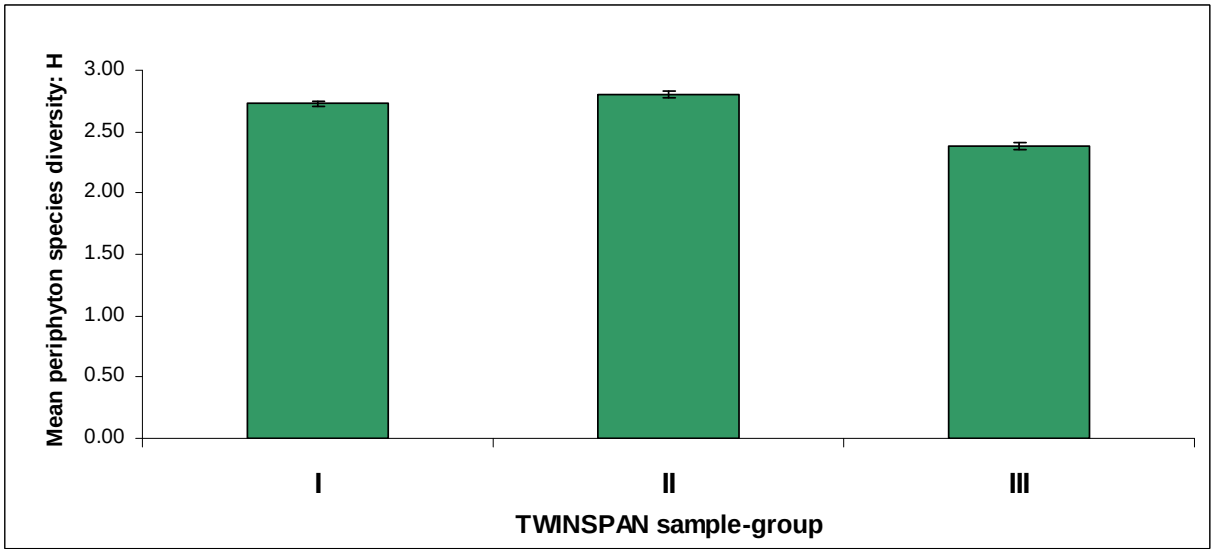


Figure 4.22 Comparison of mean values (± 1 standard error) of normally distributed periphyton species diversity per 141.52 cm^2 (data back-transformed where necessary) between TWINSPAN sample-groups I (n = 57), II (n = 52), and III (n = 54) of all naturally-occurring substrata sampled on survey dates only (n = 163): mineral particles, aquatic bryophytes and vascular submerged macrophytes.

4.5.7 Relationships between periphyton community composition, diversity and environmental habitat conditions

4.5.7.1 Periphyton community composition and diversity of short-term linoleum substrates only

Periphyton species richness and diversity were strongly and significantly positively correlated with each other from material harvested from short-term linoleum substrates (see Appendix 2h). Periphyton species richness and diversity were also positively correlated with increasing underwater light availability, streamwater pH, conductivity and temperature, as well as periphyton biomass and chlorophyll content. Periphyton species richness and diversity were negatively correlated to increasing streamwater depth and current velocity. Periphyton species dominance exhibited inverse relationships of periphyton species richness and diversity, to both of which it was negatively correlated.

4.5.7.2 Periphyton community composition and diversity of all artificial substrata

Agreeing with the former, periphyton species richness, diversity and dominance harvested from all artificial substrata (short-term linoleum, long-term linoleum, long-term Astroturf and plastic aquarium plants) during field survey campaigns (see Appendix 2i) showed similar relationships. Additionally, periphyton species richness and diversity were positively correlated to increasing streamwater concentrations of base cations (e.g. potassium, calcium and magnesium) and tended to be negatively correlated to increased concentrations of streamwater sulphate and heavy metals (e.g. lead, zinc, aluminium). Again periphyton species dominance showed the inverse of these relationships.

4.5.7.3 Periphyton community composition and diversity of all naturally-occurring substrata

Concurring with the aforementioned artificial substrates, similar relationships were found for periphyton species richness, diversity and dominance recorded from all naturally-occurring substrata (mineral particles, aquatic bryophytes, and where present vascular submerged macrophytes): see Appendix 2j.

Periphyton species richness and diversity exhibited a negative relationship with base-poor strata (e.g. granite) and tended to be positively correlated with underlying geologies possessing base-rich properties (e.g. serpentinite, Durness limestone etc.). Periphyton species richness and diversity had a positive relationship with increasing periphyton cover and were negatively correlated to increasing bare area. Periphyton species dominance showed the inverse of these relationships.

4.5.8 Predicting freshwater periphyton community composition and diversity

Data harvested from short-term linoleum substrate samplers were used to construct statistically significant full models using combinations of environmental predictor variables for predicting periphyton species diversity (H) of upland stream habitats (refer to Table 4.64). Model PERIsH1a was chosen to derive minimal models (see Table 4.65, Table 4.67, and Table 4.69) because it had a high predictive power (r^2: 49.5%) and strongly predicted temporal variation of periphyton species diversity in all months sampled, for test data sets of the Water of Dye (Table 4.66, Figure 3.44), River Girnock (Table 4.68, Figure 3.45), and Knockan Burn (Table 4.70, Figure 3.46).

Full models	Regression equations	r^2-adj (%)	P_{value}
PERIsH1a: Periphyton Species Diversity (H)	H = 1.24 + 0.183 (log_e Cond) + 0.235 (√ temp)	49.5	P<0.001***
PERIsH2a: Periphyton Species Diversity (H)	H = 2.00 + 0.129 (log_e ZeuD[1]) + 0.201 (√ temp)	38.8	P<0.001***
PERIsH3a: Periphyton Species Diversity (H)	H = 1.06 + 0.150 (pH) + 0.209 (√ temp)	37.6	P<0.001***
PERIsH4a: Periphyton Species Diversity (H)	H = 1.23 - 0.176 (log_e D) + 0.163 (pH)	35.2	P<0.001***

Table 4.64 Statistically significant full models (n = 50) for predicting temporal variation of freshwater periphyton species diversity (measured as H per area sampled: 400 cm^2) of upland stream habitats. Model codes: H: species diversity (per 400 cm^2); log_e cond: log_e streamwater conductivity (µg cm^{-1}); pH: streamwater pH; log_e D: log_e benthic depth (m); log_e ZeuD[1]: loge ratio of 1% euphotic depth to benthic depth (ZeuD[1]); √ temp: √ water temperature (°C).

Reduced (minimal) models	Regression equations	r^2-adj (%)	P_{value}
PERIsH1a: Periphyton Species Diversity (H) excluding Water of Dye November 2005 test data set	H = 1.27 + 0.178 (log_e Cond) + 0.232 (√ temp)	47.6	P<0.001***
PERIsH1a: Periphyton Species Diversity (H) excluding Water of Dye March 2005 test data set	H = 1.23 + 0.185 (log_e Cond) + 0.236 (√ temp)	47.2	P<0.001***
PERIsH1a: Periphyton Species Diversity (H) excluding Water of Dye April 2005 test data set	H = 1.18 + 0.195 (log_e Cond) + 0.267 (√ temp)	53.7	P<0.001***
PERIsH1a: Periphyton Species Diversity (H) excluding Water of Dye May 2005 test data set	H = 1.26 + 0.140 (log_e Cond) + 0.230 (√ temp)	50.1	P<0.001***
PERIsH1a: Periphyton Species Diversity (H) excluding Water of Dye July 2005 test data set	H = 1.25 + 0.184 (log_e Cond) + 0.232 (√ temp)	48.2	P<0.001***
PERIsH1a: Periphyton Species Diversity (H) excluding Water of Dye August 2005 test data set	H = 1.16 + 0.159 (log_e Cond) + 0.305 (√ temp)	58.9	P<0.001***
PERIsH1a: Periphyton Species Diversity (H) excluding Water of Dye April 2006 test data set	H = 1.17 + 0.191 (log_e Cond) + 0.245 (√ temp)	48.5	P<0.001***

Table 4.65 Statistically significant minimal models (n = 47) of PERIsH1a for predicting temporal variation of freshwater periphyton species diversity (H) of the Water of Dye data set. For model codes refer to Table 4.64.

Mean test data	Observed H: test data	Predicted H: reduced model PERIsH1a	t-statistic	P-value
Water of Dye November 2005	2.46	2.51	-0.39	NS
Water of Dye March 2005	2.44	2.42	0.10	NS
Water of Dye April 2005	2.68	2.49	2.25	NS
Water of Dye May 2005	2.15	2.42	-1.54	NS
Water of Dye July 2005	2.89	2.87	0.15	NS
Water of Dye August 2005	2.57	2.91	-3.06	NS
Water of Dye April 2006	2.51	2.40	1.53	NS
Water of Dye Mean H	**2.53**	**2.58**	**-0.65**	**NS**

Table 4.66 Comparison of mean observed and predicted values of minimal model PERIsH1a for predicting temporal variation in freshwater periphyton species diversity (H) of the Water of Dye test data set (see also Figure 4.23).

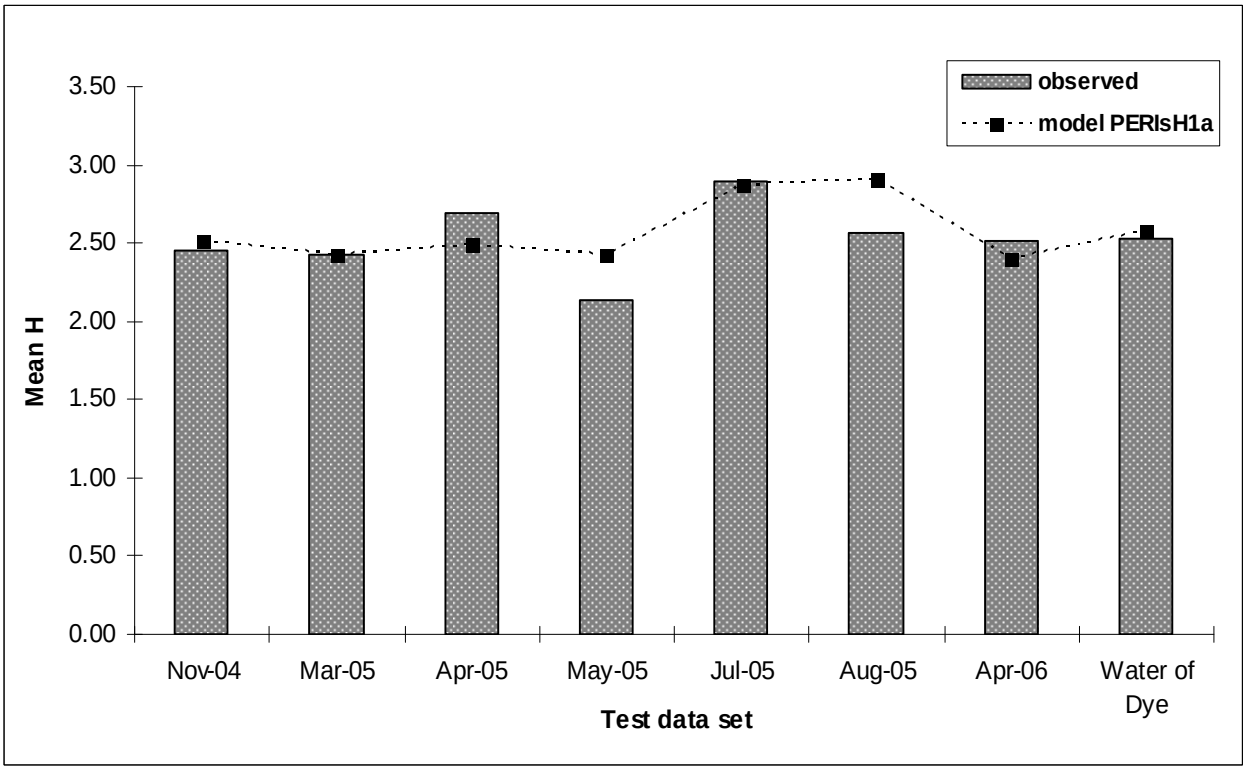


Figure 4.23 Comparison of mean observed and predicted values of minimal model PERIsH1a for predicting temporal variation in freshwater periphyton species diversity (H) of the Water of Dye test data set.

Reduced (minimal) models	Regression equations	r^2-adj (%)	P_{value}
PERIsH1a: Periphyton Species Diversity (H) excluding River Girnock April 2005 test data set	H = 1.19 + 0.194 ($\log_e$ Cond) + 0.235 (√ temp)	51.2	P<0.001***
PERIsH1a: Periphyton Species Diversity (H) excluding River Girnock May 2005 test data set	H = 1.24 + 0.176 ($\log_e$ Cond) + 0.248 (√ temp)	51.9	P<0.001***
PERIsH1a: Periphyton Species Diversity (H) excluding River Girnock July 2005 test data set	H = 1.28 + 0.195 ($\log_e$ Cond) + 0.196 (√ temp)	48.6	P<0.001***
PERIsH1a: Periphyton Species Diversity (H) excluding River Girnock August 2005 test data set	H = 1.31 + 0.187 ($\log_e$ Cond) + 0.198 (√ temp)	45.9	P<0.001***
PERIsH1a: Periphyton Species Diversity (H) excluding River Girnock April 2006 test data set	H = 1.20 + 0.196 ($\log_e$ Cond) + 0.232 (√ temp)	49.1	P<0.001***

Table 4.67 Statistically significant minimal models (n = 47) of PERIsH1a for predicting temporal variation of freshwater periphyton species diversity (H) of the River Girnock data set. For model codes refer to Table 4.64.

Mean test data	Observed H: test data	Predicted H: reduced model PERIsH1a	t-statistic	P-value
River Girnock April 2005	2.86	2.81	3.31	NS
River Girnock May 2005	2.62	2.86	-1.69	NS
River Girnock July 2005	3.23	2.93	3.18	NS
River Girnock August 2005	3.19	2.98	3.33	NS
River Girnock April 2006	2.54	2.66	-0.86	NS
River Girnock Mean H	**2.89**	**2.85**	**0.43**	**NS**

Table 4.68 Comparison of mean observed and predicted values of minimal model PERIsH1a for predicting temporal variation in freshwater periphyton species diversity (H) of the River Girnock test data set (see also Figure 4.24).

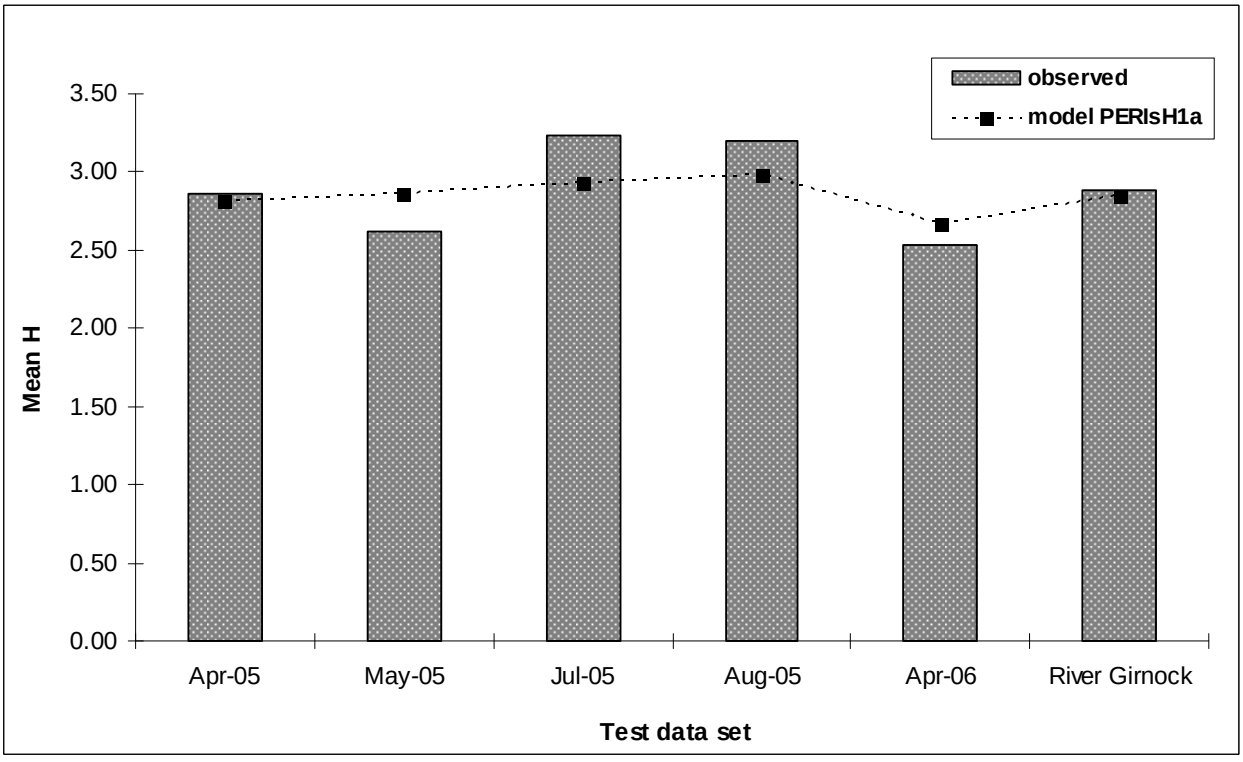


Figure 4.24 Comparison of mean observed and predicted values of minimal model PERIsH1a for predicting temporal variation in freshwater periphyton species diversity (H) of the River Girnock test data set.

Reduced (minimal) models	Regression equations	r^2-adj (%)	P_{value}
PERIsH1a: Periphyton Species Diversity (H) excluding Knockan Burn December 2005 test data set	H = 1.16 + 0.227 ($\log_e$ Cond) + 0.205 (√ temp)	50.8	P<0.001***
PERIsH1a: Periphyton Species Diversity (H) excluding Knockan Burn April 2006 test data set	H = 1.27 + 0.161 ($\log_e$ Cond) + 0.251 (√ temp)	49.7	P<0.001***
PERIsH1a: Periphyton Species Diversity (H) excluding Knockan Burn July 2006 test data set	H = 1.31 + 0.169 ($\log_e$ Cond) + 0.232 (√ temp)	41.8	P<0.001***
PERIsH1a: Periphyton Species Diversity (H) excluding Knockan Burn September 2006 test data set	H = 1.23 + 0.185 ($\log_e$ Cond) + 0.239 (√ temp)	48.2	P<0.001***
PERIsH1a: Periphyton Species Diversity (H) excluding Knockan Burn November 2006 test data set	H = 1.22 + 0.200 ($\log_e$ Cond) + 0.224 (√ temp)	51.8	P<0.001***

Table 4.69 Statistically significant minimal models (n = 47) of PERIsH1a for predicting temporal variation of freshwater periphyton species diversity (H) of the Knockan Burn data set. For model codes refer to Table 4.64.

Mean test data	Observed H: test data	Predicted H: reduced model PERIsH1a	t-statistic	P-value
Knockan Burn December 2005	2.59	2.83	-3.13	NS
Knockan Burn April 2006	2.85	2.67	1.39	NS
Knockan Burn July 2006	3.11	3.08	0.41	NS
Knockan Burn September 2006	2.89	2.95	-0.49	NS
Knockan Burn November 2006	2.47	2.76	-2.75	NS
Knockan Burn Mean H	**2.78**	**2.86**	**-0.76**	**NS**

Table 4.70 Comparison of mean observed and predicted values of minimal model PERIsH1a for predicting temporal variation in freshwater periphyton species diversity (H) of the Knockan Burn test data set (see also Figure 4.25).

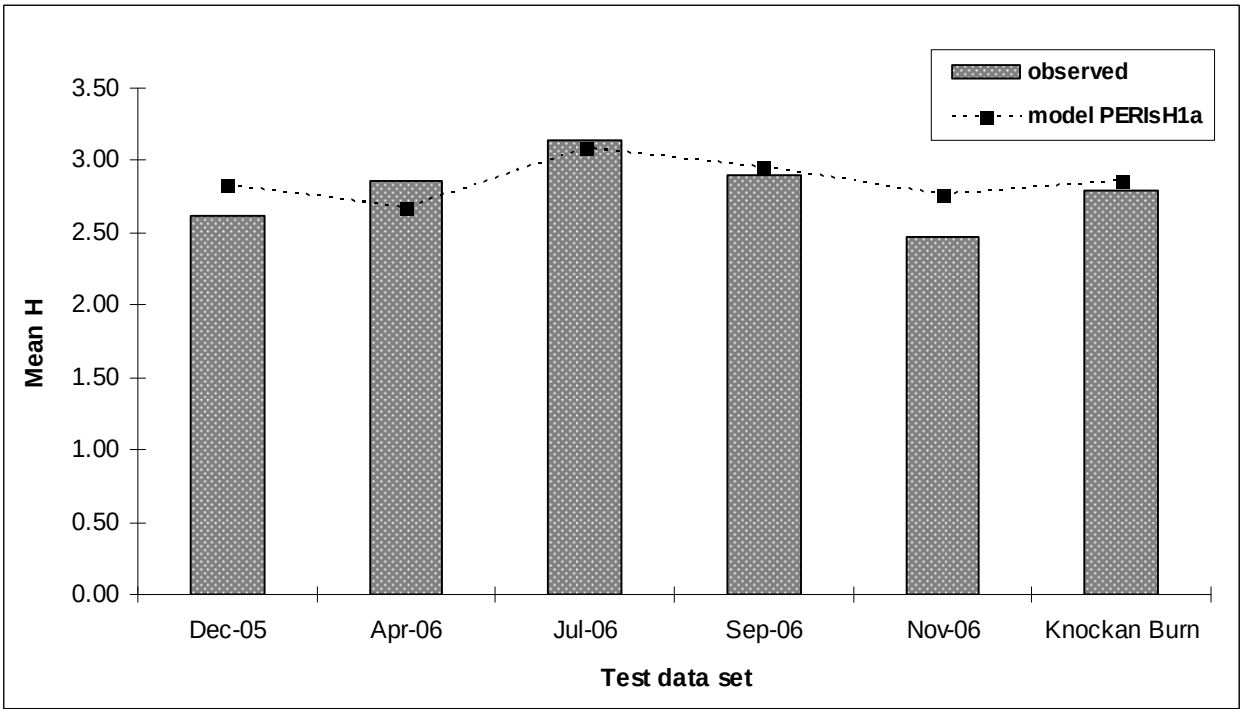


Figure 4.25 Comparison of mean observed and predicted values of minimal model PERIsH1a for predicting temporal variation in freshwater periphyton species diversity (H) of the Knockan Burn test data set.

4.5.9 Variation in aquatic bryophyte community composition and diversity in the Water of Dye, River Girnock and Knockan Burn; their sub-catchments and sites

In total seventeen aquatic bryophyte species were identified from 306 core samples including fourteen mosses: *Blindia acuta* (Hedw.) Bruch & Schimp., *Brachythecium plumosum* (Hedw.) Schimp., *Ctenidium molluscum* (Hedw.) Mitt., *Fissidens adianthoides* Hedw., *Fontinalis antipyretica* Hedw., *Hygrohypnum luridum* (Hedw.) Jenn., *Hygrohypnum ochraceum* (Turner ex Wilson) Loeske, *Mnium hornum* Hedw., *Palustriella falcata* (Brid.) Hedenäs, *Platyhypnidium riparioides* (Hedw.) Dixon, *Racomitrium aciculare* (Hedw.) Brid., *Schistidium agassizii* Sull. & Lesq., *Schistidium rivulare* (Brid.) Podp., and *Warnstorfia exannulata* (Schimp.) Loeske; and three liverworts: *Chiloscyphus polyanthus* (L.) Corda, *Pellia epiphylla* (L.) Corda, and *Scapania undulata* (L.) Dumort. Refer also to Table 4.71 for listed aquatic bryophyte flora.

Of the samples analysed for this component of the project, few lacked the presence of aquatic bryophyte vegetation. This indicated that the majority of samples (approximately between 75.6 – 93.7%) contained aquatic bryophytes meaning that a substantial proportion of the streambeds sampled in this project were occupied by aquatic bryophyte vegetation. This makes aquatic bryophytes the second most abundant stream producer after periphyton (which occurred in all habitats).

The Water of Dye was significantly richer and more diverse in terms of aquatic bryophyte species compared to the River Girnock, but did not vary significantly from Knockan Burn (although overall community composition mostly did): Table 4.72. Furthermore, although the overall species structure was different, there was no significant difference in aquatic bryophyte species richness, diversity or dominance between the River Girnock and Knockan Burn.

Aquatic bryophyte species	Synonym(s)	Family
[1]*Blindia acuta* (Hedw.) Bruch & Schimp.	-	Seligeriaceae
[1]*Brachythecium plumosum* (Hedw.) Schimp.	-	Brachytheciaceae
[1]*Ctenidium molluscum* (Hedw.) Mitt.	= *Hypnum molluscum*	Hypnaceae
[1]*Fissidens adianthoides* Hedw.	-	Fissidentaceae
[1]*Fontinalis antipyretica* Hedw.	-	Fontinalaceae
[1]*Hygrohypnum luridum* (Hedw.) Jenn.	= *Hypnum palustre*	Campyliaceae
[1]*Hygrohypnum ochraceum* (Turner ex Wilson) Loeske	= *Hypnum ochraceum*	Campyliaceae
[1]*Mnium hornum* Hedw.	-	Mniaceae
[1]*Palustriella falcata* (Brid.) Hedenäs	= *Cratoneuron commutatum* var. *falcatum*, *Palustriella communtata* var. *falcata*, *Hypnum falcatum*	Helodiaceae
[1]*Platyhypnidium riparioides* (Hedw.) Dixon	= *Rhynchostegium riparioides*, *Eurhynchium riparioides*	Brachytheciaceae
[1]*Racomitrium aciculare* (Hedw.) Brid.	-	Grimmiaceae
[1]*Schistidium agassizii* Sull. & Lesq.	= *Grimmia agassizii*	Grimmiaceae
[1]*Schistidium rivulare* (Brid.) Podp.	= *Grimmia alpicola* var. *rivularis*, *Schistidium alpicola* var. *rivulare*	Grimmiaceae
[1]*Warnstorfia exannulata* (Schimp.) Loeske	= *Drepanocladus exannulatus*, *Hypnum exannulatum*	Campyliaceae
[2]*Chiloscyphus polyanthus* (L.) Corda	= *Chiloscyphus polyanthus* var. *rivularis*	Geocalycaceae
[2]*Pellia epiphylla* (L.) Corda	-	Pelliaceae
[2]*Scapania undulata* (L.) Dumort	= *Scapania dentata*, *Scapania undulata var. aequatiformis*	Scapniaceae

Table 4.71 Aquatic bryophyte species list: [1]Moss (Smith 2004); [2]Liverwort (Paton 1999).

Mean Variable	Water of Dye		River Girnock		Knockan Burn		PANOVA
	Mean	*S.E*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Aquatic bryophyte species richness: **S**	2.96[a]	0.21	2.04[b]	0.23	2.52[ab]	0.34	P<0.05*
Aquatic bryophyte species diversity: **H**	0.89[a]	0.08	0.57[b]	0.10	0.70[ab]	0.12	P<0.05*
Aquatic bryophyte species dominance	0.59	0.04	0.62	0.06	0.56	0.06	NS

Table 4.72 Mean values (± 1 standard error) of normally distributed plant variables (including zero values, and data back-transformed where necessary): aquatic bryophyte species richness (per 19.64 cm^2), aquatic bryophyte species diversity (per 19.64 cm^2), and aquatic bryophyte species dominance (per 19.64 cm^2) between study stream sub-catchments (n = 79 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.73.

	Water of Dye						River Girnock								Knockan Burn				
Mean Variable	**BB**		**CF**		**BD**		**IB**		**HB**		**LM**		**UK**		**MK**		**LK**		**PANOVA**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Aquatic bryophyte species richness: **S**	3.33^{ad}	0.25	2.44^{b}	0.38	3.11^{ab}	0.35	2.89^{ab}	0.35	1.67^{c}	0.29	1.56^{c}	0.41	1.33^{c}	0.22	3.78^{d}	0.60	2.45^{ab}	0.38	P<0.001***
Aquatic bryophyte species diversity: **H**	1.05^{a}	0.10	0.70^{b}	0.18	0.93^{ab}	0.13	0.93^{ab}	0.12	0.36^{c}	0.15	0.43^{c}	0.18	0.17^{d}	0.05	1.11^{a}	0.17	0.82^{ab}	0.12	P<0.001**
Aquatic bryophyte species dominance	0.53^{a}	0.06	0.66^{ab}	0.09	0.58^{a}	0.07	0.51^{a}	0.06	0.82^{b}	0.08	0.54^{a}	0.14	0.84^{b}	0.14	0.37^{a}	0.07	0.48^{a}	0.06	P<0.01**

Table 4.73 Mean values (± 1 standard error) of normally distributed plant variables (including zero values, and data back-transformed where necessary): aquatic bryophyte species richness (per 19.64 cm^2), aquatic bryophyte species diversity (per 19.64 cm^2), and aquatic bryophyte species dominance (per 19.64 cm^2) between sampling sites (n = 79 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.74.

4.5.10 Seasonal variation in aquatic bryophyte community composition and diversity in the Water of Dye, River Girnock and Knockan Burn

4.5.10.1 Water of Dye

In the Water of Dye, there was no significant difference in aquatic bryophyte species richness, diversity or dominance between dates sampled (Table 4.74).

4.5.10.2 River Girnock

Also in the River Girnock, aquatic bryophyte species did not exhibit significant seasonal variation in community composition between survey dates (Table 4.75).

4.5.10.3 Knockan Burn

In Knockan Burn, aquatic bryophyte structural attributes also did not differ significantly between survey dates sampled (Table 4.76).

Mean Variable	May 2005		August 2005		April 2006		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Aquatic bryophyte species richness: **S**	3.26	0.24	2.67	0.33	2.95	0.37	NS
Aquatic bryophyte species diversity: **H**	1.00	0.07	0.75	0.15	0.92	0.17	NS
Aquatic bryophyte species dominance	0.53	0.04	0.65	0.08	0.59	0.09	NS

Table 4.74 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): aquatic bryophyte species richness (per 19.64 cm^2), aquatic bryophyte species diversity (per 19.64 cm^2), and aquatic bryophyte species dominance (per 19.64 cm^2) between sampling dates in the Water of Dye sub-catchment (n = 27 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.75.

Mean Variable	May 2005		August 2005		April 2006		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Aquatic bryophyte species richness: **S**	2.47	0.45	1.64	0.42	2.00	0.25	NS
Aquatic bryophyte species diversity: **H**	0.71	0.20	0.42	0.19	0.58	0.13	NS
Aquatic bryophyte species dominance	0.57	0.11	0.68	0.14	0.60	0.09	NS

Table 4.75 Mean values (± 1 standard error) of normally distributed plant variables (including zero values, and data back-transformed where necessary): aquatic bryophyte species richness (per 19.64 cm^2), aquatic bryophyte species diversity (per 19.64 cm^2), and aquatic bryophyte species dominance (per 19.64 cm^2) between sampling dates in the River Girnock sub-catchment (n = 27 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.76.

Mean Variable	April 2006		September 2006		November 2006		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Aquatic bryophyte species richness: **S**	2.89	0.59	2.15	0.60	2.53	0.60	NS
Aquatic bryophyte species diversity: **H**	0.77	0.19	0.69	0.20	0.62	0.24	NS
Aquatic bryophyte species dominance	0.46	0.09	0.60	0.10	0.63	0.14	NS

Table 4.76 Mean values (± 1 standard error) of normally distributed plant variables (including zero values, and data back-transformed where necessary): aquatic bryophyte species richness (per 19.64 cm^2), aquatic bryophyte species diversity (per 19.64 cm^2), and aquatic bryophyte species dominance (per 19.64 cm^2) between sampling dates in the Knockan Burn sub-catchment (n = 25 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.77.

4.5.11 Response of aquatic bryophyte community composition and diversity in the Water of Dye, River Girnock and Knockan Burn to variation in flow regime: pool, glide and riffle zones

Overall, the amalgamated data set indicated that flow regime exerted a significant effect on aquatic bryophyte species composition (Table 4.80). In general, as current velocity increased aquatic bryophyte species richness and diversity increased, whilst species dominance decreased. Aquatic bryophyte species composition was therefore richer and more diverse in riffles than in pools. However, aquatic bryophyte species assemblages occurring in glides did not appear to vary significantly from either extremely fast- or slow-flowing habitats. Although mostly these observed trends were echoed in each of the individual sub-catchment streams, no significant differences in the structural response of aquatic bryophytes were detected between the three basic flow types: Water of Dye (Table 4.77), River Girnock (Table 4.78) and Knockan Burn (Table 4.79).

Variable	Pool		Glide		Riffle		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Aquatic bryophyte species richness: **S**	2.89	0.31	2.67	0.29	3.33	0.41	NS
Aquatic bryophyte species diversity: **H**	0.88	0.15	0.79	0.13	1.00	0.16	NS
Aquatic bryophyte species dominance	0.59	0.08	0.64	0.06	0.54	0.08	NS

Table 4.77 Mean values (± 1 standard error) of normally distributed plant variables (data back-transformed where necessary): aquatic bryophyte species richness (per 19.64 cm^2), aquatic bryophyte species diversity (per 19.64 cm^2), and aquatic bryophyte species dominance (per 19.64 cm^2) between flow regime (pool, glide, riffle habitats) in the Water of Dye sub-catchment (n = 27 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.78.

Variable	Pool		Glide		Riffle		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Aquatic bryophyte species richness: **S**	1.67	0.41	1.78	0.40	2.67	0.33	NS
Aquatic bryophyte species diversity: **H**	0.39	0.17	0.48	0.17	0.85	0.14	NS
Aquatic bryophyte species dominance	0.70	0.12	0.62	0.12	0.55	0.07	NS

Table 4.78 Mean values (± 1 standard error) of normally distributed plant variables (including zero values, and data back-transformed where necessary): aquatic bryophyte species richness (per 19.64 cm^2), aquatic bryophyte species diversity (per 19.64 cm^2), and aquatic bryophyte species dominance (per 19.64 cm^2) between flow regime (pool, glide, riffle habitats) in the River Girnock sub-catchment (n = 27 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.79.

Variable	Pool		Glide		Riffle		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Aquatic bryophyte species richness: **S**	1.88	0.48	2.89	0.54	2.80	0.77	NS
Aquatic bryophyte species diversity: **H**	0.50	0.19	0.81	0.19	0.79	0.23	NS
Aquatic bryophyte species dominance	0.63	0.11	0.50	0.10	0.54	0.11	NS

Table 4.79 Mean values (± 1 standard error) of normally distributed plant variables (including zero values, and data back-transformed where necessary): aquatic bryophyte species richness (per 19.64 cm^2), aquatic bryophyte species diversity (per 19.64 cm^2), and aquatic bryophyte species dominance (per 19.64 cm^2) between flow regime (pool, glide, riffle habitats) in the Knockan Burn sub-catchment (n = 25 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.80.

Variable	Pool		Glide		Riffle		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Aquatic bryophyte species richness: **S**	2.15^{a}	0.25	2.45^{ab}	0.25	2.93^{b}	0.28	$P<0.05$*
Aquatic bryophyte species diversity: **H**	0.59^{a}	0.10	0.69^{ab}	0.10	0.88^{b}	0.10	$P<0.05$*
Aquatic bryophyte species dominance	0.64^{a}	0.06	0.59^{ab}	0.05	0.54^{b}	0.05	$P<0.05$*

Table 4.80 Mean values (± 1 standard error) of normally distributed plant variables (including zero values, and data back-transformed where necessary): aquatic bryophyte species richness (per 19.64 cm^2), aquatic bryophyte species diversity (per 19.64 cm^2), and aquatic bryophyte species dominance (per 19.64 cm^2) between flow regime (pool, glide, riffle habitats) for amalgamated sub-catchment data (the Water of Dye, River Girnock, and Knockan Burn, n = 79 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.81.

4.5.12 Response of aquatic bryophyte community composition and diversity in the Water of Dye, River Girnock and Knockan Burn to variation in substrate morphology

Generally, as the proportion of stable substrates (e.g. boulders) occurring in the streambed increased and unstable morphologies (e.g. small cobbles, gravel) decreased, aquatic bryophyte species richness and diversity increased whilst species dominance decreased (refer to Table 4.81 – Table 4.85, inclusive).

Variable	0% BO		3% BO		15.5% BO		38% BO		63% BO		88% BO		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Aquatic bryophyte species richness: **S**	0.86^{a}	0.40	1.91^{a}	0.34	2.75^{b}	0.24	3.50^{b}	0.29	2.90^{b}	0.31	3.14^{b}	0.47	P<0.01**
Aquatic bryophyte species diversity: **H**	0.12^{a}	0.22	0.44^{a}	0.16	0.74^{b}	0.09	1.23^{b}	0.10	0.76^{b}	0.14	1.03^{b}	0.41	P<0.01**
Aquatic bryophyte species dominance	0.84^{a}	0.12	0.77^{a}	0.08	0.62^{b}	0.05	0.35^{b}	0.06	0.58^{b}	0.07	0.39^{b}	0.20	P<0.01**

Table 4.81 Mean values (± 1 standard error) of normally distributed plant variables (including zero values, and data back-transformed where necessary): response of aquatic bryophyte species richness (per 19.64 cm^2), aquatic bryophyte species diversity (per 19.64 cm^2), and aquatic bryophyte species dominance (per 19.64 cm^2) to variation in the abundance (median % cover) of boulders (BO) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 79). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.82.

Variable	0% LS		3% LS		15.5% LS		38% LS		63% LS		88% LS		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Aquatic bryophyte species richness: **S**	2.10	0.96	2.28	0.30	3.04	0.21	2.32	0.28	2.73	0.43	2.57	0.38	NS
Aquatic bryophyte species diversity: **H**	0.48	0.32	0.60	0.12	1.13	0.08	0.64	0.10	0.83	0.20	0.68	0.14	NS
Aquatic bryophyte species dominance	0.76	0.17	0.67	0.08	0.36	0.04	0.62	0.06	0.48	0.10	0.65	0.08	NS

Table 4.82 Mean values (± 1 standard error) of normally distributed plant variables (including zero values, and data back-transformed where necessary): response of aquatic bryophyte species richness (per 19.64 cm^2), aquatic bryophyte species diversity (per 19.64 cm^2), and aquatic bryophyte species dominance (per 19.64 cm^2) to variation in the abundance (median % cover) of large stones (LS) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 79). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.83.

Variable	0% SS		3% SS		15.5% SS		38% SS		63% SS		88% SS		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Aquatic bryophyte species richness: **S**	4.05^{a}	0.14	3.03^{b}	0.37	2.78^{b}	0.21	2.51^{b}	0.27	1.50^{bc}	1.00	1.19^{c}	0.31	P<0.05*
Aquatic bryophyte species diversity: **H**	1.52^{a}	0.10	0.86^{b}	0.12	0.67^{b}	0.08	0.59^{b}	0.11	0.42^{bc}	0.22	0.28^{c}	0.13	P<0.05*
Aquatic bryophyte species dominance	0.20^{a}	0.05	0.52^{b}	0.06	0.63^{b}	0.04	0.66^{b}	0.06	0.73^{bc}	0.15	0.82^{c}	0.08	P<0.05*

Table 4.83 Mean values (± 1 standard error) of normally distributed plant variables (including zero values, and data back-transformed where necessary): response of aquatic bryophyte species richness (per 19.64 cm^2), aquatic bryophyte species diversity (per 19.64 cm^2), and aquatic bryophyte species dominance (per 19.64 cm^2) to variation in the abundance (median % cover) of small stones (SS) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 79). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.84.

Variable	0% GR		3% GR		15.5% GR		38% GR		63% GR		88% GR		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Aquatic bryophyte species richness: **S**	3.87^{a}	0.33	2.93^{b}	0.28	2.57^{b}	0.20	2.27^{b}	0.33	2.67^{b}	0.38	0.72^{c}	0.16	P<0.01**
Aquatic bryophyte species diversity: **H**	1.19^{a}	0.07	0.82^{b}	0.10	0.75^{b}	0.09	0.61^{b}	0.13	0.76^{b}	0.10	0.20^{c}	0.10	P<0.01**
Aquatic bryophyte species dominance	0.29^{a}	0.05	0.52^{b}	0.05	0.60^{b}	0.04	0.65^{b}	0.09	0.57^{b}	0.03	0.88^{c}	0.04	P<0.01**

Table 4.84 Mean values (± 1 standard error) of normally distributed plant variables (including zero values, and data back-transformed where necessary): response of aquatic bryophyte species richness (per 19.64 cm^2), aquatic bryophyte species diversity (per 19.64 cm^2), and aquatic bryophyte species dominance (per 19.64 cm^2) to variation in the abundance (median % cover) of gravel (GR) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 79). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.85.

Variable	0% SA		3% SA		15.5% SA		38% SA		63% SA		88% SA		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Aquatic bryophyte species richness: **S**	3.67	0.36	2.77	0.67	2.11	0.68	1.85	0.65	3.50	1.50	1.13	0.47	NS
Aquatic bryophyte species diversity: **H**	1.16	0.16	0.88	0.24	0.58	0.26	0.42	0.26	1.08	0.48	0.22	0.20	NS
Aquatic bryophyte species dominance	0.39	0.03	0.51	0.10	0.63	0.17	0.70	0.20	0.42	0.19	0.88	0.13	NS

Table 4.85 Mean values (± 1 standard error) of normally distributed plant variables (including zero values, and data back-transformed where necessary): response of aquatic bryophyte species richness (per 19.64 cm^2), aquatic bryophyte species diversity (per 19.64 cm^2), and aquatic bryophyte species dominance (per 19.64 cm^2) to variation in the abundance (median % cover) of sand (SA) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 79). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. For details of environmental habitat conditions refer to Chapter 3, Table 3.86.

4.5.13 Aquatic bryophyte community composition, diversity and environmental habitat conditions in the Water of Dye, River Girnock and Knockan Burn as determined by multivariate ordination and TWINSPAN classification

Analysis of the aquatic bryophyte species data (n = 74) using TWINSPAN revealed the existence of four primary community types, indicated by *Blindia acuta* (Group I), *Racomitrium aciculare*, *Hygrohypnum ochraceum* and *Scapania undulata* (Group II), a high abundance of *Fontinalis antipyretica* (Group III), and *Platyhypnidium riparioides*, *Hygrohypnum luridum*, *Palustriella falcata*, *Fissidens adianthoides* and *Chiloscyphus polyanthus* (Group IV): Figure 4.26. Sample-groups I, II and IV formed well-defined boundaries and were clearly separated from one another, however Group III shared some overlapping similarities with Group II in terms of aquatic bryophyte species composition (refer to Figure 4.27).

Community Type I (n = 8 samples: HBMYP05, HBMYG05, HBMYR05, HBAUP05, HBAUG05, HBAUR05, HBAPR05, LMMYR05: for key to sample codes see caption to Figure 4.27). This was the least common aquatic vegetation type, occurring in the mid- and lower basin of the River Girnock and was strongly separated from the other sample-groups with an eigenvalue of 0.821 at level 1 of the classification. This assemblage was indicated by an abundance of *Blindia acuta*, and generally supported a low diversity aquatic bryophyte community, in which few samples supported small quantities of other species such as *Schistidium agassizii* and *Racomitrium aciculare*.

Community Type II (n = 35 samples: BBMYP05, BBMYG05, BBMYR05, BBAUG05, BBAUR05, BBAPP06, BBAPG06, CFMYP05, CFMYG05, CFMYR05, CFAUP05, CFAUG05, CFAUR05, CFAPP06, CFAPG06, CFAPR06, BDMYR05, BDAUG05, BDAPR06, IBMYP05, IBMYG05, IBMYR05, IBAUP05, IBAUG05, IBAUR05, IBAPP06, IBAPR06, HBAPP06, HBAPG06, LMMYP05, LMMYG05, LMAUR05, LMAPP06, LMAPG06, LMAPR06). Group II was the commonest aquatic vegetation type occurring in the Water of Dye and also characterised part of the

River Girnock, being particularly predominant at Iron Bridge in the upper basin. This group was moderately well-delineated from the other groups (eigenvalue 0.615 at level 2 of the TWINSPAN classification). This group supported a moderately diverse bryophyte community, in which samples contained at least one or a combination of, the three indicator species: *Racomitrium aciculare*, *Hygrohypnum ochraceum* and *Scapania undulata*.

Community Type III (n = 15 samples: BBAUP05, BBAPR06, BDMYP05, BDMYG05, BDAUP05, BDAUR05, BDAPP06, BDAPG06, IBAPG06, UKAPP06, UKAPG06, UKAPR06, UKSMP06, UKNVP06, UKNVG06). This aquatic vegetation type had examples in all three streams (mainly in their upper reaches, though with 6 samples from the lowest stretch of the Water of Dye), and was strongly characterised by a low diversity community dominated almost exclusively, by one species, *Fontinalis antipyretica*, whilst other bryophytes were rare. This assemblage was separated from Group IV with an eigenvalue of 0.622 (at level 3 of the classification).

Community Type IV (n = 16 samples: MKAPP06, MKAPG06, MKAPR06, MKSMP06, MKSMG06, MKSMR06, MKNVG06, MKNVR06, LKAPP06, LKAPG06, LKAPR06, LKSMP06, LKSMG06, LKSMR06, LKNVP06, LKNVG06). Distribution of this high diversity aquatic bryophyte assemblage was restricted to the mid and lower reaches of Knockan Burn. This community was indicated by the presence of several co-occurring bryophyte species: *Platyhypnidium riparioides*, *Hygrohypnum luridum*, *Palustriella falcata*, *Fissidens adianthoides* and *Chiloscyphus polyanthus*. This group was separated from Type III with an eigenvalue of 0.622 (at level 3 of the classification).

Performing one-way ANOVA on the environmental characteristics of TWINSPAN groups I - IV, and included a fifth sample-group (V) representing samples that did not possess aquatic bryophyte vegetation (Table 4.86). This enabled me to address the following questions: "*What environmental variables drive the distribution of aquatic bryophyte assemblages in upland stream habitats*?" and furthermore, "*Why were some*

samples devoid of aquatic bryophyte vegetation?" Further evidence of aquatic bryophyte species affiliations with environmental variables could also be determined from CCA analysis (Figure 4.28).

CCA ordination of the seventeen aquatic bryophyte species, constrained by the fifty-two environmental variables used in the analysis, suggested that underlying geology, substrate morphology, water physico-chemistry factors (mainly pH and conductivity), and water chemistry parameters (heavy metals, base cations) were the primary predictors of aquatic bryophyte species occurrence within the target streams (Figure 4.28). Evidence from the outcome of the multivariate analyses, together with ANOVA and Tukey's pairwise comparisons of mean environmental data for the sample-units comprising each TWINSPAN sample-group (Table 4.86), indicated that the aforementioned variables showed significant inter-group differences between the samples comprising each community type, as did aquatic bryophyte species diversity (see Table 4.86, and also Figure 4.29).

The Group I community occurred in a streambed habitat underlain by mixed geological composition and characterised by a significantly higher proportion of large stones (compared to the other sample-groups) with fewer boulders than in Groups II and III. Generally, streamwaters associated with this group were moderately well-buffered and of circumneutral pH.

Group II showed some overlapping similarities in terms of aquatic bryophyte community composition with Groups I and III. Underlying geology associated with this sample-group was predominantly base-poor (e.g. granite) and streambed substrate morphology was mostly stable (e.g. boulder-dominated). Water chemistry was inherently base-poor and acid-sensitive: low pH (<7), conductivity, and alkalinity, coupled to accentuated sulphate levels and heavy metal availability. This particular sample-group was also prone to shading from riparian vegetation.

Group III shared many similar habitat characteristics (e.g. pH, conductivity, extent of riparian shade etc.) to the Group II community but there were some crucial differences in environmental conditions between the two sample-groups. The Group III community occurred in streambed habitats containing high cover of stable substrates (like Group II) but also abundant smaller-sized particles. Underlying geology was partly base poor (as for Group II) but also had base-rich properties. Heavy metals were less available (although similar to those associated with Group II) but base cations were significantly more abundant.

The Group IV community was composed mostly of aquatic bryophyte species exclusive to that particular assemblage and clearly distinct from the other sample-groups in terms of the stream habitat in which it occurred. Streambed substrate morphology was a diverse mixture of particle size classes. The predominance of base-rich strata markedly influenced the water chemistry associated with this sample-group which was well-buffered, had naturally high pH (>7), conductivity, alkalinity, abundance of base cations (calcium and magnesium), and suppressed levels of sulphate and heavy metals.

Group V represents the sample-group lacking aquatic bryophyte vegetation. Most notably, the samples in this group were characterised by streambed morphologies deficient in large-sized stable substrates (e.g. boulders) yet highly abundant in slighter unstable particle forms (e.g. cobbles, gravel etc.). Streamwaters were well-buffered with pH, conductivity and alkalinity generally high, as was the abundance of base cations (similar to water physico- and chemistry properties of Group IV). Extent of shade from riparian vegetation was quite pronounced (but did not differ significantly from Groups II and III).

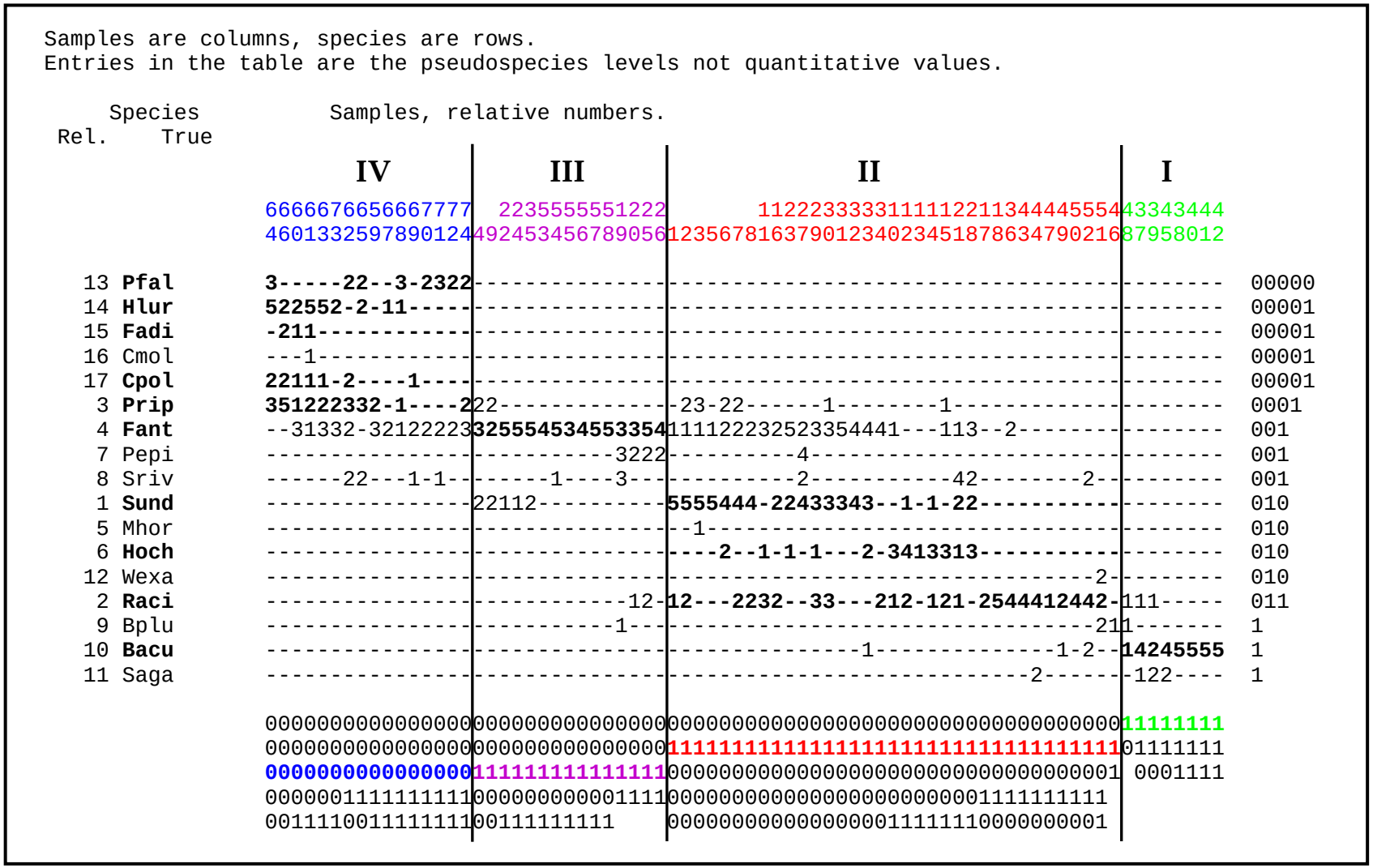


Figure 4.26 TWINSPAN output depicting 79 samples and 4 aquatic bryophyte species assemblages, with indicator species highlighted in bold font and colour-coding as appropriate for TWINSPAN sample-groups I (green), II (red), III (purple), and IV (blue). For aquatic bryophyte species codes refer to Figure 4.28.

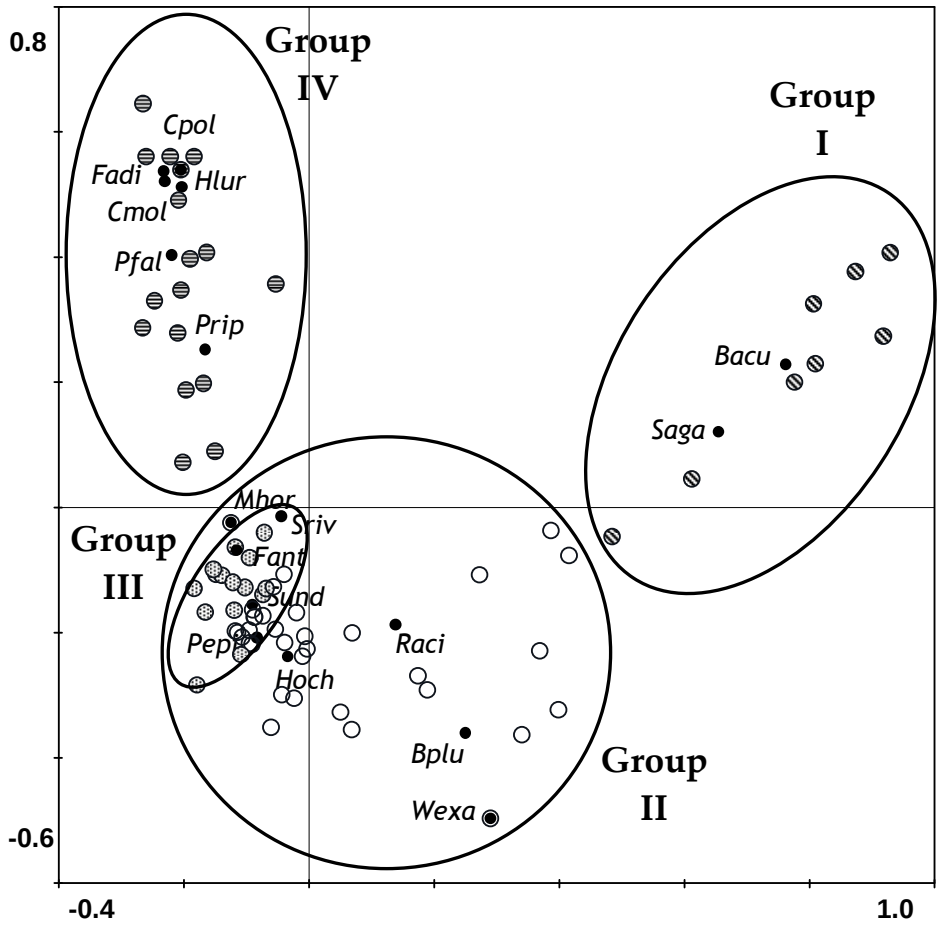


Figure 4.27 CCA ordination of 17 aquatic bryophyte species and 74 samples, with TWINSPAN sample-group boundaries overlaid. TWINSPAN sample-group identifiers as follows: Group I (n=8: HBMYP05, HBMYG05, HBMYR05, HBAUP05, HBAUG05, HBAUR05, HBAPR05, LMMYR05): diagonally striped circles ; Group II (n=35: BBMYP05, BBMYG05, BBMYR05, BBAUG05, BBAUR05, BBAPP06, BBAPG06, CFMYP05, CFMYG05, CFMYR05, CFAUP05, CFAUG05, CFAUR05, CFAPP06, CFAPG06, CFAPR06, BDMYR05, BDAUG05, BDAPR06, IBMYP05, IBMYG05, IBMYR05, IBAUP05, IBAUG05, IBAUR05, IBAPP06, IBAPR06, HBAPP06, HBAPG06, LMMYP05, LMMYG05, LMAUR05, LMAPP06, LMAPG06, LMAPR06): open circles ; Group III (n=15: BBAUP05, BBAPR06, BDMYP05, BDMYG05, BDAUP05, BDAUR05, BDAPP06, BDAPG06, IBAPG06, UKAPP06, UKAPG06, UKAPR06, UKSMP06, UKNVP06, UKNVG06): dotted circles ; and Group IV (n=16: MKAPP06, MKAPG06, MKAPR06, MKSMP06, MKSMG06, MKSMR06, MKNVG06, MKNVR06, LKAPP06, LKAPG06, LKAPR06, LKSMP06, LKSMG06, LKSMR06, LKNVP06, LKNVG06): horizontally striped circles . For sample site-codes: Water of Dye sites: Brocky Burn (BB), Charr Flume (CF) and Bogendreip (BD); River Girnock sites: Iron Bridge (IB), Hampshire's Bridge (HB) and Littlemill (LM); Knockan Burn sites: Upper Knockan (UK), Mid-Knockan (MK) and Lower Knockan (LK). Each site code is completed using code letters for survey date (AP: April; MY: May; AU: August; SM: September; NV: November), flow regime (P: Pool; G: Glide; R: Riffle) and year sampled (05: 2005; 06: 2006). Example: BBMYR05 = Brocky Burn May Riffle 2005. For aquatic bryophyte species codes and ordination statistics refer to Figure 4.28

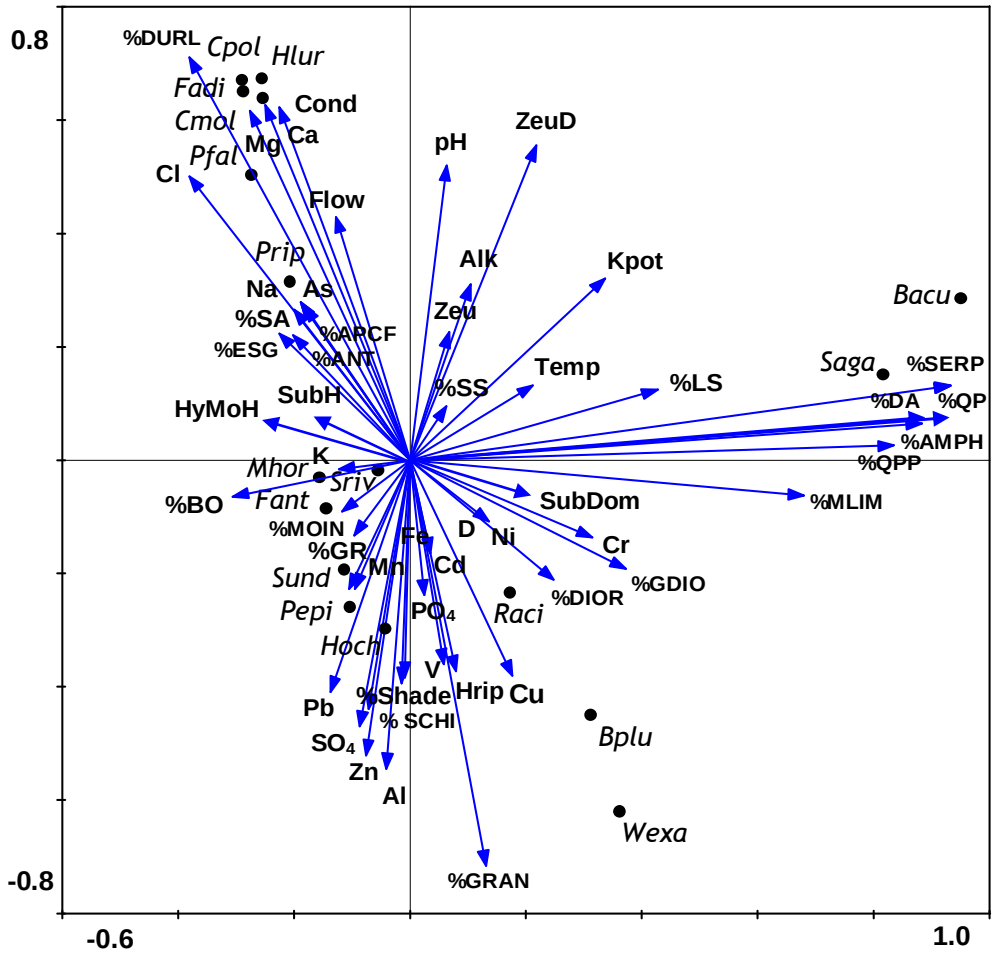


Figure 4.28 CCA ordination of aquatic bryophyte species and environmental variables. Aquatic bryophyte species codes: *Blindia acuta* (Bacu), *Brachythecium plumosum* (Bplu), *Ctenidium molluscum* (Cmol), *Fissidens adianthoides* (Fadi), *Fontinalis antipyretica* (Fant), *Hygrohypnum luridum* (Hlur), *Hygrohypnum ochraceum* (Hoch), *Mnium hornum* (Mhor), *Palustriella falcata* (Pfal), *Platyhypnidium riparioides* (Prip), *Racomitrium aciculare* (Raci), *Schistidium agassizii* (Saga), *Schistidium rivulare* (Sriv), *Warnstorfia exannulata* (Wexa), *Chiloscyphus polyanthus* (Cpol), *Pellia epiphylla* (Pepi), and *Scapania undulata* (Sund). Environmental variables: Underlying geology: Granite (%GRAN), Mica Schist (%SCHI), Granodiorite (%GDIO), Diorite (%DIOR), Quartz/Psammite (%QP), Quartz/Psammite/Pelite (%QPP), Diorite/Amphibolite (%DA), Amphibolite (%AMPH), Serpentinite (%SERP), Metamorphic Limestone (%MLIM), Durness Limestone (%DURL), Moine Schist (%MOIN), Eriboll Sandstone Group (%ESG), Applecross Formation (%APCF) and An-T'sron (%ANT). Substrate morphology: substrate particle diversity (SubH), substrate particle dominance (SubDom), hydromorphological diversity (HyMoH), streambed cover of Boulders (%BO), Large Stones (%LS), Small Stones (%SS), Gravel (%GR), and Sand (%SA). Water physico-chemistry: benthic depth (D: m), light attenuation coefficient (K: m^{-1}), euphotic depth (Zeu: m^{-1}), Zeu:D ratio, pH, alkalinity (Alk: mg/l), conductivity (Cond: µS cm^{-1}), water temperature (Temp: ^{o}C), current velocity (Flow: m s^{-1}), %Shade and height of riparian vegetation (Hrip). Water Chemistry: Phosphate (PO_4-P), Chloride (Cl), Sulphate (SO_4), Cadmium (Cd), Chromium (Cr), Copper (Cu), Lead (Pb), Nickel (Ni), Zinc (Zn), Aluminium (Al), Vanadium (V), Arsenic (As), Sodium (Na), Potassium (Kpot), Calcium (Ca), Magnesium (Mg), Iron (Fe), and Manganese (Mn). Monte Carlo significance test: Axis 1: P<0.005; all canonical axes: P<0.005. Eigenvalues: Axis 1: 0.834; Axis 2: 0.630.

	TWINSPAN sample-group										
Variable	**I**		**II**		**III**		**IV**		**V**		**P_{ANOVA}**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Aquatic bryophyte species richness: **S**	1.88^{a}	0.35	2.70^{ab}	0.20	2.03^{a}	0.25	3.48^{b}	0.30	0.0^{c}	0.00	P<0.001***
Aquatic bryophyte species diversity: **H**	0.46^{a}	0.18	0.81^{ab}	0.08	0.52^{a}	0.12	1.08^{b}	0.08	0.0^{c}	0.00	P<0.001***
Aquatic bryophyte species dominance	0.76^{a}	0.10	0.51^{ab}	0.04	0.65^{a}	0.07	0.43^{b}	0.02	0.0^{c}	0.00	P<0.001***
Substrate diversity (Simpson's D)	2.82^{a}	0.24	3.13^{ab}	0.09	3.41^{b}	0.19	3.56^{b}	0.23	2.73^{a}	0.37	P<0.05*
Hydromorphological diversity (Simpson's D)	2.94^{a}	0.25	3.20^{ab}	0.09	3.54^{b}	0.19	3.66^{b}	0.23	2.82^{a}	0.38	P<0.05*
Substrate dominance (Berger-Parker)	0.49	0.05	0.44	0.02	0.41	0.03	0.40	0.04	0.51	0.07	NS
% Granite	53.8^{a}	1.16	76.3^{b}	3.11	52.1^{a}	11.46	0.0^{c}	0.00	18.3^{c}	11.19	P<0.001***
% Granodiorite	7.6^{a}	0.34	4.5^{a}	0.98	0.9^{b}	0.93	0.0^{b}	0.00	2.1^{ab}	1.27	P<0.001***
% Diorite	0.1	0.11	0.2	0.06	0.0	0.00	0.0	0.00	0.4	0.22	NS
% Mica Schist	0.0^{a}	0.00	6.6^{b}	1.62	5.2^{b}	1.69	0.0^{a}	0.00	0.0^{a}	0.00	P<0.01**
% Amphibolite	11.3^{a}	0.09	2.7^{b}	0.85	0.0^{b}	0.00	0.0^{b}	0.00	4.8^{b}	2.92	P<0.001***
% Serpentinite	1.7^{a}	0.08	0.3^{b}	0.10	0.0^{b}	0.00	0.0^{b}	0.00	0.5^{b}	0.29	P<0.001***
% QP	1.2^{a}	0.05	0.2^{b}	0.07	0.0^{b}	0.00	0.0^{b}	0.00	0.3^{b}	0.20	P<0.001***

% DA	3.9[a]	0.18	0.7[b]	0.23	0.0[b]	0.00	0.0[b]	0.00	1.1[b]	0.66	P<0.001***
% QPP	12.6[a]	0.53	3.5[b]	1.11	0.0[b]	0.00	0.0[b]	0.00	6.5[ab]	3.99	P<0.001***
% Limestone	7.7[a]	1.11	3.0[b]	1.01	0.0[b]	0.00	0.0[b]	0.00	6.2[ab]	3.79	P<0.001***
% Durness Limestone	0.0[a]	0.00	0.0[a]	0.00	30.0[b]	9.28	75.0[c]	6.46	44.0[bc]	19.65	P<0.001***
% Eriboll Sandstone	0.0[a]	0.00	0.0[a]	0.00	4.0[a]	1.31	10.0[b]	2.58	6.0[ab]	4.00	P<0.001***
% Moine Schist	0.0[a]	0.00	0.0[a]	0.00	8.0[b]	2.62	0.0[a]	0.00	4.0[ab]	4.00	P<0.001***
% Applecross Formation	0.0[a]	0.00	0.0[a]	0.00	0.0[a]	0.00	10.0[b]	2.58	4.0[ab]	4.00	P<0.001***
% An-t'Sron	0.0[a]	0.00	0.0[a]	0.00	0.0[a]	0.00	5.0[b]	1.29	2.0[ab]	2.00	P<0.001***
% Boulders	20.2[ab]	5.90	35.4[a]	2.97	40.5[a]	6.70	41.6[a]	5.71	11.2[b]	7.22	P<0.05*
% Large Stones	53.5[a]	3.70	37.6[ab]	2.53	27.9[b]	4.41	30.0[b]	4.41	17.8[b]	10.38	P<0.01**
% Small Stones	25.8	2.96	23.7	2.36	28.6	3.62	33.5	4.01	30.2	9.39	NS
% Gravel	14.4[a]	5.71	19.0[a]	2.71	31.5[b]	4.19	26.8[b]	4.52	41.4[b]	13.09	P<0.01**
% Sand	0.0[a]	0.00	0.0[a]	0.00	7.6[ab]	3.30	16.6[b]	5.84	11.6[b]	8.27	P<0.05*
D (m)	0.12	0.15	0.16	0.09	0.19	0.13	0.14	0.11	0.09	0.29	NS
K (m^{-1})	2.87	0.53	2.72	0.25	2.57	0.41	2.75	0.36	2.70	0.94	NS
$Z_{eu}^{3\%}$ (m)	1.38	0.58	1.09	0.30	1.31	0.44	1.43	0.37	0.87	0.83	NS
Z_{eu}:$D^{3\%}$	10.71	0.63	6.12	0.30	6.20	0.38	9.80	0.36	8.79	0.98	NS
pH	7.10[ab]	0.16	6.55[a]	0.12	6.74[a]	0.19	7.68[b]	0.08	7.54[b]	0.09	P<0.001***
Conductivity (μS cm^{-1})	58.6[ab]	0.11	43.4[a]	0.05	64.5[b]	0.12	150.9[c]	0.10	119.9[c]	0.18	P<0.001***

Alkalinity (mg l^{-1})	23.08^{ab}	25.71	8.06^{a}	8.18	12.62^{a}	8.46	58.09^{b}	8.94	96.62^{b}	22.71	P<0.001***
Water Temperature (^{o}C)	13.2	0.34	8.7	0.16	7.7	0.23	9.1	0.17	10.9	0.28	NS
Flow (m s^{-1})	0.262	0.15	0.235	0.07	0.222	0.13	0.319	0.13	0.236	0.24	NS
% Shade	10.8^{ab}	8.84	26.9^{a}	4.26	20.9^{a}	4.46	5.8^{b}	1.09	40.5^{a}	17.50	P<0.05*
Height of Riparian Vegetation (m)	1.76^{ab}	1.10	2.58^{a}	0.64	3.16^{a}	0.94	0.10^{b}	0.04	3.96^{a}	2.24	P<0.05*
NH_3-N (mg l^{-1})	<0.04		<0.04		<0.04		<0.04		<0.04		NS
NO_3-N (mg l^{-1})	<0.01		<0.01		<0.01		<0.01		<0.01		NS
PO_4-P (mg l^{-1})	<0.003		<0.003		<0.003		<0.003		<0.003		NS
Cl (mg l^{-1})	7.7^{a}	4.04	8.3^{a}	0.30	11.2^{b}	0.62	13.2^{c}	0.35	11.0^{b}	0.52	P<0.001***
SO_4 (mg l^{-1})	1.04^{a}	0.36	1.81^{b}	0.25	1.53^{ab}	0.42	0.20^{c}	0.05	0.68^{ac}	0.36	P<0.01**
Cd (μg l^{-1})	0.03	0.20	0.02	0.09	0.02	0.13	0.02	0.00	0.02	0.00	NS
Cr (μg l^{-1})	0.40^{a}	0.39	0.23^{ab}	0.16	0.13^{b}	0.13	0.12^{b}	0.00	0.09^{b}	0.26	P<0.001***
Cu (μg l^{-1})	0.29^{a}	0.07	0.28^{a}	0.02	0.22^{ab}	0.05	0.12^{b}	0.03	0.28^{a}	0.10	P<0.05*
Pb (μg l^{-1})	0.14^{a}	0.28	0.36^{b}	0.14	0.21^{ab}	0.29	0.05^{c}	0.00	0.08^{ac}	0.32	P<0.001***
Ni (μg l^{-1})	0.32	0.28	0.29	0.09	0.21	0.17	0.23	0.16	0.24	0.40	NS
Zn (μg l^{-1})	1.54^{a}	0.14	2.67^{b}	0.09	1.97^{ab}	0.21	0.79^{c}	0.00	1.38^{ac}	0.34	P<0.001***
Al (μg l^{-1})	79.4^{a}	17.76	110.8^{b}	7.50	97.0^{ab}	16.79	39.5^{c}	3.07	53.1^{ac}	21.10	P<0.001***
V (μg l^{-1})	0.30^{a}	0.22	0.31^{a}	0.10	0.21^{ab}	0.17	0.13^{b}	0.06	0.19^{ab}	0.27	P<0.01**
As (μg l^{-1})	0.56	0.06	0.47	0.04	0.51	0.04	0.59	0.00	0.59	0.00	NS

Na (mg l^{-1})	4.89^{a}	0.34	4.73^{a}	0.17	5.56^{b}	0.31	5.94^{b}	0.23	5.60^{b}	0.38	P<0.01**
K (mg l^{-1})	0.63^{ab}	0.14	0.46^{a}	0.05	0.45^{a}	0.08	0.52^{ab}	0.09	0.78^{b}	0.21	P<0.01**
Ca (mg l^{-1})	1.33^{a}	0.16	0.87^{b}	0.09	1.24^{a}	0.20	2.48^{c}	0.13	2.34^{c}	0.21	P<0.001***
Mg (mg l^{-1})	1.53^{a}	0.13	0.89^{b}	0.07	1.66^{a}	0.24	7.23^{c}	0.16	5.09^{c}	0.38	P<0.001***
Fe (mg l^{-1})	0.26^{a}	0.08	0.28^{a}	0.11	0.21^{ab}	0.17	0.15^{b}	0.13	0.17^{b}	0.25	P<0.05*
Mn (mg l^{-1})	0.009^{ab}	0.06	0.016^{a}	0.15	0.009^{ab}	0.20	0.007^{b}	0.10	0.006^{b}	0.12	P<0.05*

Table 4.86 Mean values (± 1 standard error) of normally distributed plant variables (including zero values, and data back-transformed where necessary): aquatic bryophyte species richness per 19.64 cm^2 (n = 79), aquatic bryophyte species diversity per 19.64 cm^2 (n = 79), aquatic bryophyte species dominance per 19.64 cm^2 (n = 79), and environmental habitat variables (n = 79) between TWINSPAN sample-groups I (n = 8), II (n = 35), III (n = 15), and IV (n = 16) with the 'no bryophytes' sample-group V (n = 5) encompassing all other samples lacking aquatic bryophyte vegetation. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values are quoted for non-normal variables compared using Kruskal-Wallis test.

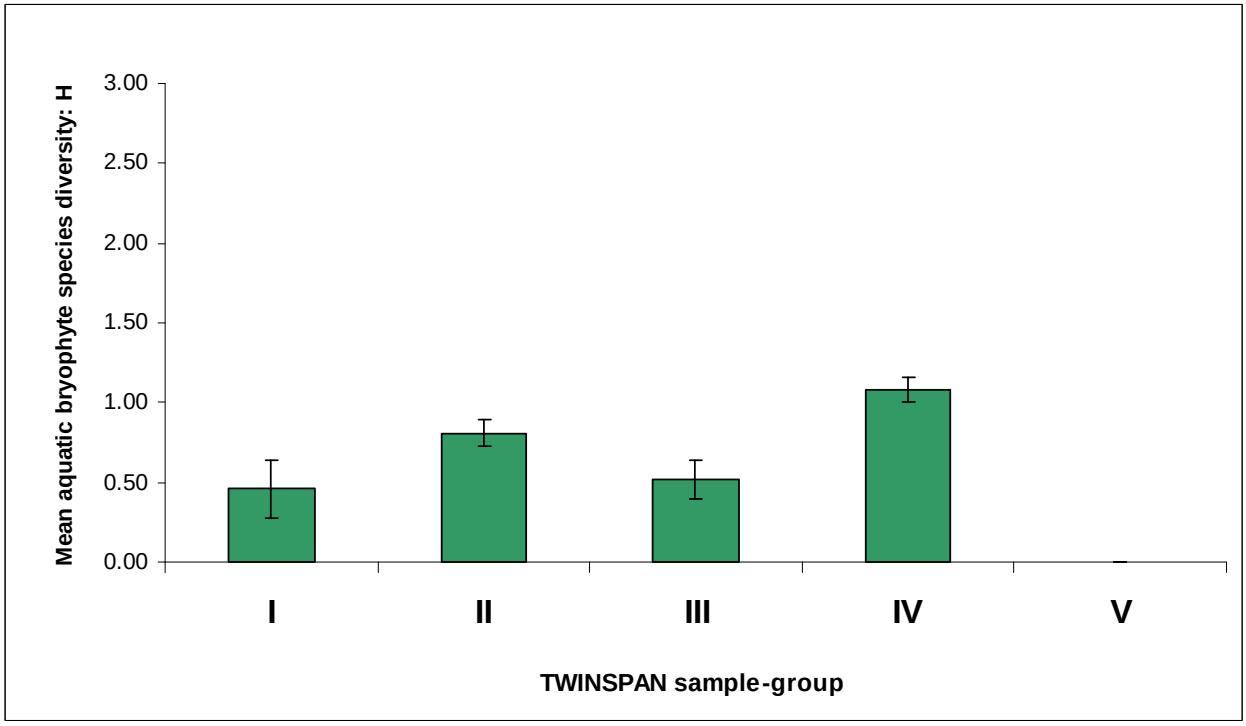


Figure 4.29 Comparison of mean values (± 1 standard error) of normally distributed plant variables (including zero values, and data back-transformed where necessary): aquatic bryophyte species diversity per 19.64 cm^2 (n = 79) between TWINSPAN sample-groups I (n = 8), II (n = 35), III (n = 15), and IV (n = 16) with the 'no bryophytes' sample-group V (n = 5) encompassing all other samples lacking aquatic bryophyte vegetation.

4.5.14 Relationships between aquatic bryophyte community composition, diversity and environmental habitat conditions

Aquatic bryophyte species richness and diversity were strongly and significantly positively correlated with each other and negatively correlated to species dominance (refer to Appendix 2k). In general, aquatic bryophyte species richness and diversity were positively correlated to stable substrates (e.g. boulder-dominated morphology) and negatively correlated to the prevalence of loose streambed particles (e.g. small stones, gravel). Aquatic bryophyte species richness and diversity also exhibited a positive relationship to increasing streamwater sulphate levels, heavy metal content (e.g. lead, zinc, aluminium) and current velocity. Streamwaters influenced by base-rich geologies tended to predominated by fewer aquatic bryophyte species and were therefore negatively correlated with species richness and diversity.

4.5.15 Predicting freshwater aquatic bryophyte community composition and diversity

Several full models were developed for predicting aquatic bryophyte species diversity (H) of upland stream habitats using various combinations of environmental predictor variables (refer to Table 4.87). The selected model AqBRYOsH1a was chosen because it produced the highest r^2 value (47.5%) and gave rise to variant minimal models with similar predictive power (see Table 4.88) which quite strongly predicted the response variable, mean aquatic bryophyte species diversity of the third and final field surveys, for test data sets of the Water of Dye (Table 4.89, Figure 4.30), River Girnock (Table 4.90, Figure 4.31), and Knockan Burn (Table 4.91, Figure 4.32).

Full models	Regression equations	r^2-adj (%)	P_{value}
AqBRYOsH1a: Aquatic Bryophyte Species Diversity (H)	H = 1.62 + 0.086 (BO) - 0.201 (pH) + 0.548 (√ flow)	47.5	P<0.001***
AqBRYOsH2a: Aquatic Bryophyte Species Diversity (H)	H = 1.84 + 0.106 (BO) - 0.198 (pH)	38.5	P<0.01**
AqBRYOsH3a: Aquatic Bryophyte Species Diversity (H)	H = 0.214 + 0.100 (BO) + 0.545 (√ flow)	25.6	P<0.01**

Table 4.87 Statistically significant full models (n = 79) using combinations of environmental variable(s) for predicting freshwater aquatic bryophyte species diversity (measured as H per area sampled: 19.64 cm^2) of upland stream habitats. Model codes: H: species diversity (per 19.64 cm^2); BO: boulder cover (%); pH: streamwater pH; √ flow: √ current velocity (m s^{-1}).

Reduced (minimal) models	Regression equations	r^2-adj (%)	P_{value}
AqBRYOsH1a: Aquatic Bryophyte Species Diversity (H) excluding Water of Dye April 2006 test data set	H = 1.56 + 0.0938 (BO) - 0.196 (pH) + 0.633 (√ flow)	46.5	P<0.001***
AqBRYOsH1a: Aquatic Bryophyte Species Diversity (H) excluding River Girnock April 2006 test data set	H = 1.72 + 0.0866 (BO) - 0.209 (pH) + 0.512 (√ flow)	44.8	P<0.001***
AqBRYOsH1a: Aquatic Bryophyte Species Diversity (H) excluding Knockan Burn November 2006 test data set	H = 1.70 + 0.0604 (BO) - 0.204 (pH) + 0.574 (√ flow)	43.3	P<0.001***

Table 4.88 Statistically significant minimal models (n = 70) of AqBRYOsH1a for predicting freshwater aquatic bryophyte species diversity (H) of the Water of Dye, River Girnock and Knockan Burn test data sets. For model codes refer to Table 4.87.

Mean test data	Observed H: test data	Predicted H: reduced model AqBRYOsH1a	t-statistic	P-value
Brocky Burn (BB)	1.28	1.13	0.33	NS
Charr Flume (CF)	0.45	0.80	-1.11	NS
Bogendreip (BD)	1.02	0.96	0.08	NS
Water of Dye (WoD) April 2006	0.92	0.96	-0.22	NS

Table 4.89 Comparison of mean observed and predicted values of minimal model AqBRYOsH1a for predicting freshwater aquatic bryophyte species diversity (H) of the Water of Dye April 2006 test data set (see also Figure 4.30).

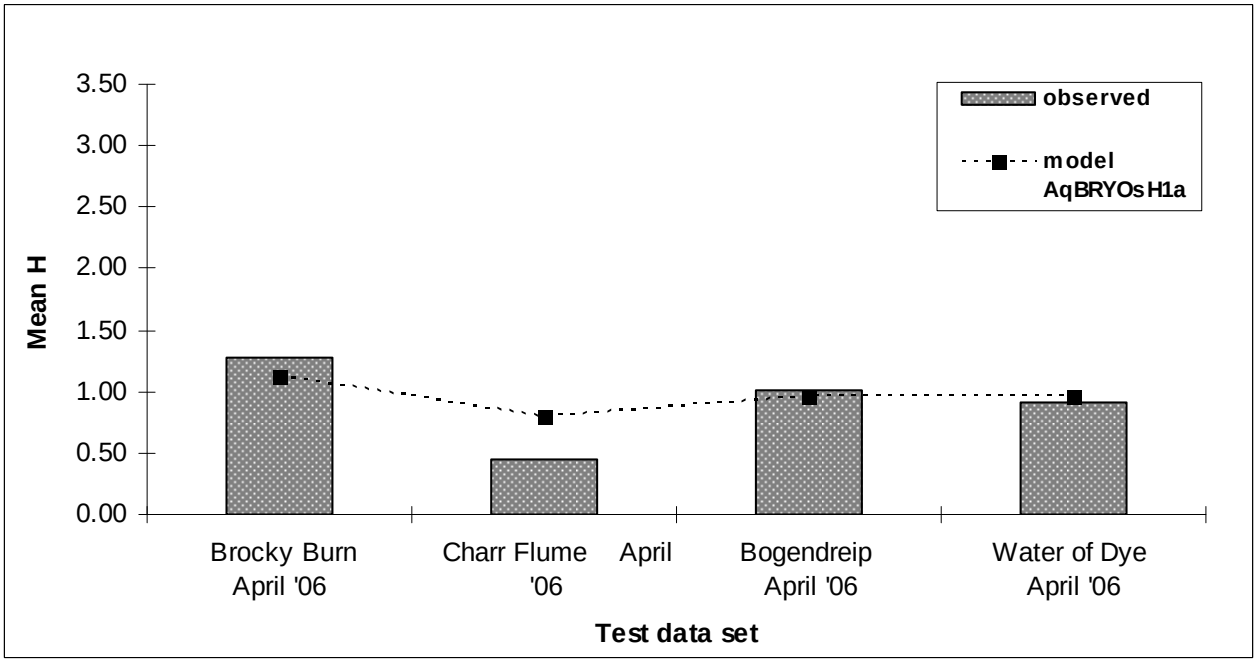


Figure 4.30 Comparison of mean observed and predicted values of minimal model AqBRYOsH1a for predicting freshwater aquatic bryophyte species diversity (H) of the Water of Dye April 2006 test data set.

Mean test data	Observed H: test data	Predicted H: reduced model AqBRYOsH1a	t-statistic	P-value
Iron Bridge (IB)	0.61	0.94	-2.23	NS
Hampshire's Bridge (HB)	0.42	0.63	-0.89	NS
Littlemill (LM)	0.71	0.66	0.14	NS
River Girnock April 2006	0.58	0.74	-1.09	NS

Table 4.90 Comparison of mean observed and predicted values of minimal model AqBRYOsH1a for predicting freshwater aquatic bryophyte species diversity (H) of the River Girnock April 2006 test data set (see also Figure 4.31).

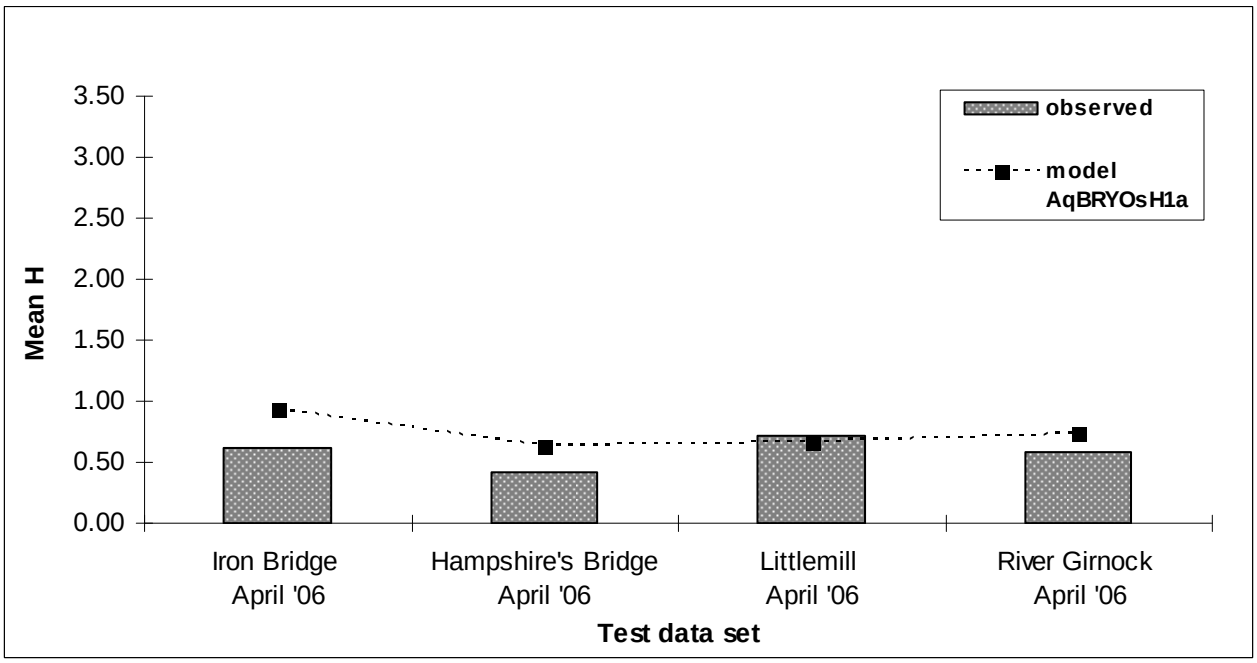


Figure 4.31 Comparison of mean observed and predicted values of minimal model AqBRYOsH1a for predicting freshwater aquatic bryophyte species diversity (H) of the River Girnock April 2006 test data set.

Mean test data	Observed H: test data	Predicted H: reduced model AqBRYOsH1a	t-statistic	P-value
Upper Knockan (UK)	0.11	0.45	-2.67	NS
Mid Knockan (KM)	0.97	0.53	0.73	NS
Lower Knockan (LK)	0.78	0.67	0.01	NS
Knockan Burn November 2006	0.62	0.55	0.05	NS

Table 4.91 Comparison of mean observed and predicted values of minimal model AqBRYOsH1a for predicting freshwater aquatic bryophyte species diversity (H) of the Knockan Burn November 2006 test data set (see also Figure 4.32).

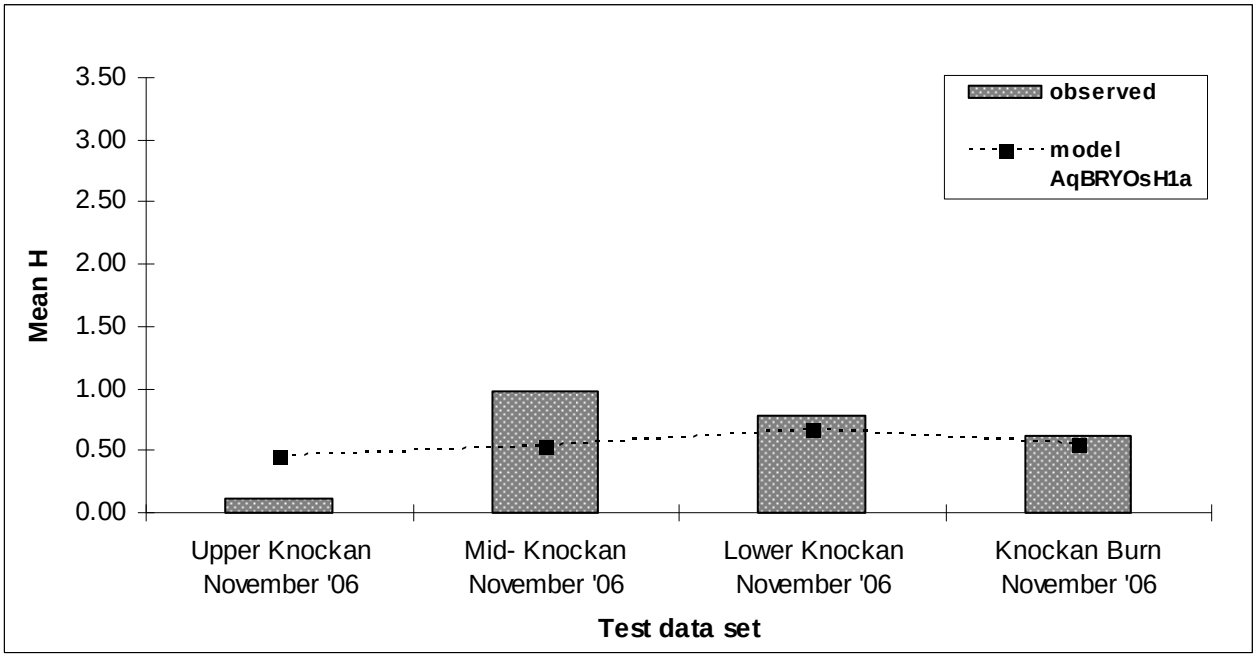


Figure 4.32 Comparison of mean observed and predicted values of minimal model AqBRYOsH1a for predicting freshwater aquatic bryophyte species diversity (H) of the Knockan Burn November 2006 test data set.

4.5.16 Variation in vascular submerged macrophyte community composition and diversity in the Knockan Burn sub-catchment and its sites

Four species of vascular submerged macrophytes were found to occur in Knockan Burn: *Potamogeton polygonifolius* Pourret, *Eleogiton fluitans* (L.), *Ranunculus flammula* L., and *Myriophyllum alterniflorum* DC. One macrophytic characean algal species *Chara globularis* var. *globularis* Thuill., was also identified from this sub-catchment stream and integrated with the vascular plant dataset as it is often referred to in literature dealing specifically with aquatic macrophytes (e.g. Haslam 1978, 2006). Furthermore, *Chara* was readily distinguishable by the naked eye, unlike other forms of macroalgae included in this project which required comprehensive microscopic analysis to obtain their identification (Whitton 1975). Furthermore, in this particular study, the charophyte grew submerged amongst stands of other aquatic macrophytes and could not be easily separated from vascular plants. Refer to Table 4.92 for listed aquatic macrophyte flora.

Of the samples analysed for this component of the project, the majority lacked the presence of aquatic macrophyte vegetation. This indicated that few samples (approximately between 7.4 – 12.7%) actually contained submerged macrophytes meaning that a minor proportion of the streambeds sampled in this project were occupied by aquatic macrophyte vegetation. This makes submerged macrophytes the least abundant stream producer of the study.

There were significant differences in vascular submerged macrophyte species richness and diversity between the three sub-catchment streams (Table 4.93). Vascular submerged macrophytes occurred only in Knockan Burn and furthermore, community composition varied between the upper and lower study sites (refer to Table 4.94 for details). Please note that species dominance could not be calculated by the software package due to the limited data set available for vascular submerged macrophytes in this study and will therefore not be further referred to in this section.

Aquatic macrophyte species	Synonym(s)	Family
[1]*Eleogiton fluitans* (L.)	-	Cyperaceae
[1]*Myriophyllum alterniflorum* DC.	-	Haloragaceae
[1]*Potamogeton polygonifolius* Pourret	-	Potamogetonaceae
[1]*Ranunculus flammula* L.	-	Ranunculaceae
[2]*Chara globularis* var. *globularis* Thuill.	-	Characeae

Table 4.92 Aquatic macrophyte species list: [1]Vascular submerged macrophyte (Haslam 1975); [2]Macrophytic characean alga (Moore 1986).

Variable	Water of Dye		River Girnock		Knockan Burn		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Vascular submerged macrophyte species richness: **S**	0.00		0.00		1.33		P<0.001***
Vascular submerged macrophyte species diversity: **H**	0.00		0.00		0.35		P<0.01**

Table 4.93 Mean values (± 1 standard error) of normally distributed plant variables (including zero values, and data back-transformed where necessary): vascular submerged macrophyte species richness (per 400 cm^2), and vascular submerged macrophyte species diversity (per 400 cm^2) between study stream sub-catchments (n = 79 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values are quoted for non-normal variables compared using Kruskal-Wallis test. For details of environmental habitat conditions refer to Chapter 3, Table 3.93.

	Water of Dye						River Girnock						Knockan Burn						
Variable	**BB**		**CF**		**BD**		**IB**		**HB**		**LM**		**UK**		**MK**		**LK**		**P**ANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Vascular submerged macrophyte species richness: **S**	0.0		0.0		0.0		0.0		0.0		0.0		3.00		0.0		1.00		P<0.001***
Vascular submerged macrophyte species diversity: **H**	0.0		0.0		0.0		0.0		0.0		0.0		1.05		0.0		0.00		P<0.001***

Table 4.94 Mean values (± 1 standard error) of normally distributed plant variables (including zero values, and data back-transformed where necessary): vascular submerged macrophyte species richness (per 400 cm^2), and vascular submerged macrophyte species diversity (per 400 cm^2) between sampling sites (n = 79 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values are quoted for non-normal variables compared using Kruskal-Wallis test. For details of environmental habitat conditions refer to Chapter 3, Table 3.94.

4.5.17 Seasonal variation in vascular submerged macrophyte community composition and diversity in Knockan Burn

Although overall there appeared to be a peak in vascular submerged macrophyte richness and diversity in September 2006 compared to April and November 2006, these differences in the Knockan Burn sub-catchment between dates surveyed were not found to be significant (Table 4.95).

Variable	April 2006		September 2006		November 2006		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Vascular submerged macrophyte species richness: **S**	1.00		2.00		1.00		NS
Vascular submerged macrophyte species diversity: **H**	0.25		0.50		0.30		NS

Table 4.95 Mean values (± 1 standard error) of normally distributed plant variables (including zero values, and data back-transformed where necessary): vascular submerged macrophyte species richness (per 400 cm^2), and vascular submerged macrophyte species diversity (per 400 cm^2) between sampling dates in the Knockan Burn sub-catchment (n = 16 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values are quoted for non-normal variables compared using Kruskal-Wallis test. For details of environmental habitat conditions refer to Chapter 3, Table 3.95.

4.5.18 Response of vascular submerged macrophyte community composition and diversity in Knockan Burn to variation in flow regime: pool, glide and riffle zones

There was a significant response of vascular submerged macrophyte community composition to flow regime in the Knockan Burn sub-catchment (Table 4.96). Most notably vascular submerged macrophyte vegetation was lacking from fast-flowing, riffle habitats. Further, it may be possible to interpret that vascular submerged macrophyte richness and diversity did not vary significantly between pools and glides, but was significantly higher in glide habitats than in riffle zones.

Variable	Pool		Glide		Riffle		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Vascular submerged macrophyte species richness: **S**	1.00		3.00		0.00		P<0.05*
Vascular submerged macrophyte species diversity: **H**	0.30		0.75		0.00		P<0.05*

Table 4.96 Mean values (± 1 standard error) of normally distributed plant variables (including zero values, and data back-transformed where necessary): vascular submerged macrophyte species richness (per 400 cm^2), and vascular submerged macrophyte species diversity (per 400 cm^2) between flow regime (pool, glide, riffle habitats) in the Knockan Burn sub-catchment (n = 16 samples). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values are quoted for non-normal variables compared using Kruskal-Wallis test. For details of environmental habitat conditions refer to Chapter 3, Table 3.96.

4.5.19 Response of vascular submerged macrophyte community composition and diversity in Knockan Burn to variation in substrate morphology

In general, vascular submerged macrophyte richness and diversity were negatively correlated with streambeds characterised by a predominance of coarse substrate particles (e.g. high boulder cover), and tended to increase as substrate composition was replaced with increasing proportions of fine substrate particles (Table 4.97 – Table 4.101, inclusive).

Variable	0% BO		3% BO		15.5% BO		38% BO		63% BO		88% BO		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Vascular submerged macrophyte species richness: **S**	4.00		4.00		0.00		0.00		0.00		0.00		P<0.01**
Vascular submerged macrophyte species diversity: **H**	1.10		1.00		0.00		0.00		0.00		0.00		P<0.01**

Table 4.97 Mean values (± 1 standard error) of normally distributed plant variables (including zero values, and data back-transformed where necessary): response of vascular submerged macrophyte species richness (per 400 cm^2), and vascular submerged macrophyte species diversity (per 400 cm^2) to variation in the abundance (median % cover) of boulders (BO) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 79). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values are quoted for non-normal variables compared using Kruskal-Wallis test. For details of environmental habitat conditions refer to Chapter 3, Table 3.97.

Variable	0% LS		3% LS		15.5% LS		38% LS		63% LS		88% LS		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Vascular submerged macrophyte species richness: **S**	4.00		4.00		0.00		0.00		0.00		0.00		P<0.01**
Vascular submerged macrophyte species diversity: **H**	1.15		0.95		0.00		0.00		0.00		0.00		P<0.01**

Table 4.98 Mean values (± 1 standard error) of normally distributed plant variables (including zero values, and data back-transformed where necessary): response of vascular submerged macrophyte species richness (per 400 cm^2), and vascular submerged macrophyte species diversity (per 400 cm^2) to variation in the abundance (median % cover) of large stones (LS) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 79). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values are quoted for non-normal variables compared using Kruskal-Wallis test. For details of environmental habitat conditions refer to Chapter 3, Table 3.98.

Variable	0% SS		3% SS		15.5% SS		38% SS		63% SS		88% SS		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Vascular submerged macrophyte species richness: **S**	0.00		0.00		0.00		1.00		4.00		3.00		P<0.01**
Vascular submerged macrophyte species diversity: **H**	0.00		0.00		0.00		0.50		0.90		0.70		P<0.05*

Table 4.99 Mean values (± 1 standard error) of normally distributed plant variables (including zero values, and data back-transformed where necessary): response of vascular submerged macrophyte species richness (per 400 cm^2), and vascular submerged macrophyte species diversity (per 400 cm^2) to variation in the abundance (median % cover) of small stones (SS) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 79). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values are quoted for non-normal variables compared using Kruskal-Wallis test. For details of environmental habitat conditions refer to Chapter 3, Table 3.99.

Variable	0% GR		3% GR		15.5% GR		38% GR		63% GR		88% GR		P_{ANOVA}
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Vascular submerged macrophyte species richness: **S**	0.00		0.00		0.00		3.00		4.00		2.00		P<0.05*
Vascular submerged macrophyte species diversity: **H**	0.00		0.00		0.00		0.65		1.00		0.45		P<0.05*

Table 4.100 Mean values (± 1 standard error) of normally distributed plant variables (including zero values, and data back-transformed where necessary): response of vascular submerged macrophyte species richness (per 400 cm^2), and vascular submerged macrophyte species diversity (per 400 cm^2) to variation in the abundance (median % cover) of gravel (GR) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 79). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values are quoted for non-normal variables compared using Kruskal-Wallis test. For details of environmental habitat conditions refer to Chapter 3, Table 3.100.

Variable	0% SA		3% SA		15.5% SA		38% SA		63% SA		88% SA		PANOVA
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	
Vascular submerged macrophyte species richness: **S**	0.00		0.00		0.00		1.00		4.00		3.00		P<0.01**
Vascular submerged macrophyte species diversity: **H**	0.00		0.00		0.00		0.20		1.00		0.90		P<0.01**

Table 4.101 Mean values (± 1 standard error) of normally distributed plant variables (including zero values, and data back-transformed where necessary): response of vascular submerged macrophyte species richness (per 400 cm^2), and vascular submerged macrophyte species diversity (per 400 cm^2) to variation in the abundance (median % cover) of sand (SA) from amalgamated sub-catchment data (the Water of Dye, River Girnock and Knockan Burn, n = 79). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values are quoted for non-normal variables compared using Kruskal-Wallis test. For details of environmental habitat conditions refer to Chapter 3, Table 3.101.

4.5.20 Vascular submerged macrophyte community composition, diversity and environmental habitat conditions in Knockan Burn as determined by TWINSPAN classification

TWINSPAN analysis of the vascular submerged macrophyte data set suggested two community types were present in the Knockan Burn sub-catchment stream (refer to Figure 4.33).

Community Type I (n = 5 samples; UKAPP06, UKAPG06, UKSMP06, UKSMG06, UKNVG06: for key to sample codes see caption to Figure 4.27). Group I was restricted to the upper realm of Knockan Burn, and was strongly separated from Group II with an eigenvalue of 0.928 at level 1 of the classification. This assemblage represented a moderately diverse aquatic macrophyte community indicated by the presence of *Potamogeton polygonifolius*, co-occurring with several associated species including *Chara globularis* var. *globularis*, *Eleogiton fluitans* and *Ranunculus flammula*.

Community Type II (n = 5 samples: LKAPP06, LKAPG06, LKSMG06, LKNVP06, LKNVG06). Group II was a low diversity assemblage found only in the lower section of Knockan Burn, characterised by a predominance of *Myriophyllum alterniflorum* and mostly the absence of other aquatic macrophyte species.

CCA analysis was not performed due to the insufficient data set available. However, it was possible to perform one-way ANOVA on the environmental characteristics of TWINSPAN groups I and II, as well as a third sample-group (III) which encompassed the remainder of samples that did not possess vascular submerged macrophyte vegetation (refer to Table 4.102). This enabled me to address the following questions: "*What environmental variables drive the distribution of these two vascular submerged macrophyte assemblages in Knockan Burn*?" and, "*Why are the majority of samples across the three study streams, distinctly lacking in aquatic macrophyte vegetation*?" Substrate morphology factors (prevalence of small-sized particles) coupled to streamwater physico-chemical properties (e.g. pH, buffering capacity etc.), which are themselves influenced by the underlying basic geology, showed

significant inter-group differences (Table 4.102) as did species diversity (Figure 4.34) and most probably act as the principal drivers determining the occurrence and composition of vascular submerged macrophyte communities in near-pristine upland streams.

Although assemblages I and II shared some common habitat characteristics, there appeared to be significant differences in ecological preferences of the vascular submerged macrophyte species comprising the two communities therein.

The Group I community occurred in a stream habitat characterised almost entirely by an abundance of fine substrate particles (e.g. small cobbles, gravel and sand) and hear-homogenous base-rich geology (e.g. Durness limestone). Streamwaters experienced moderate shade, and were generally well-buffered, of pH <7.5, with moderate concentrations of heavy metals and base cations.

The Group II community was principally associated with a streambed substrate composition strewn with fine substrate particles (similar to Group I) but that also contained some coarser materials and less sand. Underlying geology corresponded partly to that of Group I but generally was of mixed composition and more abundant in highly calcareous strata (e.g. An-t'Sron). Consequently, streamwaters were extremely well-buffered with a pH >7.5, coupled to inherently low sulphate and heavy metal levels, high base cation content, and riparian shade was least compared to the other two sample-groups.

Group III represents the sample-group entirely lacking vascular submerged macrophyte flora. Most notably, the samples in this group were characterised by a hard resistant geology (e.g. granite) and streambed morphologies predominated by coarse substrates (e.g. boulders, cobbles) in which small-sized particles (e.g. gravel, sand) were scarce. Streamwaters were inherently acid-sensitive: pH <7, of elevated sulphate and heavy metal content together with low conductivity, alkalinity and abundance of base cations. Heavy shade pressure from riparian vegetation was experienced in parts.

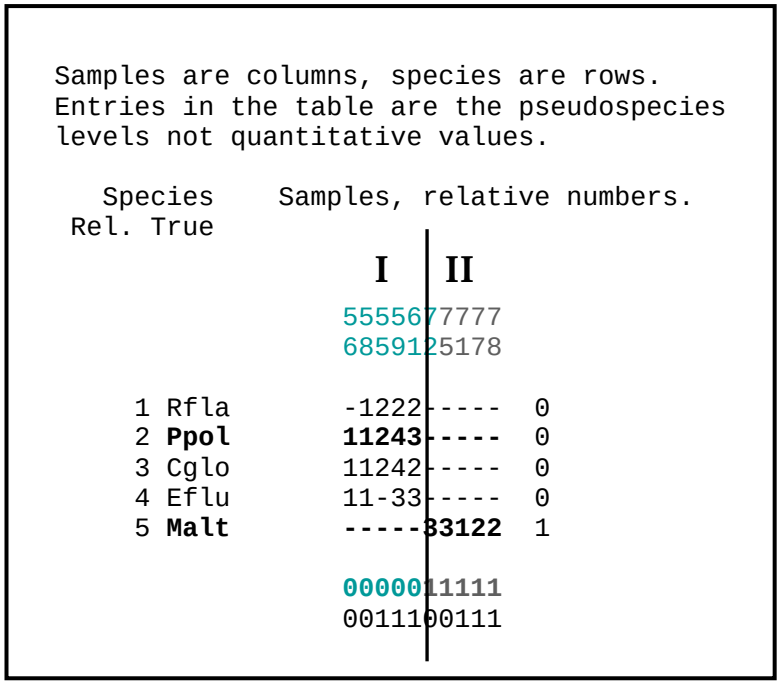


Figure 4.33 TWINSPAN output depicting 10 samples and 2 vascular submerged macrophyte species assemblages, with indicator species highlighted in bold font and colour-coding as appropriate for TWINSPAN sample-groups I (teal), and II (grey). Vascular submerged macrophyte species codes: *Ranunculus flammula* (Rfla), *Potamogeton polygonifolius* (Ppol), *Chara globularis* var. *globularis* (Cglo), *Eleogiton fluitans* (Eflu) and *Myriophyllum alterniflorum* (Malt).

	TWINSPAN sample-group						
Variable	**I**		**II**		**III**		**PANOVA**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Vascular submerged macrophyte species richness: **S**	3.00		1.00		0.0		P<0.001***
Vascular submerged macrophyte species diversity: **H**	1.10		0.00		0.00		P<0.001***
Substrate diversity (Simpson's D)	3.93[a]	0.32	3.96[a]	0.21	3.11[b]	0.08	P<0.01**
Hydromorphological diversity (D)	4.02[a]	0.32	4.04[a]	0.21	3.21[b]	0.08	P<0.001***
Substrate dominance (Berger-Parker)	0.33[a]	0.02	0.35[a]	0.02	0.45[b]	0.02	P<0.01**
% Granite	0.0[a]	0.00	0.0[a]	0.00	57.6[b]	4.18	P<0.001***
% Granodiorite	0.0	0.00	0.0	0.00	3.5	0.61	NS
% Diorite	0.0	0.00	0.0	0.00	0.1	0.04	NS
% Mica Schist	0.0	0.00	0.0	0.00	4.5	0.95	NS
% Amphibolite	0.0	0.00	0.0	0.00	3.0	0.63	NS
% Serpentinite	0.0	0.00	0.0	0.00	0.4	0.08	NS
% QP	0.0	0.00	0.0	0.00	0.3	0.07	NS
% DA	0.0	0.00	0.0	0.00	0.9	0.19	NS
% QPP	0.0	0.00	0.0	0.00	3.7	0.77	NS
% Limestone	0.0	0.00	0.0	0.00	2.9	0.65	NS
% Durness Limestone	70.0[a]	0.00	50.0[b]	0.00	18.2[c]	4.34	P<0.001***
% Eriboll Sandstone	10.0[a]	0.00	20.0[b]	0.00	1.5[c]	0.59	P<0.001***
% Moine Schist	20.0[a]	0.00	0.0[b]	0.00	0.6[b]	0.41	P<0.001***
% Applecross Formation	0.0[a]	0.00	20.0[b]	0.00	1.2[a]	0.57	P<0.001***
% An-t'Sron	0.0[a]	0.00	10.0[b]	0.00	0.6[a]	0.28	P<0.001***
% Boulders	7.3[a]	3.92	34.7[b]	8.96	59.7[c]	2.43	P<0.001***
% Large Stones	26.6[a]	6.96	23.9[a]	4.72	37.8[b]	2.18	P<0.01**
% Small Stones	31.2[ab]	6.43	43.8[a]	2.59	25.8[b]	1.73	P<0.05*
% Gravel	37.5[a]	5.85	33.2[a]	4.27	22.2[b]	2.26	P<0.01**
% Sand	30.2[a]	4.43	13.5[b]	6.78	3.2[c]	1.49	P<0.001***
D (m)	0.17	0.34	0.20	0.13	0.14	0.08	NS
K (m^{-1})	2.96	0.74	2.23	0.68	2.73	0.18	NS
$Z_{eu}^{3\%}$ (m)	1.36	0.81	1.82	0.86	1.26	0.21	NS
Z_{eu}:$D^{3\%}$	7.55	0.65	9.29	0.57	7.26	0.22	NS

pH	7.31[a]	0.03	7.87[b]	0.09	6.84[c]	0.09	P<0.001***
Conductivity (μS cm^{-1})	115.0[a]	0.11	201.9[b]	0.09	58.6[c]	0.07	P<0.001***
Alkalinity (mg l^{-1})	46.6[a]	14.78	79.8[b]	14.04	14.2[c]	6.22	P<0.001***
Water Temperature (°C)	8.6	0.34	9.3	0.25	9.1	0.12	NS
Flow (m s^{-1})	0.170	0.18	0.215	0.16	0.261	0.06	NS
% Shade	13.6[ab]	6.16	1.8[a]	0.25	22.6[b]	2.97	P<0.05*
Height of Riparian Vegetation (m)	0.37[a]	0.28	0.02[a]	0.02	2.48[b]	0.44	P<0.01**
NH_3-N (mg l^{-1})	<0.04		<0.04		<0.04		NS
NO_3-N (mg l^{-1})	<0.01		<0.01		<0.01		NS
PO_4-P (mg l^{-1})	<0.003		<0.003		<0.003		NS
Cl (mg l^{-1})	12.5[a]	0.77	12.9[a]	0.72	9.5[b]	0.30	P<0.001***
SO_4 (mg l^{-1})	0.10[a]	0.00	0.20[a]	0.00	1.45[b]	0.17	P<0.001***
Cd (μg l^{-1})	0.02	0.00	0.02	0.00	0.03	0.01	NS
Cr (μg l^{-1})	0.14	0.02	0.12	0.00	0.19	0.04	NS
Cu (μg l^{-1})	0.13	0.63	0.08	0.35	0.20	0.12	NS
Pb (μg l^{-1})	0.10[ab]	0.41	0.05[a]	0.00	0.21[b]	0.14	P<0.05*
Ni (μg l^{-1})	0.29	0.49	0.26	0.30	0.26	0.09	NS
Zn (μg l^{-1})	1.65[ab]	0.45	0.79[a]	0.00	1.96[b]	0.08	P<0.05*
Al (μg l^{-1})	54.8[ab]	0.38	32.4[a]	0.06	76.0[b]	0.08	P<0.05*
V (μg l^{-1})	0.20[ab]	0.37	0.12[a]	0.10	0.25[b]	0.08	P<0.05*
As (μg l^{-1})	0.59	0.00	0.59	0.00	0.51	0.03	NS
Na (mg l^{-1})	5.71	0.43	6.17	0.39	5.10	0.13	NS
K (mg l^{-1})	0.57	0.27	0.50	0.14	0.49	0.04	NS
Ca (mg l^{-1})	8.95[a]	0.23	16.37[b]	0.15	3.48[c]	0.09	P<0.001***
Mg (mg l^{-1})	5.73[a]	0.27	9.89[b]	0.15	1.46[c]	0.11	P<0.001***
Fe (mg l^{-1})	0.17	0.32	0.13	0.21	0.24	0.08	NS
Mn (mg l^{-1})	0.005	0.16	0.006	0.16	0.012	0.10	NS

Table 4.102 Mean values (± 1 standard error) of normally distributed (including zero values, and data back-transformed where necessary) vascular submerged macrophyte species richness per 400 cm^2 (n = 79), vascular submerged macrophyte species diversity per 400 cm^2 (n = 79), and environmental habitat variables (n = 79) between TWINSPAN sample-groups I (n = 5) and II (n = 5), with the non-vascular submerged macrophyte sample-group III (n = 69) encompassing all other samples lacking aquatic macrophyte vegetation. Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values are quoted for non-normal variables compared using Kruskal-Wallis test.

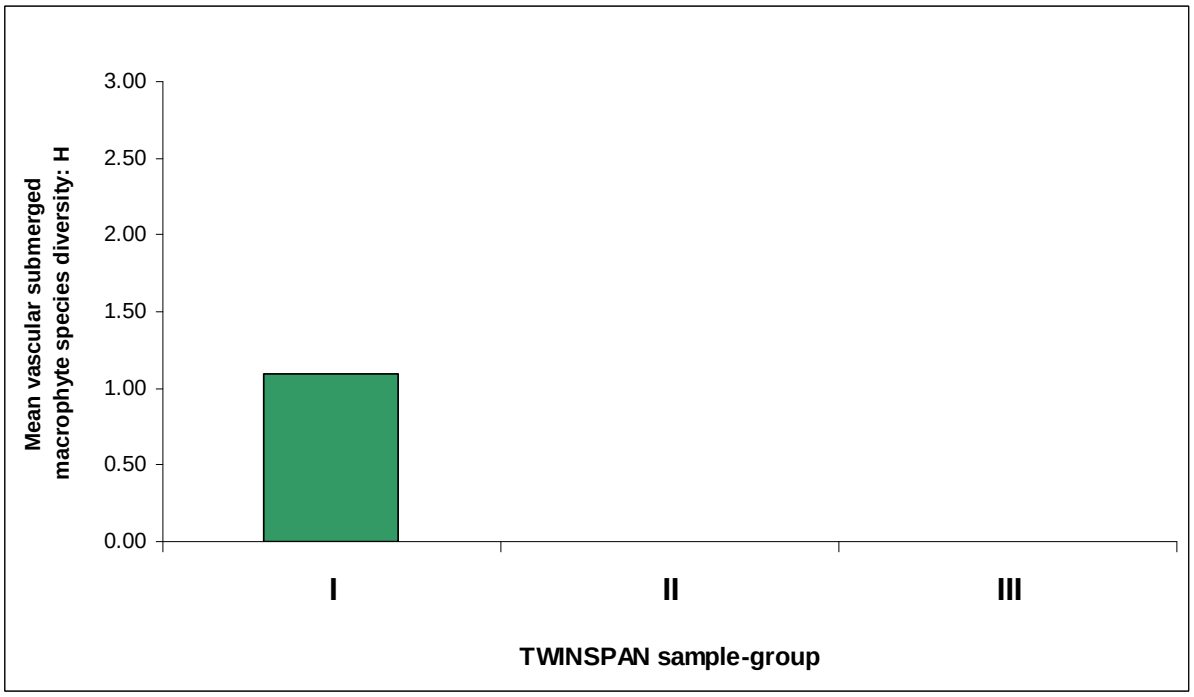


Figure 4.34 Comparison of mean values (± 1 standard error) of normally distributed (including zero values, and data back-transformed where necessary) vascular submerged macrophyte species diversity per 400 cm^2 (n = 79) between TWINSPAN sample-groups I (n = 5) and II (n = 5), with the non-vascular submerged macrophyte sample-group III (n = 69) encompassing all other samples lacking aquatic macrophyte vegetation.

4.5.21 Relationships between vascular submerged macrophyte community composition, diversity and environmental habitat conditions

Vascular submerged macrophyte species richness and diversity were strongly and significantly positively correlated with each other (see Appendix 2l). Also vascular submerged macrophyte species richness and diversity tended to be positively correlated with well-buffered base-rich streamwaters characterised by a pebbled substrate composition, particularly abundant in fine gravelly and sandy particles. Vascular submerged macrophytes tended to be entirely absent and therefore negatively correlated with streambeds characterised by hard, impenetrable substrate morphologies (e.g. boulders, largely cobbled) resultant of the resistant base-poor, and often acid-sensitive geologies (e.g. granite) which formed them.

4.5.22 Predicting freshwater vascular submerged macrophyte community composition and diversity

The limited data set available for vascular submerged macrophyte vegetation (n = 10 samples) meant that multiple regression could not be performed and conclusions could only be drawn from correlations undertaken with environmental variables (refer back to 4.5.21).

It is most probable that substrate morphology factors (prevalence of small-sized particles) coupled to streamwater physico-chemical properties (e.g. pH, buffering capacity etc.), which are direct products of underlying geology, would be the principal drivers determining the occurrence and composition of vascular submerged macrophyte communities in upland streams.

4.5.23 The three-tier approach to characterising upland stream habitat conditions by combining freshwater vegetation assemblages: periphyton, aquatic bryophyte and vascular submerged macrophyte community composition and diversity

To determine potential environmental drivers controlling differences in the structural response of freshwater vegetation assemblages, an integrated three-tier approach was utilised to characterise variation in community composition and diversity of freshwater vegetation in relation to stream habitat conditions by combining three groups of aquatic plants: periphyton, aquatic bryophytes and (where present) vascular submerged macrophytes.

4.5.23.1 Freshwater vegetation community composition, diversity and environmental habitat conditions as determined by multivariate ordination and TWINSPAN classification

Three major communities (plus two component sub-assemblages) were identified from TWINSPAN classification of freshwater vegetation data set (n = 79) harvested from naturally-occurring mineral substrata during the field survey campaigns: Figure 4.35. These were mainly indicated by the presence of *Fragilaria pulchella* (Group I), *Gomphonema acuminatum* and *Blindia acuta* (Group II), and *Frustulia rhomboides* var. *rhomboides* (Group III). The three sample-groups overlapped partially in the centre of the CCA diagram where several commonly occurring epilithic periphyton species (e.g. *Achnanthidium minutissima*, Amin; *Fragilaria capucina* var. *gracilis*, Fcgr; *Fragilaria capucina* var. *vaucheriae*, Fcva; *Synedra ulna*, Suln; *Tabellaria flocculosa*, Tfloc; *Mougeotia* sp., Moug; and *Ulothrix* sp., Ulox) and aquatic bryophyte species (e.g. *Fontinalis antpyretica*, Fant) ubiquitous to almost all samples, were ordinated (refer to Figure 4.36).

Community Type I (n = 25 samples: UKAPP06, UKAPG06, UKAPR06, UKSMP06, UKSMG06, UKNVP06, UKNVG06, MKAPP06, MKAPG06, MKAPR06, MKSMP06,

MKSMG06, MKSMR06, MKNVP06, MKNVG06, MKNVR06, LKAPP06, LKAPG06, LKAPR06, LKSMP06, LKSMG06, LKSMR06, LKNVP06, LKNVG06, LKNVR06: for key to sample codes see caption to Figure 4.36). The Group I freshwater vegetation community type represented the Knockan Burn sub-catchment stream and was indicated by an abundance of the diatom *Fragilaria pulchella* in every sample. However, several other diatom species were considered as co-dominants of this particular TWINSPAN sample-group: *Cocconeis placentula*, *Didymosphenia geminata*, *Cymbella lanceolata, Gomphonema olivaceum* and *Gomphonema olivaceum* var. *olivaceoides*. The rhodophyte, *Batrachospermum* sp. was also quite abundant in occurrence. Four aquatic bryophytes species were exclusive to the Group I community: *Fissidens adianthoides*, *Hygrohypnum luridum*, *Palustriella falcata*, and *Chiloscyphus polyanthus*, as were the five macrophytes described previously (refer back to section 4.5.20). At level 1 of the TWINSPAN classification, an eigenvalue of 0.476 split this assemblage from the other two sample-groups.

Community Type II (n = 21 samples: IBAUP05, IBAUG05, IBAUR05, HBMYP05, HBMYG05, HBMYR05, HBAUP05, HBAUG05, HBAUR05, HBAPP06, HBAPG06, HBAPR06, LMMYP05, LMMYG05, LMMYR05, LMAUP05, LMAUG05, LMAUR05, LMAPP06, LMAPG06, LMAPR06). Species indicators of this TWINSPAN sample-group were *Gomphonema acuminatum* (diatom) and *Blindia acuta* (aquatic bryophyte). The occurrence of this community type was confined mostly to Hampshire's Bridge and Littlemill in the River Girnock, separated from Group III with an eigenvalue of 0.332 (at level 2 of the classification).

Community Type III (n = 33 samples: BBMYP05, BBMYG05, BBMYR05, BBAUP05, BBAUG05, BBAUR05, BBAPP06, BBAPG06, BBAPR06, CFMYP05, CFMYG05, CFMYR05, CFAUP05, CFAUG05, CFAUR05, CFAPP06, CFAPG06, CFAPR06, BDMYP05, BDMYG05, BDMYR05, BDAUP05, BDAUG05, BDAUR05, BDAPP06, BDAPG06, BDAPR06, IBMYP05, IBMYG05, IBMYR05, IBAPP06, IBAPG06, IBAPR06). This community type was indicated by a predominance of the diatom *Frustulia rhomboides* var. *rhomboides* in almost every sample, characterising the Water of Dye

and usually also, Iron Bridge in the upper River Girnock. Additional inspection of the TWINSPAN output suggested that Group III could be further divided into two composite ecological sub-assemblages, namely IIIa (n = 9) and IIIb (n = 24) from an eigenvalue of 0.225 at level 3 of the classification. In doing so, it could be determined that sub-assemblage IIIa (Brocky Burn) was indicated by a high abundance of *Frustulia rhomboides* var. *rhomboides* and the aquatic liverwort, *Scapania undulata*, and almost exclusively characterised Brocky Burn of the upper Water of Dye. As the underlying environmental gradient(s) progressed, species turnover and shifts in community composition resulted in the development of sub-assemblage IIIb, which largely encompassed Charr Flume, Bogendreip and Iron Bridge and tended to be indicated by the presence of the aquatic moss, *Hygrohypnum ochraceum* and often the diatom, *Gomphonema clavatum*.

Gradually, the advancing sub-assemblage IIIb began to exhibit an overlapping community structure with that of Group II. Similarly, as Group II progressed in response the underlying environmental driver(s), the extreme ends of the species composition began to bear resemblance to those characterising the ecological community of Group I.

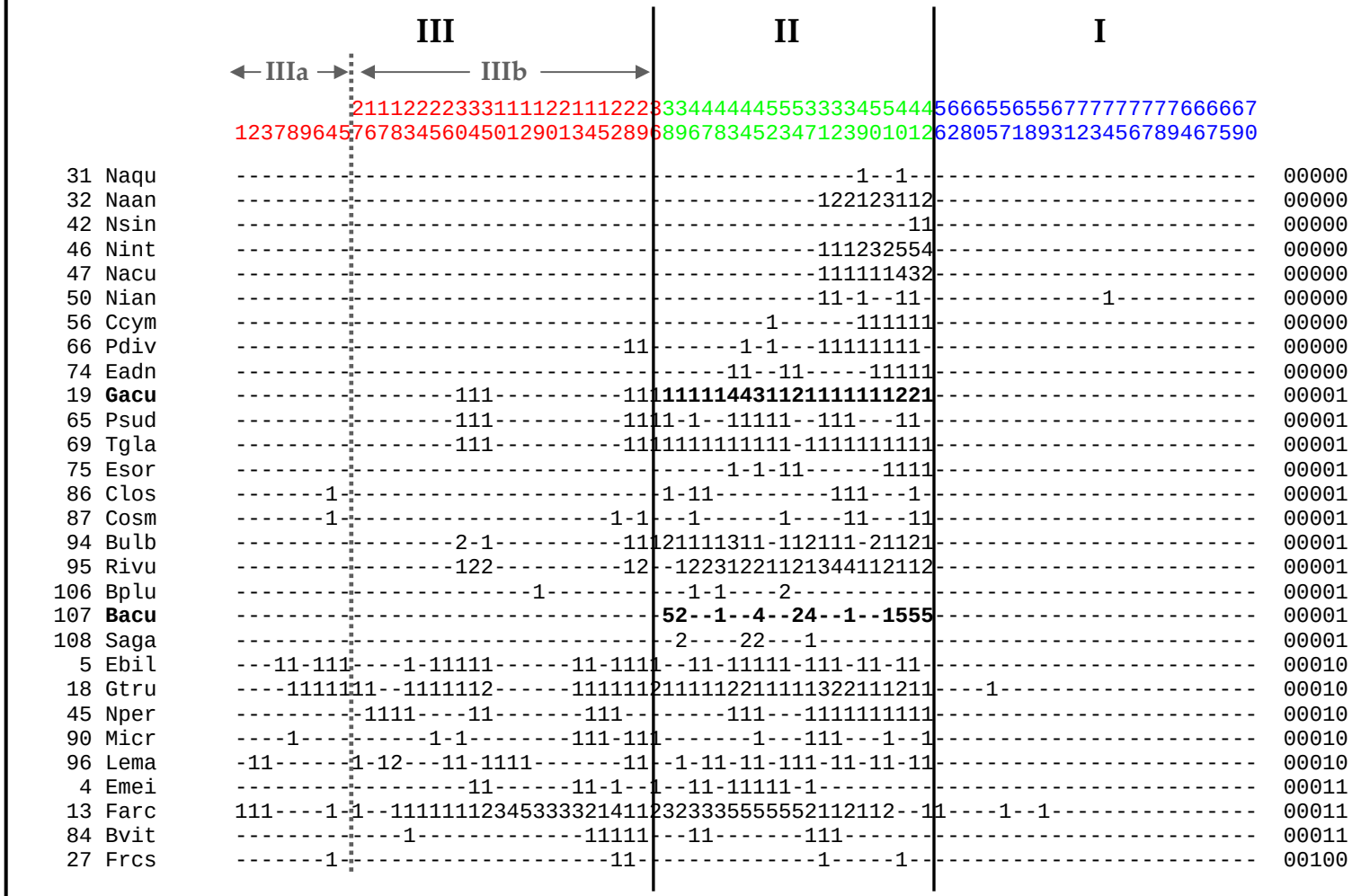
CCA ordination of the 119 freshwater vegetation species (85 diatom species, 12 other algal genera, 17 aquatic bryophytes and 5 submerged macrophytes) constrained by the fifty-four environmental variables used in the analysis suggested that streamwater pH, conductivity, alkalinity and water chemistry constituents (heavy metals vs. base cations) were the principal drivers structuring species composition of freshwater vegetation (Figure 4.37) and showed significant inter-group differences between the samples comprising each community type (Table 4.103). Substrate morphology factors may also have played an important role in structuring the freshwater vegetation communities, especially for aquatic bryophytes. The diversity of freshwater vegetation also varied significantly between the sample-groups (see Table 4.103, and also Figure 4.38) and their respective sub-assemblages (see Table 4.104, and also Figure 4.39). Together, CCA output (Figure 4.37), ANOVA and

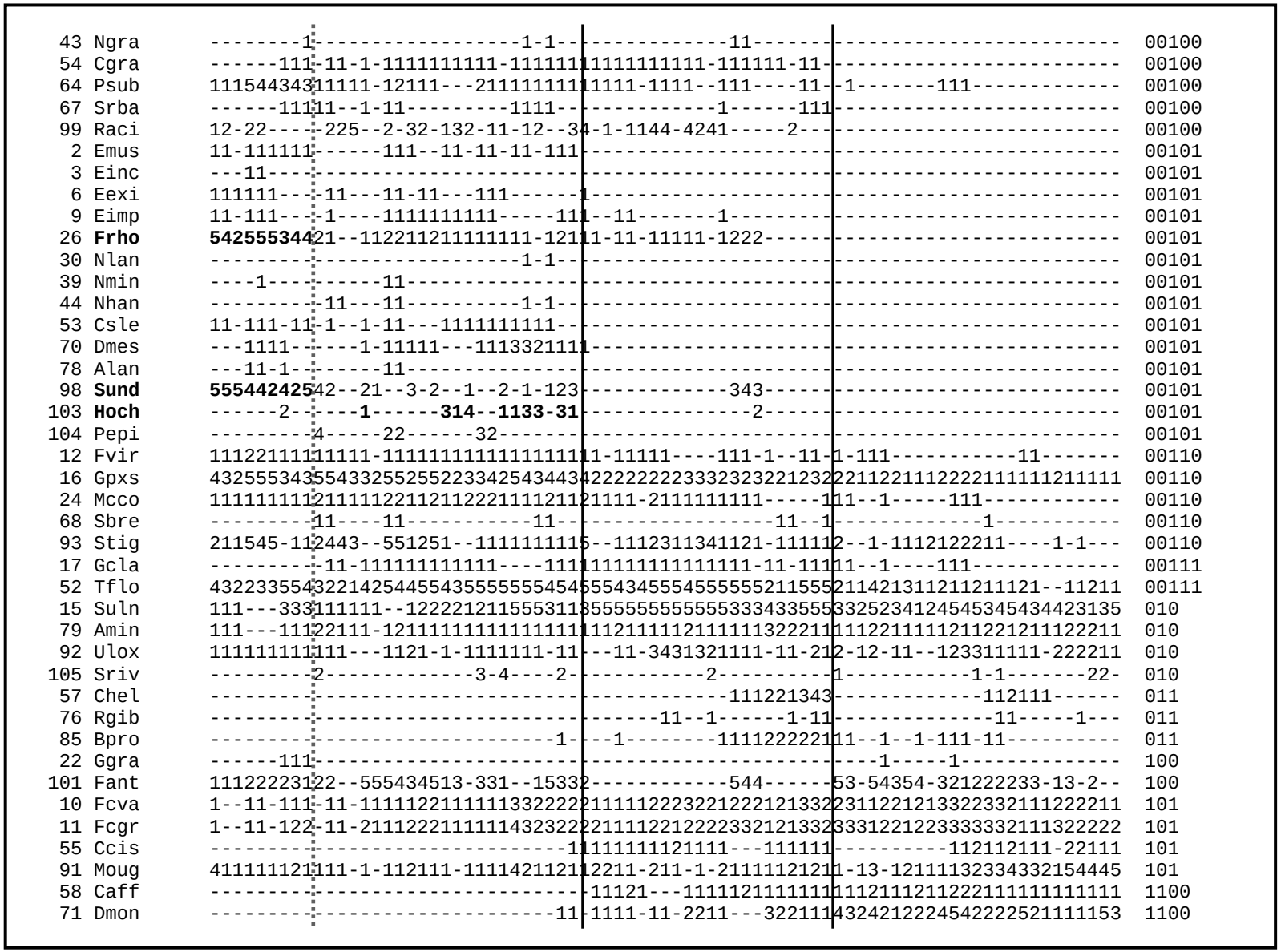
Tukey's pairwise comparisons of mean environmental data for the sample-units comprising each TWINSPAN sample-group (Table 4.103) and sub-assemblage (Table 4.104) indicated that first and foremost in near-pristine reference habitat conditions, underlying geological composition was the overriding factor constraining the community composition and diversity of freshwater vegetation by pre-determining stream substrate morphology and water (physico-) chemistry attributes, affecting the ecological preferences of individual species and their predisposed response to form certain assemblages associated with a given set of environmental habitat conditions.

The Group I assemblage exhibited a distinct preference for near-pristine base-rich habitat conditions, and generally occurred in streamwaters with a pH >7, of high buffering capacity and bed littered with petite substrate particles. In contrast, the Group III community type characterised near-pristine base-poor and inherently acid-sensitive habitat conditions, associated with predominantly robust substrate morphology and streamwaters of pH <7 with accentuated sulphate and heavy metal (especially lead, zinc and aluminium) content. The Group II community usually occurred in near-pristine circumneutral habitat conditions, with moderately-well buffered streamwaters containing curbed levels of sulphate, heavy metals and base cations (compared to Group III), and highly cobbled substrate morphology.

Samples are columns, species are rows.
Entries in the table are the pseudospecies levels not quantitative values.

Species Samples, relative numbers.
Rel. True





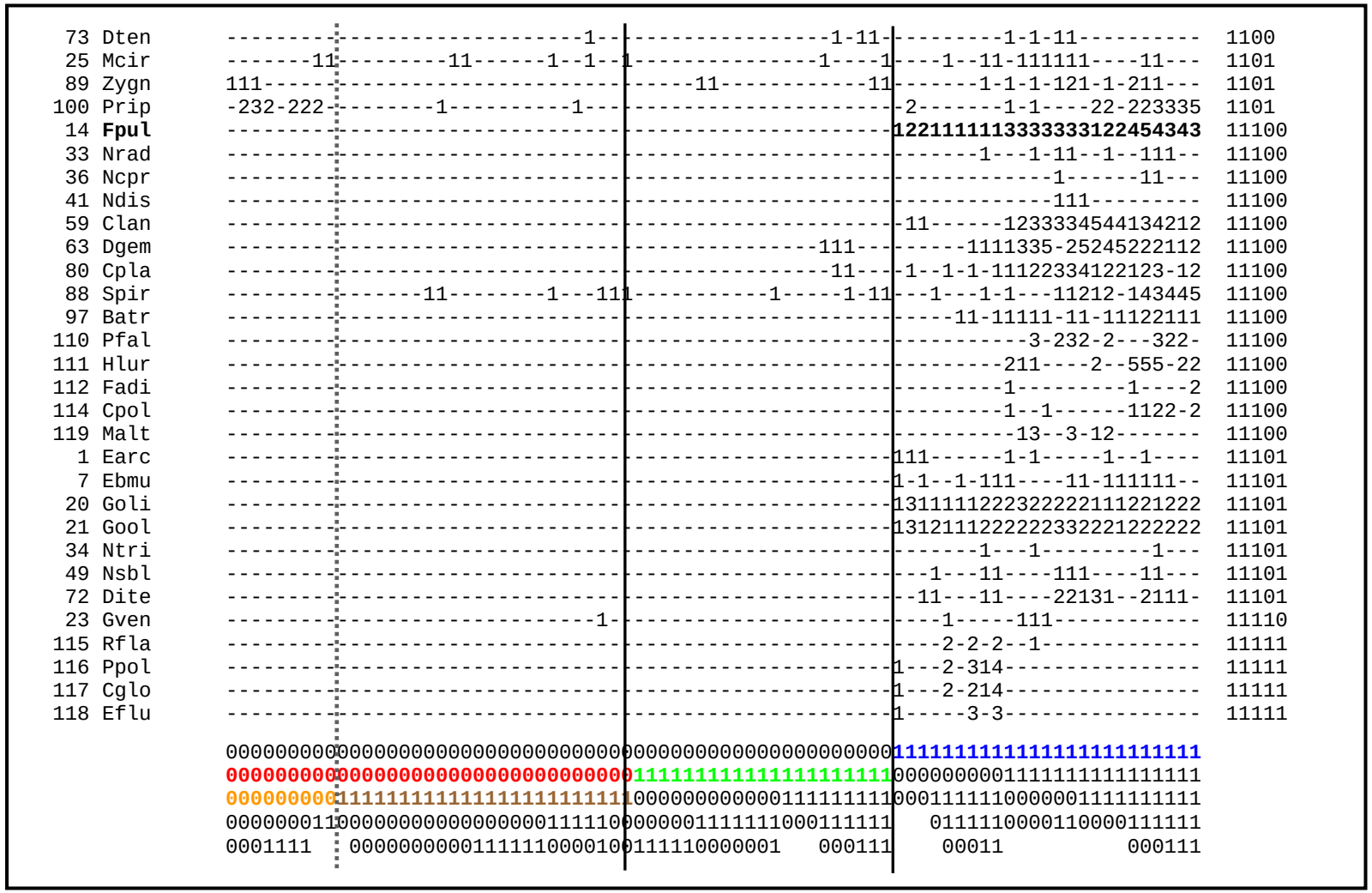


Figure 4.35 TWINSPAN output depicting 79 samples and 3 freshwater vegetation species assemblages, with indicator species highlighted in bold font and colour-coding as appropriate for TWINSPAN sample-groups I (blue), II (green), and III (red), plus two TWINSPAN sub-assemblages IIIa (orange) and IIIb (brown). For epilithic periphyton, aquatic bryophyte and vascular submerged macrophyte species codes refer to Figure 4.37.

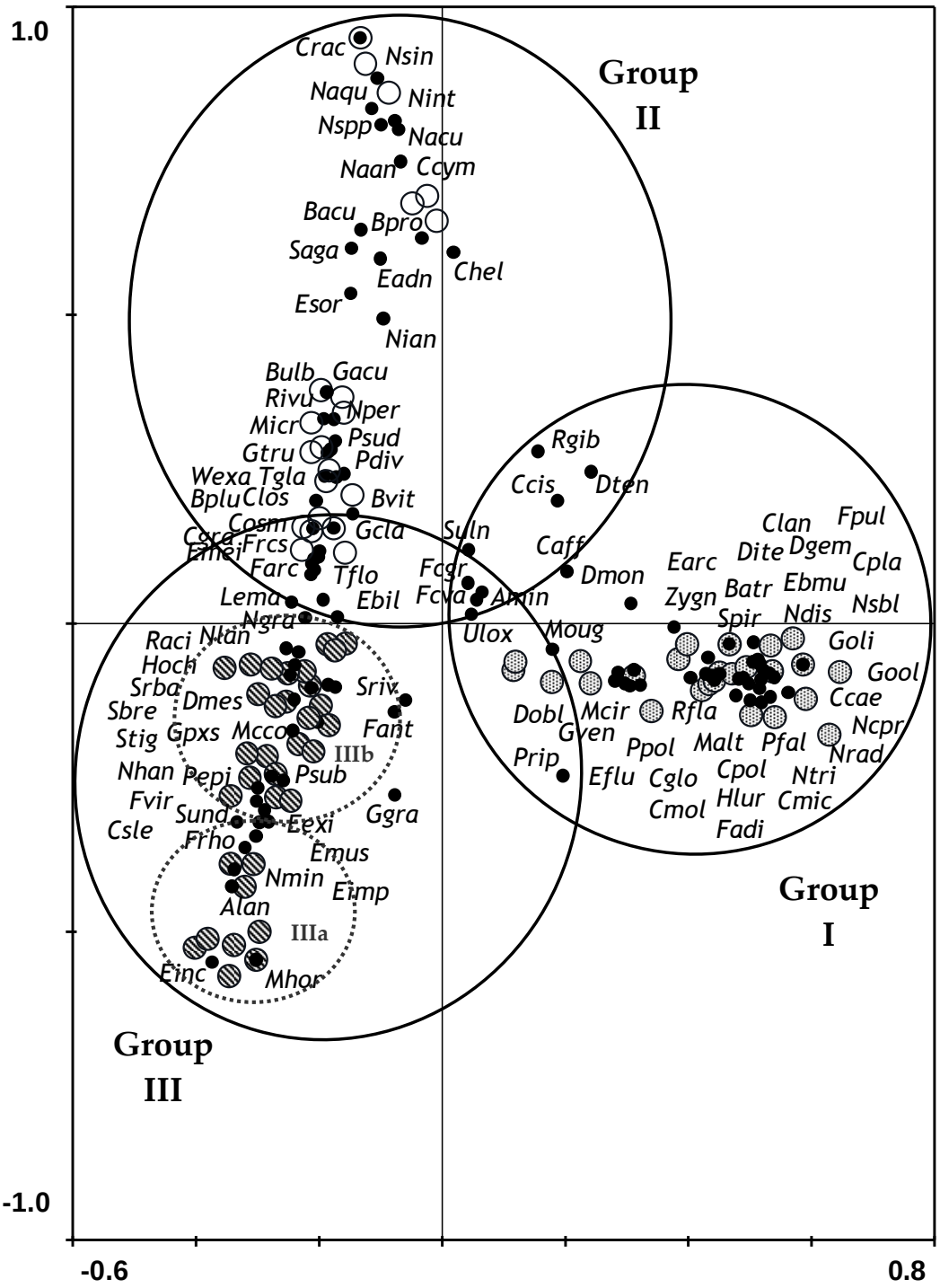


Figure 4.36 CCA ordination of 119 epilithic periphyton (on naturally-occurring mineral substrata), aquatic bryophyte and vascular submerged macrophyte species and 79 samples, with TWINSPAN sample-group boundaries overlaid. TWINSPAN sample-group identifiers as follows: Group I (n=25: UKAPP06, UKAPG06, UKAPR06, UKSMP06, UKSMG06, UKNVP06, UKNVG06, MKAPP06, MKAPG06, MKAPR06, MKSMP06, MKSMG06, MKSMR06, MKNVP06, MKNVG06, MKNVR06, LKAPP06, LKAPG06, LKAPR06, LKSMP06, LKSMG06, LKSMR06, LKNVP06, LKNVG06, LKNVR06): dotted circles ; Group II (n=21: IBAUP05, IBAUG05, IBAUR05, HBMYP05, HBMYG05, HBMYR05, HBAUP05, HBAUG05, HBAUR05, HBAPP06, HBAPG06, HBAPR06, LMMYP05, LMMYG05, LMMYR05, LMAUP05, LMAUG05, LMAUR05, LMAPP06, LMAPG06, LMAPR06): open circles ; Group III (n=33: BBMYP05, BBMYG05, BBMYR05, BBAUP05, BBAUG05, BBAUR05, BBAPP06, BBAPG06,

BBAPR06, CFMYP05, CFMYG05, CFMYR05, CFAUP05, CFAUG05, CFAUR05, CFAPP06, CFAPG06, CFAPR06, BDMYP05, BDMYG05, BDMYR05, BDAUP05, BDAUG05, BDAUR05, BDAPP06, BDAPG06, BDAPR06, IBMYP05, IBMYG05, IBMYR05, IBAPP06, IBAPG06, IBAPR06): diagonally striped circles , with sub-assemblages IIIa (n=9) and IIIb (n=24) encircled by dashed TWINSPAN boundaries. For sample site-codes: Water of Dye sites: Brocky Burn (BB), Charr Flume (CF) and Bogendreip (BD); River Girnock sites: Iron Bridge (IB), Hampshire's Bridge (HB) and Littlemill (LM); Knockan Burn sites: Upper Knockan (UK), Mid-Knockan (MK) and Lower Knockan (LK). Each site code is completed using code letters for survey date (AP: April; MY: May; AU: August; SM: September; NV: November), flow regime (P: Pool; G: Glide; R: Riffle), year sampled (05: 2005; 06: 2006). Example: BBMYR05 = Brocky Burn May Riffle 2005. For periphyton, aquatic bryophyte, vascular submerged macrophyte species codes and ordination statistics refer to Figure 4.37.

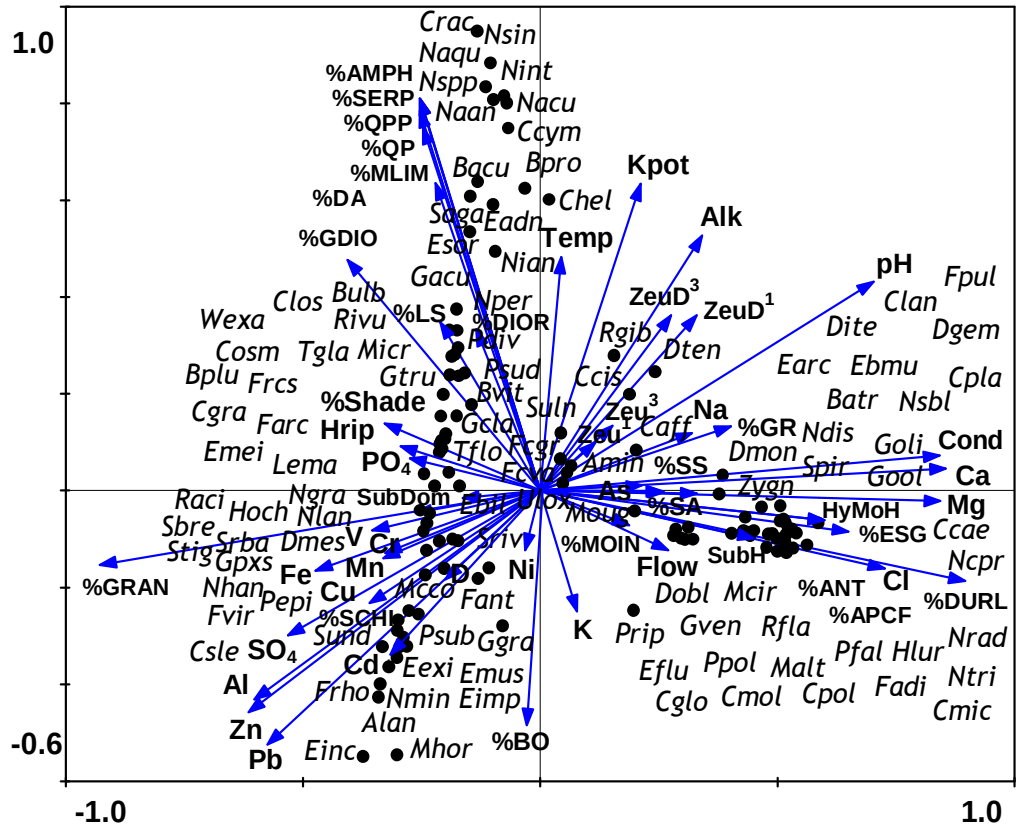


Figure 4.37 CCA ordination of epilithic periphyton (on naturally-occurring mineral substrata), aquatic bryophyte, vascular submerged macrophyte species and environmental variables. **Periphyton species codes: *Eunotia arcus* sensu (Earc), *Eunotia muscicola* var. *tridentula* (Emus), *Eunotia* cf. *incisa* (Einc), *Eunotia meisteri* (Emei), *Eunotia bilunaris* var. *linearis* (Ebil), *Eunotia exigua* (Eexi), *Eunotia bilunaris* var. *mucophila* (Ebmu), *Eunotia serra* (Eser), *Eunotia implicata* (Eimp), *Fragilaria capucina* var. *vaucheriae* (Fcva), *Fragilaria capucina* var. *gracilis* (Fcgr), *Fragilaria virescens* (Fvir), *Fragilaria arcus* (Farc), *Fragilaria pulchella* (Fpul), *Synedra ulna* (Suln),*Gomphonema* cf. *parvulum* var. *exilissimum* (Gpxs), *Gomphonema clavatum* (Gcla), *Gomphonema truncatum* (Gtru), *Gomphonema acuminatum* (Gacu), *Gomphonema olivaceum* (Goli), *Gomphonema olivaceum* var. *olivaceoides* (Gool), *Gomphonema gracile* (Ggra), *Gomphonema ventricosum* (Gven), *Meridion circulare* var. *constrictum* (Mcco), *Meridion circulare* (Mcir), *Frustulia rhomboides* var. *rhomboides* (Frho), *Frustulia rhomboides* var. *crassinervia* (Frcs), *Frustulia vulgaris* (Fvul), *Navicula rhynchocephala* (Nrhy), *Navicula lanceolata* (Nlan), *Navicula* cf. *aquaedurae* (Naqu), *Navicula angusta* (Naan), *Navicula radiosa* (Nrad), *Navicula tripunctata* (Ntri), *Navicula* cf. *gregaria* (Ngre), *Navicula capitatoradiata* (Ncpr), *Navicula* cf. *pygmaea* agg. (Npyg), *Navicula jaernefeltii* (Njae), *Navicula minima* (Nmin), *Craticula acidoclinata* (Crac), *Nitzschia dissipata* (Ndis), *Nitzschia sinuata* (Nsin), *Nitzschia gracilis* (*Ngra*), *Nitzschia hantzschiana* (Nhan), *Nitzschia perminuta* agg. (Nper), *Nitzschia intermedia* agg. (Nint), *Nitzschia* cf. *acula* (Nacu), *Nitzschia palea* agg. (Npal), *Nitzschia sublinearis* (Nsbl), *Nitzschia angustata* (Nian), *Nitzschia* undefined sp. (Nspp), *Tabellaria flocculosa* (Tflo), *Cymbella silesiaca* (Csle), *Cymbella gracilis* (Cgra), *Cymbella cistula* (Ccis), *Cymbella cymbiformis* (Ccym), *Cymbella helvetica* (Chel), *Cymbella affinis* (Caff), *Cymbella lanceolata* (Clan), *Cymbella caespitosa* (Ccae), *Cymbella naviculiformis***

(Cnav), *Cymbella microcephala* (Cmic), *Didymosphenia geminata* (Dgem), *Pinnularia subcapitata* (Psub), *Pinnularia* cf. *sudetica* (Psud), *Pinnularia* cf. *divergens* (Pdiv), *Surirella roba* (Srba), *Surirella brebissonii* (Sbre), *Tetracyclus glans* (Tgla), *Diatoma mesodon* (Dmes), *Diatoma moniliformis* (Dmon), *Diatoma tenuis* (Dite), *Denticula tenuis* (Dten), *Epithemia adnata* (Eadn), *Epithemia sorex* (Esor), *Rhopalodia gibba* (Rgib), *Nedium bisulcatum* (Nebi), *Achnanthes lanceolata* (Alan), *Achnanthidium minutissima* (Amin), *Cocconeis placentula* (Cpla), *Diploneis* cf. *elliptica* (Dell), *Diploneis marginestriata* (Dmar), *Diploneis oblongella* (Dobl), *Brachysira vitrea* (Bvit), *Brachysira procera* (Bpro), *Closterium* sp. (Clos), *Cosmarium* sp. (Cosm), *Spirogyra* sp. (Spir), *Zygnema* sp. (Zygn), *Microspora* sp. (Micr), *Mougeotia* sp. (Moug), *Ulothrix* sp. (Ulox), *Stigeoclonium* sp. (Stig), *Bulbochaete* sp. (Bulb), *Rivularia* sp. (Rivu), *Lemanea fluviatilis* (Lema), *Batrachospermum* sp. (Batr). Aquatic bryophyte species codes: *Blindia acuta* (Bacu), *Brachythecium plumosum* (Bplu), *Ctenidium molluscum* (Cmol), *Fissidens adianthoides* (Fadi), *Fontinalis antipyretica* (Fant), *Hygrohypnum luridum* (Hlur), *Hygrohypnum ochraceum* (Hoch), *Mnium hornum* (Mhor), *Palustriella falcata* (Pfal), *Platyhypnidium riparioides* (Prip), *Racomitrium aciculare* (Raci), *Schistidium agassizii* (Saga), *Schistidium rivulare* (Sriv), *Warnstorfia exannulata* (Wexa), *Chiloscyphus polyanthus* (Cpol), *Pellia epiphylla* (Pepi), and *Scapania undulata* (Sund). Vascular submerged macrophyte species codes: *Potamogeton polygonifolius* (Ppol), *Chara globularis* var. *globularis* (Cglo), *Eleogiton fluitans* (Eflu), *Ranunculus flammula* (Rfla), and *Myriophyllum alterniflorum* (Malt). Environmental variables: Underlying geology: Granite (%GRAN), Mica Schist (%SCHI), Granodiorite (%GDIO), Diorite (%DIOR), Quartz/Psammite (%QP), Quartz/Psammite/Pelite (%QPP), Diorite/Amphibolite (%DA), Amphibolite (%AMPH), Serpentinite (%SERP), Metamorphic Limestone (%MLIM), Durness Limestone (%DURL), Moine Schist (%MOIN), Eriboll Sandstone Group (%ESG), Applecross Formation (%APCF) and An-T'sron (%ANT). Substrate morphology: substrate particle diversity (SubH), substrate particle dominance (SubDom), hydromorphological diversity (HyMoH), streambed cover of Boulders (%BO), Large Stones (%LS), Small Stones (%SS), Gravel (%GR), and Sand (%SA). Water physico-chemistry: benthic depth (D: m), light attenuation coefficient (K: m^{-1}), euphotic depth 1% (Zeu^1: m^{-1}), $Zeu:D^1$ ratio 3%, euphotic depth 3% (Zeu^3: m^{-1}), $Zeu:D^3$ ratio 1%pH, alkalinity (Alk: mg/l), conductivity (Cond: µS cm^{-1}), water temperature (Temp: ^{O}C), current velocity (Flow: m s^{-1}), %Shade and height of riparian vegetation (Hrip). Water Chemistry: Phosphate (PO_4-P), Chloride (Cl), Sulphate (SO_4), Cadmium (Cd), Chromium (Cr), Copper (Cu), Lead (Pb), Nickel (Ni), Zinc (Zn), Aluminium (Al), Vanadium (V), Arsenic (As), Sodium (Na), Potassium (Kpot), Calcium (Ca), Magnesium (Mg), Iron (Fe), and Manganese (Mn). Monte Carlo significance test: Axis 1: P<0.005; all canonical axes: P<0.005. Eigenvalues: Axis 1: 0.543; Axis 2: 0.363.

	TWINSPAN sample group						
Variable	**I**		**II**		**III**		**P_{ANOVA}**
	Mean	***S.E.***	***Mean***	***S.E.***	***Mean***	***S.E.***	
Freshwater vegetation species richness: **S**	26.2[a]	1.08	32.9[b]	1.30	24.3[a]	1.12	P<0.001***
Freshwater vegetation species diversity: **H**	2.87[a]	0.05	2.91[a]	0.05	2.61[b]	0.05	P<0.001***
Freshwater vegetation species dominance	0.14[a]	0.01	0.14[a]	0.01	0.19[b]	0.01	P<0.001***
D (m)	0.14	0.13	0.14	0.11	0.16	0.12	NS
K (m^{-1})	2.72	0.30	2.45	0.31	2.82	0.27	NS
$Z_{eu}^{1\%}$ (m)	0.36	0.33	0.26	0.42	0.30	0.28	NS
Z_{eu}:$D^{1\%}$	2.84[a]	0.37	2.07[b]	0.55	2.12[b]	0.32	P<0.001***
$Z_{eu}^{3\%}$ (m)	1.27	0.31	0.90	0.43	1.05	0.29	NS
Z_{eu}:$D^{3\%}$	10.02[a]	0.33	6.54[b]	0.51	6.50[b]	0.31	P<0.001***
pH	7.58[a]	0.06	7.11[b]	0.10	6.33[c]	0.13	P<0.001***
Conductivity ($\mu S\ cm^{-1}$)	137.7[a]	0.09	58.7[b]	0.07	41.0[c]	0.05	P<0.001***
Alkalinity ($mg\ l^{-1}$)	55.96[a]	5.49	25.48[b]	8.89	5.58[c]	6.65	P<0.001***
Water Temperature (^oC)	8.9	0.10	10.7	0.21	8.6	0.18	NS
Flow ($m\ s^{-1}$)	0.289	0.06	0.204	0.06	0.247	0.05	NS
% Shade	7.3[a]	1.35	34.9[b]	7.98	21.8[c]	2.68	P<0.001***
Height of Riparian Vegetation (m)	0.14[a]	0.06	4.40[b]	0.98	2.34[c]	0.58	P<0.001***
Substrate diversity	3.48[a]	0.19	3.16[ab]	0.12	3.03[b]	0.10	P<0.05*
Substrate dominance	0.41[a]	0.03	0.44[ab]	0.03	0.48[b]	0.02	P<0.05*
Hydromorphological diversity	3.59[a]	0.19	3.27[ab]	0.11	3.13[b]	0.10	P<0.05*
% Granite	0.0[a]	0.00	43.6[b]	2.95	84.8[c]	1.84	P<0.001***
% Granodiorite	0.0[a]	0.00	7.9[b]	0.63	2.2[c]	0.96	P<0.05*
% Diorite	0.0[a]	0.00	0.4[b]	0.10	0.0[a]	0.00	P<0.001***
% Mica Schist	0.0[a]	0.00	0.0[a]	0.00	9.4[b]	1.61	P<0.001***
% Amphibolite	0.0[a]	0.00	10.0[b]	0.85	0.0[a]	0.00	P<0.001***
% Serpentinite	0.0[a]	0.00	1.3[b]	0.15	0.0[a]	0.00	P<0.001***
% QP	0.0[a]	0.00	0.9[b]	0.09	0.0[a]	0.00	P<0.001***
% DA	0.0[a]	0.00	3.0[b]	0.27	0.0[a]	0.00	P<0.001***

% QPP	0.0[a]	0.00	12.2[b]	0.80	0.0[a]	0.00	P<0.001***
% Limestone	0.0[a]	0.00	9.5[b]	1.26	0.0[a]	0.00	P<0.001***
% Durness Limestone	73.3[a]	2.48	0.0[b]	0.00	0.0[b]	0.00	P<0.001***
% Eriboll Sandstone	10.0[a]	1.47	0.0[b]	0.00	0.0[b]	0.00	P<0.001***
% Moine Schist	6.7[a]	1.22	0.0[b]	0.00	0.0[b]	0.00	P<0.001***
% Applecross Formation	6.7[a]	0.82	0.0[b]	0.00	0.0[b]	0.00	P<0.001***
% An-t'Sron	3.3[a]	0.41	0.0[b]	0.00	0.0[b]	0.00	P<0.001***
% Boulders	13.9[a]	3.07	25.0[b]	3.15	45.2[c]	3.33	P<0.001***
% Large Stones	23.1[a]	3.27	45.0[b]	3.04	30.0[a]	2.83	P<0.001***
% Small Stones	30.6[a]	3.04	27.0[ab]	2.96	21.8[b]	2.08	P<0.05*
% Gravel	30.1[a]	4.33	18.5[b]	3.13	20.5[b]	3.03	P<0.05*
% Sand	6.1[a]	1.42	0.0[b]	0.00	0.0[b]	0.00	P<0.001***
NH_3-N (mg l^{-1})	<0.04		<0.04		<0.04		NS
NO_3-N (mg l^{-1})	<0.01		<0.01		<0.01		NS
PO_4-P (mg l^{-1})	<0.003		<0.003		<0.003		NS
Cl (mg l^{-1})	12.86[a]	0.25	8.38[b]	0.30	8.60[b]	0.37	P<0.001***
SO_4 (mg l^{-1})	0.17[a]	0.03	1.18[b]	0.17	2.19[c]	0.28	P<0.001***
Cd (μg l^{-1})	0.02	0.00	0.02	0.09	0.03	0.10	NS
Cr (μg l^{-1})	0.13[a]	0.02	0.19[ab]	0.23	0.23[b]	0.16	P<0.05*
Cu (μg l^{-1})	0.17[a]	0.04	0.25[ab]	0.03	0.30[b]	0.02	P<0.05*
Pb (μg l^{-1})	0.06[a]	0.09	0.15[b]	0.17	0.48[c]	0.12	P<0.001***
Ni (μg l^{-1})	0.25	0.12	0.30	0.14	0.27	0.10	NS
Zn (μg l^{-1})	1.00[a]	0.10	1.79[b]	0.10	2.96[c]	0.09	P<0.001***
Al (μg l^{-1})	45.6[a]	5.65	73.7[b]	8.54	117.1[c]	8.76	P<0.001***
V (μg l^{-1})	0.15[a]	0.08	0.25[ab]	0.13	0.33[b]	0.09	P<0.05*
As (μg l^{-1})	0.59	0.00	0.47	0.06	0.50	0.05	NS
Na (mg l^{-1})	5.73[a]	0.15	4.94[b]	0.21	4.94[b]	0.20	P<0.01**
K (mg l^{-1})	0.53	0.14	0.64	0.17	0.42	0.08	NS
Ca (mg l^{-1})	11.19[a]	0.08	3.87[b]	0.09	2.02[c]	0.08	P<0.001***
Mg (mg l^{-1})	6.87[a]	0.09	1.46[b]	0.08	0.78[c]	0.06	P<0.001***
Fe (mg l^{-1})	0.16[a]	0.08	0.24[ab]	0.15	0.29[b]	0.08	P<0.05*
Mn (mg l^{-1})	0.006[a]	0.07	0.012[ab]	0.14	0.016[b]	0.10	P<0.05*

Table 4.103 Mean values (± 1 standard error) of normally distributed plant variables (including zero values, and data back-transformed where necessary): response of freshwater vegetation species

richness (per 141.52 cm^2), freshwater vegetation species diversity (per 141.52 cm^2), and freshwater vegetation species dominance (per 141.52 cm^2) between TWINSPAN sample-groups I (n = 25), II (n = 21) and III (n = 33): for combined periphyton, aquatic bryophyte and (where present) vascular submerged macrophyte assemblages (n = 79). Significance testing: one-way ANOVA and application of Tukey's mean separation test. For variables with significant outcomes only, mean values sharing a superscript letter in common are not significantly different. Note also that median values are quoted for non-normal variables compared using Kruskal-Wallis test.

	TWINSPAN sub-assemblage				
Variable	**IIIa**		**IIIb**		**P**ANOVA
	Mean	***S.E.***	***Mean***	***S.E.***	
Freshwater vegetation species richness: **S**	22.0	1.03	26.7	1.45	P<0.05*
Freshwater vegetation species diversity: **H**	2.51	0.05	2.69	0.06	P<0.05*
Freshwater vegetation species dominance	0.21	0.01	0.17	0.02	P<0.05*
D (m)	0.12	0.10	0.19	0.08	NS
K (m^{-1})	4.38	0.36	2.39	0.22	P<0.001***
$Z_{eu}^{1\%}$ (m)	0.18	0.36	0.35	0.22	NS
Z_{eu}:$D^{1\%}$	0.64	0.38	1.25	0.23	NS
$Z_{eu}^{3\%}$ (m)	1.96	0.38	2.18	0.22	NS
Z_{eu}:$D^{3\%}$	5.96	0.37	6.70	0.23	NS
pH	5.87	0.18	6.59	0.12	P<0.01**
Conductivity (μS cm^{-1})	38.4	0.07	44.4	0.06	P<0.01**
Alkalinity (mg l^{-1})	2.49	9.80	8.55	8.46	P<0.01**
Water Temperature (^oC)	8.7	0.30	8.4	0.17	NS
Flow (m s^{-1})	0.256	0.06	0.240	0.04	NS
% Shade	20.1	7.32	23.7	2.58	NS
Height of Riparian Vegetation (m)	1.50	0.23	3.15	0.71	P<0.01**
Substrate diversity (Simpson's D)	3.06	0.16	3.00	0.13	NS
Substrate dominance (Berger-Parker Index)	0.45	0.02	0.50	0.03	NS
Hydromorphological diversity (D)	3.15	0.17	3.11	0.13	NS
% Granite	100.0	0.00	79.7	1.22	P<0.001***
% Granodiorite	0.0	0.00	3.1	1.26	P<0.05*
% Mica-Schist	0.0	0.00	11.5	1.74	P<0.001***
% Boulders	42.8	4.98	46.7	3.94	NS
% Large Stones	30.6	4.48	28.5	3.48	NS
% Small Stones	20.9	3.85	22.6	2.52	NS
% Gravel	17.6	4.43	23.3	3.77	NS
NH_3-N (mg l^{-1})	<0.04		<0.04		NS
NO_3-N (mg l^{-1})	<0.01		<0.01		NS
PO_4-P (mg l^{-1})	<0.003		<0.003		NS
Cl (mg l^{-1})	8.38	0.53	8.68	0.47	NS

SO_4 (mg l^{-1})	3.00	0.73	1.38	0.25	P<0.01**
Cd (μg l^{-1})	0.04		0.02		P<0.01**
Cr (μg l^{-1})	0.25	0.26	0.21	0.19	NS
Cu (μg l^{-1})	0.27	0.07	0.32	0.03	NS
Pb (μg l^{-1})	1.21	0.11	0.33	0.06	P<0.001***
Ni (μg l^{-1})	0.44	0.19	0.20	0.11	P<0.001***
Zn (μg l^{-1})	4.60	0.25	2.51	0.10	P<0.001***
Al (μg l^{-1})	140.8	10.07	103.0	11.20	P<0.01**
V (μg l^{-1})	0.45		0.31		P<0.01**
As (μg l^{-1})	0.72		0.59		P<0.01**
Na (mg l^{-1})	4.57	0.50	5.08	0.30	NS
K (mg l^{-1})	0.35	0.07	0.44	0.04	NS
Ca (mg l^{-1})	1.47	0.25	2.28	0.16	P<0.01**
Mg (mg l^{-1})	0.58	0.15	0.87	0.06	P<0.01**
Fe (mg l^{-1})	0.50	0.18	0.22	0.05	P<0.001***
Mn (mg l^{-1})	0.027	0.03	0.013	0.05	P<0.001***

Table 4.104 Mean values (± 1 standard error) of normally distributed freshwater vegetation species richness (per 141.52 cm^2), freshwater vegetation species diversity (per 141.52 cm^2), freshwater vegetation species dominance (per 141.52 cm^2), and environmental habitat variables (including zero values, and data back-transformed where necessary) between TWINSPAN sample-group III sub-assemblages IIIa (n = 9) and IIIb (n = 24): for combined periphyton, aquatic bryophyte and (where present) vascular submerged macrophyte assemblages (n = 33). Significance testing: one-way ANOVA. Note also that median values are quoted for non-normal variables compared using Kruskal-Wallis test.

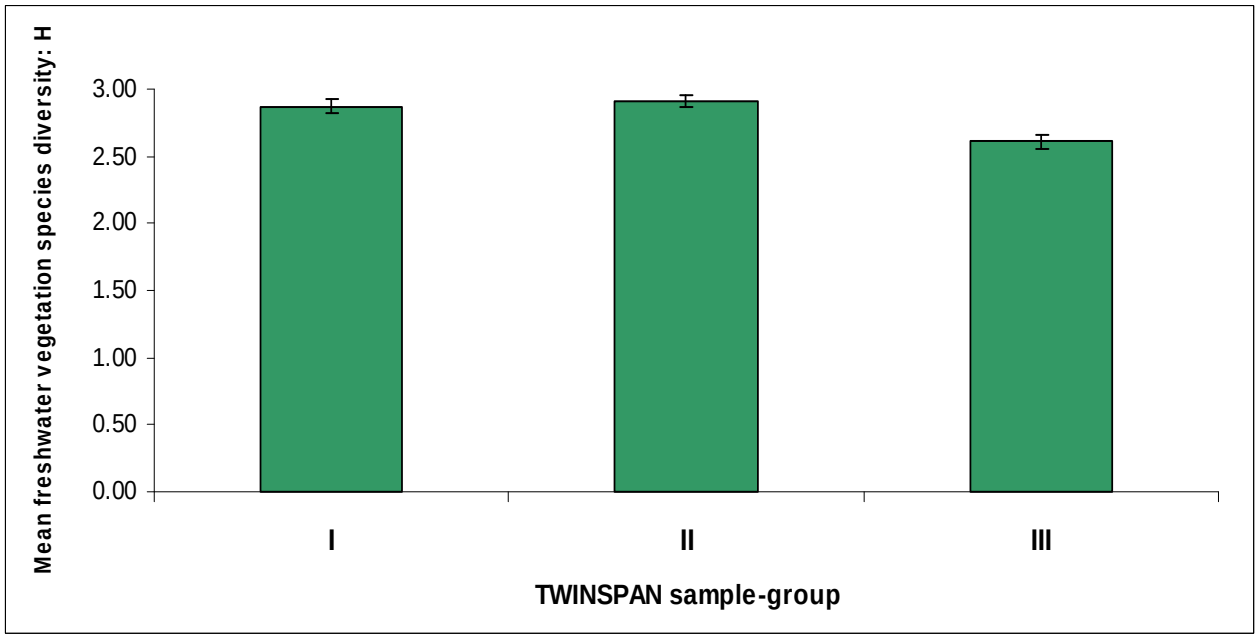


Figure 4.38 Comparison of mean values (± 1 standard error) of normally distributed freshwater vegetation species diversity per 141.52 cm^2 (including zero values, and data back-transformed where necessary) between TWINSPAN sample-groups I (n = 25), II (n = 21) and III (n = 33): for combined periphyton, aquatic bryophyte and (where present) vascular submerged macrophyte assemblages (n = 79).

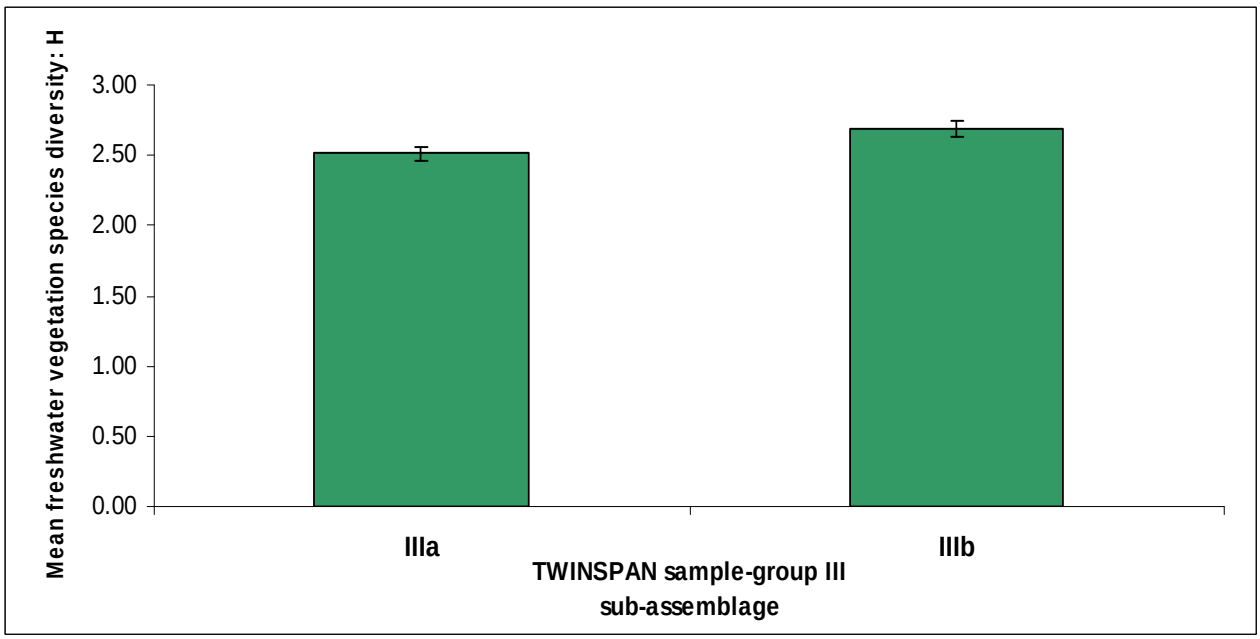


Figure 4.39 Comparison of mean values (± 1 standard error) of normally distributed freshwater vegetation species diversity per 141.52 cm^2 (including zero values, and data back-transformed where necessary) between TWINSPAN sample-group III sub-assemblages IIIa (n = 9) and IIIb (n = 24): for combined periphyton, aquatic bryophyte and (where present) vascular submerged macrophyte assemblages (n = 33).

4.5.23.2 Relationships between freshwater vegetation community composition, diversity and environmental habitat conditions

Freshwater vegetation species richness and diversity were strongly and significantly positively correlated with each other (see Appendix 2m). Freshwater vegetation species richness and diversity were also positively correlated to streamwaters influenced by base-rich geologies, increasing streamwater temperature and substrate complexity. Whereas streamwaters of base-poor and acid-sensitive character, as well as increasing substrate dominance and bare area showed negative relationships with freshwater vegetation species richness and diversity. Furthermore, freshwater vegetation species dominance was negatively correlated with both species richness and diversity, and exhibited the inverse relationships of these.

4.5.23.3 Predicting freshwater vegetation community composition and diversity

Due to the small data set collected for vascular submerged macrophytes in this research project, it was not appropriate to integrate this information together with the more comprehensive data sets belonging to periphyton and aquatic bryophytes as tool for predicting the species diversity of freshwater vegetation.

4.6 Discussion

4.6.1 Periphyton

4.6.1.1 Variation in periphyton community composition and diversity in the Water of Dye, River Girnock and Knockan Burn; their sub-catchments and sites

Although there were some instances where indices of periphyton species richness and diversity were closely-similar between the three streams, species composition of the periphytic algal assemblages were certainly quite different. For further discussion refer to section 4.6.1.6.

The Water of Dye and Knockan Burn were characterised by contrasting species-assemblages of periphyton, whilst the River Girnock supported a diverse community containing elements of both the naturally acidic and calcareous streams, together with a handful of species which appeared to be exclusive to this river (e.g. *Epithemia* spp., *Navicula angusta*, *Nitzschia* cf. *acula*, *Nitzschia intermedia* agg., *Tetracyclus glans*). Several diatom species (e.g. *Achnanthidium minutissima*, *Fragilaria capucina* var. *gracilis*, *Fragilaria capucina* var. *vaucheriae*, *Synedra ulna*, *Tabellaria flocculosa*) were ubiquitous in their occurrence and similarly most of the green filamentous algae (e.g. *Mougeotia*, *Spirogyra*, *Ulothrix*,) were common to all three streams. Variation in water chemistry appeared to be the major influential factor driving differences in periphyton community structure, especially with respect to the formation of distinct diatom species-assemblages. This will be discussed in more detail in section 4.6.1.6.

4.6.1.2 Temporal and seasonal variation in periphyton community composition and diversity in the Water of Dye, River Girnock and Knockan Burn

In general, significant temporal and seasonal variations in periphyton species richness, diversity and dominance were observable in each of the three target

streams. Throughout most of the sampling year, baseline periphyton production was characterised by an assemblage of diatom species distinct to the water chemistry of each subcatchment stream. However in all three streams, distinct peaks or troughs in periphyton species diversity were often most apparent during summer baseflows or spring spates, respectively.

Diversity indices can provide a useful indication of how periphyton assemblages respond to environmental disturbances. However, to begin with it is imperative to understand the natural course of successional development in periphytic algal communities before considering the parameters responsible for driving ecological shifts in community composition therein. Initially in streams and rivers, bare substrata undergo conditioning by bacteria, fungi and organic matter which coat the surface with a thin biofilm. This attracts rapid pioneer invasion of the substratum surface by prostrate diatom species, usually comprising short growth forms (e.g. *Cocconeis*, *Acthnanthidium*), typically the first to colonise an open niche and inherently disturbance-resistant. This low stature, low biomass understorey community can then be overgrown by adnate diatoms which become apically-attached to the substratum surface using mucilage pads (e.g. *Fragilaria*, *Synedra*). This tightly adhering layer of prostrate and adnate growth forms can be superceded by erectly-growing stalked diatoms (e.g. *Cymbella*, *Didymosphenia*, *Gomphonema*) protruding above the understorey, and forming a loosely attached canopy layer, together with an overstorey of chain-forming diatoms (e.g. *Tabellaria*) and filamentous green algae (e.g. *Mougeotia*, *Ulothrix*). This community is usually indicative of a successional climax in the periphyton. Succession of periphyton communities can be considered analogous to a forest structure, in which the tightly adhering microscopic adnate-prostrate layer forms the scrubby understorey and ground cover herbaceous layer, above which the loosely attached macroscopic stalked-filamentous layer forms the overstorey canopy (Hoagland *et al*. 1982, Lamb & Lowe 1987, Stevenson *et al.* 1996). Natural disturbance pressures (e.g. flow, grazing) regulate the diversity and taxonomic composition of periphyton communities in streams and rivers (Stevenson *et al.* 1996). Essentially

it is the timing and intensity of these disturbances which manipulate the displacement and replacement of periphyton species, thereby governing successional development from a relatively flat low diversity community maintained under heavy disturbance pressure, progressing to a complex multi-dimensional species-rich assemblage when disturbance pressures are relaxed (Stevenson *et al.* 1996).

Thus the results of my study reinforce the fact that periphyton communities have complex dynamics, and patterns of successional development are strongly regulated by disturbance from high-velocity events (Peterson & Stevenson 1992). Peak discharges exerted marked effects on the structure and function of periphyton assemblages by dramatically reducing species diversity and thereby community biomass. Flow-pertubations interrupt community succession in periphytic algae by selecting for a predominance of pioneer growth forms adapted to stress or disturbance (SR-strategist: Grime 1979) like tightly adhering prostrate (e.g. *Cocconeis placentula*, *Acthnanthidium minutissimum*) or adnate (e.g. *Fragilaria capucina* var. *vaucheriae*, *Synedra ulna*) morphologies, which provide the necessary resilience attributes to endure high-velocity scours (Peterson & Stevenson 1992, Blenkinsopp & Lock 1994, Biggs 1995, Biggs & Thomsen 1995, Stevenson *et al*. 1996, Passay 2007). Stalked, filamentous or other canopy-forming morphologies of loosely attached diatoms and green algae (e.g. *Gomphonema* sp., *Tabellaria* sp., *Mougeotia* sp., *Spirogyra* sp.) which protrude into the water column, are less hydraulically stable and more susceptible to becoming dislodged under high flows exceeding the threshold capacity (Hynes 1970, Uehlinger 1991, Steinman *et al*. 1991, Peterson & Stevenson 1992, Biggs 1995, Biggs & Thomsen 1995, Stevenson *et al*. 1996, Biggs *et al*. 1998). Therefore there is an apparent ecological trade-off between early coloniser scour-resistant growth forms (e.g. prostrate diatoms) and late successional communities developing large biomasses of green filamentous morphologies which are vulnerable to flow (Biggs 1995, Biggs & Thomsen 1995) and grazer disturbance (Marks *et al*. 2000, Rosemond *et al*. 2000).

Throughout most of the sampling year each stream was characterised by a background minimum or baseline of periphyton production consisting of a thin layer of biofilm (usually, <2 mm thick) dominated by low-profile non-filamentous diatoms (usually, >90% of the total population sampled). This indicated that periphytic algal succession was stalled in the pioneer phase (or early colonisation stage) by frequent flow disturbances and high-velocity spates (Biggs 1995, Biggs & Thomsen 1995, Stevenson *et al*. 1996), thus constraining periphyton diversity to a minimum during the autumn-winter-spring period.

However, the combination of stable baseflows and high light intensity encouraged structurally more-complex periphyton to develop over the summer months in each target stream. During this time green filamentous algae (e.g. *Mougeotia*, *Spirogyra*, *Ulothrix*) overgrew the understorey of tightly attached prostrate and adnate diatoms (Biggs & Thomsen 1995), indicating that the more stable summer baseflow conditions had enabled periphyton communities to reach a successional climax, or a condition at least close to climax. Even so, diatoms remained a substantial component (usually, >70% of the total population) showing an increased abundance of some growth forms (e.g. stalked, filamentous chains) and overall greater diversity attributed to the recent appearance of species during the summer which were rarely found to be present at any other time of the sampling year. For example solitary motile diatoms of the genus *Navicula* and *Nitzschia* are generally poorly attached (occurring usually on epipelic or epipsammic substrata) and are easily scoured (Ghosh & Gaur 1998), thus explaining their increased frequency under low velocity baseflows. Notably the composition of diatom species assemblages reflected prevailing water chemistry characteristics and is discussed elsewhere (see section 4.6.1.6). Also in my study I found that at sampling sites experiencing heavy shade from riparian vegetation the occurrence of filamentous chlorophytes was notably suppressed during the summer. Futhermore diatoms tended to predominate under low irradiances and accrued fairly small (often near-negligible) quantities of biomass but were overgrown by an abundance of green filamentous algae where light conditions were sufficiently

high. This agrees with the findings of many other studies on the ecology of stream periphyton communities (e.g. Lowe *et al*. 1986, Lamberti *et al*. 1989, Steinman *et al*. 1989, Duncan & Blinn 1989, Wellnitz *et al*. 1996, Mosisch *et al*. 2001, Kiffney *et al*. 2003). Despite having high light requirements similar to other green filamentous algae found in my study, *Mougeotia* seemed capable of persisting under the low irradiances at Littlemill (albeit considerably less abundantly compared to unshaded sites further upstream). These observations agree with the findings of Graham *et al*. (1996a), whilst *Ulothrix* (Graham & Kranzfelder 1985) and *Spirogyra* (Graham *et al*. 1995) are known to be less tolerant of light deprivation, and did not occur under heavily shaded conditions in my study. Shade is known to limit green filamentous algal growth and delay the onset of community climax under laboratory stream conditions (McIntire & Phinney 1965, Steinman & McIntire 1987). In contrast, diatom community composition appeared to be largely unaffected by changes in light intensity and appeared tolerant of low irradiances (Lowe *et al*. 1986, DeNicola *et al*. 1992, Bourassa & Cattaneo 2000, Rier *et al*. 2006). Genetic predisposition notwithstanding, environmental drivers may significantly modify wild populations of diatoms in other ways. For example recent research suggests that diatom morphology and life cycle periodicity are strongly influenced by temperature variation (Potapova & Snoeijs 1997), whilst others have found sexual responsiveness to be affected by nitrate levels (Jewson 1992, Poulícková & Mann 2008). However, interactions of light and nutrients on the community structure of stream periphyton is discussed thoroughly elsewhere in this thesis (see Chapter 3, section 3.6.1.2), and hence will not be repeated.

Grazing pressure can directly affect (e.g. selective consumption) and indirectly impact (e.g. preventing accumulation of detritus, habitat disturbance, nutrient cycling) the assemblage structure of periphyton communities (Hill & Knight 1987, 1988b, Hill & Harvey 1990, McCormick & Stevenson 1989, 1991). It has been shown in several experimental studies that intense grazing pressure favours the predominance of scour-resistant prostrate diatoms (e.g. *Achnanthidium minutissimum*, *Cocconeis placentula*) and specialised basal cells of some filamentous

algae (e.g. *Stigeoclonium tenue*, *Batrachospermum moniliforme*). Ungrazed periphyton communities often comprise diverse growth morphologies (Steinman *et al*. 1989, 1991, Mulholland *et al*. 1991, Marks *et al*. 2000, Jones *et al*. 2000a). By comparison heavily grazed algal assemblages are characterised by relatively low species richness and may show complicated interactions with available levels of light (e.g. Lamberti *et al*. 1989, Steinman 1992, Wellnitz & Ward 1998) and/or nutrients (e.g. Mulholland *et al*. 1991, McCormick & Stevenson 1989, 1991). Therefore as the assemblage and abundance of macroinvertebrates was not incorporated into this particular study, it is unknown whether grazing pressure exerted significant impacts on the community structure and diversity of stream periphyton in each of the three target streams. Therefore although I accept there will have been some unquantified losses of periphyton species to herbivory throughout the course of this study, existing biomonitoring protocols take account of natural disturbance mechanisms (e.g. flow, grazing) by sampling the net community composition (concerned primarily with changes in communities in relation to anthropogenic pressures), and therefore do not intend to discuss the matter further.

In the early autumn the onset of heavy precipitation produced variable high flows which removed the periphytic climax community by detaching canopy-forming diatoms and more weakly-attached green filamentous algae vulnerable to the effects of flow disturbance in streams. This underlines the paramount importance of flow scour in punctuating the diversity and successional development of periphyton communities in streams and rivers (Peterson & Stevenson 1992, Biggs & Thomsen 1995). Come winter, substrates were almost entirely denuded except for a near-negligible biomass of tightly adhering scour-resistant diatoms, indicating that the periphyton communities had been restored to an early successional phase. Post-disturbance, periphytic algal succession may recover and resume development but critically this depends upon prevailing environmental conditions, specifically the prevalence and strength of flood disturbances. This suggests that successional climax of periphytic algal assemblages in streams and

rivers will be constrained to summer baseflows, or perhaps other periods of unusually low flow.

The observed temporal and seasonal variation in periphyton community structure described in this study is typical of the ecology of attached algae inhabiting frequently disturbed streams as documented elsewhere (e.g. Welch *et al*. 1988, Lohman *et al*. 1992, Biggs 1995, Stevenson *et al*. 1996). Fluctuations in periphyton diversity were complemented by changes in species dominance, with dramatic reductions in species richness mostly attributable to high-velocity scours in which disturbance-resistant diatoms typically governed community composition. On the whole, the findings of my study underpin the suggestion that the physical effects of flow disturbance are the principal mechanism regulating community composition and ecological succession of periphytic algal assemblages in unregulated fast-flowing streams (Sousa 1984). However primacy of the flow disturbance regime appeared to be seasonally interchangeable with other environmental factors (e.g. daylength, light intensity, temperature), which became more important in influencing periphytic algal species composition and diversity when current velocities were relaxed. For example, during the summer baseflows large algal standing crops developed that were characterised by diverse assemblages of periphyton species selectively driving the formation of the climax community under well-lit, slow flowing habitat conditions. Thereafter variable high flows and predominance of nutrient-poor conditions maintained a diatom dominated community that reflected prevailing streamwater chemistry throughout the winter-spring period. There is some evidence from my study that mild nutrient enrichment was responsible for driving an ecological shift in the species composition and effectively overrode the effects of physical flow disturbance in controlling community structure of stream periphyton. A spontaneous P-loading phenomenon has been described to occur in upland river systems during the early spring in other parts of the UK (e.g. Turner *et al*. 2003, Ellwood *et al*. 2008). From data gathered in April 2006 (most notably in the River Girnock) the findings of my study tend to support evidence of this phenomenon of

P-inputs experienced in comparable upland stream habitats of the Scottish highlands during the spring snow melt. The ecological implication of this P-loading phenomenon was that mild P-enrichment tended to select for an abundance of algae known to be responsive to increased nutrient levels (e.g. *Stigeoclonium*, *Nitzschia*) and particularly favour an ecological dominance of taxa capable of utilising available P (e.g. *Rivularia*). The filamentous cyanobacteria *Rivularia* is known to exhibit phosphatase activity in response to nutrient inputs (Turner *et al*. 2003). Therefore the observed dominance of *Rivularia* in the River Girnock supports general theory that the organism is taking advantage of increased availability of P released during the snowmelt flush. Furthermore this cyanobacteria develops 'hairs' (most probably a gene 'switching on') in response to P-deficiency to increase the surface area available for sequestration from the environment (B. Whitton, pers. comm.).

4.6.1.3 Response of periphyton community composition and diversity in the Water of Dye, River Girnock and Knockan Burn to variation in flow regime: pool, glide and riffle zones

Fast velocity riffles were least diverse in terms of periphyton community composition. This indicated that the critical threshold capacity had been exceeded and vulnerable forms of algae had mostly been removed from the biofilm to leave behind a predominance of scour resistant (e.g. prostrate, erect-adnate and stalked) growth forms which also tended to be higher in abundance (data not shown). Firmly attached disturbance-resistant strategists, chiefly low profile diatoms, adapted for surviving extremely fast current velocities (but probably poorer competitors under low velocity conditions) acquired niche gaps exposed by flow scour within which vulnerable, loosely-packed filamentous forms (otherwise competitively-dominant) were removed from the biofilm (Biggs *et al*. 1998), thus reducing overall community diversity. Adaptations to high current velocity habitats were probably species-specific. Similar to the findings of Lamb & Lowe (1987), slow-flowing pools were characterised by greater algal densities and more

diverse species assemblage mostly attributed to a more developed canopy and higher incidence of rare species. In contrast algal cells are expelled from fast-flowing waters and immigration is impeded (Stevenson 1983, Peterson & Stevenson 1989). Generally there was a significant decline in periphyton community diversity in response to increasing flow velocity, with at least a 20% reduction in species richness from pool to riffle habitats. However, my findings also demonstrate that to an extent periphyton communities exhibited a degree of in-built resistance to shear effects under moderate flow patterns (glides) and in terms of overall community structure and diversity were mostly similar to assemblages congregating in pools despite substantial biomass losses.

4.6.1.4 Response of periphyton community composition and diversity in the Water of Dye, River Girnock and Knockan Burn to variation in substrate morphology

Although periphyton species richness and diversity exhibited a humpback response to more heterogeneous streambed morphologies, this probably reflects the ecological response of freshwater diatoms to an underlying water chemistry gradient, in which streamwaters of circumneutral pH supported a more diverse species assemblage of periphytic algae (see also section 4.6.1.6).

4.6.1.5 Comparison of periphyton community composition and diversity between artificial and naturally-occurring substrata: do artificial substrates make good surrogates for naturally-occurring microhabitats?

Overall, artificial substrate samplers showed similar patterns of variation in periphyton community composition and diversity in respect to most aspects of the study in which they could be compared. Furthermore, the majority of artificial substrate samplers accumulated periphytic algal assemblages which exhibited a high degree of similarity in species composition compared with those growing on

their respective naturally-occurring substrates, although relative abundances tended to vary (data not shown; but see comments in Chapter 3, section 3.6.1.5), usually within a few weeks of exposure (Bergey & Weaver 2004).

This study adhered to recommendation that (at least) a four week interval of exposure to in-situ environmental habitat conditions should be allowed for a representative periphyton community to become established upon artificial substrate samplers (Aloi 1990, Kelly *et al*. 1998, 2001). The work of Schagerl & Donabaum (1998) in the River Danube emphasizes possible repercussions of insufficient interval of exposure (<14 days), where artificial samplers possessed a largely different periphyton compared to naturally-occurring gravel beds. Other studies have also reported low similarities between periphyton assemblages colonising naturally-occurring and artificial substrata (e.g. Tippett 1970, Brown 1976, Siver 1977, Antoine & Benson-Evans 1985, Fisher & Dunbar 2007), but commonly for a different reason: each of these studies used glass or perspex microscope slides as surrogate microhabitat. Biggs (1988) suggested that if glass or perspex microscope slides are to be used as artificial substrates, then these should be sufficiently textured (e.g. etched) to mimic naturally-occurring substrate surfaces and capture a representative periphytic algal community. Aloi (1990) stressed a similar point in a later review of field methods for sampling periphyton.

My findings tend to support the neutral substrate hypothesis (Cattaneo & Kalff 1979) and debate theory of species-specific interactions (e.g. Blindow 1987) between epiphytes and aquatic plant surfaces upon which they accrue, as periphytic algal assemblages occurring on the fronds of plastic aquarium plants strongly resembled those colonising vascular submerged macrophytes. This agrees with the work of Morin (1986) who found that periphyton assemblages growing on the apices of *Myriophyllum heterophyllum* and morphologically similar artificial plants were mostly similar. Furthermore an experimental study conducted under controlled laboratory conditions which used real aquatic plants and plastic replicas, found little evidence of species specificity between epiphytes

and submerged macrophytes, demonstrating instead that plant architecture and environmental factors (e.g. grazing) were more influential in shaping periphyton assemblage structure (Jones *et al*. 2000a).

In conclusion, each of the artificial substrate samplers utilised in this study proved to be good surrogates for supporting a periphyton community composition acceptably comparable to those inhabiting their respective naturally-occurring microhabitat. A number of other ecological studies of lotic periphyton have also found this to be the case (e.g. Grzenda & Brehmer 1960, Lowe & Gale 1980, Khan *et al.* 1987, Biggs 1988, Eulin & Le Cohu 1998, Lane *et al*. 2003). The results also reassure me that replicate samples collected from each sampling location produces similar information concerning periphytic species assemblage and therefore water quality status. Some recent studies (e.g. Ndiritu *et al.* 2003, 2006) have suggested that diatoms colonising artificial substrata are more accurate indicators of water quality than naturally-occurring assemblages. Overall, I feel this justifies integrating the use of artificial substrates in sampling protocols for monitoring water quality where gathering information of community composition and diversity is the main objective. One can also be assured the results obtained from the artificial substrate samplers proposed in this study (i.e. without endorsing smooth surface glass or perspex slides) are reproducible and accurately reflect the naturally-occurring communities. Furthermore, artificial substrate samplers may be of particular benefit for sampling inland waters when logistically it may be problematic or inappropriate to directly sample the naturally-occurring microhabitat (Kelly *et al*. 1998). Above all, utilising artificial substrates between sampling locations, ensures fair, comparable and replicable results (Hurlbert 1984). However, the other side of the coin raises some concerns regarding whether periphytic standing crops harvested from artificial substrate samplers accurately reflect those occurring on the surfaces of naturally-occurring substrata (refer back to Chapter 3, section 3.6.1.5).

4.6.1.6 Periphyton community composition, diversity and environmental habitat conditions in the Water of Dye, River Girnock and Knockan Burn as determined by multivariate ordination and TWINSPAN classification

The diatom species and other periphytic algal flora described in this study are typical of the freshwater communities found throughout the British Isles (Kelly 2000, Kelly *et al*. 2005, John *et al*. 2002).

Variation in periphyton community composition between the TWINSPAN sample-groups highlighted differences in ecological habitat preferences of the species comprising those assemblages. Furthermore, all three multivariate analyses (refer back to section 4.5.6) produced similarly clustered periphyton sample-group species assemblages for the various types of artificial and naturally-occurring substrata sampled.

Although a certainly a major constraint on periphyton production, on the whole flow velocity was not identified as a significant environmental factor responsible for shaping periphyton community structure between the three TWINSPAN sample-groups. Furthermore periphyton assemblages neither appeared to exhibit specific substrate requirements (though streambed substrate particle composition was an inherent characteristic of the predominant underlying geology: refer back to Chapter 2, section 2.7.1), nor accounted for species distribution. It is most probable that an ecological shift in the community composition of periphyton species between the three sample-group assemblages was more strongly influenced by variation in streamwater chemistry than an underlying disturbance-stability gradient. This contrasts with the distribution of other vegetation communities in these streams (e.g. aquatic bryophytes and vascular submerged macrophytes) which were equally as importantly constrained by the effects of flow-substrate interactions and water chemistry (see section 4.6.2.5 and 4.6.3.5, respectively). This suggests that in streams or rivers of near-pristine water quality, periphyton communities tend to be true reflectors of the water physico-chemistry, whereas aquatic bryophytes (section 4.6.2.5) and vascular submerged

macrophytes (section 4.6.3.5) integrate prevailing physical and chemical environmental habitat conditions.

I shall, for now, explore the possibility that variation in periphyton community composition between the TWINSPAN sample-groups highlighted differences in species morphology comprising those assemblages relates to their life strategies and ecological habitat preferences. It is commonly understood that the morphology of aquatic bryophytes (Suren & Ormerod 1998) and vascular submerged macrophytes (Riis & Biggs 2001) provides a fundamental predictor of life strategy. However, referring specifically to the species richness v. standing crop scatterplots (Figure 3.55, Figure 3.56, and Figure 3.57: Chapter 3, section 3.6.1.6) and proposed conceptual habitat-template model (see Figure 4.41, this chapter), I am not convinced that this concept is wholly applicable to periphytic algal assemblages found in my study. My findings do not tend to support Connell's (1978) Intermediate Disturbance Hypothesis and Grime's (1979) C-S-R theory, even though the abundance and diversity of periphyton exhibited a slight hump-back response to environmental gradients of streambed disturbance-stability and streamwater chemistry, this relationship was weak and the proportion of explained variance low (<10%: refer back to Chapter 3, scatterplots in section 3.6.1.6). I postulate that this was due to the fact that the headwaters I studied were of similarly turbulent, frequently disturbed and predominantly oligotrophic character. Perhaps this relationship would have been more apparent across a disturbance gradient of nutrient enrichment, and is discussed elsewhere (refer back to Chapter 3, section 3.6.1.6). Instead, with particular reference to diatoms, my findings were that a range of C-S-R strategists (see Morin *et al*. 2008) spanned the relatively low periphyton production gradient, probably because the physical forces of flow disturbance frequently punctuated algal succession, and when current velocity restraints were alleviated then oligotrophic character of the streams generally capped the upper threshold for potential production. Further to this, I consider that periphyton species richness was specifically a function of water chemistry: circumneutral streamwaters supported a greater diversity of

diatom species (Group II) accommodating a range of acidophilic, indifferent and alkaliphilic taxa indicative of nutrient-poor status. By comparison the streamwaters characterising the Group III and I species-assemblages, fell below and rose above pH 7, respectively, thus placing restrictions upon the ecological distributions of pH-sensitive diatom taxa. This could explain the humpback trend or apparent peak in periphyton diversity (attributed mostly to the diatom flora), and consequently the 'drop off' in species richness at either side of the water chemistry spectrum as one steers away from circumneutral, weakly calcareous, moderately well-buffered waters, towards either acid-sensitive (Group III) or base-rich (Group I) stream habitat conditions. Altogether, the results of my study suggest that water chemistry variables such as pH, are the principal environmental drivers behind variation in the community composition of periphytic algae, especially diatoms, in these near-pristine upland streams. I will outline further supporting evidence of this, subsequently in this section of my thesis.

The moderately-diverse Group I community was mostly restricted to the base-rich streamwaters of Knockan Burn and indicated by the presence of *Fragilaria pulchella*, co-occurring with an abundance of *Cocconeis placentula*, *Didymosphenia geminata*, *Cymbella lanceolata*, *Gomphonema olivaceum* and *Gomphonema olivaceum* var. *olivaceoides*. Many of the diatom species encompassing the Group I assemblage (e.g. *Cocconeis placentula*, *Fragilaria pulchella*, *Cymbella affinis*, *Cymbella lanceolata*, *Denticula tenuis*, *Diatoma tenuis*, *Diatoma moniliformis*, *Didymosphenia geminata*, *Gomphonema olivaceum*, *Gomphonema olivaceum* var. *olivaceoides*, *Meridion circulare, Rhopalodia gibba*) are considered alkaliphilous taxa (preferring pH >7) and indicators of oligotrophic basic streamwaters (Cholnoky 1968, Kelly 2000, Kelly *et al*. 2005, Taylor *et al*. 2007a), in agreement with the habitat conditions allied to this particular periphyton sample-group in my study. Similarly in northern England (U.K.), *Didymosphenia geminata* occurs typically in streams draining upland peat underlain by base-rich strata producing high pH and calcium content (Ellwood & Whitton 2007, Whitton *et al*. 2009). This species is also mostly thought to be

associated with near-pristine reference conditions in other parts of the world (e.g. Potapova & Charles 2007), which again is consistent with the findings of my study. However, more recent evidence points towards a broader ecological tolerance of *Didymosphenia geminata* with mounting reports of excessive growths expanding within its native geographical range, becoming particularly extensive in rivers subject to flow regulation and/or eutrophication (e.g. Kawecka & Sanecki 2003, Kelly 2006, Spaulding & Elwell 2007). The diatom itself has also become a nuisance invasive species in parts of the Southern Hemisphere (Kilroy 2004, Blanco & Ector 2009). Further evidence of the nutrient-poor status of Knockan Burn streamwaters comes from the incidence of species of Epithemiaceae (e.g. *Rhopalodia gibba*, *Denticula tenuis*), the ecology of which tends to be favoured by nitrogen-limitation because their frustules encase endosymbiotic cyanobacteria capable of fixing inorganic N (Geitler 1977, Fairchild & Lowe 1984, Mulholland *et al*. 1991, DeYoe *et al*. 1992). In particular, the presence of *Brachysira vitrea* is a sole indication of oligotrophic and calcareous water chemistry (Lange-Bertalot & Moser 1994). *Fragilaria pulchella* can occur in slightly brackish inland waters (Kelly 2000, Potapova & Charles 2003, Kelly *et al*. 2005, Taylor *et al*. 2007a) and may therefore explain its apparent affinity with chloride on the CCA diagrams shown in Figure 4.17 and Figure 4.21. The diatom *Eunotia bilunaris* var. *mucophila* is frequently epiphytic upon *Batrachospermum* filaments growing in stretches of fast-flowing, clear water streams (Krammer & Lange-Bertalot 1986 - 1991), also supporting findings of my study. Several other studies have described a diatom flora of similar composition to the species assemblage characterising Knockan Burn for comparable unpolluted calcareous mountain streams in other parts of the U.K. (e.g. Jones 1949, Pentecost 1991, Lewis *et al*. 2007), and elsewhere in Europe (e.g. Sabater & Roca 1992, Leira & Sabater 2005, Tison *et al*. 2005).

Group II supported a high diversity periphyton assemblage indicated by an abundance of *Gomphonema acuminatum*, and was mostly associated with the mid- and lower portions of the River Girnock but also Iron Bridge during the summer. This particular assemblage characterised moderately well-buffered streamwaters

of circumneutral pH and weak calcareous chemistry, of which *Gomphonema acuminatum* is a typical indicator species (Taylor *et al*. 2007a). Under these circumneutral habitat conditions, other diatoms (e.g. *Synedra ulna*, *Tabellaria flocculosa, Fragilaria arcus, Fragilaria capucina* var. *gracilis* and *F. capucina* var. *vaucheriae*) common all to three TWINSPAN sample-groups also occurred abundantly, and are generally indicative of high ecological status (Kelly *et al*. 2008). Diatom community composition overlapped with that of the Group I (alkaliphilous) and Group III (acidophilous) assemblages, but also had a distinct microflora of its own (e.g. *Navicula angusta*, *Nitzschia* cf. *acula*, *Nitzschia intermedia*, *Tetracyclus glans*). *Navicula angusta* is one of the few *Navicula* species characteristic of high water quality status (Taylor *et al*. 2007a, Kelly, M. G., pers. comm). In fact, many of the diatom species comprising the Group II community were indicative of low nutrient status and pristine water quality such as *Achnanthidium minutissima* (Potapova & Hamilton 2007) and *Brachysira* spp. (Lange-Bertalot & Moser 1994). Additionally, several species of Epithemiaceae (e.g. *Epithemia adnata and E. sorex*, *Rhopalodia gibba*, *Denticula tenuis*) characterised the Group II assemblage, and are also indicative of oligotrophic habitat conditions because of their microscopic endosymbiotic cyanobacteria, which fix N_2 thus conferring a competitive advantage in low nutrient streams (Mulholland *et al.* 1991). Most notably, the occurrence of particular diatom species (e.g. *Brachysira vitrea*, *Cocconeis placentula*, *Cymbella affinis*, *Didymosphenia geminata*, *Diatoma moniliformis, Diatoma tenuis*) indicated an ecological transition of the outermost Group II assemblage (usually associated with summer baseflow conditions in the River Girnock) inclining towards the Group I community type, which characterised the calcareous Knockan Burn.

Group III consisted of a relatively low diversity periphyton community, characterising the acid-sensitive streamwaters mostly of the Water of Dye but also the upper Girnock during the spring and was indicated by a predominance of *Frustulia rhomboides* var. *rhomboides*. Chiefly the diatom species comprising the Group III assemblage (e.g. *Frustulia rhomboides*, *Tabellaria flocculosa*, *Pinnularia*

subcapitata, *Eunotia exigua*, *Gomphonema parvulum* var. *exilissimum*) are renowned acidophilous taxa (preferring pH <7) and indicators of oligotrophic base-poor streamwaters (Cholnoky 1968, Kelly 2000, Potapova & Charles 2003, Kelly *et al*. 2005, Taylor *et al*. 2007a), agreeing with the habitat conditions associated with this particular periphyton sample-group in my study. Further to this, some morphologically abnormal forms of *Fragilaria* (e.g. *F. capucina* var. *vaucheriae*, *F. capucina* var. *gracilis*, *F. arcus*, and *F. ulna*) occurred infrequently with notches or twists in their frustules (e.g. Figure 4.40) in the periphyton populations, most notably during or shortly thereafter major discharge events, particularly the early spring snow melt. During these spates dramatic disturbances to the poorly-buffered water chemistry occurred (refer back to Chapter 2, section 2.6.2 and 2.7.2) differentiated by reductions in pH and accentuated heavy metal concentrations: changes which are known to cause abnormalities in diatom cell walls (McFarland *et al*. 1997, Sgro *et al*. 2007). It is of general consensus that abnormal diatom valves pinpoint heavy metal contamination (Kelly 2000). However, in my study the incidence of distorted diatom valves was consistently <1% indicating that Group III sampling sites were minimally-impacted by acidification, whereas an incidence of >10% abnormal valves would tend to suggest that heavy metal contaminants were exerting significant effects on the population (Kelly, M. G., pers. comm). Furthermore overland run-off events affected diatom microstructure in other more subtle ways: an evident peak in the abundance of acidophilous (and acidobiontic) taxa (e.g. *E. exigua*, *F. rhomboides*, *P. subcapitata*) was indicative of extremely acidic episodes under which conditions these taxa temporarily flourished to dominate community composition. The co-occurrence of the aforementioned taxa resemble the characteristic flora described as inhabiting streams impacted by acid mine drainage (e.g. Verb & Vis 2000). Therefore it may be difficult to distinguish between the effects of low pH and elevated heavy metals as differential drivers of diatom community composition in streams, as these environmental factors may synergistically structure species assemblages (EA 2008). However, Hirst *et al*. (2004) showed that transplantations of substratum from circumneutral streamwaters to acidic streamwaters encouraged rapid accumulation of *Eunotia*

exigua and was primarily driven by changes in water chemistry. Similarly, *F. arcus* and *D. mesodon* have been reported as potential reflectors of heavy metals in the upper tributaries of the Animas River system in Colorado previously disturbed by mining activity (e.g. Sgro *et al.* 2007), whilst others remark that these diatoms prefer low streamwater conductivities (e.g. Potapova & Charles 2003) and are generally indicators of high ecological status (e.g. Juttner *et al.* 2003). Furthermore, a number of other studies have reported diatom-dominated communities comparable to the Group III community type of my study as occurring in oligotrophic, relatively base-poor streams draining mountainous habitats worldwide, including elsewhere in the U.K. (e.g. Lewis *et al*. 2007, Kelly *et al*. 2008), Australia (e.g. Chessman 1986), and Finland (e.g. Eloranta & Soininen 2002).

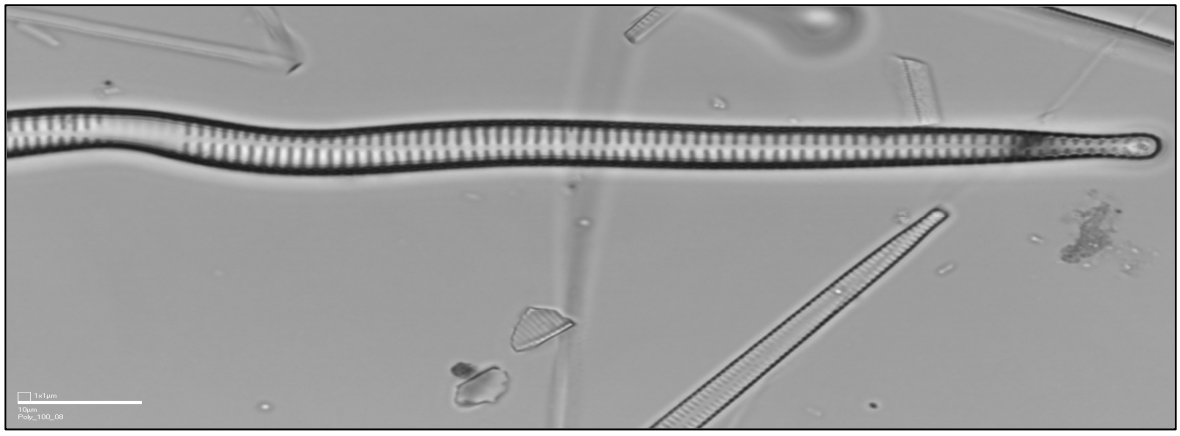


Figure 4.40 Abnormal *Fragilaria* valve

It has been known for a long time that diatoms exhibit preferential habitat occurrences relating to pH and nutrient status (Kelly 2000). In my study water chemistry parameters (other than nutrient status), especially pH and closely-related attributes (e.g. conductivity, alkalinity) coupled to variation in base cation composition (e.g. Ca^{2+}, Mg^{2+}) were the strongest drivers of diatom community composition in the oligotrophic streams. The results presented show that diatom species assemblages were distributed along gradients of water chemistry characteristics, particularly pH and ionic composition, and are consistent with findings elsewhere which have also shown these to be the major environmental variables structuring freshwater diatom communities in streams in rivers throughout Europe (e.g. Vilbaste & Truu 2003: Estonia; Soininen 2004, Soininen &

Könönen 2004: Finland; Sabater & Roca 1992, Leira & Sabater 2005, Blanco *et al*. 2008, Urrea & Sabater 2009: Spain; Feio *et al*. 2009: Portugal), the U.S.A. (e.g. Munn *et al*. 2002, Potapova & Charles 2002, 2003, Charles *et al*. 2006), Canada (e.g. Winter & Duthie 2000, Griffith *et al.* 2002, Wunsam *et al*. 2002, Lavoie *et al*. 2004), Africa (e.g. Cholnoky 1968, De la Rey *et al*. 2004, Taylor & Lange-Bertalot 2006, Taylor *et al*. 2007bc, Archibald & Taylor 2007, Ndiritu *et al.* 2003, 2006, van Vuuren *et al*. 2008), Nepal and northern India (e.g. Juttner *et al*. 2003). The majority of these studies examined streams and rivers subject to various anthropogenic influences, most commonly from sources of nutrient enrichment. Therefore such reports of strong conductivity and hardness gradients driving diatom species assemblages may not be at all surprising, since these are often associated with eutrophication. There are a few recent UK (e.g. Kelly *et al*. 2008) and European-based studies (e.g. Cantonati *et al*. 2001, Rimet *et al*. 2004, Tison *et al*. 2005, Bona *et al*. 2007), comparable to the approach undertaken in my study, which have attempted to distinguish benchmark diatom communities characterising reference headwaters and the principal environmental parameters driving their formation. For the most part, these benchmark studies found that variation in water chemistry gradients, particularly carbonate hardness and closely-related environmental factors (e.g. pH, alkalinity, conductivity, Ca^{2+}), were the most important environmental constraints determining diatom species composition in streams of near-pristine reference condition.

The findings of my study revealed that diatom species assemblages were most profoundly distinct in streamwaters of contrasting water chemistry, with pH and closely-related factors (e.g. conductivity, alkalinity) identified as the major determinants of diatom community structure, though other mineral components (e.g. Ca, Mg, Cl) were also of importance. Accordingly, diatom communities were most ecologically divergent between base-poor, acid-sensitive and base-rich, calcareous stream habitat conditions: corresponding to the Group III community type which was characterised mostly by acidophilic diatoms unlike the Group I assemblage distinguishable from a predominance of alkaliphilic taxa. It could

further be inferred that Group II functioned as an ecological bridge between the other two assemblages, encompassing a diverse diatom microflora occurring also in Group III and I, as well as a distinct assembly of other species characteristic of moderately acid, weakly calcareous streamwaters. A number of other studies have also described a distinct shift in diatom species composition in pristine streamwaters of contrasting water chemistry, from naturally acidic to well-buffered habitat conditions (e.g. Rimet *et al*. 2004, Lewis *et al*. 2007, Bona *et al*. 2007, Urrea & Sabater 2009), and assemblages resembling those of Groups III and I of my study, respectively. Collectively, this underpins the importance of the underlying geology as a predetermining factor controlling diatom community composition by influencing prevailing water chemistry characteristics in streams of near-pristine status (reference conditions). Feasible explanations as to why diatoms exhibit ecological preferences for pH may reflect species affinity for forms of prevailing carbon (e.g. CO_2, HCO_3^-, CO_3^{2-}). Thus carbonate hardness may directly select for C-user status, or possibly implications for nutrient acquisition (e.g. Ca^{2+}) in diatoms. This is generally speculative, as causal mechanisms tipping the balance of species assemblages are not yet fully understood and knowledge of inorganic carbon acquisition in diatoms is particularly limited (reviewed in Roberts *et al*. 2007). However, it is known that the function of the underlying geology in governing water chemistry characteristics in streams of reference conditions is overridden in rivers exposed to the effects of nutrient enrichment (see Tison *et al*. 2005, Bona *et al*. 2007, Urrea & Sabater 2009). River diatom communities representative of impacted water qualities, those disturbed by eutrophication, are characterised by an abundance of *Navicula* and *Nitzschia* species (e.g. *Navicula lanceolata*, *Navicula gregaria*, *Nitzschia dissipata*, *Nitzschia palea*). A high abundance of these motile diatoms indicates degraded water quality, and many of the assemblages resemble impacted Scottish rivers (e.g. River Clyde catchment, reviewed in Lang & Krokowski 2010). Thus the scarceness of motile *Navicula* and *Nitzschia* taxa (usually <2%) tends to support my contention that the streams studied were of (at least) good, if not, high ecological status (Kelly, M. G., pers. comm).

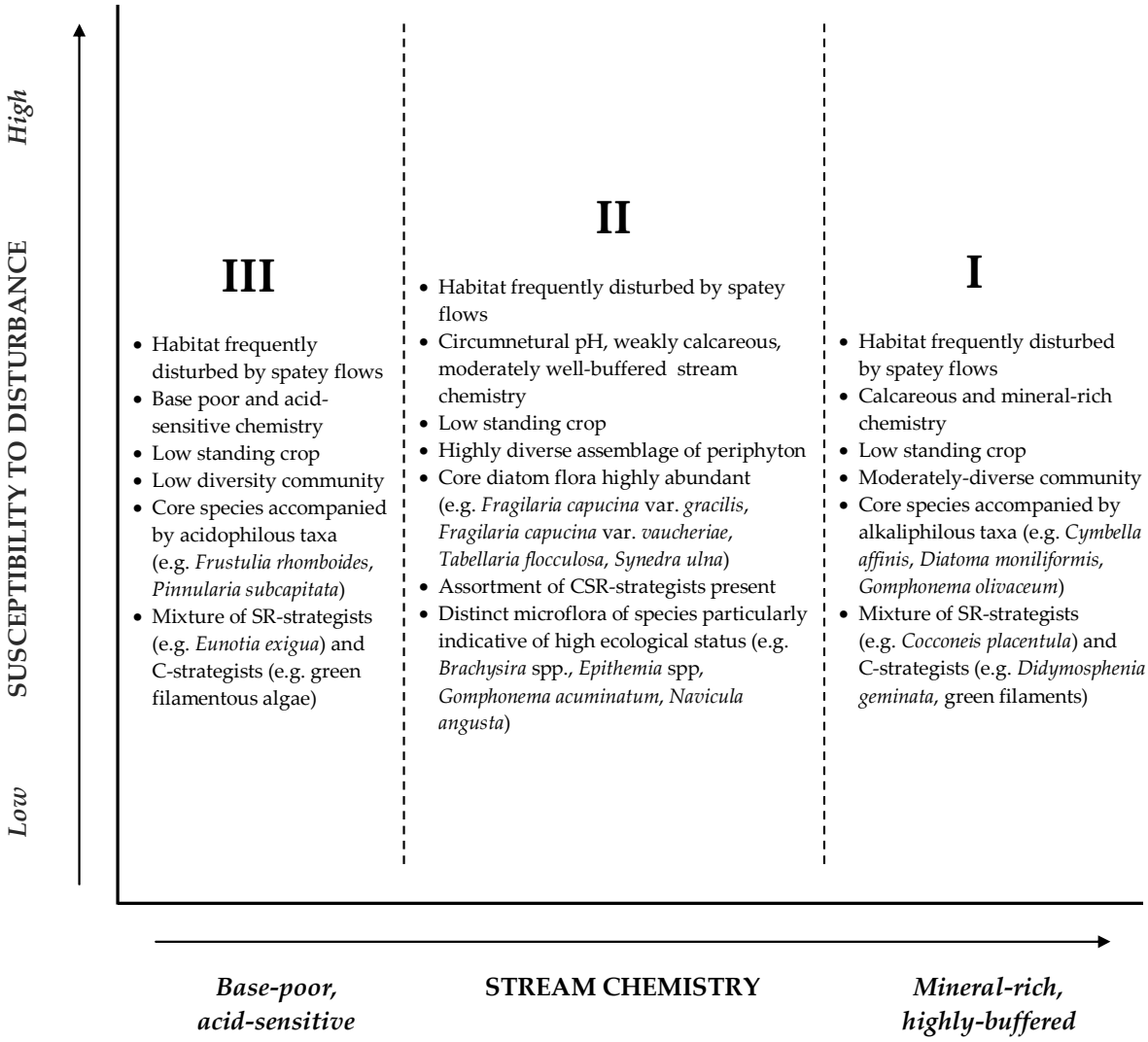


Figure 4.41 Conceptual habitat-template model (adapted from Morin *et al*. 2008) of the TWINSPAN sample-groups indicating their position on the axes in relation to environmental gradients of streamwater chemistry and streambed disturbance, showing variation in diversity, community composition and abundance of periphyton in the target streams of this study.

The three TWINSPAN sample-groups overlapped with each other due to the presence of a background periphyton community composed of several ubiquitous species characteristically common to almost all samples (e.g. *Achnanthidium minutissima*, *Fragilaria capucina* var. *gracilis*, *F. capucina* var. *vaucheriae*, *Synedra ulna*, *Tabellaria flocculosa*, *Mougeotia* sp., and *Ulothrix* sp.). Perhaps it could be postulated that in response to contrasting water chemistry, this 'core' periphyton community diverged into two distinct assemblages (I and III) accompanied by a composition of diatom species exhibiting their own ecological habitat preferences. Therefore this 'core' periphyton assemblage comprised of many diatom species ordinated

close to the centre of the CCA diagram, mostly preferencing circumneutral pH but distributed over ranging alkalinities, may be representative of high or (at least) good ecological status (Kelly *et al*. 2008). The central ordination of the green filamentous algae is suggestive of cosmopolitan distribution (John *et al*. 2002). For example, it is known that *Mougeotia* can be highly abundant in both acid and alkaline waters, having a broad range of tolerance to pH and heavy metals (Graham *et al*. 1996b), the ecology of which was never attributed to different species of the algae. I have given thought to the possibility that a clear pattern did not emerge from the the green filamentous algal community because most were not identified to species taxonomic level, which may have revealed more specific ecological information in regards to favoured environmental habitat conditions. This is attributed to difficulty of ascertaining confident species identification for green filaments, particularly Zygnematacean algae (e.g. *Mougeotia*, *Spirogyra*) if they were not sexually reproducing at the time of sampling (characteristic features associated with conjugation patterns and spore production are usually required to identify green filaments to species level: see John *et al*. 2002) and therefore filaments in their vegetative state are not generally useful for this purpose. Foerster *et al*. (2004) successfully showed that benthic algae, other than diatoms, have potential bioindicator value from specific species assemblages formed in relation to water chemistry characteristics of near-pristine rivers in Germany. Therefore I may have lost some precision within multivariate ordinations because filamentous Zygnematacean algae could only be identified to generic level.

With special reference to the *Achnanthidium minutissima* type, this species itself is probably part of a larger species complex, in other words, an aggregation of morphologically similar phenotypes, and is currently undergoing substantial taxonomic revision (as are many other diatom aggregates e.g. *Navicula* senso). Characteristically, *Achnanthidum minutissima* is often regarded as an indicator of good ecological status and ubiquitous to nutrient-poor habitats (Kelly & Whitton 1995, Potapova & Charles 2007, Ponader & Potapova 2007, Potapova & Hamilton 2007, Kelly *et al*. 2008). More recently for species aggregates of *Achnanthidum*

minutissima, it has been shown that various morphotypes exhibited ecological preferences for certain water chemistries. Potapova & Hamilton (2007) illustrated that the undifferentiated *A. minutissima* type formed the core 'species' and from this various morphotypes branched out relating principally to variation in pH, conductivity, ionic content and nutrient status. This emphasizes how critical it is to identify diatoms to species level as accurately as possible, and in a consistently manner, even for difficult taxa such as those of the *Achnanthidum minutissima* complex because this may have implications for evaluating environmental water quality. This may help explain why the *Achnanthidum minutissima* type occurred as a centrally-distributed species in my study, which in fact may have been comprised of several morphologically similar species aggregates. Even amongst expert diatomists, species belonging to the *Achnanthidium minutissima* complex are notoriously difficult to distinguish using solely the light microscope due to their tendency to share ambiguous characteristics (e.g. size, shape, striation density and pattern) (Cantonati & Lange-Bertalot 2006, Ponader & Potapova 2007, Potapova & Hamilton 2007). I have therefore considered the possibility that the *Achnanthidum minutissima* type of Knockan Burn may be *A. dolomiticum*, recently identified from oligotrophic, mineral rich spring waters draining Dolomitic limestone catchments in the Italian Alps (Cantonati & Lange-Bertalot 2006). Although the habitat conditions there are certainly similar to those of Knockan Burn, it would be necessary to scan specimens with electron microscopy to ascertain whether morphological characteristics correspond with those of *Achanthidium dolomiticum* described by Cantonati & Lange-Bertalot (2006).

To summarise, in these near-pristine upland stream habitats in Scotland, underlying geology was identified as the major macro-scale factor influencing the distribution of periphyton, especially diatom species, by pre-determining the inherent properties of meso-scale factors, principally water chemistry gradients (e.g. pH, conductivity, alkalinity, mineral cations). In contrast, physical micro-scale factors (e.g. flow, substrate morphology) were not particularly useful as explanatory drivers of the formation of stream diatom assemblages (Taylor *et al*.

2005). The conventional Baas Becking (1934) principle asserts that for micro-organisms like diatoms, species distributions are ubiquitous, in other words, 'everything is everywhere'. This concept postulates that regional factors (e.g. biogeography, climate) do not constrain species dispersal, rather, local processes (e.g. disturbance, competition) determine micro-organismal community composition (Vanormelingen *et al*. 2008). Thus similar habitats are expected to support similar species irrespective of geographic location. In my study, the three periphyton assemblages were characterised by a proportion of cosmopolitan diatom species which probably diverged from a regional species pool in response to variation in streamwater chemistry. Although local-scale elements (e.g. competition under base flows, intense disturbance under turbulent flows) can regulate species diversity, regional-scale factors (e.g. environmental gradients) were clearly important drivers shaping diatom community composition. Therefore interactions between local and regional processes appear to structure diatom assemblages and species richness in flowing waters (Soininen *et al*. 2009, Passay 2009). Clearly this is an area that would benefit from further research.

Upland oligotrophic stream habitats of acid-sensitive and base-poor water quality (Group III) were characterised by acidophilous species (e.g. *Frustulia rhomboides*, *Eunotia exigua, Pinnularia subcapitata*), whereas calcareous and mineral-rich streamwaters (Group I) were characterised by known alkaliphilous species (e.g. *Cocconeis placentula*, *Cymbella lanceolata*, *Diatoma moniliformis*, *Gomphonema olivaceum*). Species-assemblages III and I represent two main groups of periphyton occurring in near-pristine oligotrophic headwater streams of contrasting character (e.g. underlying geology, water chemistry, buffer capacity) for upland stream habitats in Scotland. Further to this, the Group II community type formed an ecological bridge between the two diverging water chemistries and embraced a number of diatom species particularly distinctive of high water quality (e.g. *Brachysira* spp., *Navicula angusta*).

Overall, the findings of this study have proven diatoms to be especially useful as bioindicators of water quality by providing baseline assemblages across a ranging geology, and responding directly to the chemical properties of the reference streamwaters studied. In this respect, more attention has been paid to diatoms, whilst few other studies (e.g. Foerster *et al*. 2004) have attempted to elucidate the usefulness of entire periphytic algal assemblages in a biomonitoring role for assessing water quality under the WFD.

Therefore the results of this study may contribute to further development of an already existing approach in ecological biomonitoring tools (e.g. DARLEQ) implemented under the WFD by providing specific knowledge of indicator species characterising near-pristine habitat conditions and the principal environmental drivers responsible for their formation. This information could be utilised to determine the extent to which periphyton species-assemblages of impacted rivers deviate from benchmark reference communities of similar river typology, for ascertaining water quality status of streams and rivers throughout Scotland, and whole of the UK.

4.6.1.7 Predicting freshwater periphyton community composition and diversity

As expected, a combination of water chemistry (e.g. conductivity) with variation in water physico-chemistry (e.g. temperature) variables strongly predicted temporal variation in periphyton species diversity from their role as environmental factors driving periphytic algal succession and community composition. Incorporation of logger flow data rather than snap shot current velocity measurements (see comments in Chapter 3, section 3.6.1.7) may have improved the predictive power of the model and helped to more accurately predict the response variable.

4.6.2 Aquatic Bryophytes

4.6.2.1 Variation in aquatic bryophyte community composition and diversity in the Water of Dye, River Girnock and Knockan Burn; their sub-catchments and sites

In general, the observed variation in aquatic bryophyte species richness and diversity reflected the predominant growth forms and life strategies of the assemblages present, which in turn assisted in characterising the environmental habitat conditions for each of the sampling stretches in the target streams comprising the study. Aquatic bryophyte species morphology and habitat ecology are addressed in more detail in subsequent sections (e.g. 4.6.2.3, 4.6.2.4, 4.6.2.5) of this chapter.

4.6.2.2 Seasonal variation in aquatic bryophyte community composition and diversity in the Water of Dye, River Girnock and Knockan Burn

Aquatic bryophytes are characteristically slow-growing, sessile producer organisms which often require many years to become established within a stream ecosystem following their initial attachment (Biggs 1996). Furthermore aquatic bryophyte species composition is not expected to change over short time periods and often the species that become established are markers of the physical habitat conditions in place (Suren & Ormerod 1998). Therefore it is not surprising that aquatic bryophyte species richness and diversity did not exhibit significant seasonal variation in each of sub-catchment streams, reinforcing aquatic bryophytes as stable persistent communities indicative of the integrated environmental habitat conditions in which they occur.

Notably however, aquatic bryophyte species richness and diversity tended to be lower, though not significantly so, in the summer season in each of the streams (refer back to section 4.5.10). This can probably be attributed to the sampling regime which was based on recording the occurrence of aquatic bryophytes

beneath or close to the water surface at the time of sampling. Therefore when the water level dropped (e.g. during summer base flow conditions) obligate aquatic species were more conspicuous in submerged habitats, and I probably lost records of other species such as turfed facultative or semi-aquatic forms anchoring mainly to the terrestrial microhabitat of the tops of large boulders situated well above the water line at the time of sampling. This further supports other theory of vertical zonation (e.g. Muotka & Virtanen 1995, Virtanen 1995, Virtanen *et al*. 2001) stream bryophytes and subsequent discussion in this chapter (see sections 4.6.2.3, 4.6.2.4 and 4.6.2.5).

4.6.2.3 Response of aquatic bryophyte community composition and diversity in the Water of Dye, River Girnock and Knockan Burn to variation in flow regime: pool, glide and riffle zones

The effects of substrate stability and hydrological regime are often considered together, because of the physical association that exists between substrate morphology and flow patterns in streams (refer to Chapter 2, section 2.7.3). In turbulent streams, like those of this study, disturbance of aquatic bryophyte communities probably occurs in two forms: spates (flow intensity, propensity of substratum repositioning) and fluctuating water levels (drought, submergence, vertical zonation) as discussed by Muotka & Virtanen (1995), Suren & Duncan 1999, and Virtanen *et al*. (2001).

The interactions between streambed morphology, flow patterns and bryophyte occurrence are well-known, and boulder-riffle zones are often described as the principal habitat colonised by aquatic bryophytes (e.g. Hynes 1970, Haslam 1978, 2006, Suren 1996, Nikora *et al*. 1998, Suren & Duncan 1999, Suren *et al*. 2000, Linhart *et al*. 2002ab). Generally, my preceding findings on this indicate that aquatic bryophyte species diversity (section 4.5.11) and abundance (see Chapter 3, section 3.5.11) increased as current velocity increased, with most occurrences in stable habitats characterised by riffles as opposed to slow-flowing pools

characterised by deposits of unstable substrate particles, in which stream bryophyte occurrence was, at best, patchy. Fundamentally, the work of this study has separated the overriding influential effect of streambed substrate morphology (stability) from within-stream flow variations (pool-glide-riffle habitats) in determining aquatic bryophyte community composition in upland streams in Scotland.

Aquatic bryophytes are a successful group of plants, occupying a set of niches unavailable to most other macrophytes (with the notable exception of the Podostemaceae, which tend to replace bryophytes in the disturbed fast-flowing stream habitats of tropical upland rivers: Cook 1990), by utilising their specialised rhizoids to anchor themselves securely to a suitable substrate (Hynes 1970, Biggs 1996, Stream Bryophyte Group 1999). Hence stream bryophytes are generally well-adapted to turbulent habitats, and are morphologically constructed to withstand the forces of intense flows (Muotka & Virtanen 1995, Suren 1996, Suren *et al*. 2000). However, the effect of flow on aquatic bryophyte production is another matter and my results in relation to this issue are discussed in detail elsewhere in this thesis (refer back to Chapter 3, section 3.6.2.3).

Rather than flow intensity (which is a function of discharge, velocity and spatiness) pinpointed by other research as a key factor affecting species composition and abundance in flow-regulated rivers (e.g. Englund *et al*. 1997, Vanderpooten & Klein 1999a, Vanderpooten & Klein 2000), the findings of this study suggest that substrate morphology is a major constraining factor of the distribution and diversity of aquatic bryophytes in upland streams in Scotland, as discussed in the following section (4.6.2.4).

The aquatic liverwort *Chiloscyphus polyanthos* appeared to dominate high-velocity stretches of streams in north-eastern Finland (Muotka & Virtanen 1995), and in this study also commonly occurred in fast-flowing habitats (see CCA ordination: Figure 4.28). However, this was probably more attributable to the boulder-riffle

effect that is indicative of substrate stability, rather than the effects of flow regime itself (as discussed next in section 4.6.2.4).

4.6.2.4 Response of aquatic bryophyte community composition and diversity in the Water of Dye, River Girnock and Knockan Burn to variation in substrate morphology

Generally, as the size and therefore stability of the streambed substrate particles increased, the quantity of supported aquatic bryophyte species grew, whereas smaller and less stable substrate particle sizes tended to harbour fewer species. Therefore in agreement with theory and observations elsewhere, the results of this study reiterate the critical role of substratum stability in providing suitable habitat for aquatic bryophytes in the turbulent conditions typical of mountain streams (Haslam 1978, 2006, Suren & Ormerod 1998, Suren & Duncan 1999, Virtanen *et al*. 2001, Linhart *et al*. 2002ab, Heino *et al*. 2005, Scarlett & O'Hare 2006). Mostly only substrate particles large enough to withstand the disturbance mobilisation forces occurring during spates in such streams form sufficiently stable habitats for aquatic bryophyte colonisation and establishment (Slack & Glime 1985, Englund 1991, Suren 1991, Steinman & Boston 1993, Muotka & Virtanen 1995, Suren 1996, Suren & Ormerod 1998, Duncan *et al*. 1999, Suren & Duncan 1999, Stream Bryophyte Group 1999). Hence smaller substrate particles (e.g. cobbles, gravel and sand) due to their inherent instability and predisposition to dislodgement during spates usually provide inadequate surfaces for the attachment of aquatic bryophytes (Glime & Clemons 1972, Haslam 1978, 2006, Suren 1996, Suren & Duncan 1999).

Large streambed structures, principally boulders (and consequently riffle habitats: refer back to Chapter 2, section 2.7.3) were identified as stream microhabitats sustaining particularly high diversity species-assemblages of aquatic bryophytes. To an extent substrate stability is certainly a contributory factor (as seen from unstable substrates lacking bryophyte flora) and thereby determines the

possibility of occurrence of bryophytes in the first place. However, the size or more specifically, height or profile of boulders protruding above the surface (and thus propensity to create 'riffles') in shallow streams (like those of this study) is a supplementary key factor determining the diversity of aquatic bryophyte species present by providing a moisture gradient from microhabitats mostly continuously submersed to intermittently inundated by streamwater. This is often termed '*vertical zonation*' (Muotka & Virtanen 1995, Virtanen 1995, Virtanen *et al*. 2001) and explains why boulders in particular embraced a rich aquatic bryophyte flora, comprising both obligatory aquatic species (e.g. *Fontinalis antipyretica*, *Platyhypnidium riparioides*: restricted to lower down, characteristically submerged habitats) as well as facultative and semi-aquatic species (e.g. *Racomitrium aciculare*, *Schistidium agassizii*, *Schistidium rivulare*: often limited to the transitional splash zones and emerging higher up, on the more exposed parts of the substrate). The results of my study support theory and the observations of other work (e.g. Ormerod *et al*. 1994, Muotka & Virtanen 1995, Virtanen 1995, Suren 1996, Virtanen *et al*. 2001) regarding the importance of substrate morphology (size and composition) in structuring aquatic bryophyte species-assemblages in headwater streams.

4.6.2.5 Aquatic bryophyte community composition, diversity and environmental habitat conditions in the Water of Dye, River Girnock and Knockan Burn as determined by multivariate ordination and TWINSPAN classification

The species recorded in this study are typical of the bryophyte flora associated with moist habitats, and in particular upland streams occurring in mostly temperate to sub-arctic regions of the UK, Europe, and similar high-latitude regions elsewhere (Hynes 1970, Watson 1981, Paton 1999, Smith 2004, Scarlett & O'Hare 2006, Hill *et al*. 1991, 1992a and 1994).

Variation in aquatic bryophyte community composition between the TWINSPAN sample-groups highlighted differences in morphologies of the species comprising those assemblages in relation to their life strategies and ecological habitat preferences. It is commonly understood that aquatic bryophyte morphology provides a fundamental link to life strategy (Muotka & Virtanen 1995, Englund *et al*. 1997, Suren & Ormerod 1998, Suren & Duncan 1999, Virtanen *et al*. 2001). Referring specifically to the species richness v. standing crop scatterplot (Figure 3.59: Chapter 3, section 3.6.2.5) and proposed conceptual habitat-template model (Figure 4.42, this chapter), the results of this study agree with the predictions of the Intermediate Disturbance Hypothesis of Connell (1978) and Grime's (1979) C-S-R theory: the abundance and diversity of aquatic bryophytes exhibit a hump-back response to environmental gradients of streambed disturbance-stability and streamwater chemistry.

These findings are similar to those outlined in other studies (e.g. Muotka & Virtanen 1995, Suren & Ormerod 1998, Suren & Duncan 1999, Virtanen *et al*. 2001), depicting an ecological shift in the community composition and standing crop of aquatic bryophyte species probably in relation to a stream disturbance-stability gradient. However, I also found that the species-assemblage and functional attributes of stream bryophytes are not only constrained by the effects of flow-substrate interactions (e.g. scour, streambed stability) but are also strongly influenced by streamwater chemistry. Therefore according to the disturbance-stability gradient, the most unstable and highly disturbed habitats are characterised by a limited number of turfed SR-strategists (Group I community-type: *Blindia acuta*, *Schistidium agassizii*) and a low standing crop, whereas the most stable habitats are often dominated by a single C-strategist (with few, if any, co-occurring species) and possess a naturally high standing crop, a functional attribute of its trailing carpet morphology (Group III community-type: *Fontinalis antipyretica*). The niche continuum existing between either extreme on the disturbance gradient is characterised by a range of intermediate environmental disturbances thus supporting a moderate standing crop of diverse species-

assemblages (Group II and IV community-types), in which neither superior competitors nor subordinate disturbance-tolerators manage to attain dominance. However, it appears that a combination of stream substrate morphology and water chemistry determines the species composition of aquatic bryophytes occupying these mostly stable and frequently disturbed habitats. The stream habitats to which I refer (those inhabited by assemblages II and IV) were often characterised by core partner species, *Fontinalis antipyretica* and *Platyhypnidium riparioides*, two widespread obligate aquatic mosses commonly found together, generally known to occur in closely-similar niches and wide-ranging habitats in streams (Hynes 1970, Watson 1981, Kelly & Whitton 1987, López *et al*. 1997, Whitton 1999, Smith 2004, Scarlett & O'Hare 2006) and lakes (Karttunen & Toivonen 1995). Although embracing these core similarities, the two assemblages (II and IV) were distinctly segregated in their composition of accompanying aquatic bryophytes, each with a mixture of C-S-R strategists, exhibiting their own ecological habitat preferences. Critically, it is the composition of these (perhaps specialist) accompanying species that prove useful in defining the environmental habitat conditions of the streams in which these assemblages occur. In Group II, core species *Fontinalis antipyretica* and *Platyhypidium riparioides* were accompanied by typically acidophilous species (e.g. *Scapania undulata*, *Hygrohypnum ochraceum*), whereas in Group IV both mosses are often accompanied by known calcicole species (e.g. *Chiloscyphus polyanthus*, *Hygrohypnum luridum*, *Palustriella falcata*). Suren & Ormerod (1998) also found that together, substrate stability and water quality (other than nutrient status e.g. pH, conductivity, base cation concentrations particularly Ca^{2+} and Mg^{2+}, buffer capacity) structured the aquatic bryophyte communities of Himalayan streams in Nepal. Although few studies have exclusively examined aquatic bryophyte communities occurring in near-pristine habitat conditions (and therefore often not without built-in anthropogenic influence), there are some similar findings in streams of England and Wales in the UK (e.g. Scarlett & O'Hare 2006), France, Germany and Switzerland (e.g. Thiebaut *et al*. 1998, Vanderpooten & Klein 1999ab, Stetzka & Baumann 2002), Northern Spain (e.g. García-Álvaro *et al*. 2000), Western Canada (e.g. Vitt *et al*. 1986), and

New Zealand (e.g. Suren 1996). Specifically for the UK, Scarlett & O'Hare (2006) described stream bryophyte species-assemblages similar to those supported in this study occurring in comparable habitats, and an ecological shift in the community composition of aquatic bryophytes characterising softwater upland streams and calcareous lowland rivers in England and Wales. Furthermore, at least thirteen of the aquatic bryophyte species occurring in my study were also listed in the British river dataset of Scarlett & O'Hare (2006), indicating that my dataset shared >75% floristic similarity with their work. In keeping with the results of this study (Group II), Scarlett & O'Hare (2006) found that elsewhere in the UK, *Scapania undulata* commonly occurred in streams characterised by hard geology and base-poor, acid-sensitive water chemistry, as did the work of Ormerod *et al*. (1987). A number of aquatic bryophyte species that co-occurred with *Scapania undulata* in the work of Scarlett & O'Hare (2006), also frequently accompanied the liverwort in my study (e.g. *Hygrohypnum ochraceum*, *Pellia epiphylla*, *Racomitrium aciculare*, *Brachythecium plumosum*, and *Mnium hornum*), most of which are acidophilous (Watson 1981, Hill *et al*. 1991, 1992a & 1994, Paton 1999, Smith 2004). On the other hand, in streams characterised by softer geology and base-rich, well-buffered water chemistry, Scarlett & O'Hare (2006) identified an aquatic bryophyte community composed of *Platyhypnidium riparioides* and several co-occurring species (e.g. *Hygrohypnum luridum*, *Chiloscyphus polyanthos*, and *Palustriella falcata* [= *Cratoneuron commutatum*]), many of which are known calcicoles (Watson 1981, Hill *et al*. 1992a & 1994, Smith 2004) and akin to those found in similarly calcareous habitats of my study (Group IV). Perhaps it is also worth mentioning that a few turfed forms, namely *Blindia acuta*, *Schistidium agassizii* and *Racomitrium aciculare*, tended to occur high up on the gradient of the 'steep slope' arrow on the CCA digram of Scarlett & O'Hare (2006). This supports general theory that these particular aquatic bryophyte species characteristically occur in more disturbed stream habitats like those of Group I community in my study.

Group I comprised a low diversity aquatic bryophyte community indicated by the turf moss *Blindia acuta*, together with occasional clumps of other low carpet forms:

Schistidium agassizii and *Racomitrium aciculare*. The incidence of these turfed life forms and absence (or rarity) of obligate aquatic species (e.g. *Fontinalis antipyretica*, *Platyhypnidium riparioides*) in the streambeds of Hampshire's Bridge and Littlemill in the River Girnock suggests habitat conditions are flashy and frequently disturbed (a common feature of each of the target streams in this study) but crucially that the substratum is predominantly unstable and susceptible to the effects of turbulence during spates. Characteristically, turfed forms of aquatic bryophytes are good stress/ disturbance-tolerators or SR-strategists (Grime 1979). They are fast-colonising pioneer species capable of withstanding arduous conditions (not least, by virtue of their small size, which means they can utilise micro-habitats, such as crevice refugia, unavailable to most other bryophyte life forms) and characteristically thrive in unpredictable habitats (Watson 1981, Muotka & Virtanen 1995, Suren & Duncan 1999, Virtanen *et al*. 2001, Smith 2004). *Blindia* plants are hydrodynamically-streamlined in morphology (Suren *et al*. 2000) and are recognised as good disturbance-tolerators in a number of other studies (e.g. Muotka & Virtanen 1995, Suren & Duncan 1999). *Schistidium agassizii* and *Racomitrium aciculare* are also semi-aquatic bryophytes with naturally-robust disturbance resistance cushion growth morphology (e.g. thin-branched streamlined morphology, small bushy apical leaves and wiry stems). Often such semi-aquatic species are prolific in unstable stream habitats, tightly hugging the substrate surface and commonly out-competed by other aquatic bryophytes lower down in the zonation (Watson 1981, Muotka & Virtanen 1995, Virtanen *et al*. 2001, Smith 2004). True aquatic forms such as *Fontinalis antipyretica* and *Platyhypnidium riparioides*, known to occur in the upper River Girnock (e.g. Iron Bridge), were unsuccessful in becoming established further downstream in that river. Sexual sporulation is considered to be a rare occurrence in obligatory aquatic bryophytes like *Fontinalis* spp., opting for vegetative fragmentation (usually under high flows) and downstream rhizoid dispersal as the normal mode of reproduction (Suren & Duncan 1999, Stream Bryophyte Group 1999, Siebert *et al*. 1996, Davies 2007, Muotka & Syrjanen 2007). Therefore it is assumed that had stable habitat conditions been available downstream of Iron Bridge in the River Girnock, then

submerged life forms such as *Fontinalis antipyretica* and *Platyhypnidium riparioides* would have been there also. However the lack of obligatory aquatic bryophytes coupled to the incidence of turfed semi-aquatic species points towards the Hampshire's Bridge and Littlemill stretches of the River Girnock, as being particularly highly disturbed and unstable stream habitats, compared to the upstream stretch. In the Water of Dye where streambed morphology is relatively homogenous throughout, aquatic bryophyte community composition was also more homogenous in the three stretches sampled.

Group II encompassed a moderately diverse aquatic bryophyte community of mixed morphologies including some canopy-formers (e.g. *Fontinalis antipyretica* and *Hygrohypnum ochraceum*) and an abundance of short mat formers (e.g. *Scapania undulata*, *Racomitrium aciculare*, *Schistidium rivulare*). This aquatic bryophyte community characterised mostly the Water of Dye and upper River Girnock (which harboured an overlapping species composition with Group I). The predominance of large boulders meant that streambed morphology was mostly stable and could accommodate vertical zonation of aquatic bryophytes, explaining why the species-assemblage was characterised by a variety of life forms. Concurring with the base-poor and acid sensitive ecology of the Group II assemblage described in this study, others have found a comparable species composition of aquatic bryophytes, indicated by an abundance of *Scapania undulata*, occurring in characteristically similar stream habitats in the UK (e.g. Ormerod *et al*. 1987, Holmes *et al*. 1999a, Paton 1999, Scarlett & O'Hare 2006) and other parts of Europe (e.g. Thiebaut *et al*. 1998, Vanderpooten & Klein 1999b, Stetzka & Baumann 2002). Consistent with these findings, it has been reported elsewhere that the liverwort *Scapania undulata* has the ability to regulate the proton (H^+) content of its protoplast and is therefore well-adapted to highly acidic habitat conditions including tolerance of elevated levels of heavy metals, particularly aluminium (Satake *et al*. 1989a, Thiebaut *et al*. 1998). Furthermore, *Scapania undulata* has shown an affinity for heavy metal uptake in other work (e.g. Satake *et al*. 1989b, Yoshimura *et al*. 1998, Vázquez *et al*. 1999, Vincent *et al*. 2001). In this

study, *Scapania undulata* was commonly associated with other acidophilous bryophytes, such as *Racomitrium aciculare* and *Brachythecium plumosum* in poorly buffered streams characterised by low mineral concentrations, which is also consistent with the observations of Vanderpooten & Klein (1999b) and Scarlett & O'Hare (2006). Acidophilous *Hygrohypnum ochraceum* accompanied the species-assemblage in both this study (Group II) and the other UK-based study (Scarlett & O'Hare 2006). In France and Germany, *Hygrohypnum duriusculum* was the co-occurring species in the equivalent community of the European study (Vanderpooten & Klein 1999b). Therefore these two *Hygrohypnum* species may exhibit differential geographical distributions but nevertheless may occupy a functionally-similar habitat niche in different parts of Europe. Elsewhere in the Vosges Mountains (France), specifically in streams draining the granitic bedrock of the Rouge-Rupt river basin, *Hygrohypnum ochraceum* was indeed widespread and tended to occur with *Pellia epiphylla* in particularly acid conditions (Claveri *et al*. 1995), corresponding with the Group II species-assemblage of my study. Also recorded as frequently occurring together in other central European work, *Scapania undulata*, *Platyhypnidium riparioides* and *Fontinalis antipyretica*, constituent species of the Group II community my study, characterised the aquatic bryophyte assemblages of streams draining similarly base-poor geologies including the Ore Mountains in Germany (Samecka-Cymerman *et al*. 2002), and Tatra Mountains in Poland (Samecka-Cymerman *et al*. 2007). Therefore Group II probably represents a near-pristine aquatic bryophyte community, for an extensively-occurring type of upland streams in Scotland (and possibly other low-order temperate river systems in high-latitude Europe), containing a species-assemblage characterising catchments draining resistant base-poor geologies, with inherently oligotrophic, weakly-buffered and acid-sensitive streamwater chemistry, experiencing elevated levels of sulphate and heavy metals.

Group III was dominated by the occurrence of *Fontinalis antipyretica*, existing as a near-monoculture moss lawn with few, if any, co-occurring species. *Fontinalis antipyretica* occurred as a true core or generalist species (Muotka & Virtanen 1995,

Virtanen 1995, Heino & Virtanen 2006) located at the heart of the CCA diagram (see Figure 4.27), indicating that this particular aquatic bryophyte is capable of colonising widespread environmental habitat conditions (Hill *et al*. 1994). This concept, that the stream habitats of *Fontinalis antipyretica* vary considerably, is further supported by the fact that the moss-carpeted stable bedrock of Bogendreip in the Water of Dye, subject to fast-flow, spatey conditions also grew in profusion on smaller substrate particles in the upper stretch of Knockan Burn, where fast flows and spates are much less likely owing to its relatively low slope, and short distance from source, in conditions ranging from acidic and base-poor to calcareous and mineral rich, respectively. My results thus are in agreement with the suggestion that in less disturbed, more stable conditions (such as lowland canals: Murphy & Eaton 1983), some aquatic bryophytes such as *Fontinalis* may form extensive mats in lotic habitats characterised by smaller particle size substrata, because of the lower risk of damage due to substrate dislodgement, whereas in faster flowing systems *Fontinalis* usually prefers large, stable substrata for attachment (Glime & Clemons 1972, Chambers *et al*. 2008). In the current study when *Fontinalis antipyretica* dominated the stream bryophyte flora, it was observed that the substrates to which the plants were attached were mostly situated below the water surface, thus eliminating the occurrence of facultative and semi-aquatic species from the niche. However, neither depth nor underwater light regime played a significant environmental role in shaping the distribution of stream bryophyte species in this study (refer back to Figure 4.28). *Fontinalis antipyretica* is widely recognised as a strong competitor or C-strategist (Grime 1979) capable of monopolising space under relatively stable conditions, at least in part due to its canopy morphology and aggressive clonal reproduction powers, which allow the moss to out-compete and thereby exclude other aquatic bryophyte species from the habitat (Muotka & Virtanen 1995, Virtanen *et al*. 2001). Furthermore, *Fontinalis antipyretica* is capable of utilising both free carbon dioxide as a C-source in acid streamwaters, and bicarbonate in solution in more alkaline conditions (Peñuelas 1985), the latter being an unusable source of carbon for most other C_3 aquatic

bryophytes (Bain & Proctor 1980, Raven *et al*. 1985, Ballesteros *et al*. 1998) emphasizing the niche breadth of this particular moss species.

Group IV supported a high diversity of aquatic bryophyte vegetation characterised by weft-carpet formers, namely *Platyhypnidium riparioides* and to a lesser extent *Fontinalis antipyretica*, as well as other bryophyte morphologies (e.g. carpet-turfs such as *Palustriella falcata*, *Hygrohypnum luridum*, and short-statured species like *Chiloscyphus polyanthos* and *Fissidens adianthoides*). This assemblage mostly characterised the mid- and lower sampling stretches of Knockan Burn, wherein a more diverse streambed morphology occurred, compared with upstream of these sites, containing an assortment of substrate particle sizes. This provided a range of stream habitat conditions which in some areas favoured almost exclusively submerged species like *Fontinalis antipyretica* and *Platyhypidium riparioides* yet also accommodated colonisation of facultative and semi-aquatic species (e.g. *Hygrohypnum luridum*, *Palustriella falcata*, *Fissidens adianthioides*) where vertical zonation was possible upon coarser substrates. In agreement with the well-buffered mineral-rich ecology of the Group IV assemblage described in this study, others have found a comparable species composition of aquatic bryophytes, indicated by an abundance of *Chiloscyphus polyanthos*, tending to occur in characteristically similar stream habitats in the UK (e.g. Hill *et al*. 1991, Scarlett & O'Hare 2006) and other parts of Europe (e.g. Vanderpooten & Klein 1999b). Elsewhere it is documented that the liverwort *Chiloscyphus polyanthos* is sensitive to acid habitat conditions, as streamwater protons (H^+) inhibit protonema development in this particular species (Tremp & Kohler 1993, Thiebaut *et al*. 1998). In this study, *Chiloscyphus polyanthos* was occurred frequently with *Platyhynidium riparioides*, and a known calcicole species *Hygrohypnum luridum* in highly buffered streams characterised by high mineral concentrations, particularly Ca^{2+} and Mg^{2+}, which is also consistent with the observations of Vanderpooten & Klein (1999b) and Scarlett & O'Hare (2006). *Palustriella falcata* is also a strongly calcicole species preferring mostly calcareous stream habitats (Watson 1981, Hill *et al*. 1994, Smith 2004), which commonly co-occurred with the aforementioned species-assemblage

in Group IV community of this study and other UK-based study (Scarlett & O'Hare 2006). However in the work conducted between France and Germany, this particular species was replaced by *Cratoneuron filicinum*, which occupied a similar niche in the European-based study (Vanderpooten & Klein 1999b). *Platyhypnidium riparioides* is a widely distributed species, occurring in a broad range of habitat conditions (Watson 1981, Kelly & Whitton 1987, Hill *et al*. 1994, García-Álvaro *et al*. 2000, Smith 2004). However in this study, *Platyhypnidium riparioides* was particularly more abundant in calcareous stream habitats, which mirrors observations made elsewhere (e.g. Hill *et al*. 1994, Scarlett & O'Hare 2006). In Britain, Pentecost (1991) noted that the aquatic bryophyte flora of an oligotrophic calcareous stream of the Yorkshire Dales was co-dominated by *Platyhypnidium riparioides* and *Palustriella falcata*. Further evidence that the Group IV species-assemblage is analogous to other parts of Europe as well as the UK, comes from a study in the Vosges Mountains (France), wherein *Chiloscyphus polyanthos* frequently occurred with *Platyhypnidium riparioides* in habitats characterised by a pH >6, and also exhibited relatedness to streamwater Ca^{2+} and Mg^{2+} concentrations (Thiebaut *et al*. 1998). Elsewhere, in variably human-modified river catchments such as the Rhine in Switzerland and Germany, and the Walloon network in Belgium, which experience both flow regulation and nutrient pollution, often *Hygrohypnum luridum* and *Palustriella commutata* (= *Palustriella falcata*), or *Cratoneuron filicinum*, co-occurred with *Fontinalis antipyretica* and *Platyhypnidium riparioides* in remnant regions of these watercourses characterised by undisturbed oligotrophic conditions (Vanderpooten & Klein 1999a, Vanderpooten 1999a). In their study of 11 montane streams in the Canadian Rocky Mountains, Vitt *et al*. (1986) also found that *Hygrohypnum luridum* and *Palustriella* (= *Cratoneuron*) *commutatum* characterised the aquatic bryophyte assemblages of the more calcareous streams on the eastern slopes, and were partitioned from communities inhabiting less calcareous habitats on the western slopes, mainly by variation in water chemistry, particularly streamwater Ca^{2+} and Mg^{2+} concentrations. Therefore Group IV probably comprises a near-pristine aquatic bryophyte community typical of a widely distributed set of upland

streams in Scotland (and possibly elsewhere), containing a species-assemblage characterising catchments draining more weatherable base-rich geologies, with inherently oligo-trophic, highly-buffered and calcareous streamwater chemistry, experiencing suppressed levels of sulphate and heavy metals.

Group V represents the sample-group entirely devoid of aquatic bryophytes. As attributes of water chemistry and riparian shade did not vary significantly from those characterising other species-assemblages, these could not be attributed as causal factors explaining why aquatic bryophytes were absent from this sample-group. A common feature of the samples in this group was that they lacked stable substrates and were characterised by physically unstable habitat conditions. Clearly at the micro-scale, the physical structure (substrate morphology and stability) of the streambed is a decisive factor determining whether aquatic bryophytes are firstly capable of colonising the available niche. This agrees with the '*minimal stability threshold*' concept proposed by Suren (1996), from research conducted on aquatic bryophyte distribution in New Zealand streams. Sample-group V was restricted to unstable, easily perturbed stretches of streambed that could not be successfully exploited by any C, S or R strategist species, and therefore was characterised by the distinct absence of aquatic bryophytes from this type of habitat. These results concur with findings elsewhere (e.g. Suren 1996, Suren & Ormerod 1998).

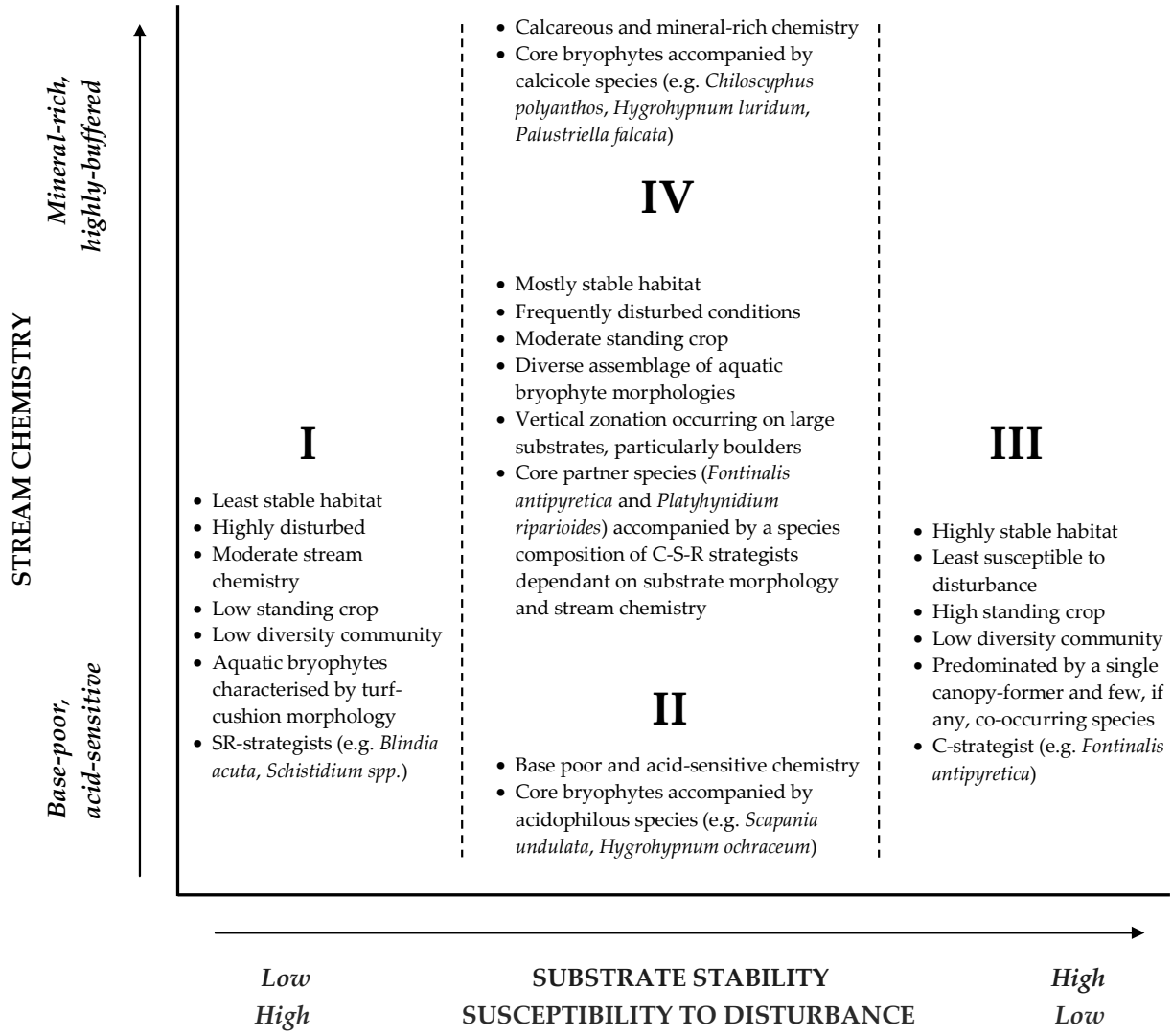


Figure 4.42 Conceptual habitat-template model (adapted from Suren & Ormerod 1998) of the TWINSPAN sample-groups indicating their position on the axes in relation to environmental gradients of streambed disturbance-stability and streamwater chemistry, showing variation in diversity, community composition and abundance of aquatic bryophytes in the target streams of this study.

In agreement with other works (e.g. Muotka & Virtanen 1995, Virtanen 1995, Heino & Virtanen 2006), *Fontinalis antipyretica* emerged from this study as a true core species occurring in a range of stream habitat conditions. I did also consider the possibility of whether the *Fontinalis antipyretica* specimens characterising the Group II and Group IV aquatic bryophyte communities were actually (at least) two varieties of the moss, exhibiting differing habitat ecologies. In the hope of confirming this I forwarded the specimens to *Fontinalis* expert Ron Porley (Natural England) for further detailed inspection. *Fontinalis* members can prove

notoriously difficult to identify because phenotypic chararcteristics (e.g. leaf morphology) can be highly variable in streams habitats of differing current velocities and are therefore susceptible to environmental modification (Crum & Anderson 1981, Biehle *et al*. 1998, Shaw & Allen 2000, Bleuel *et al*. 2005). Moreover, *Fontinalis antipyretica* varieties tend to intergrade morphologically, further complicated by their indeterminate ecologies and rather weak literature base, in which the topic is addressed only in a handful of publications (e.g. Welch 1960, Watson 1981, Smith 2004). As anticipated, elucidation of *Fontinalis antipyretica* specimens to variety level was not easy despite examining several diagnostic characters (e.g. leaf size and shape, cell size and shape, keeled vs. channel structure). It was proposed that the Water of Dye and Iron Bridge sample specimens mostly corresponded to that of *Fontinalis antipyretica* var. *gracilis* (Group II), depicted as usually occurring '*on rocks in fast-flowing montane streams*' (Smith 2004). The other sample specimens from Knockan Burn (Group IV) fitted more within the range of variation usually observed in *Fontinalis antipyretica* var. *antipyretica*, usually known to occur *'on rocks in neutral or basic streams'* (Smith 2004). It should be noted that few specimens were identified to variety level with a high degree of certainty and that the findings were based on a limited sample size. A large number of entire *Fontinalis* samples would have needed to be provided to account for the variation occurring within and between plants, if varieties were to be established with confidence. I therefore decided the most logical approach would be to pursue multivariate analysis of the aquatic bryophyte data set excluding variety level of *Fontinalis antipyretica* from the main body of results in the thesis (refer back to section 4.5.13). However, I also conducted TWINSPAN and CCA analyses on the aquatic bryophyte dataset which included *Fontinalis antipyretica* var. *gracilis* and *F. antipyretica* var. *antipyretica*, to examine what affect incorporating a lower taxonomic level of the moss would exert upon the sample classification and species ordination (refer to Appendix 4). The outcome was that following inclusion of the *Fontinalis antipyretica* variants, TWINSPAN classification partitioned the 74 samples into three sample group species-assemblages: Group I (n = 22) was strongly separated from the remaining

samples (n = 52) at level 1 of the classification by an eigenvalue of 0.878, indicated by *Fontinalis antipyretica* var. *antipyretica*, *Chiloscyphus polyanthos*, *Hygrohypnum luridum*, *Palustriella falcata*, and *Platyhypnidium riparioides*, representing Knockan Burn. Group II (n = 41) was partitioned from Group III (n = 11) at level 2 of the classification by an eigenvalue of 0.773. Group II was indicated by an abundance of *Fontinalis antipyretica* var. *gracilis*, *Hygrohypnum ochraceum*, and *Scapania undulata*, representing mostly the Water of Dye and upper River Girnock. Group III was indicated by the presence of *Blindia acuta* and *Schistidium agassizii*, mostly occurring at Hampshire's Bridge and Littlemill, downstream sampling sites of the River Girnock. Overall, this provided a better reflection of the clustered environmental habitat conditions previously characterised (see Chapter 2, section 2.6.1) and supported findings comparable to the three neat groups arising from multivariate analysis of periphyton communities on naturally-occurring substrata, as detailed elsewhere in this chapter (see section 4.5.6). This implements a key role for molecular investigation as a tool in determining *Fontinalis antipyretica* varieties: to my current knowledge no such genetic studies on this moss have yet been undertaken (except for Shaw & Allen (2000) who examined phylogeny and geographic speciation in the Fontinalaceae). Overall the findings of the study clearly support the contention of Smith (2004) that these alleged *Fontinalis antipyretica* varieties (or whatever taxonomic level they may be) are of '*uncertain status and not necessarily confined to one type of habitat*'. Clearly, this is an area that would benefit from further research.

To summarise, in these near-pristine upland stream habitats in Scotland, the underlying geology was identified as the major macro-scale factor influencing the distribution of aquatic bryophytes by pre-determining the inherent properties of meso-scale (e.g. water chemistry) and micro-scale (e.g. substrate size-profile morphology, composition and stability) factors. Together substrate morphology and water quality were recognised as driving the formation of aquatic bryophyte species-assemblages in these streams.

Further to this, core aquatic bryophytes, *Fontinalis antipyretica* and *Platyhypnidium riparioides* were common partner species in most of the reasonably stable stream habitats sampled. However, it was the composition of accompanying aquatic bryophyte species which determined the water quality status of the stream habitats in which the core partner species occurred. Upland oligotrophic stream habitats of acid-sensitive and base-poor water quality (Group II) were characterised by acidophilous species (e.g. *Scapania undulata*, *Hygrohypnum ochraceum*), whereas calcareous and mineral-rich streamwaters (Group IV) were characterised by known calcicole species (e.g. *Chiloscyphus polyanthus*, *Hygrohypnum luridum*). Species-assemblages II and IV represent two main groups of aquatic bryophytes occurring in near-pristine oligotrophic headwater streams characterised by relatively stable substrate morphologies of contrasting character (e.g. underlying geology, water chemistry, buffer capacity) for upland stream habitats in Scotland.

The results of this study may contribute information, perhaps as a precursor to further development of LEAFPACS and other biomonitoring protocols, to assist environment agencies (e.g. SEPA, EA) to build a more robust classification system that utilises aquatic bryophytes for assessing the trophic status of inland waters in the UK.

4.6.2.6 Predicting freshwater aquatic bryophyte community composition and diversity

Together streamwater pH, flow velocity and substrate morphology (e.g. predominance of boulders) variables were strong predictors of aquatic bryophyte diversity (H) in the upland stream habitats of the study using model AqBRYOsH1a. This supports prior discussion of streambed stability and boulder-riffle zones supporting high diversity of aquatic bryophyte vegetation.

4.6.3 Vascular Submerged Macrophytes

4.6.3.1 Variation in vascular submerged macrophyte community composition and diversity in the Knockan Burn sub-catchment and its sites

In general, the observed variation in species richness and diversity provide an initial indication as to whether or not vascular submerged macrophytes were actually present in the streams sampled, and then if so, a hint as to how varied these communities were (or could become). Both the Water of Dye and River Girnock lacked the occurrence of true river plants. The five aquatic macrophyte species found in this study occurred only in two sampling stretches, the upper and lower parts, of one stream: Knockan Burn.

4.6.3.2 Seasonal variation in vascular submerged macrophyte community composition and diversity in Knockan Burn

Generally the vascular submerged macrophyte species-assemblage of Knockan Burn was stable and persistent, without significant change between sampling seasons or visibly during other routine fieldwork, which is common to river systems minimally-impacted by humans (Holmes *et al*. 1998). This suggests similarly to aquatic bryophytes, that vascular submerged macrophytes are reliable indicators of the integrated environmental habitat conditions in which they occur and build-in this response over time (Daniel & Haury 1996, Lancaster *et al*. 1996, Ali *et al*. 1999, Ellwood *et al*. 2008).

Notably however, vascular submerged macrophyte species richness and diversity tended to be higher, though not significantly so, in the summer season in Knockan Burn (refer back to section 4.5.17). This can probably be attributed to the sampling regime which was based on recording the occurrence of aquatic macrophytes submerged below, floating on, or growing up through the water surface at the time of sampling. Therefore when the water level dropped (e.g. during summer base flow conditions) less conspicuous submerged species growing in relatively

low abundance amongst the crowded, often co-dominated stands of *Potamogeton polygonifolius* and *Chara globularis* var. *globularis* were more easily singled out, and I probably gained records of other species usually hidden deep within the vegetation at this time of year.

4.6.3.3 Response of vascular submerged macrophyte community composition and diversity in Knockan Burn to variation in flow regime: pool, glide and riffle zones

Vascular submerged macrophytes tended to be completely absent from high-velocity riffle habitats in Knockan Burn, which indicated that most growth forms in my study were vulnerable to the effects of scour but most probably displacement under conditions of fast-flow (Biggs 1996, Riis & Biggs 2003, Sand-Jensen 2003, Schutten *et al*. 2005, Riis *et al*. 2008). While some aquatic plants occurred in slow-flowing pools, species richness and diversity was generally (though not significantly) higher in glide habitats. For example, submerged *Myriophyllum* plants tended to occur more frequently in glides and may have been better adapted to resist moderate swift-flows from the flexible stem morphology, a characteristic attributed to several other watermilfoil species (Sand-Jensen 2003). This trait confers tolerance to flood disturbance and an abundance of cobbled substrate particles supports theory that *Myriophyllum alterniflorum* withstood hydraulically-disturbed habitat conditions unsuitable to other, perhaps less-resilient macrophytes of my study. The *Potamogeton-Chara* dominated community tended to occur much further upstream and mostly in sluggishly-flowing waters, perhaps constrained by their susceptibility to breakage or dislodgement under high drag forces, substrate preferences (e.g. fine muddy, silty sediments), or a combination of both physical factors. Furthermore, glides were often characterised by a more heterogeneous substrate particle composition (refer back to Chapter 2, section 2.6.4.1). This probably encouraged a greater number of aquatic macrophyte species to co-occur together because the diverse overlapping diverse physical habitat characteristics broadened the width for niche occupancy,

tilting the balance away from competitive exclusion (Baattrup-Pedersen & Riis 1999).

4.6.3.4 Response of vascular submerged macrophyte community composition and diversity in the Water of Dye, River Girnock and Knockan Burn to variation in substrate morphology

On the whole, vascular submerged macrophytes were excluded from streambeds characterised by a dominance of hard, impenetrable and coarse substrate morphology, tending to grow in habitats characterised by finer substrate particles and soft sediments which could more easily be penetrated by plant roots (Biggs 1996, Baattrup-Pedersen & Riis 1999). Furthermore variation in substrate particle composition between the upper and lower parts of Knockan Burn may also help explain why the aquatic macrophyte communities appeared to diverge between the two sampling sites (see following discussion: section 4.6.3.5).

4.6.3.5 Vascular submerged macrophyte community composition, diversity and environmental habitat conditions in the Water of Dye, River Girnock and Knockan Burn as determined by TWINSPAN classification

The species documented in this study are generally characteristic of the river plants occurring in upland freshwater habitats of the UK and temperate parts of Europe (Haslam 1978, 1987, 2006, Palmer 1999, Holmes *et al*. 1999a). In particular, the occurrences of *Potamogeton polygonifolius*, *Eleogiton fluitans* and *Myriophyllum alterniflorum* are indicative of nutrient-poor reference conditions in British rivers (Holmes *et al*. 1998, 1999a) and standing waters (Palmer *et al*. 1992). Additionally, Murphy (2002) listed the four vascular macrophytes of this study, as species recorded from softwater lakes of northern Europe. Charophytes are also considered to be reliable indicators of clear, nutrient-poor waters (Krause 1981), and susceptible to the effects of eutrophication (Blindow 1992).

It is most likely that several strong environmental gradients were responsible for driving the distribution and species-assemblage of aquatic macrophytes in this study. Above all however, the type of underlying geology reigned as the primary controlling factor which influenced streambed substrate morphology and streamwater physico-chemistry, together predetermining the incidence and composition of vascular submerged macrophyte communities in upland stream habitats. The results of my study underpin findings of other work that an abundance of fine substrate particles and mineral-rich habitat conditions, particularly Ca^{2+}, support the occurrence of aquatic plants in riverine habitats (e.g. Haury 1996, Wilby *et al*. 1998, Dodkins *et al*. 2005).

The freshwater angiosperms *Potamogeton polygonifolius* and *Myriophyllum alterniflorum*, representative of oligotrophic conditions, have been documented as commonly co-occurring together in other European rivers (e.g. Holmes *et al*. 1999a, Palmer 1999) and standing waters (e.g. Spence 1967, Palmer *et al*. 1992, Heegard *et al*. 2001). However, in this particular study the two species were differentiated as indicators of vascular submerged macrophyte communities separated spatially from each other, in the upper and lower parts, of the same sub-catchment stream. Although some habitat characteristics (e.g. depth, underwater light regime, flow, nutrient status) coincided between the two river plant communities, significant differences in substrate morphology and water chemistry (particularly Ca^{2+} and Mg^{2+}) probably reflect ecological habitat preferences and may explain why the two species-assemblages diverged as they did in Knockan Burn. It is generally known that *Potamogeton polygonifolius* and *Eleogiton fluitans* exhibit similar habitat preferences for mostly oligotrophic waters, of slow-swift flow, characterised by fine sands and silts (Butcher 1933, Haslam 1975, Triest 2006). *Myriophyllum alterniflorum* seems to have similar ecological preferences to the aforementioned plant species, but tends to occur in river habitats strewn with coarser substrate particles (Butcher 1933, Haslam 1975). These observations tend to support the findings of my study, which suggested that in Knockan Burn the assorted aquatic macrophyte community of the upper section (dominated by *Potamogeton*

polygonifolius and *Chara globularis*) was restricted to habitats characterised by fine sands and soft muddy sediments, while in the lower basin *Myriophyllum alterniflorum* inhabited a more cobbled streambed. This suggests that physical habitat characteristics partitioned the vascular macrophyte communities in Knockan Burn, and that plant species showed differential patterns of distribution principally in relation to spatial variation in streambed substrate morphology (reviewed in French & Chambers 1996). Elsewhere in the UK and also Europe, flow velocity and substrate morphology are amongst the major physical environmental factors structuring the distribution of submerged macrophyte vegetation assemblages in streams and rivers (e.g. Haslam 1978, 2006, Baattrup-Pedersen & Riis 1999, Riis *et al*. 2000, Kuhar *et al*. 2007). Nevertheless, it may be probable where flow and substrate morphology habitat characteristics overlap (i.e. are sufficiently heterogeneous) then *Potamogeton polygonifolius* and *Myriophyllum alterniflorum* may be expected to occur together. Following their full-length macrophyte survey of Knockan Burn conducted during the summer of 2009, Tapia Grimaldo & Murphy (pers comm.) noted that the *Potamogeton-Chara* dominated community and *Myriophyllum alterniflorum* occurred in discrete habitats and further confirmed that the two communities (as identified in my study) remained segregated from one another to the most extreme upper and lower parts of the stream. These recent findings are at odds with my hypothesis and suggest that sections of the river in between these two sampling points in Knockan Burn do not share overlapping habitat characteristics, meaning that it was not possible for these species to coexist in a similar niche. Notoriously, *Myriophyllum alterniflorum* tends to occur less frequently with other macrophytes than it does on its own, typically in 'more fast flowing rocky reaches' (Rodwell 1995). Therefore contrasting physical habitat preferences probably explains its distinct distribution from the *Potamogeton-Chara* dominated community which grew mostly in more sluggish waters underlain with a silty-sandy substrate.

It is also quite possible that variation in streamwater chemistry played a role in structuring the two aquatic plant communities of Knockan Burn. For example, in

their macrophytes survey of mountain lakes in the eastern Pyrenees, Gacia *et al*. (1994) found that *Myriophyllum alterniflorum* preferentially occurred in waters characterised by high values of pH and conductivity, concurring with the findings of my study. Similarly, Palmer *et al*. (1992) found that water chemistry (pH, conductivity and alkalinity) factors were most important in explaining the distribution of aquatic macrophytes in standing waters in Britain. In their study of Danish lowland streams, Riis *et al*. (2000) found that variation in alkalinity concentrations was one of the main environmental drivers explaining the distribution of submerged vegetation, in which *Myriophyllum alterniflorum* occurred in streams of lower alkalinity than the *Potamogeton* community. Loss of natural habitat conditions from anthropogenic disturbances, especially eutrophication and aquatic weed management (e.g. cutting, dredging), have exerted a profound impact on the species composition of aquatic plants occurring in Danish streams over the last 100 years (Riis *et al*. 2000, Riis & Sand-Jensen 2001). The *Potamogeton* community described by Riis *et al*. (2000) was characterised by an abundance of *P. crispus*, *P. natans*, *P. pectinatus* and *P. perfoliatus* and notably lacked *P. polygonifolius*. Presumably, *Potamogeton polygonifolius* was displaced by better-adapted *Potamogeton* species as nutrient enrichment replaced prior oligotrophic conditions in which it had preferentially occurred (Riis & Sand-Jensen 2001). Other European-based studies have documented that *Myriophyllum alterniflorum* has disappeared from (at least, threatened) freshwater habitats affected by acidification (e.g. Arts 2002, Murphy 2002), but also that the species has become a reputed nuisance plant following exposure to liming elsewhere (e.g. Brandrud 2002). It is most likely that *Myriophyllum alterniflorum* is sensitive to disturbance of the dissolved inorganic carbon pool brought about by changes in alkalinity from human intervention. Together, my findings and the work of several other studies (e.g. Riis *et al*. 2000, Feijoo & Lombardo 2007, Baattrup-Pedersen *et al*. 2008) indicate that amongst the various chemical parameters measured, alkalinity is probably the most influential factor governing aquatic plant distribution in freshwater habitats. Closely associated with pH and conductivity, alkalinity is especially renowned as a useful surrogate for

measurements of dissolved inorganic carbon, as its concentration determines whether prevailing forms in freshwaters occur as free-CO_2, bicarbonate or carbonate ions (Drever 1982). In turn, it is the composition and abundance of available carbon sources which shapes the species-assemblage of freshwater macrophytes depending on their physiological capacity to utilise HCO_3^- for photosynthesis, which is particularly advantageous in conditions where free-CO_2 may be scarce (Carr *et al*. 1997, Riis *et al*. 2000, Dodkins *et al*. 2005, Feijoo & Lombardo 2007). Many vascular submerged macrophytes can use only free-CO_2 and consequently distribution is constrained (to low alkalinity or frequently turbulent waters) by their inability to sequester other forms of carbon for photosynthesis (Carr *et al*. 1997, Riis *et al*. 2000). However, some aquatic plant species are equipped with HCO_3^- acquisition mechanism to alleviate carbon limitation (Carr *et al*. 1997, Riis *et al*. 2000, Dodkins *et al*. 2005, Feijoo & Lombardo 2007). For example, *Myriophyllum alterniflorum* is capable of using HCO_3^- as an alternative inorganic carbon source (Riis *et al*. 2000, Schneider 2007), explaining its occurrence in a wide range of water chemistries, of contrasting pH and bicarbonate concentrations, in Scottish freshwater lochs (Spence 1967). Similarly, some *Potamogeton* species, particularly those having morphologies furnished with both floating and sub-surface leaves (e.g. *P. gramineus*) possess the added ecological advantage of exploiting HCO_3^- to meet their photosynthetic requirements by using H^+ polarity of submerged leaves to convert bicarbonate into a more readily usable form of carbon dioxide and aerial leaves to sequester free-CO_2 from the atmosphere (Frost-Christensen & Sand-Jensen 1995). However, there seems to be some degree of uncertainty regarding whether *Potamogeton polygonifolius* is a capable bicarbonate-user, with one study alleging there is some evidence of HCO_3^- proficiency (e.g. Frost-Christensen & Sand-Jensen 1995) and others stating HCO_3^- user-status is not viable (e.g. Maberly & Spence 1983, Schneider 2007) in this particular species. The results of my study tend to support latter belief, or at least that *Potamogeton polygonifolius* is considerably less efficient than *Myriophyllum alterniflorum* in using bicarbonate as an inorganic carbon source for photosynthesis. My basis for this rests on their disjunct distribution of the two

vascular submerged macrophyte communities in Knockan Burn: *Potamogeton polygonifolius* was restricted to the low alkalinity waters of the upper catchment and was not found to occur downstream further than the point in which the river passed through a band of highly calcareous strata (An-t'Sron: Salterella Grit and Fucoid Bed), consequently in the lower part of catchment *Myriophyllum alterniflorum* was probably better-adapted to endure the high alkalinity conditions given its known affinity for bicarbonate and highly-dissected leaf morphology increasing the surface area available for free-CO_2 uptake (Chambers *et al*. 2008).

Overall in my study, vascular submerged macrophytes were excluded from streams characterised by hard resistant geologies with an ensuing base-poor, acid-sensitive streamwater chemistry (pH <7) and boulder-dominated streambeds not suitable for root penetration. All of these habitat features comprised the Group III sample-group which entirely lacked vascular submerged macrophyte flora, and reinforces prior discussion as to the reasons why aquatic plants were generally confined to streams draining soft calcareous geologies, characterised by mineral rich chemistry and an abundance of fine sediment particles. Spatial variability in physical habitat characteristics as well as water chemistry, especially alkalinity and base cation (e.g. Ca^{2+}, Mg^{2+}) concentrations probably act coherently as the major controlling factors constraining macrophyte distribution and community structure in Scottish Highland streams, and generally agrees with findings elsewhere (e.g. Haslam 1978, 2006, Riis *et al*. 2000). In particular two macrophyte species comprising my study, *Potamogeton polygonifolius* and *Myriophyllum alterniflorum* either separately or together, are often recognised as indicators of oligotrophic water quality in several EU-member state countries besides the UK (e.g. Meilinger *et al*. 2005, Haury *et al*. 2006, Triest 2006, Baattrup-Pedersen *et al*. 2008). Occasionally in other eastern-European streams and rivers, *Potamogeton coloratus* seems to replace *Potamogeton polygonifolius* by filling the niche as the indicator of nutrient-poor reference conditions (e.g. Schorer *et al*. 2000), which may reflect patterns in the natural distribution range of these functionally-similar *Potamogeton* species.

In summary, this study has shown that discrete submersed macrophyte species-assemblages were identifiable based on a relatively small dataset and that these community types appeared to be affiliated with particular sets of physical and chemical habitat conditions in the Knockan Burn catchment.

Finally, had more data been available for vascular submerged macrophytes (i.e. if this plant group had occurred as abundantly as periphyton and aquatic bryophytes) in this study, then it certainly would have been worthwhile analysing the relationship between species richness and standing crop, as I did for periphyton (e.g. Figure 3.55, Figure 3.56,Figure 3.57) and aquatic bryophytes (e.g. Figure 3.59). The proposed approach is similar to the work of Willby *et al*. (2001), who found that in British canal systems aquatic plant assemblages responded unimodally to boat-trafficking and waterway management activities, in which habitats subjected to intermediate levels of human-disturbance maintained the highest species diversity. Similarly in Danish lowland streams, Riis *et al*. (2008) demonstrated that aquatic macrophyte species richness and diversity exhibited a bell-shaped response-curve as a function of natural hydrological disturbance.

Therefore it would be interesting to model the response of submerged macrophytic vegetation in this study to environmental-disturbance (e.g. flow-stability) gradients in upland streams, comparable to findings presented elsewhere in this chapter for periphyton and aquatic bryophytes (see again scatterplots of Chapter 3, sections 3.6.1.6 and 3.6.2.5, respectively). For example, I have given some consideration to the possibly that similarly to other watermilfoil species (reviewed in Riis & Biggs 2001), *Myriophyllum alterniflorum* might be a CR-strategist, as conveyed from an apparent combination of disturbance resistance (e.g. flexible stems) and competitive (e.g. bicarbonate-user, dense canopy growth, large surface area) traits. A conceptual habitat model is proposed for vascular submerged macrophyte communities responding to increased flow and substrate disturbances (see Figure 4.43). I acknowledge this model does not fit IDH theory that would have predicted a humpback response of species diversity to

intermediate levels of disturbance, which may be because it is based on a limited dataset. Instead, the observed trend is a reduction species diversity following exposure to increasing habitat disturbance and unsuitable substrate composition, but this outcome concurs with the main findings of Riis & Biggs (2003) and Riis *et al*. (2008).

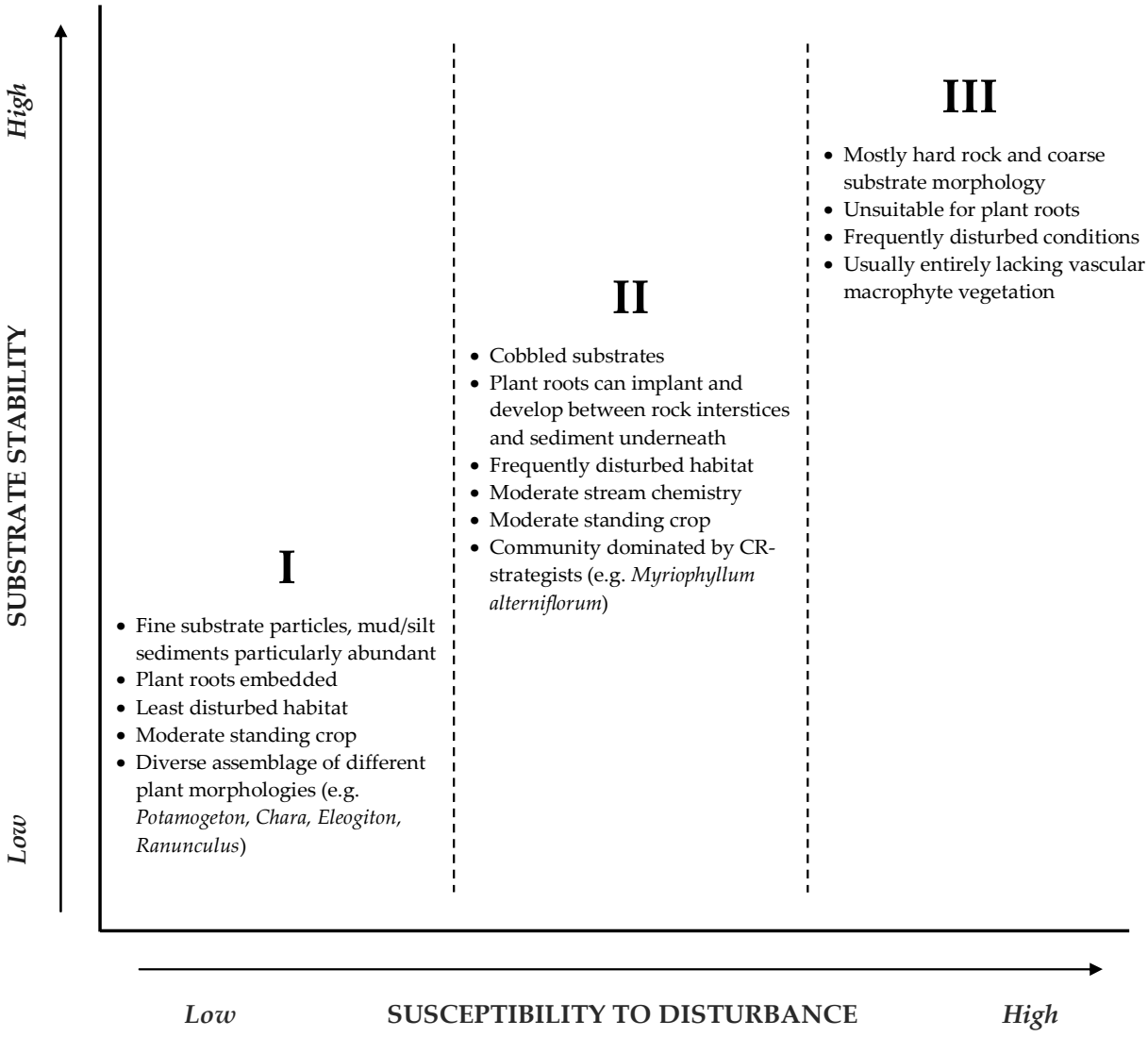


Figure 4.43 Conceptual habitat-template model (adapted from Riis & Biggs 2001) of the TWINSPAN sample-groups indicating their position on the axes in relation to environmental gradients of streambed disturbance and substrate stability, showing variation in diversity, community composition and abundance of vascular submerged macrophytes in the target streams of this study.

4.6.3.6 Predicting freshwater vascular submerged macrophyte community composition and diversity

Again I suggest that it would be possible to create a model using combinations of environmental variables (probably alkalinity, Ca^{2+} and substrate particle composition) to predict the diversity of vascular submerged macrophytes occurring in upland stream habitats in the UK. However, it is clear that a much larger dataset would be required to refine this approach. The dataset presented herewith is really too small to enable me to conduct further analysis than has been included and discussed elsewhere in this thesis regarding true river plants.

In this study, nutrient status (e.g. N, P) was not found to be significantly correlated with aquatic macrophyte diversity, unlike the work of Murphy *et al*. (2003) who demonstrated that phosphate content of the water predictably reduced macrophyte diversity over a range of waterbodies within the riverine floodplain of the upper Rio Paraná, Brazil. This is most probably because the streams sampled in my study varied considerably less in terms of water quality, more specifically nutrient status, compared with those of comprising the Rio Paraná river channel system surveyed by Murphy *et al.* (2003). Particularly for rooted aquatic macrophytes, interactions with the sediment may be important (Ali *et al*. 1999). Furthermore, sediment nutrient characteristics can be highly spatially variable and may be influential in structuring the rooted macrophyte communities of lowland rivers in the UK (Clarke & Wharton 2001). Therefore it may also be a sensible approach to assess mineral and nutrient content, as well as redox conditions of the sediment, rather than solely relying on streamwater concentrations as predictor variables of aquatic macrophyte functional variables or community composition. For example, Ali *et al*. (1995) found that sediment P was a better predictor than streamwater P of rooted submerged macrophyte species-assemblages occurring in regulated waterbodies in Egypt. Elsewhere in Brazil, aquatic macrophyte distribution was also strongly related to sediment P, and other environmental variables (particularly light penetration) in the Itaipu Reservoir (e.g. Bini *et al*. 1999).

4.6.4 The three-tier approach to characterising upland stream habitat conditions by combining freshwater vegetation assemblages: periphyton, aquatic bryophyte and vascular submerged macrophyte community composition and diversity

4.6.4.1 Freshwater vegetation community composition, diversity and environmental habitat conditions as determined by multivariate ordination and TWINSPAN classification

Overall, adopting the three-tier approach in this study made it possible to distinguish between three major communities of freshwater vegetation (plus two component sub-assemblages) and furthermore, to characterise the environmental habitat conditions driving the formation of these species assemblages in upland streams of Scotland. Water chemistry characteristics (e.g. pH, conductivity, alkalinity, composition of base cations and heavy metals) were the principal environmental drivers structuring the species composition and diversity of freshwater vegetation communities as a whole. However, substrate morphology factors played a key role in structuring the species assemblages of hydrophytes but were not particularly affiliated with periphyton community composition which responded principally to variation in streamwater physico-chemistry (a function of the underlying geology). This concurs with the work Paavola *et al*. (2003) which used multiple taxonomic groups for classifying headwater streams in Finland, and found that the community structure of aquatic bryophytes, macroinvertebrates and fish responded to different environmental factors.

Upland oligotrophic stream habitats of acid-sensitive and base-poor water quality (Group III) were characterised by acidophilous species of diatoms (e.g. *Frustulia rhomboides*, *Eunotia exigua, Pinnularia subcapitata*) and aquatic bryophytes (e.g. *Scapania undulata*, *Hygrohypnum ochraceum*). Whereas more calcareous and mineral-rich streamwaters (Group I) were characterised by alkaliphilous diatoms (e.g. *Cocconeis placentula, Cymbella lanceolata, Diatoma moniliformis*, *Gomphonema olivaceum*), known calcicole species of aquatic bryophytes (e.g. *Hygrohypnum luridum*, *Palustriella falcata* and *Chiloscyphyus polyanthos*) and the appearance of

vascular submerged macrophytes (e.g. *Potamogeton polygonifolius*, *Myriophyllum alterniflorum*). Species-assemblages III and I represent two main groups of freshwater vegetation communities occurring in near-pristine oligotrophic headwater streams of contrasting character (e.g. underlying geology, water chemistry, buffer capacity) for upland stream habitats in Scotland. Additionally, Group III could be sub-divided into two ecological sub-assemblages: IIIa and IIIb. Sub-assemblage IIIa was indicated by a high abundance of the diatom *Frustulia rhomboides* var. *rhomboides* and the acidophilous liverwort species *Scapania undulata* which characterised extremely acid-sensitive streamwaters (e.g. pH <6, accentuated levels of sulphate and heavy metal cations). The presence of the aquatic moss, *Hygrohypnum ochraceum* and often the diatom, *Gomphonema clavatum* denoted an ecological shift in community composition of freshwater vegetation from sub-assemblage IIIa towards IIIb as conditions became less acid (pH 6-7). This step change was also indicative of an initial ecological transition into the Group II community, particularly under circumneutral and weakly alkaline conditions. Similarly, an ecological transition of the outermost Group II assemblage bearing resemblance towards the Group I community type was indicated from the occurrence of some diatom species (e.g. *Cocconeis placentula*, *Didymosphenia geminata*, and *Diatoma moniliformis*). Therefore with particular reference to diatoms, the Group II community type shared an overlapping species composition with both Groups III and I, thus forming an ecological bridge between the two diverging water chemistries.

Altogether my results contribute new information by providing valuable benchmarks of (at least) good ecological integrity for upland headwater streams of near-pristine reference condition and contrasting water chemistry in the Scottish Highlands, against which other rivers could potentially be compared as a means of assessing water quality status in the UK. To my current knowledge, this study is the first of its kind for Scottish Highland streams to integrate periphyton, aquatic bryophyte and vascular submerged macrophyte communities. Other

studies have attempted similar work elsewhere (e.g. Sherwood *et al*. 2000, Schaumburg *et al*. 2004) though not for the UK.

Climate change is a major threat to global biodiversity, habitat resilience, and environmental sustainability. Attempts have been made to predict the effects of climate change scenarios in Britain and Ireland, including a case study of the Scottish highlands (see MONARCH 3: Berry *et al*. 2007). Therefore it is probably worth mentioning that the communities of freshwater vegetation characterising near-pristine headwater streams in this study may alter in the future as a consequence of climatic effects on the environmental factors driving their formation or governing species distribution.

4.6.4.2 Predicting freshwater vegetation community composition and diversity

The lack of sufficient aquatic macrophyte data made it impracticable to incorporate this information together with that of periphyton and aquatic bryophytes to build a sensible model. It may have been feasible to construct a model based on a much larger data set and use combinations of probable environmental drivers (e.g. water chemistry, substrate morphology) to predict the species diversity of freshwater vegetation occurring in upland stream habitats in the UK.

4.7 Conclusions

- Three primary periphyton species assemblages emerged from TWINSPAN classification. The results of CCA ordination (together with ANOVA of sample-group mean environmental data) showed that in particular diatom species assemblages were distributed along gradients of water chemistry characteristics, revealing pH, conductivity, alkalinity and base cation composition (e.g. Ca^{2+}, Mg^{2+})

to be the major environmental drivers structuring freshwater diatom communities in the oligotrophic streams studied. Accordingly, diatom communities were most profoundly distinct between streamwaters of contrasting water chemistry and furthermore may have diverged ecologically from a ubiquitous core periphyton community into two distinct species assemblages representative of upland oligotrophic stream habitats of acid-sensitive and base-poor water quality (Group III) characterised by acidophilous species (e.g. *Frustulia rhomboides*, *Eunotia exigua, Pinnularia subcapitata*), and calcareous and mineral-rich streamwaters (Group I) characterised by known alkaliphilous species (e.g. *Cocconeis placentula*, *Cymbella lanceolata*, *Diatoma moniliformis*, *Gomphonema olivaceum*).

- Four major aquatic bryophyte community types emerged from TWINSPAN classification, plus a fifth sample-group entirely devoid of aquatic bryophyte vegetation characterised by physically unstable environmental habitat conditions. Otherwise, between sample-groups I – IV there was an evident ecological shift in the community composition and standing crop of aquatic bryophyte species in relation to environmental gradients of streambed disturbance-stability (flow, substrate composition) and streamwater chemistry (e.g. pH, conductivity, Ca^{2+}). Ultimately, stream micro-scale disturbance-stability gradients determined the predominant growth morphologies of aquatic bryophytes present as a functional attribute of life strategy, for example the least stable habitats (Group I) were characterised by stress/ disturbance-tolerator turfs (e.g. *Blindia*, *Racomitrium*, *Schistidium*) whilst highly stable streambeds (Group III) were often dominated by competitive canopy-formers (e.g. *Fontinalis antipyretica*). However, stream meso-scale variation in water chemistry (a function of the underlying geology) strongly influenced the species composition of aquatic bryophytes present, for example although core aquatic bryophytes, *Fontinalis antipyretica* and *Platyhypnidium riparioides* were common partner species in most of the reasonably stable stream habitats sampled, it was the composition of accompanying aquatic bryophyte species which determined the water quality status of the stream habitats in which the core partner species occurred. Generally, upland oligotrophic stream habitats

of acid-sensitive and base-poor water quality (Group II) were characterised by acidophilous species (e.g. *Scapania undulata*, *Hygrohypnum ochraceum*), whereas more calcareous and mineral-rich streamwaters (Group IV) were characterised by known calcicole species (e.g. *Chiloscyphus polyanthus*, *Hygrohypnum luridum*).

- As Knockan Burn was characterised by an abundance of fine sediment particles (e.g. silt, sand and gravel) and calcareous water chemistry it was additionally able to support river macrophytes. In this stream, the vascular submerged macrophytes diverged into two distinct communities: I (co-dominated by *Potamogeton polygonifolius* and *Chara globularis*) and II (*Myriophyllum alterniflorum*) characterising the upper and lower sections of the river, respectively. Spatial variability in physical habitat characteristics (e.g. substrate composition, flow disturbance) as well as water chemistry, especially alkalinity and base cation (e.g. Ca^{2+}, Mg^{2+}) concentrations probably acted together as the major controlling factors constraining macrophyte species distribution in this particular river.

- Overall by adopting the three-tier approach, three major species-assemblages of freshwater vegetation emerged which reflected the three neat clusters of environmental habitat characteristics obtained in Chapter 2. This shows that in upland oligotrophic streams, aquatic plant communities of periphyton, aquatic bryophytes and (where present) vascular submerged macrophytes are structured principally by environmental gradients of water chemistry and (where applicable) substrate morphology. Altogether, this contributes new information by providing valuable benchmarks of (at least) good ecological integrity for upland headwater streams of near-pristine reference condition and contrasting water chemistry in the Scottish Highlands, against which other rivers could potentially be compared as a means of assessing water quality status in the UK.

- In streams or rivers of near-pristine water quality, periphyton communities, especially diatoms tend to be true reflectors of the water physico-chemistry properties, whereas aquatic bryophytes and vascular submerged macrophytes integrate prevailing physical and chemical environmental habitat conditions.

- Overall, freshwater plant species diversity was more strongly and accurately predicted using the minimal models proposed in this study than plant production (Chapter 3). Plant diversity responded reasonably in a predictable manner to water chemistry variables and (where relevant) substrate morphology factors were also important functional drivers of the species present. However, a major limitation of these models is that they predict only the number of species present (as diversity) but do not provide an indication of ecological shifts in species composition in response to environmental variation.

Chapter 5. General Discussion and Conclusions

This final chapter integrates findings of the three main results chapters, summarises their main conclusions and discusses the potential implementation of the results of this study, as well as the scope for future research.

5.1 Summary of main objectives, findings and outcomes

This section provides a reminder of the main objectives of the project and summarises the main findings of the research, with the aim of addressing specific questions presented at the outset.

1. To categorise and characterise the environmental habitat conditions of the target streams of the study.

The environmental habitat conditions of the three upland streams in this study were characterised in detail, largely following the methodology of the River Habitat Survey, used in combination with multivariate approaches. From PCA ordination and cluster variable analyses, three primary clusters of stream environmental habitat conditions emerged which were separated by significant differences in water chemistry properties and streambed morphology features driven by variation in the underlying geology.

- *Can knowledge of the environmental characteristics of upland stream habitat be used to predict the abundance and composition of plant communities expected to occur in upland streams?*

It was expected that sampling sites grouped within-clusters would support more functionally-similar aquatic vegetation communities than between-clusters.

The first cluster materialised from sampling sites belonging to the Water of Dye (Brocky Burn, Charr Flume, Bogendreip) and the upper River Girnock (Iron

Bridge) characterised as streams of base-poor, acid-sensitive water chemistry and low buffering capacity, dominated by stable boulder morphology. Assemblages of periphytic diatoms and aquatic bryophytes were chiefly characterised by acidophilous species, in which extremely acidic conditions were indicated by a dominance of *Frustulia rhomboides* var. *rhomboides* and *Scapania undulata*. Generally, the number of plant species present tended to be quite low. However, a more diverse and moderately productive community of aquatic bryophyte vegetation developed upon boulders as a result of strong vertical zonation, supporting a variety of morphologies from obligatory aquatic canopy-formers (e.g. *Fontinalis antipyretica*, *Platyhypnidium riparioides*) to semi-aquatic short turfs (e.g. *Racomitrium aciculare*, *Schistidium rivulare*).

The second cluster was formed from the remaining sampling sites of the River Girnock (Hampshire's Bridge and Littlemill) which were characterised by weakly calcareous water chemistry and highly cobbled streambed morphology. Periphytic diatom assemblages were relatively diverse and embraced a number of species particularly distinctive of high water quality (e.g. *Brachysira* spp., *Navicula angusta*). Aquatic bryophyte species richness was generally low for few species were capable of tolerating the physically unstable habitat conditions, except for some small mosses equipped with disturbance-resistant traits (e.g. *Blindia acuta*, *Racomitrium aciculare*, *Schistidium agassazii*).

The third cluster comprised the three sampling sites of Knockan Burn, a stream characterised by a mineral-rich, well-buffered water chemistry and fine substrate morphology. In addition to diverse species-assemblages of periphytic diatoms (e.g. *Cocconeis placentula*, *Cymbella affinis*, *Diatoma moniliformis*, *Gomphonema olivaceum*,) and aquatic bryophytes (e.g. *Chiloscyphus polyanthus*, *Hygrohypnum luridum*) characteristic of moderately calcareous streamwaters, this stream was in some parts, also able to support vascular submerged macrophytes (e.g. *Potamogeton polygonifolius*, *Myriophyllum alterniflorum*), usually indicative of nutrient-poor reference conditions in British rivers.

Thus it can be demonstrated that the composition of aquatic plant communities and their functional attributes (e.g. biomass, morphology) reflected prevailing environmental habitat conditions present, and also that sample-groups were more similar within than between each of the clusters. Meso-scale water chemistry and (where relevant) micro-scale substrate morphology gradients (functions of macro-scale underlying geology) acted as the principal drivers of the production, abundance, species composition and diversity of freshwater plant communities in upland oligotrophic streams in Scotland.

2. To determine the relative importance of environmental processes potentially driving freshwater plant production, species-assemblage and diversity in upland stream habitats.

- *How is the growth of each of the three target aquatic plant groups (periphytic algae, bryophytes and vascular submerged macrophytes) affected by environmental variation?*

Periphytic algal growth was principally governed by the effects of flow disturbance, and constrained production to low standing crops characterised by communities of scour-resistant diatoms. However, usually during the summer flow constraints were relaxed and other environmental factors (e.g. light, temperature) became important secondary drivers of periphytic algal production, particularly by advancing succession and encouraging the growth of filamentous green algae. Combined with the effects of flow, P-limitation probably helped establish the upper limit of periphytic algal growth in the streams studied, as indicated from increases in the abundance of some known nutrient-responsive taxa (e.g. *Rivularia*, *Stigeoclonium*, *Nitzschia palea*) during P-flushes characterising periods of spring melt. Altogether, the abundance and diversity periphyton species-assemblages in each of the streams was controlled by interchangeable environmental factors, the prevalence of which varied seasonally, but consistently across the three target streams.

The growth of aquatic bryophytes appeared to be strongly influenced by physical habitat characteristics (e.g. flow, substrate morphology), with the largest standing crops accumulating in fast-flowing riffles dominated by large boulders, and lowest production occurring in slowing-flowing pools characterised by unstable substrates. Stable substrates resist flow-induced streambed movements and offer persistent microhabitat for aquatic bryophytes, particularly by providing large-diameter substrates such as boulders which permit the development of a vertically-zoned bryophyte community, facilitating a greater diversity of aquatic bryophyte species; and by the high velocity 'riffle effect' (which thins boundary layers and increases CO_2 diffusion).

The dataset collected for vascular submerged macrophytes was small but provided some indications that physical habitat characteristics (e.g. flow, substrate morphology) were probably also important to the abundance of river plants. Vascular macrophytes were markedly absent from high velocity stretches of streambed characterised by coarse substrates (i.e. the principal aquatic bryophyte habitat). Though in this context, large stands of aquatic plants are capable of manipulating microhabitat conditions by altering near-bed flow regimes (thus acting as sediment traps) which may partly explain why vascular macrophytes often grew in slow-flowing waters dominated by fine substrate particles. Critically however, the summer peak of aquatic macrophyte growth was probably missed (sampling April, September and November) hence no significant seasonal differences in standing crop were found. Therefore future monitoring protocols to include the sampling of aquatic macrophytes should occur during the temperate summer (e.g. June – August) to capture peak growth.

- *What sets of environmental habitat conditions drive the formation of these freshwater vegetation assemblages, and plant species diversity, in such streams?*

To summarise, in these near-pristine upland stream habitats in Scotland, underlying geology was identified as the major macro-scale factor influencing the distribution and species diversity of freshwater vegetation assemblages of

periphytic diatoms, aquatic bryophytes and vascular submerged macrophytes, by pre-determining the inherent properties of meso-scale factors, principally water chemistry gradients (e.g. pH, conductivity, alkalinity, mineral cations). Physical micro-scale factors (e.g. flow, substrate morphology) were important environmental drivers forming hydrophyte assemblages but were not particularly useful explanatory factors for diatoms or other periphytic algae. Thus in streams or rivers of near-pristine water quality, periphyton communities, especially diatoms tend to be true reflectors of the water physico-chemistry properties, whereas aquatic bryophytes and vascular submerged macrophytes integrate prevailing physical and chemical environmental habitat conditions.

3. To characterise the stream habitat conditions associated with the communities of freshwater vegetation present; to identify potential indicator species and/or plant assemblages indicative of high environmental quality; and to use this information to determine near-pristine (reference) conditions for use in the implementation of biomonitoring protocols to assess environmental quality for upland stream habitats in Scotland.

- *Can the data be used to establish type-specific reference conditions (Annex II of WFD)?*

Overall, three communities of freshwater vegetation emerged from the combined dataset which reflected the three clusters of environmental habitat conditions (identified in Chapter 2). Together this helps prove that in near-pristine upland streams in Scotland, the distribution of diatom, aquatic bryophyte and vascular submerged macrophyte species-assemblages are spatially-organised in relation to environmental gradients, especially water chemistry and (where relevant) substrate morphology.

The three communities (and potential indicator species therein) of freshwater vegetation indicative of high environmental quality, characterised oligotrophic headwater streams of the Scottish Highlands across gradients of environmental habitat conditions, principally water chemistry and substrate morphology (for a

summary of this refer to Table 5.1). Essentially, each species-assemblage of periphytic diatoms, aquatic bryophytes and (where present) vascular submerged macrophytes could potentially function as an ecological benchmark of near-pristine (reference) conditions. This complies with Annex II of the Directive, having established type-specific conditions to act as a point of reference for communities of aquatic plants occurring in disturbed rivers of similar typology to facilitate WFD classification of water quality status in Scotland.

To summarise, the findings of the three main results chapters merge together to form a coherent piece of research by characterising sets of habitat conditions and major environmental gradients driving the abundance and diversity of freshwater plants in upland streams of near-pristine water quality in Scotland (see again Table 5.1). The availability of improved knowledge could help identify marker species characterising suites of environmental habitat conditions, for possible future implementation in biomonitoring schemes for upland rivers, appropriate under WFD and similar legislation worldwide. The work undertaken in this study represents the first study of its kind (probably for the UK and certainly for Scotland), to provide information which could potentially be used as a prerequisite and potential feeder strategy for WFD progression *a propos* river biomonitoring protocols utilising communities of freshwater vegetation.

- *Can data derived from this project be used to develop a minimal model system to effectively predict reference (near-pristine) conditions for upland stream habitats in Scotland?*

Several minimal models were constructed to assess whether it was feasible to accurately predict the response (e.g. standing crop, diversity) of freshwater vegetation using combinations of potentially influential environmental predictor variables chosen from outputs of correlation matrices and/or lengthy arrows on multivariate ordination diagrams. The diversity of periphyton and aquatic bryophyte communities was quite accurately predicted from water chemistry

variables and (where relevant) substrate morphology factors, but production was generally less well predicted (especially for periphytic algae).

As for minimal models in general, the use of test data sets in this study emphasizes the limited envelope of applicability of minimal models (Scheffer & Beets 1994); these being restricted to a defined set of environmental conditions from which they were developed. The models proposed in this study may be useful for predicting plant production of upland streams of near-pristine reference condition. However, they are unlikely to function particularly well for lowland rivers (where environmental drivers of aquatic vegetation, such as flow regime and substrate morphology, will likely differ from headwater habitat conditions) or systems disturbed by nutrient enrichment, thus overriding the envelope of applicability of the models.

Another criticism of the work undertaken is that even the strongest minimal models produced are not very powerful probably because the work is based on just three streams. What I have demonstrated is that the approach utilised is viable, but obviously more sites would be required to build more robust models suitable for use as a management tool for water quality assessment.

4. To determine the value or otherwise of artificial substrate sampling procedures for assessing periphyton production and community composition, in comparison with direct sampling of naturally-occurring microhabitats in upland stream habitats.

- *Do artificial substrate samplers make effective surrogates for naturally-occurring microhabitats and periphyton colonisation?*

The use of artificial substrate samplers benefited the project mainly by ensuring fairness of comparability between sub-catchments (and their sites) and allowing reproducible samples of periphyton to be collected. Furthermore it enabled me to contribute to an ongoing debate in the literature regarding the effectiveness of their usage for sampling periphyton from streams and rivers. Generally my

findings were that artificial substrate samplers of linoleum, Astroturf and plastic aquarium plants made good surrogates for supporting a periphyton community composition acceptably comparable to those inhabiting their respective naturally-occurring microhabitat (unlike glass or Perspex slides used in a number of other studies). However, I could not warrant similar confidence for periphyton production which appeared to be underestimated using artificial substrate samplers. This is most probably due to the highly heterogeneous texture (e.g. porosity, roughness) of naturally-occurring substrate surfaces compared to surrogate samplers.

	TWINSPAN assemblage		
Stream environmental habitat characteristics	**III**	**II**	**I**
Underlying Geology	Predominated by resistant, base-poor strata	Mixed geological composition, presence of both base-poor and base-rich strata	Predominated by weatherable calcareous, base-rich strata
Streambed Substrate Morphology	Stable, boulder dominated, characterised by robust particles	Moderately stable, highly cobbled	Unstable, predominated by fine substrate particles
Water Quality	High status: oligotrophic, near-pristine reference conditions	High status: oligotrophic, near-pristine reference conditions	High status: oligotrophic, near-pristine reference conditions
Water Physico-Chemistry	Low buffering capacity and acid sensitive: low pH, conductivity and alkalinity Susceptible to acid-induced	Moderate buffering capacity: circumneutral pH, moderate conductivity and alkalinity	High buffering capacity: high pH, conductivity and alkalinity

	events (e.g. spates, atmospheric deposition, snowmelt)		
Water Chemistry	Base-poor: low Ca^{2+} and Mg^{2+}	Moderately base-rich: intermediate Ca^{2+} and Mg^{2+}	Base-rich: high Ca^{2+} and Mg^{2+}
	Elevated SO_4 levels	Moderate SO_4 levels	Reduced SO_4 levels
	Heavy metal availability: high prevalence of Pb, Zn, Al, V, Fe and Mn	Moderate metal availability: intermediate prevalence of Pb, Zn, Al, V, Fe and Mn	Supressed heavy metal availability: low prevalence of Pb, Zn, Al, V, Fe and Mn
Species Assemblage	Both periphyton and aquatic bryophytes co-dominant	Periphyton dominant producer, bryophytes less abundant	Periphyton and aquatic bryophytes present
	Lacks vascular macrophyte flora	Mostly lacks vascular macrophyte flora, but probable scope for colonisation in more habitable regions of streambed	Appearance of vascular submerged macrophytes (e.g. *Potamogeton polygonifolius*, *Myriophyllum alterniflorum*)
	Low species richness and diversity	High species richness and high diversity	Low-moderate species richness and high diversity
	Indicated by the presence of	Indicated by occurrence of	Indicated primarily by *Fragilaria*

	Frustulia rhomboides var. *rhomboides* (diatom) in almost every sample	*Blindia acuta* (bryophyte) and *Gomphonema acuminatum* (diatom), but also evidence of overlapping ecology with Groups III and I	*pulchella* (diatom) but also contains several bryophytes exclusive to this sample-group particularly: *Hygrohypnum luridum*, *Palustriella falcata* and *Chiloscyphyus polyanthos*
Sub-assemblages	Group III comprised of two sub-assemblages:- **IIIa**: *Frustulia rhomboides* (diatom) and *Scapania undulata* (bryophyte), typically occurred in extremely acid-sensitive conditions, characterised by pH <6, accentuated levels of sulphate and heavy metal cations **IIIb**: *Hygrohypnum ochraceum* (bryophyte), and appearance of *Gomphonema clavatum* (diatom), in moderately-acid conditions pH 6-7, some base-rich strata	No distinct sub-assemblages to mention However notable occurrence of some diatoms (e.g. *Cocconeis placentula*, *Didymosphenia geminata*, and *Diatoma moniliformis*) indicates ecological transition of outermost Group II to Group I community type.	No distinct sub-assemblages to mention

	present. Species composition indicates initial ecological transition to Group II.		
Primary Production	Low periphyton production	Low periphyton production	Low periphyton production
	Moderate aquatic bryophyte production, canopy formers and turfs	Low aquatic bryophyte production, cushions and turfs	High aquatic bryophyte production, predominance of canopy formers
	Negligible vascular submerged macrophyte production	Near-negligible vascular submerged macrophyte production	Moderate vascular submerged macrophyte production
	Moderately-low freshwater vegetation production	Low freshwater vegetation production	Moderately-high freshwater vegetation production

Table 5.1 Summary of stream environmental habitat characteristics and associated assemblages of freshwater vegetation present, including indicator species.

5.2 Potential Future Work

As with nearly all scientific research projects and their main findings (outlined in section 5.1), there remains potential scope to develop future directions of the research theme from questions or ideas which have arisen as products of the work conducted here. An intensive survey approach was adopted to address the main aims of the project, which mean that fewer sampling sites were surveyed, though in greater depth, than would have been possible for an extensive survey (less attention to detail but broader range of sampling sites surveyed). Further work is certainly required as part of the ongoing development strategy to feed knowledge in support of WFD objectives relating specifically to improved biomonitoring and assessment metrics of water quality status in Scotland. Ultimately with more time and resources, I would recommend expanding to a national scale the approach undertaken in this project using unpolluted headwater streams to build more robust models for the wider context of application in assessing impacts of eutrophication and climate change.

Some specific applications of future directed work, especially constructing more effective models could be to:

- Establish complementary laboratory experiments using continuous flow-through channels to quantify the structural and functional response of aquatic vegetation to variation in current velocity for comparison with field acquired data.

- Quantify the effects of macroinvertebrate grazing pressure on the production and diversity of periphyton assemblages using suitably constructed exclosures (e.g. petroleum gel, mesh, cages) which do not interfere with natural surface hydraulics yet would capably withstand flow scour.

- Employ nutrient-diffusing substrata to predict the extent to which the functional and structural attributes of these communities deviate from their reference state in response to disturbance from eutrophication.

- Clarify whether organic P enrichment is a more influential driver of changes in diatom community composition in disturbed rivers, than inorganic P. It has recently been disputed that some diatom species (e.g. *Didymosphenia geminata*) exhibit localised alkaline phosphatase activity (involved in nutrient uptake) and are therefore responding directly to organic P enrichment (Ellwood & Whitton 2007, Whitton *et al*. 2009). A concern is that currently environment agencies do not measure organic P and water quality metrics (e.g. TDI) therefore only relate ecological shifts in diatoms to eutrophication driven by inorganic P.

One particular area of research which I personally feel deserves further attention is to determine whether benthic diatom morphology responds predictably to changing environmental stress (due to both natural and anthropogenic drivers) in streams. A further recommendationwould hence be to assess the potential of such an approach as the basis for developing a river biomonitoring metric based on simple-to-measure morphometric data for common benthic diatom species, to complement existing diatom assemblage-based metrics already in use (e.g. TDI) for evaluating water quality. The underlying rationale for such work is that little is known about the environmental cues that influence the observed (and often extensive) variations in frustule morphology (e.g. valve size, shape, striation orientation and density) within individual diatom species (Snoeijs *et al*. 2002). Prior research has more commonly focussed on the life cycle of single species of diatoms (e.g. Potapova & Snoeijs 1997), rather than examining size variation within several co-occurring species of the same community. Furthermore, these studies have usually been confined to marine diatoms (e.g. Busse & Snoeijs 2002, Snoeijs *et al*. 2002), and restricted their investigation to a limited number of environmental variables. The proposed research would aim to examine natural variation in valve morphology of several wild diatom species over substantially longer periods than in most other documented studies (Potapova & Snoeijs 1997). Moreover, compared to detailed accounts of reproductive behaviour determined from culture studies (in an artificial environment) little research has been conducted on naturally-occurring diatom populations in this respect (Potapova &

Snoeijs 1997). Reproduction in diatoms is both asexual, involving mitotic cell divisions, and sexual, through means of auxosporulation (Mann 1993). During vegetative reproduction, the predominant reproductive strategy throughout the diatom life cycle, and often exceeding more than a year in duration, diatom cell size is reduced with each successive generation of cell divisions until a critical minimal size (usually 30-40% of maximum cell size) is reached. Thereupon, a sexual phase is initiated, necessary to restore larger cell size and facilitate genetic diversity in naturally-occurring diatom populations (Lewis 1984, Potapova & Snoeijs 1997). Parent cells undergo meiosis producing gametangia for conjugation to form a fertilised zygote or auxospore, which grows to full size and resumes the asexual life cycle (Mann 1993). The asexual phase in diatoms may extend several years, referred to as supra-annual life cycle (Mann 1988) and is highly variable between species (Amato *et al*. 2005). By comparison sexual episodes are infrequent in diatoms and such events can occur extremely rapidly, with sexual forms occurring at naturally low abundances in wild populations (Mann 1988, Potapova & Snoeijs 1997). The concept of the diatom 'sex clock' infers that an overriding genetic factor regulates sexual intervals and is entirely independent of environmental cues (Lewis 1984). However, recent research suggests that diatom morphology and life cycle periodicity is strongly influenced by environmental drivers such as temperature variation (Potapova & Snoeijs 1997), whilst others found sexual responsiveness was affected by nitrate levels (Jewson 1992, Poulícková & Mann 2008). This suggests that genetic predisposition notwithstanding life cycles in wild populations of diatoms are modified by environmental pressures (Potapova & Snoeijs 1997).

5.3 Conclusions

The main findings of the results chapters of this thesis are summarised as follows:

- The habitat characteristics of the nine sampling sites were categorised into three clusters representing environmental gradients of water chemistry and substrate morphology in upland streams of nutrient-poor reference status, with habitat conditions ranging from base-poor and acid sensitive to mineral-rich and calcareous.

- Periphyton production was principally governed by the physical forces of flow disturbance and P-limitation but light and temperature were important secondary environmental factors. Standing crops of aquatic bryophytes and vascular submerged macrophytes were largely determined by flow-substrate interactions.

- Underlying geology was the major macro-scale factor pre-determining environmental habitat characteristics by directly influencing inherent properties of meso-scale factors especially water chemistry gradients (e.g. pH, conductivity, alkalinity, mineral cations) and (where relevant) physical micro-scale factors (e.g. substrate morphology), which were the principal drivers of species-assemblages of freshwater vegetation in upland streams of near-pristine reference condition.

In conclusion, the work of this thesis integrates environmental habitat characteristics (e.g. water chemistry, hydro-morphology), together with the structural and functional ecology of freshwater plant species-assemblages (e.g. periphyton, aquatic bryophytes and vascular submerged macrophytes) in reference condition streams, of which previous knowledge was scarce. In particular, the project offers new contributions by defining benchmark communities of freshwater vegetation characterising suites of environmental habitat conditions and species-assemblages indicative of high water quality status across water chemistry and substrate morphology gradients in near-pristine upland streams of the Scottish Highlands. This provides fundamental knowledge for possible future development of baseline monitoring tools as part of WFD implementation for assessing water quality status in Scotland.

Appendix 1. Iconography of Diatom Specimens

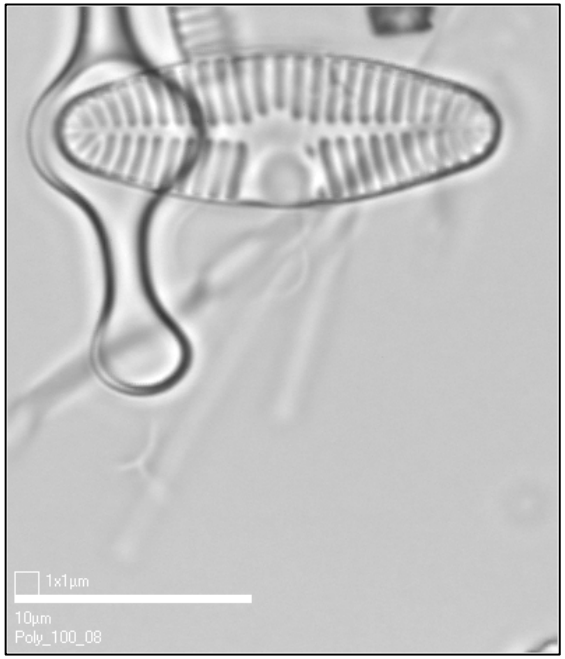
Here presented is an iconography of the diatom species sampled from the upland stream habitats of the Water of Dye, River Girnock and Knockan Burn, in Northern Scotland.

The majority of diatom images were captured at a magnification of x1000, with the exception of those photographs taken at a magnification of x400 [indicated throughout]. For purposes of consistency, diatom species were identified mostly from Krammer & Lange-Bertalot (1986-1991).

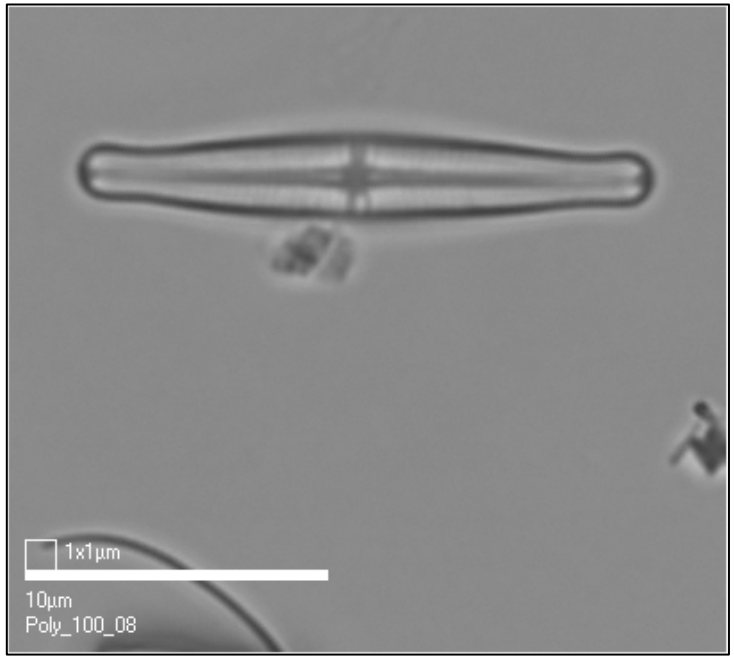
Samples comprised two of the three main classes of diatoms: the Bacillariophyceae (raphid pennate diatoms) and Fragilariophyceae (araphid pennate diatoms). Centric diatoms were not found to be present in any of the samples analysed. Diatom species marked with 'cf.' indicates some uncertainty of identification, using the nearest equivalent as presented in Krammer & Lange-Bertalot (1986-1991), or published elsewhere (refer back to section 4.3.1.2).

BACILLARIOPHYCEAE

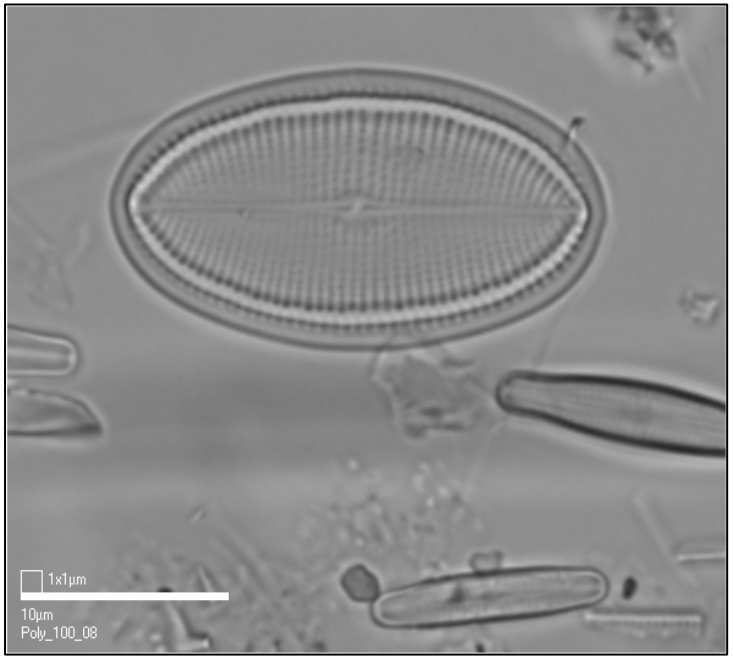
a) ACHNANTHALES



Achnanthes lanceolata

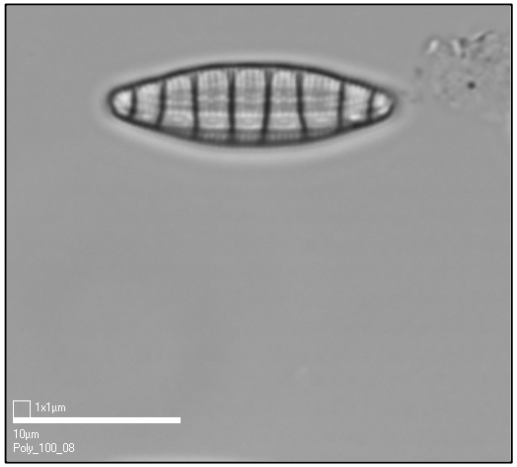


Achnanthidium minutissimum

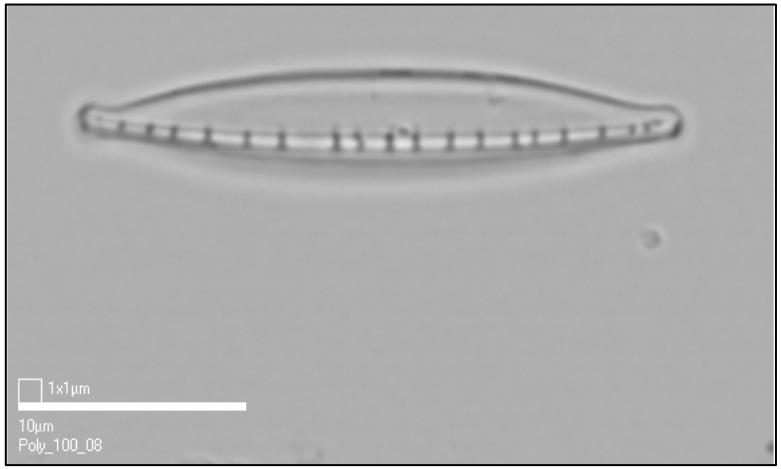


Cocconeis placentula

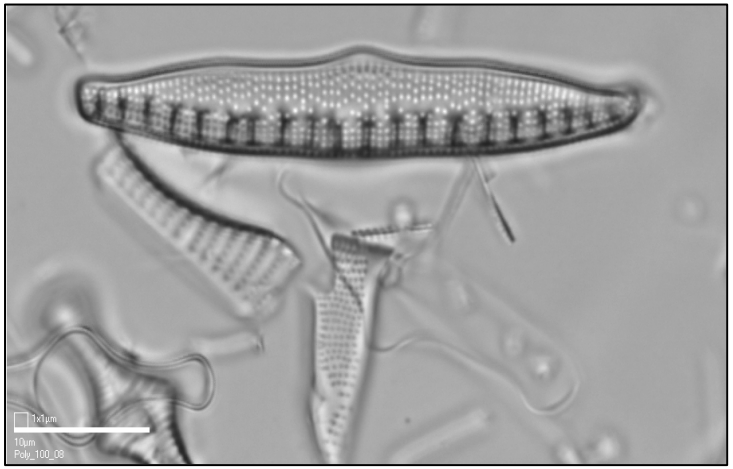
b) BACILLARIALES



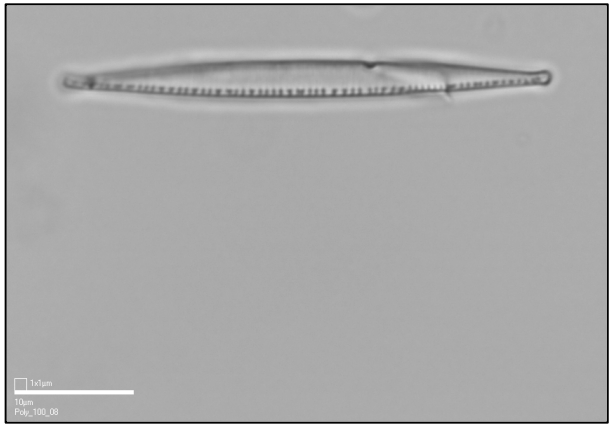
Denticula tenuis



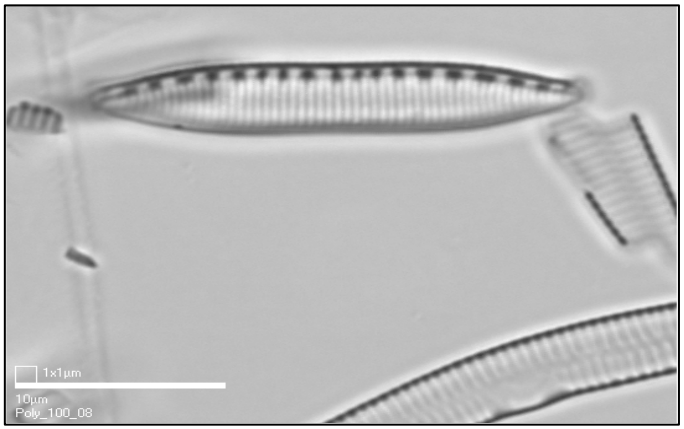
Nitzschia dissipata



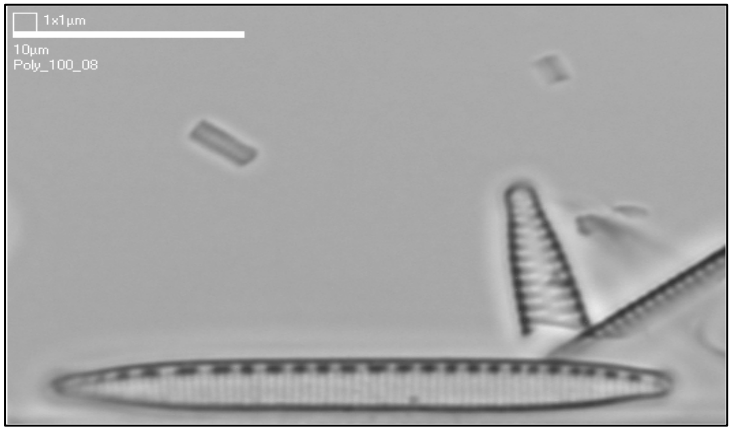
Nitzschia sinuata



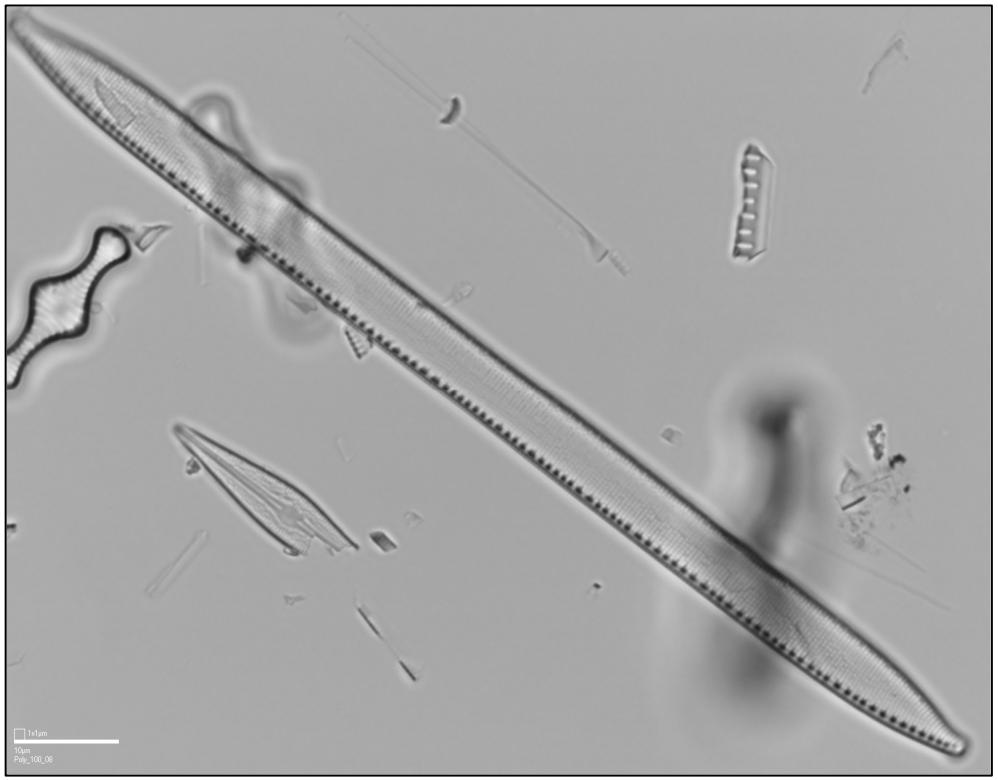
Nitzschia gracilis



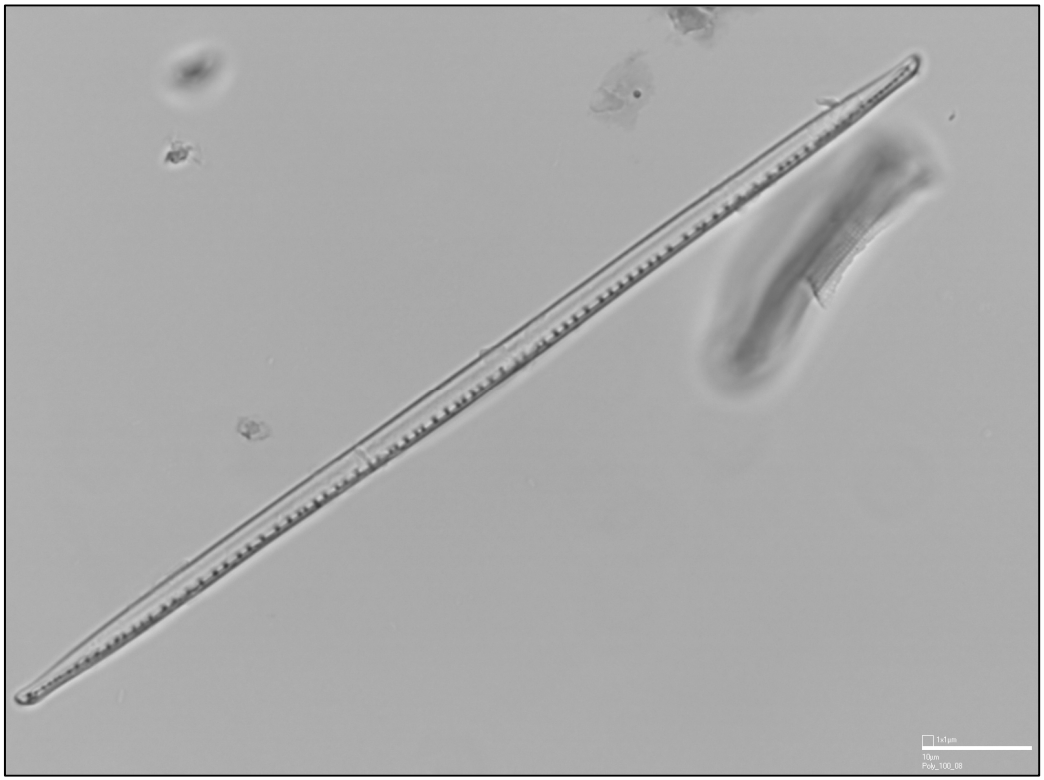
Nitzschia hantzschiana



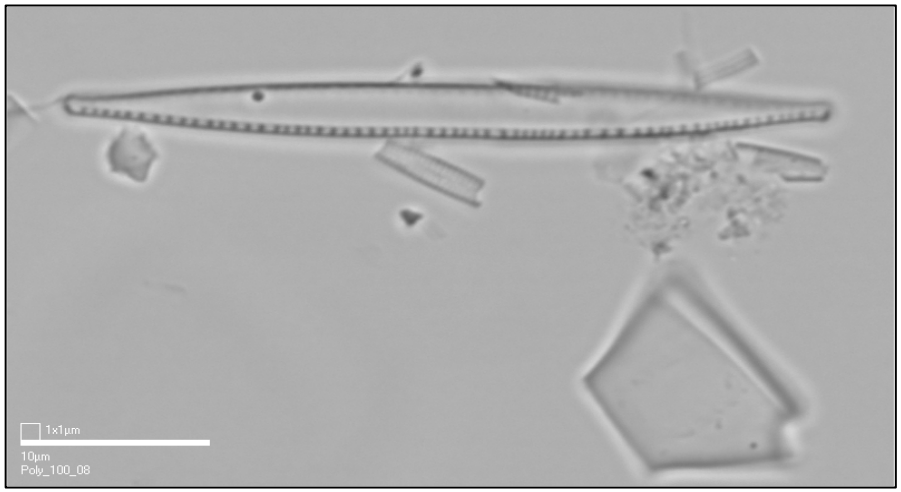
Nitzschia perminuta **agg.**



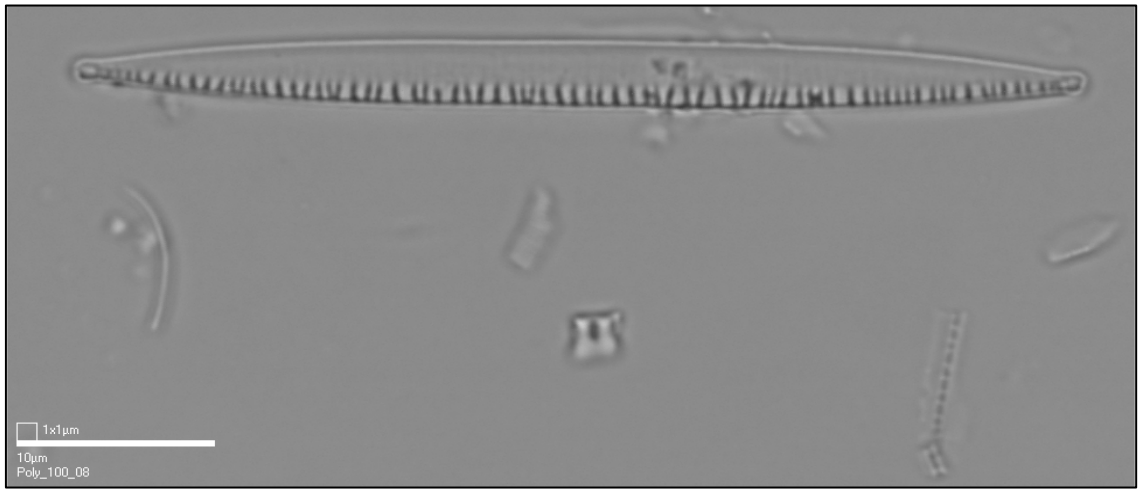
Nitzschia intermedia **agg.**



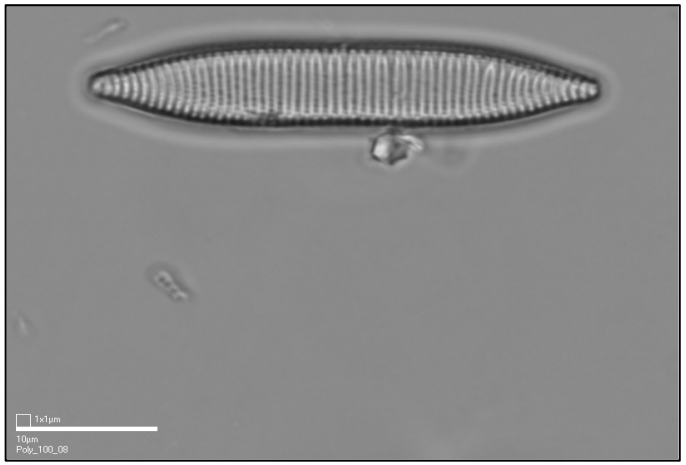
Nitzschia **cf.** *acula*



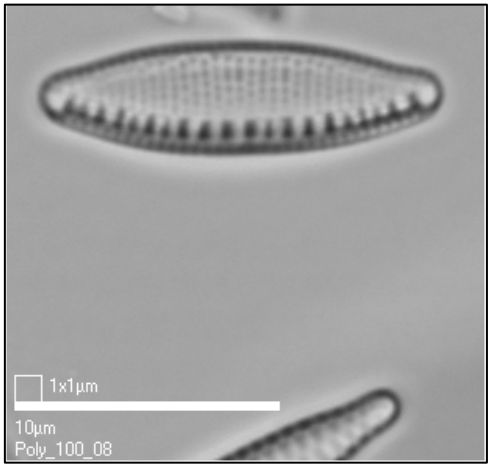
***Nitzschia palea* agg.**



Nitzschia sublinearis

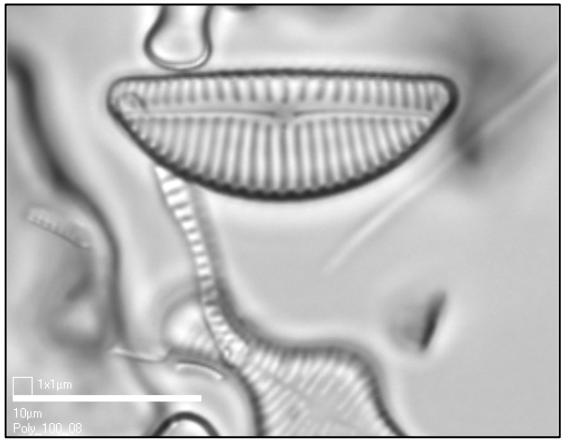


Nitzschia angustata

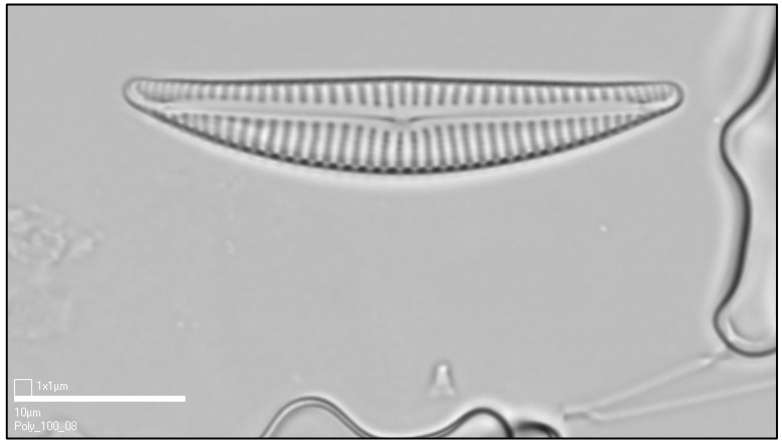


***Nitzschia* unknown (sp. 1)**

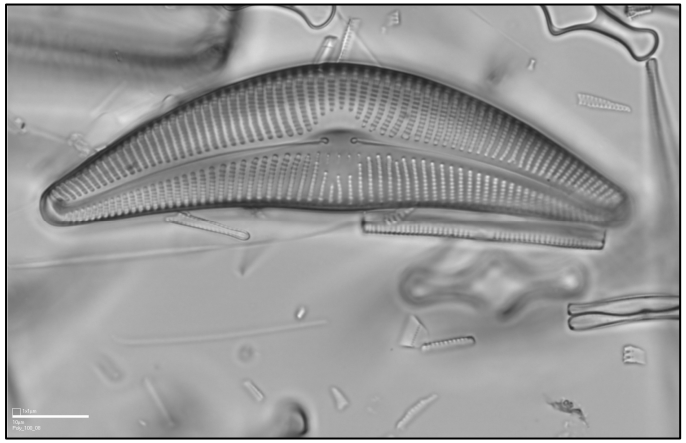
c) CYMBELLALES



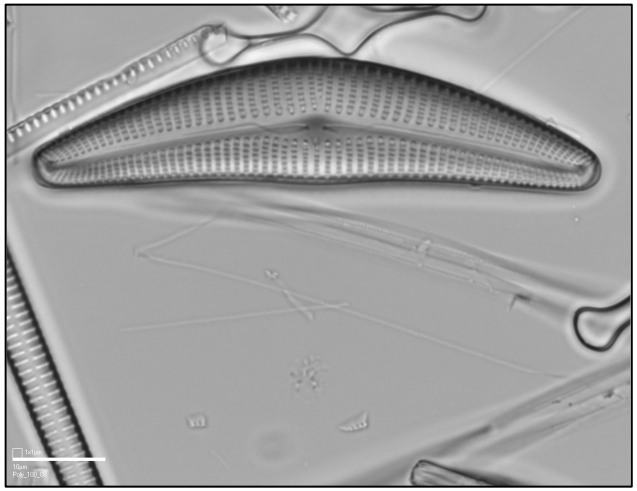
Cymbella silesiaca



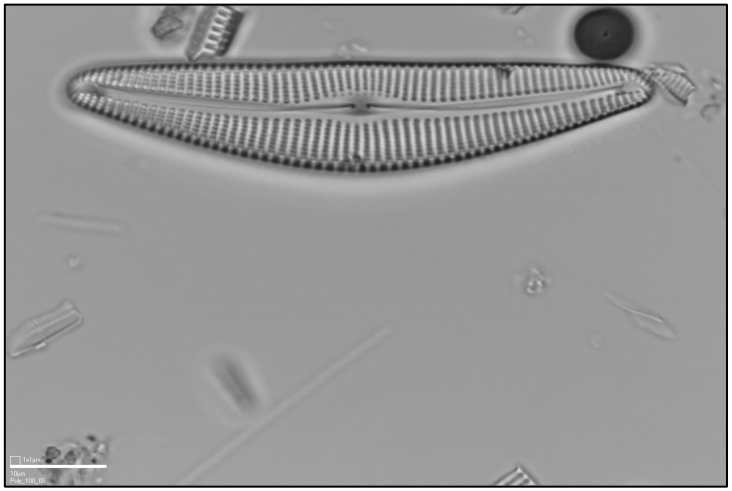
Cymbella gracilis



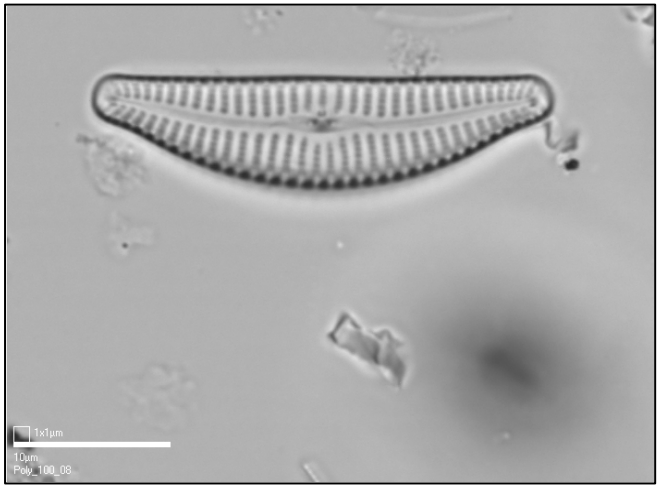
Cymbella cistula



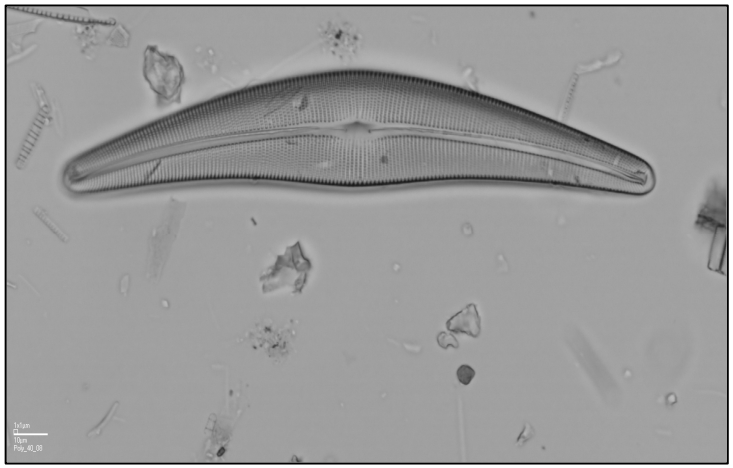
Cymbella cymbiformis



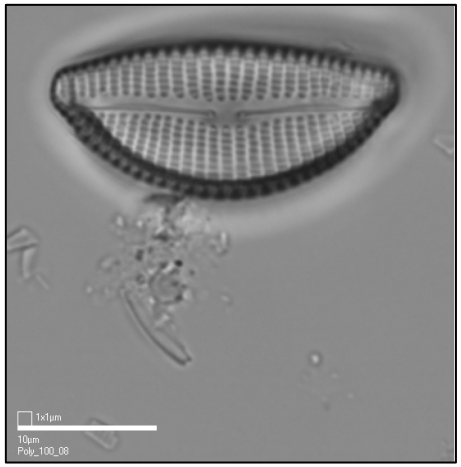
Cymbella helvetica



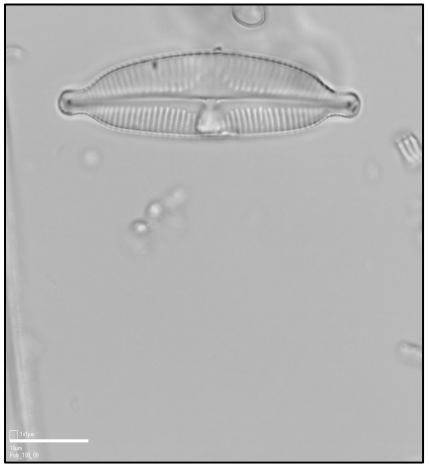
Cymbella affinis



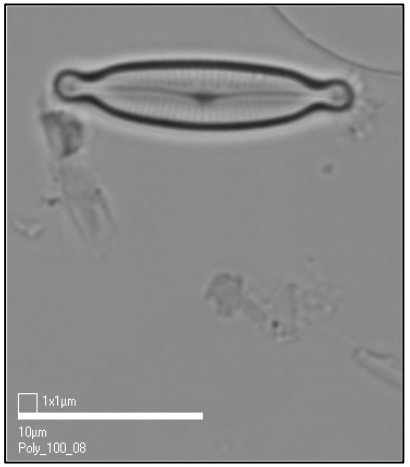
Cymbella lanceolata **[x400]**



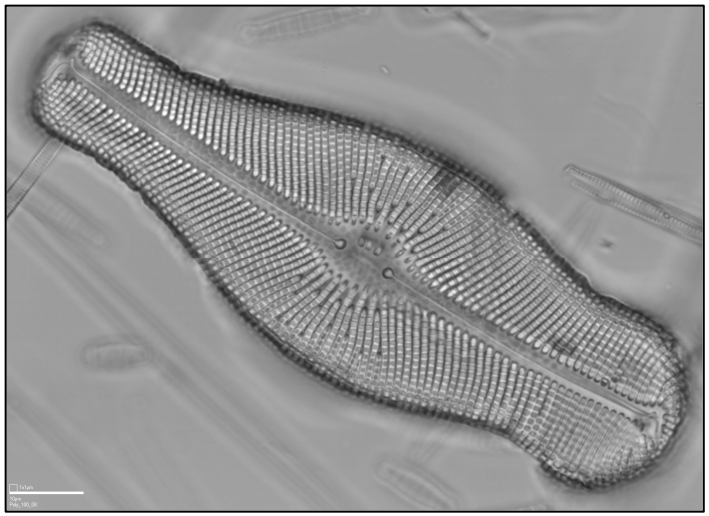
Cymbella caespitosa



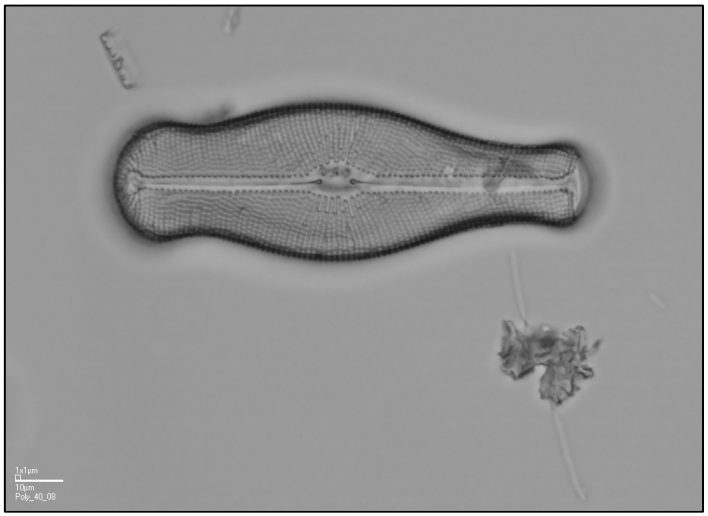
Cymbella naviculiformis



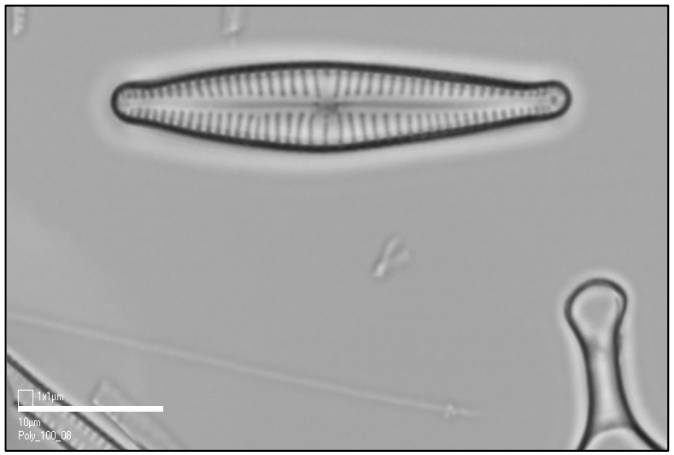
Cymbella microcephala



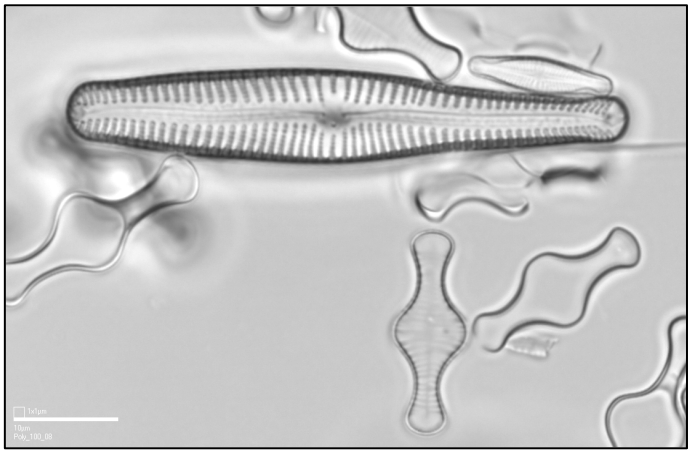
Didymosphenia geminata



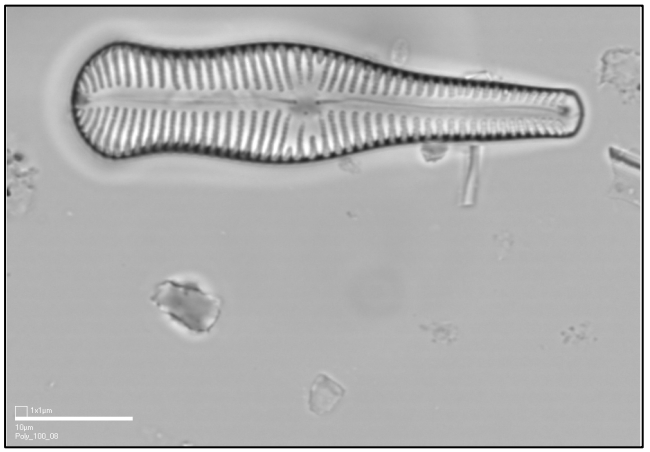
*Didymosphenia geminata***[x400]**



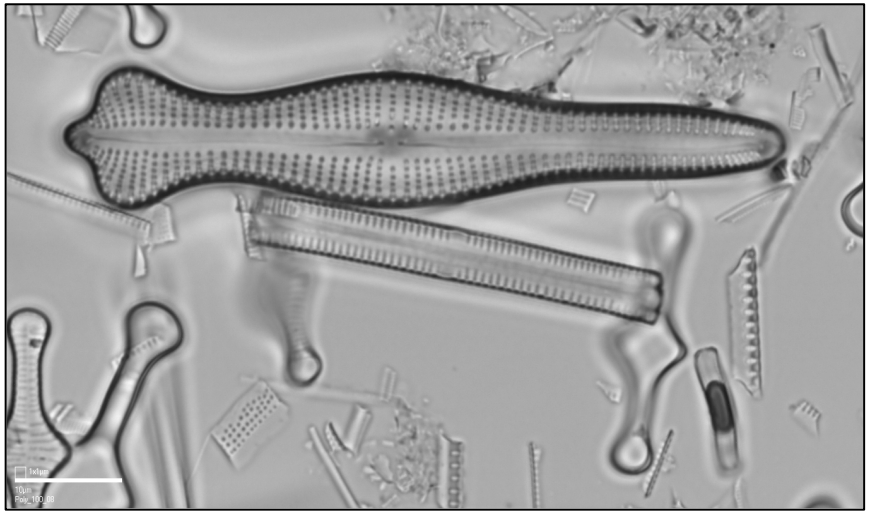
Gomphonema **cf.** *parvulum* **var.** *exilissimum*



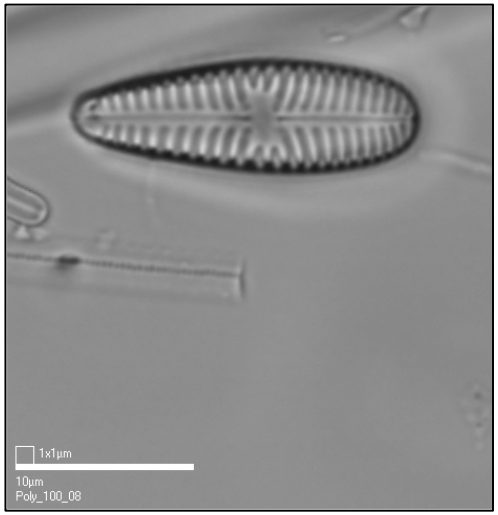
Gomphonema clavatum



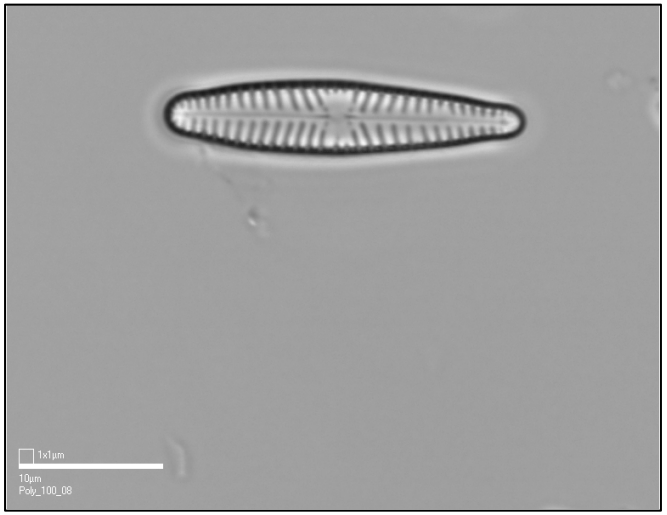
Gomphonema truncatum



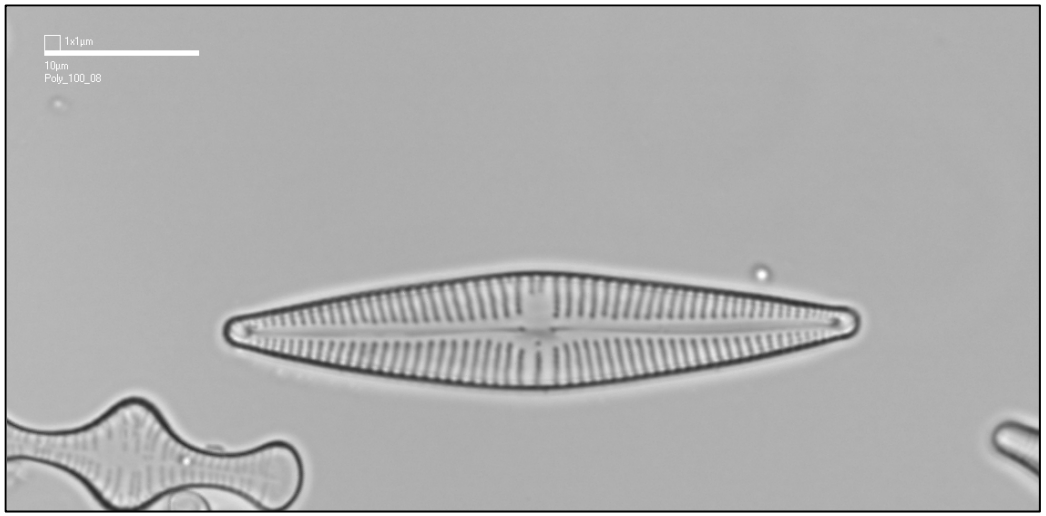
Gomphonema acuminatum



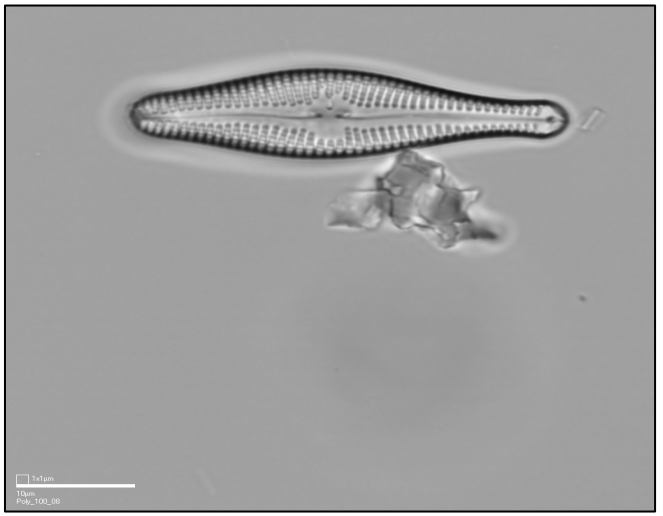
Gomphonema olivaceum



Gomphonema olivaceum **var.** *olivaceoides*

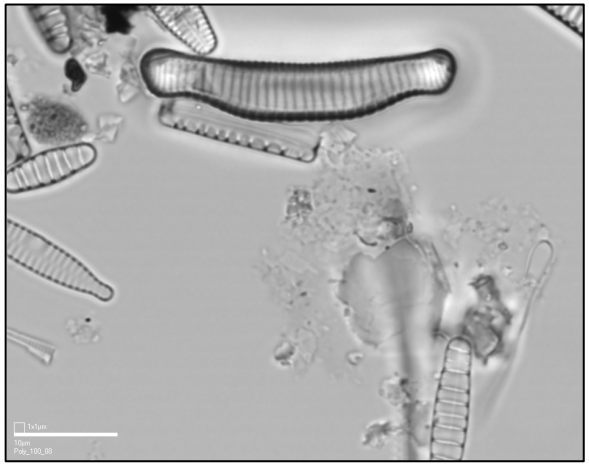


Gomphonema gracile

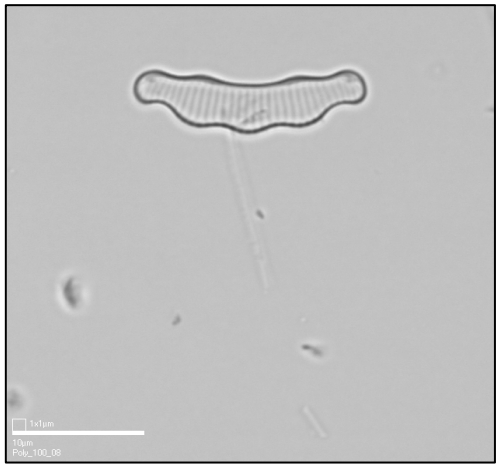


Gomphonema ventricosum

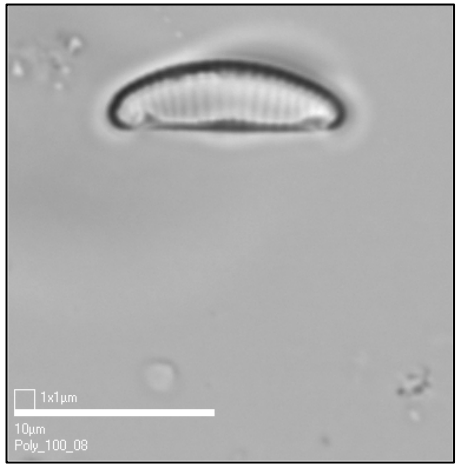
d) EUNOTIALES



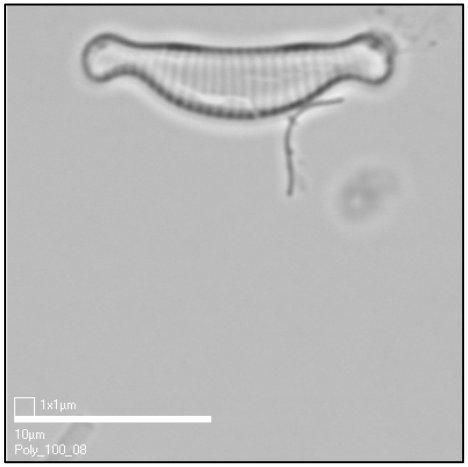
***Eunotia arcus* sensu**



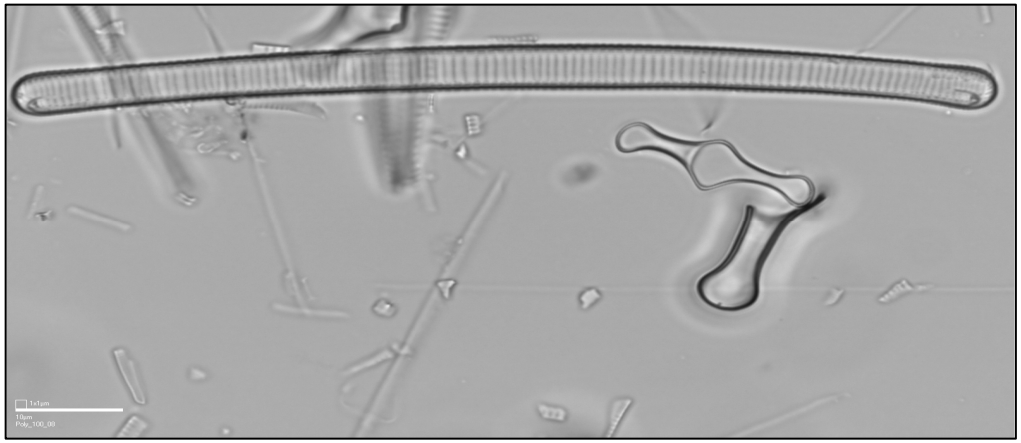
Eunotia muscicola* var. *tridentula



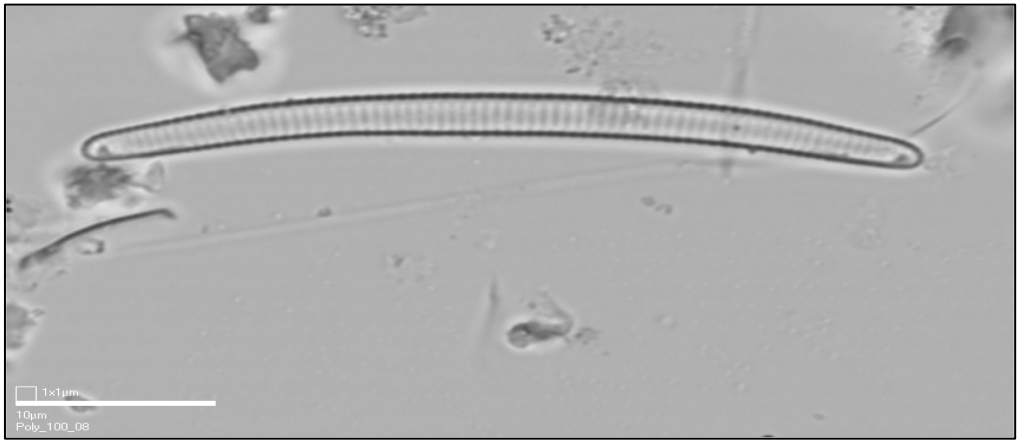
Eunotia* cf. *incisa



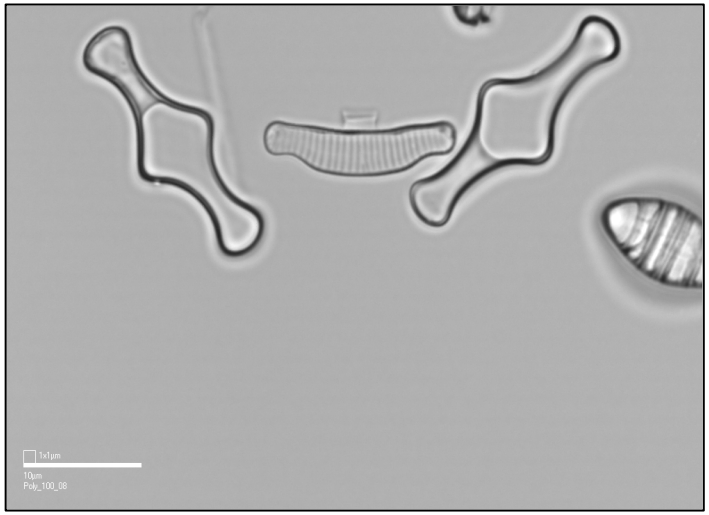
Eunotia meisteri



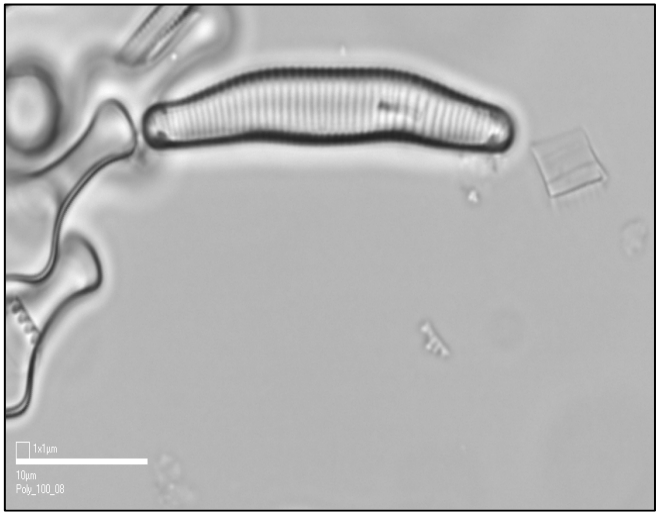
Eunotia bilunaris* var. *linearis



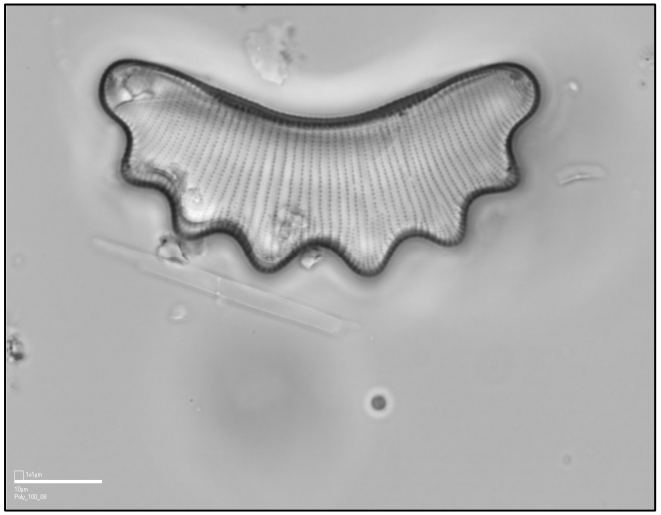
Eunotia bilunaris* var. *mucophila



Eunotia exigua

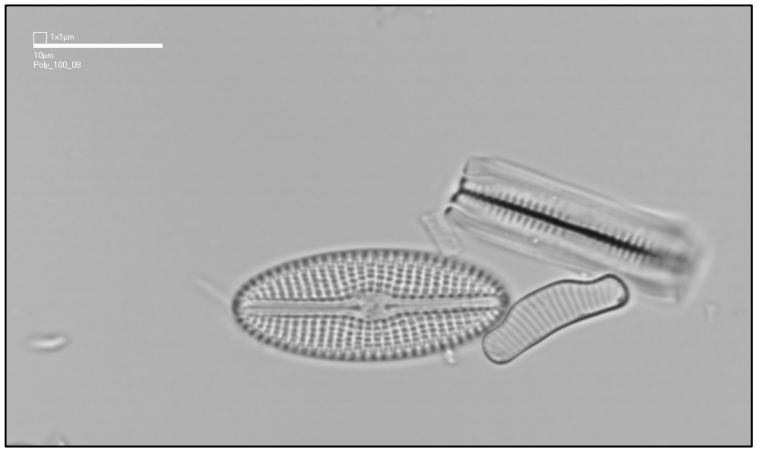


Eunotia implicata

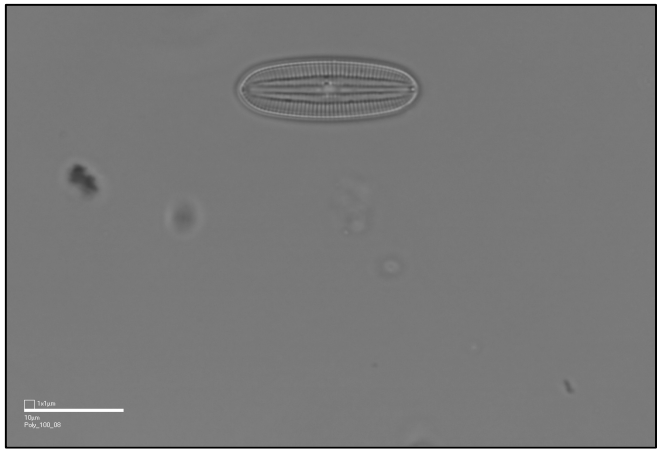


Eunotia serra **var.** *diadema*

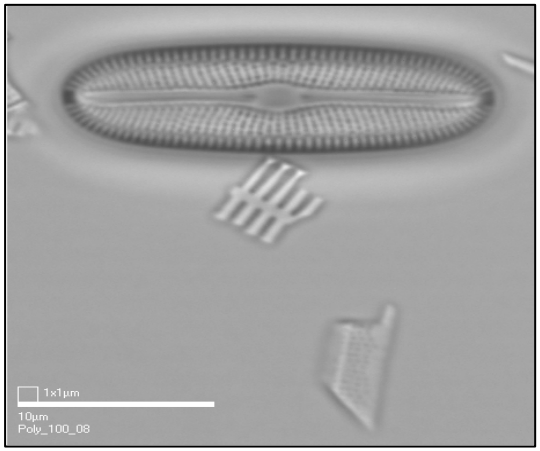
e) NAVICULALES



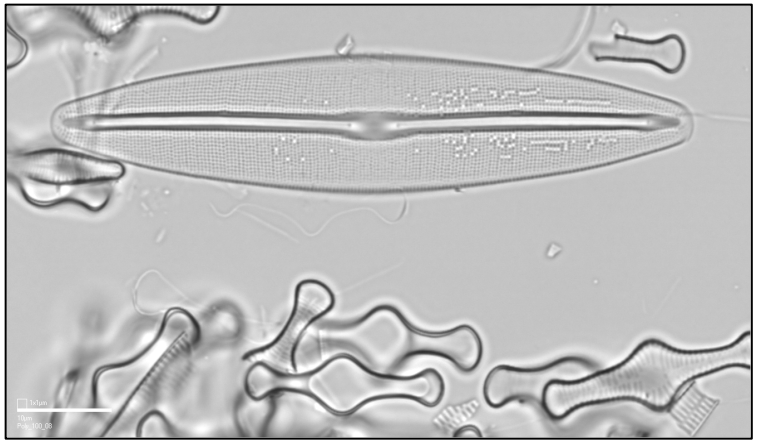
Diploneis* cf. *elliptica



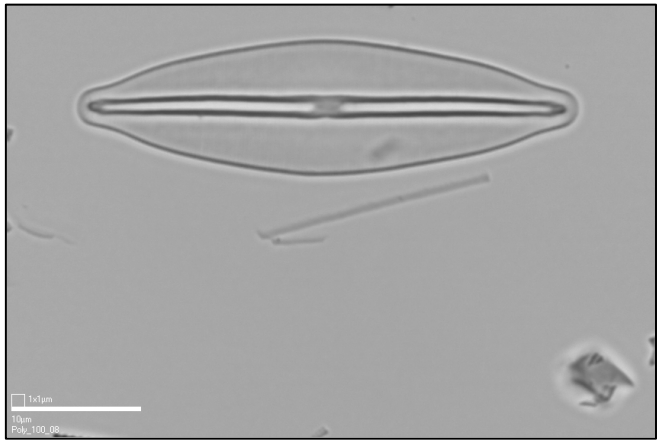
Diploneis marginestriata



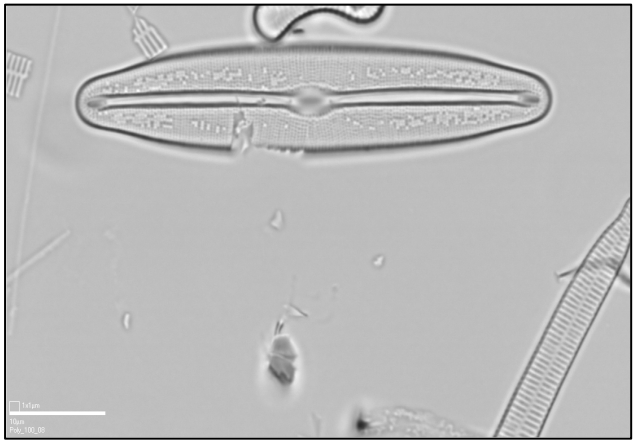
Diploneis oblongella



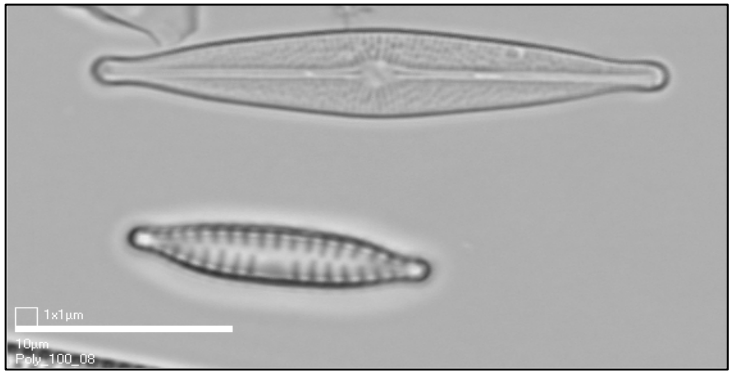
Frustulia rhomboides* var. *rhomboides



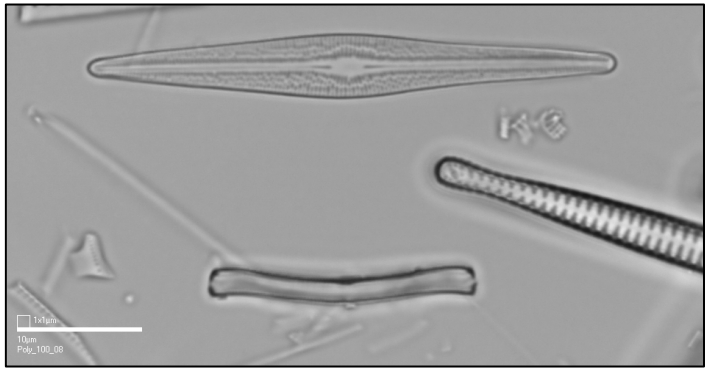
Frustulia rhomboides* var. *crassinervia



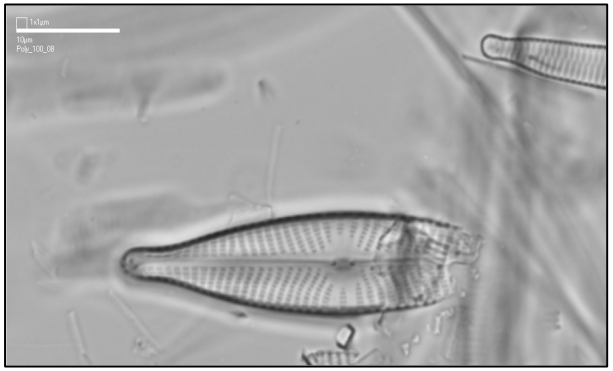
Frustulia vulgaris



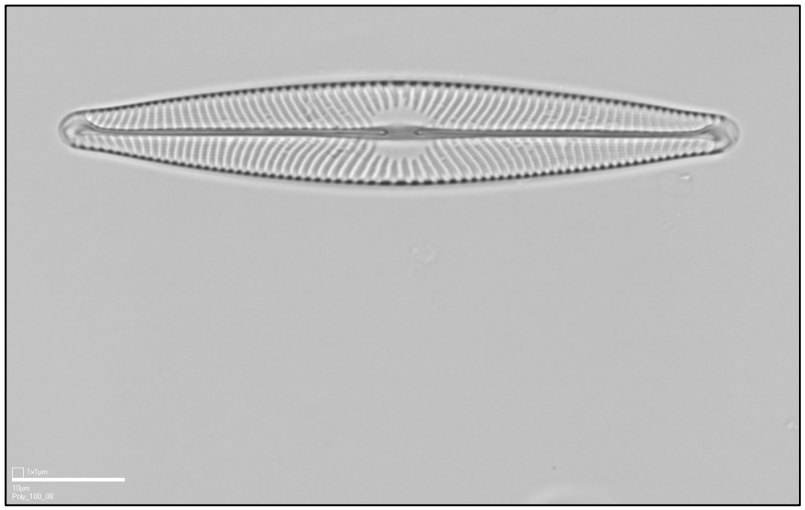
Brachysira vitrea



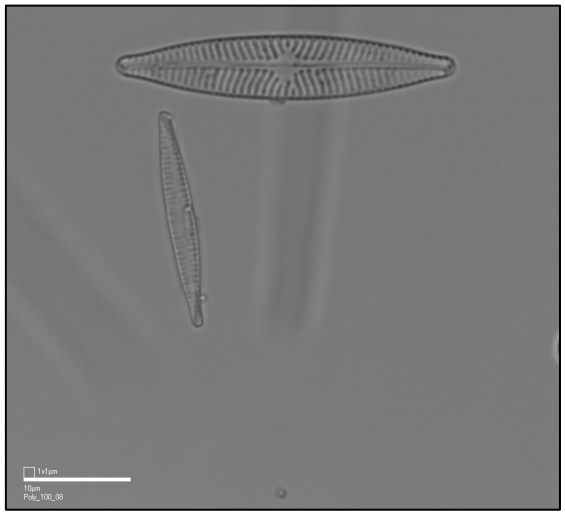
Brachysira procera



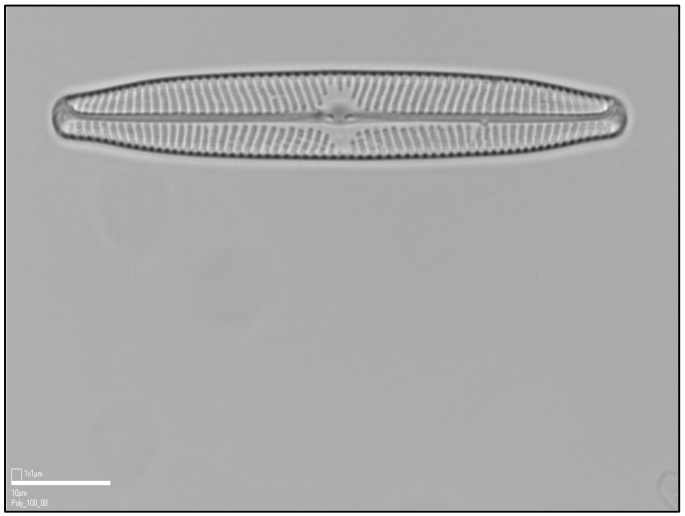
Navicula rhynchocephala



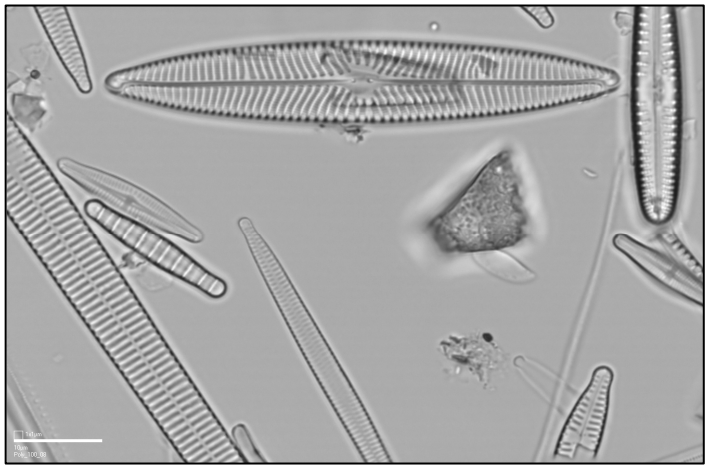
Navicula lanceolata



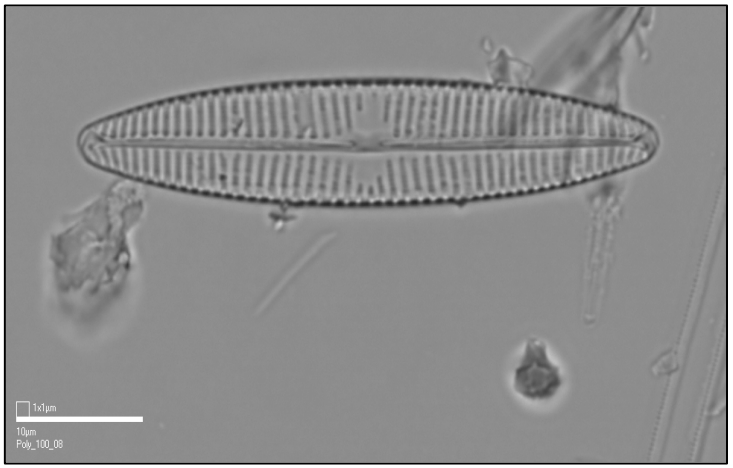
Navicula cf. *aquaedurae*



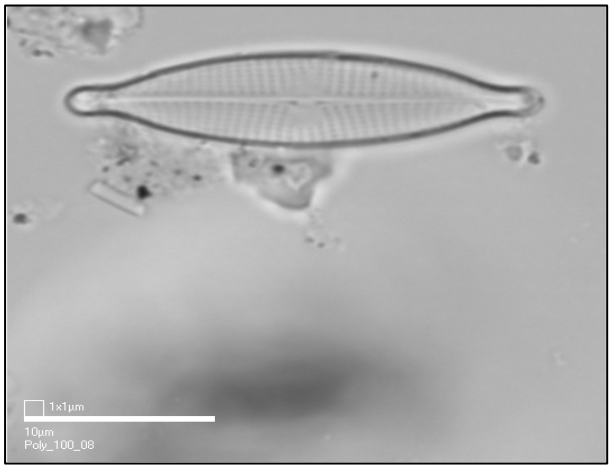
Navicula angusta



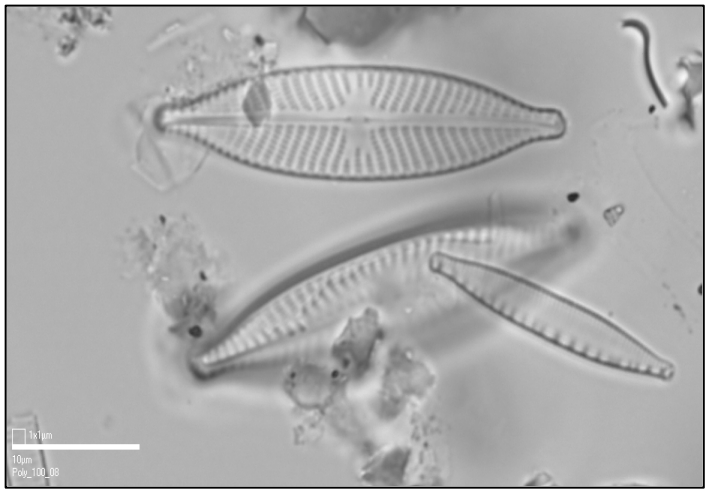
Navicula radiosa



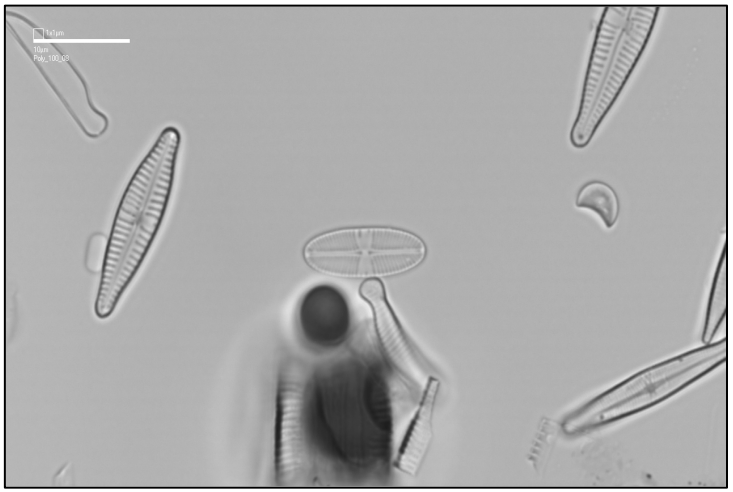
Navicula tripunctata



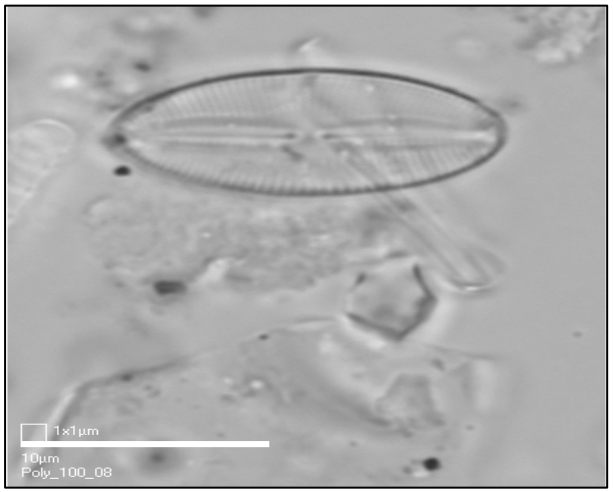
Navicula **cf.** *gregaria*



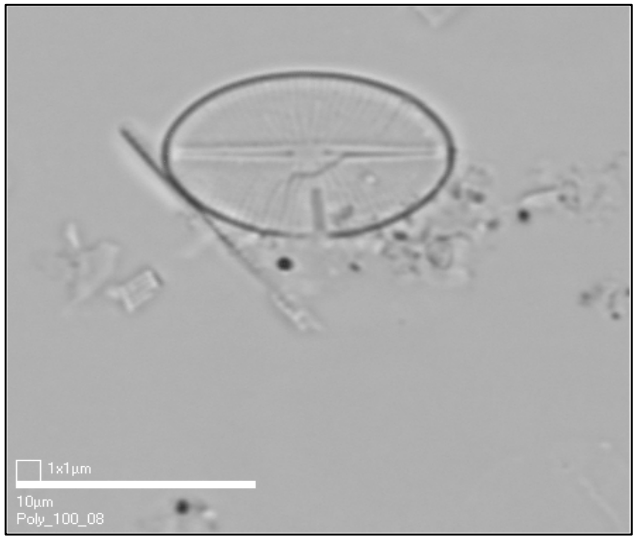
Navicula capitatoradiata



Navicula minima

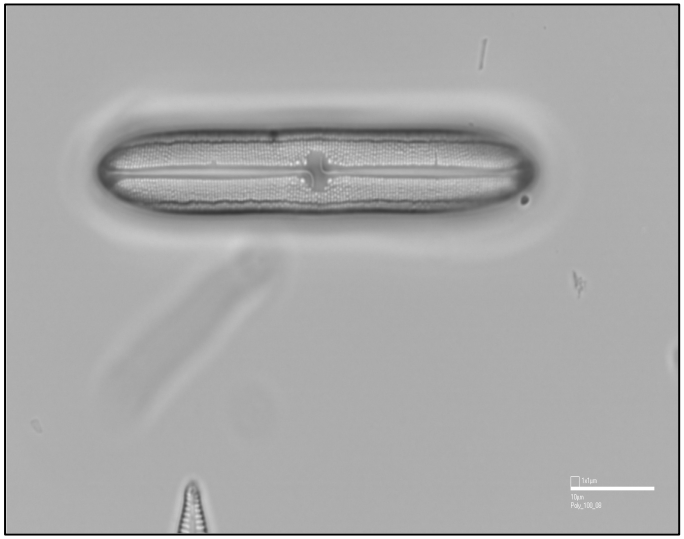


Navicula **cf.** *pygmaea* **agg.**

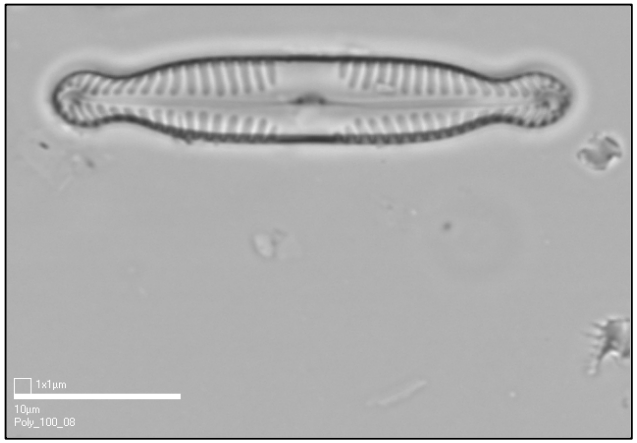


Cavinula jaernefeltii

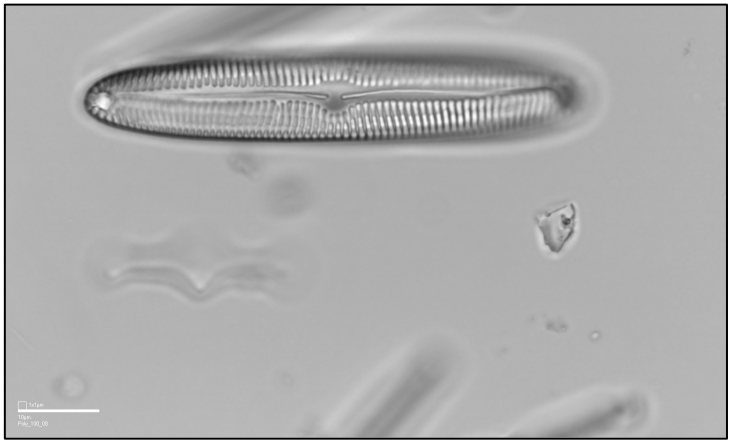
Craticula acidoclinata



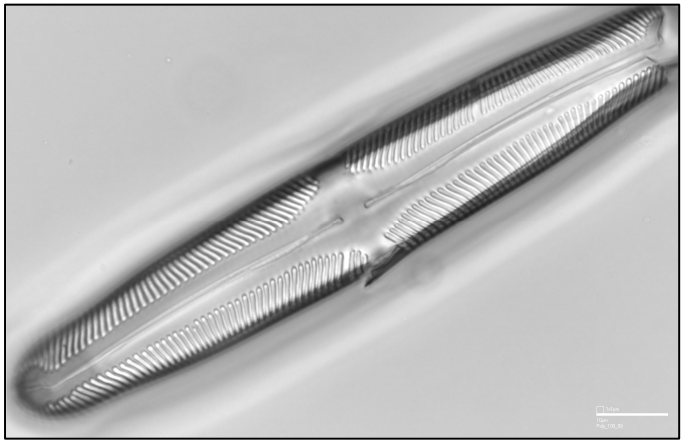
Nedium bisulcatum



Pinnularia subcapitata

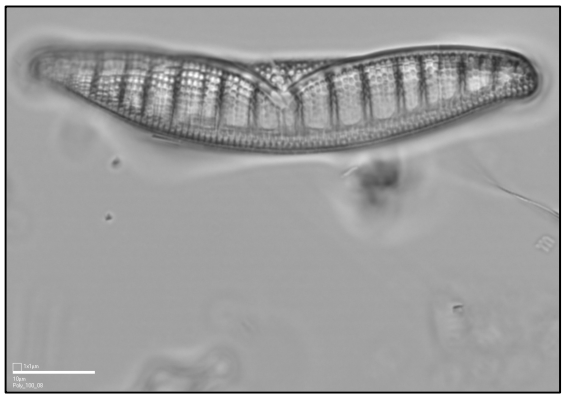


Pinnularia cf. *sudetica*

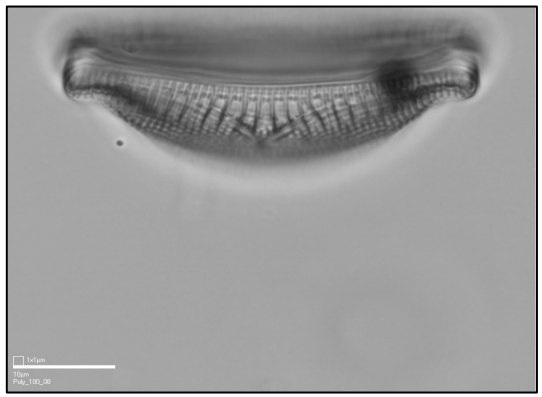


Pinnularia cf. *divergens*

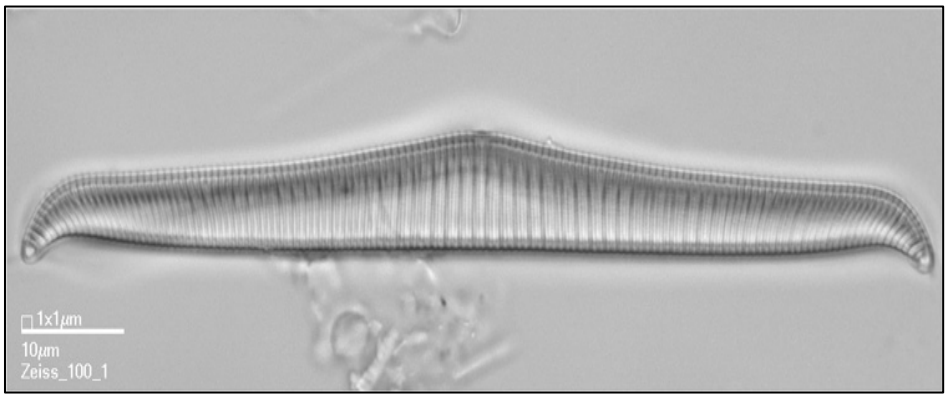
f) RHOPALODIALES



Epithemia adnata

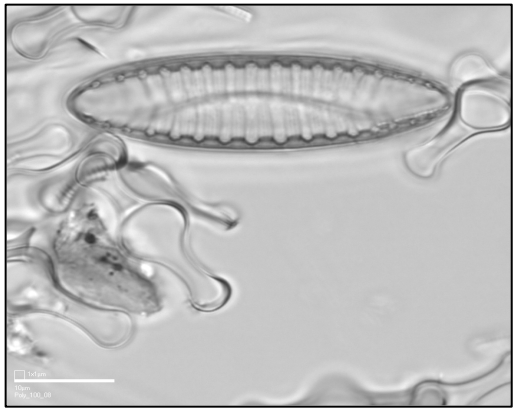


Epithemia sorex

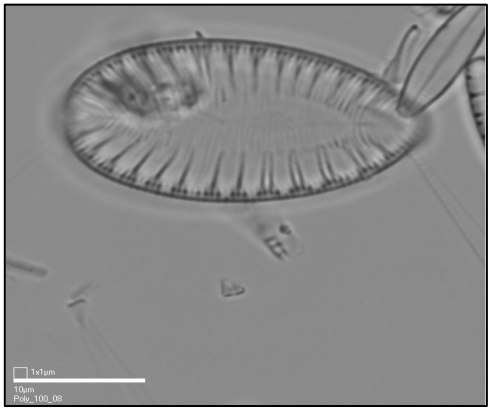


Rhopalodia gibba

g) SURIRELLALES



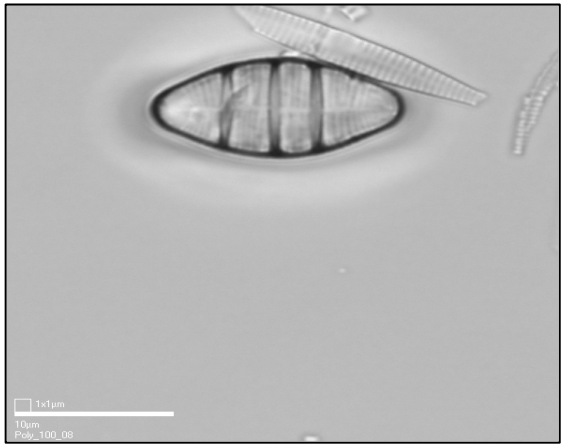
Surirella roba



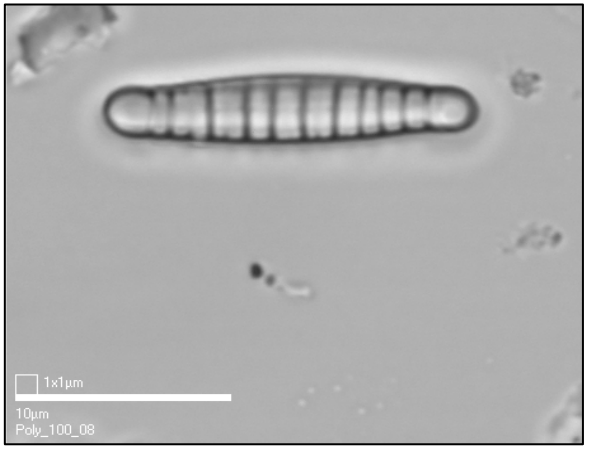
Surirella brebissonii

FRAGILARIOPHYCEAE

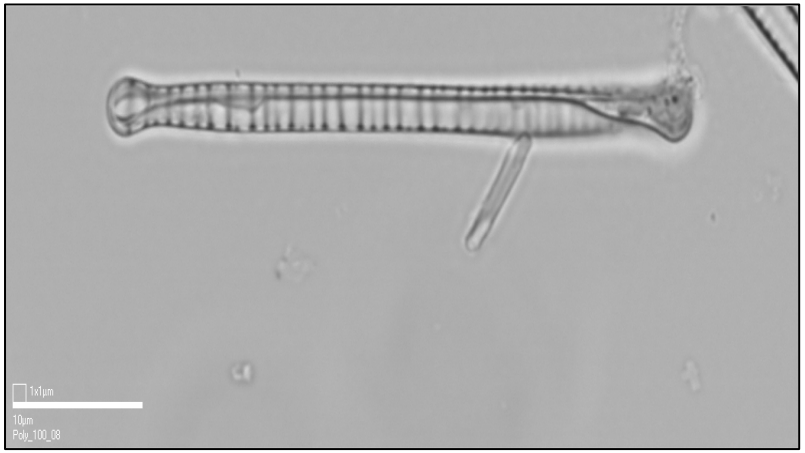
a) FRAGILARIALES



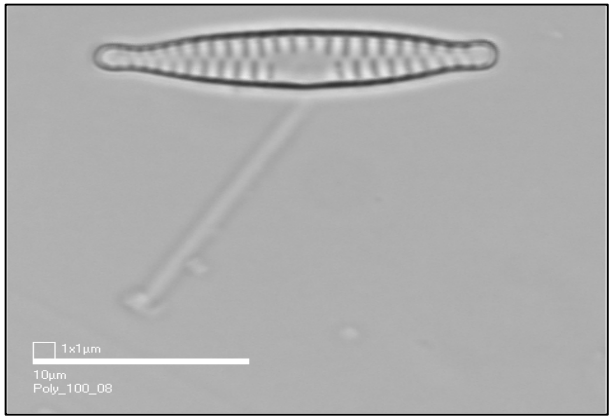
Diatoma mesodon



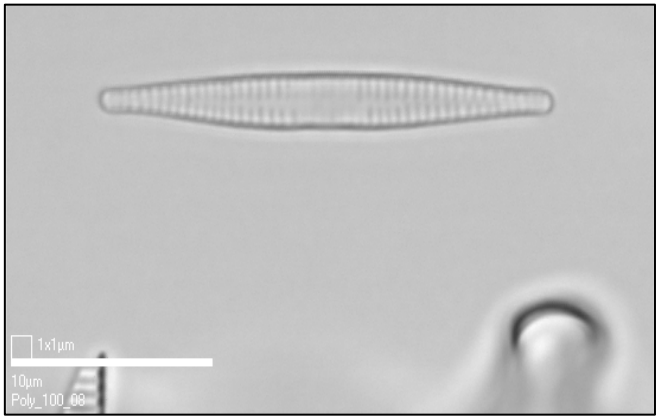
Diatoma moniliformis



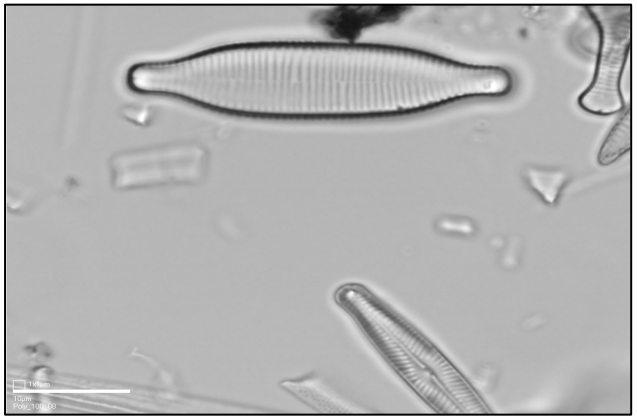
Diatoma tenuis



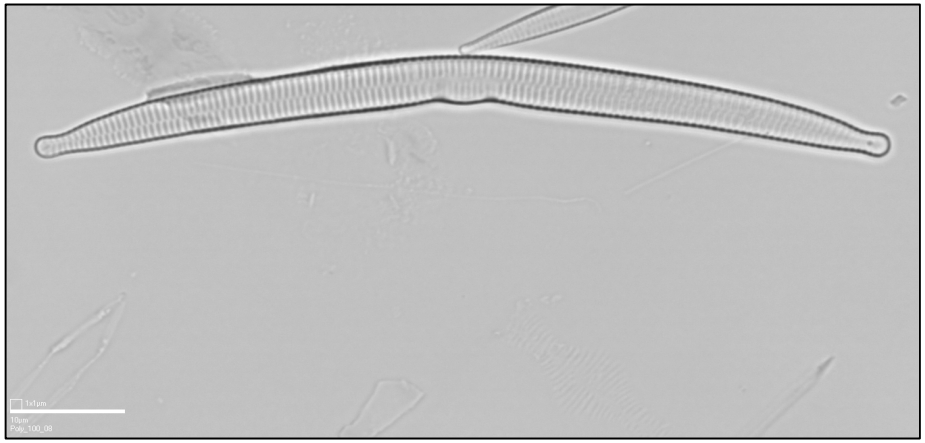
Fragilaria capucina **var.** *vaucheriae*



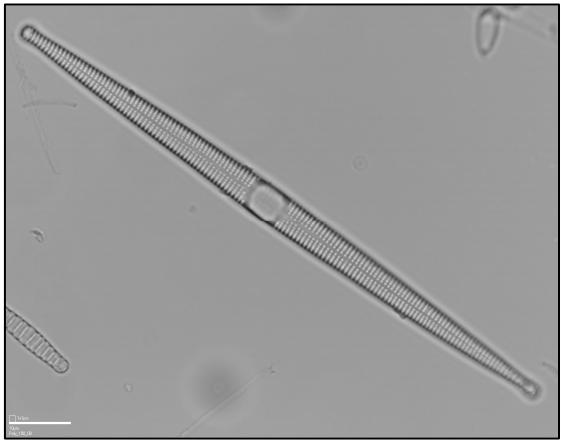
Fragilaria capucina **var.** *gracilis*



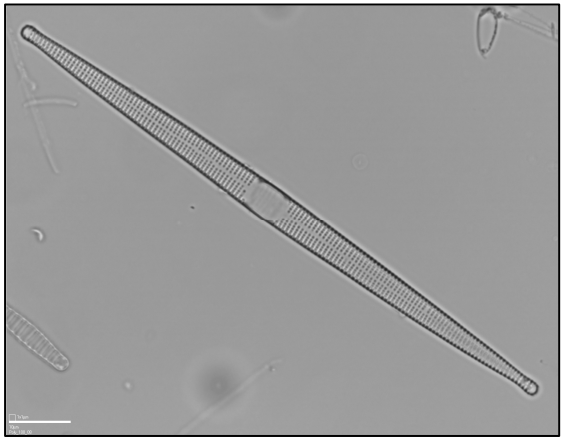
Fragilaria virescens

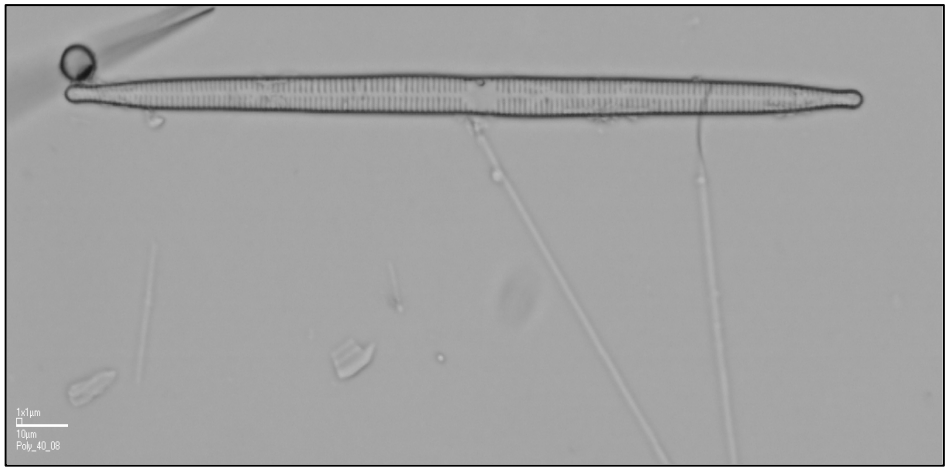


Fragilaria arcus

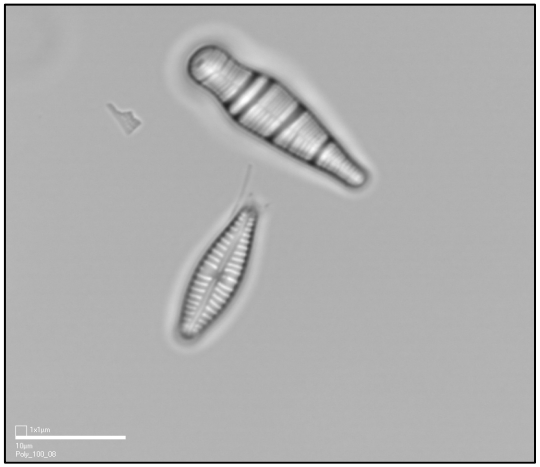


Fragilaria pulchella

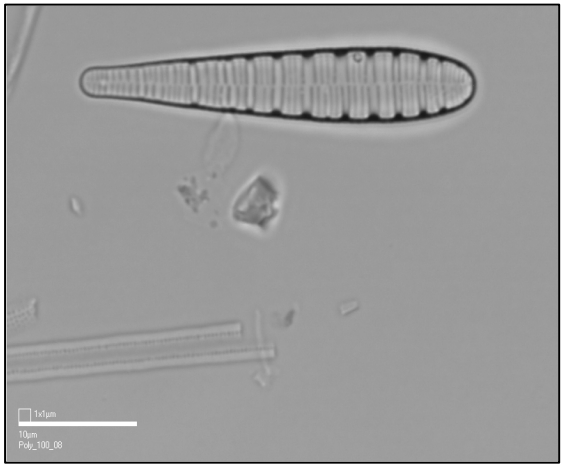




***Synedra ulna* [x400]**

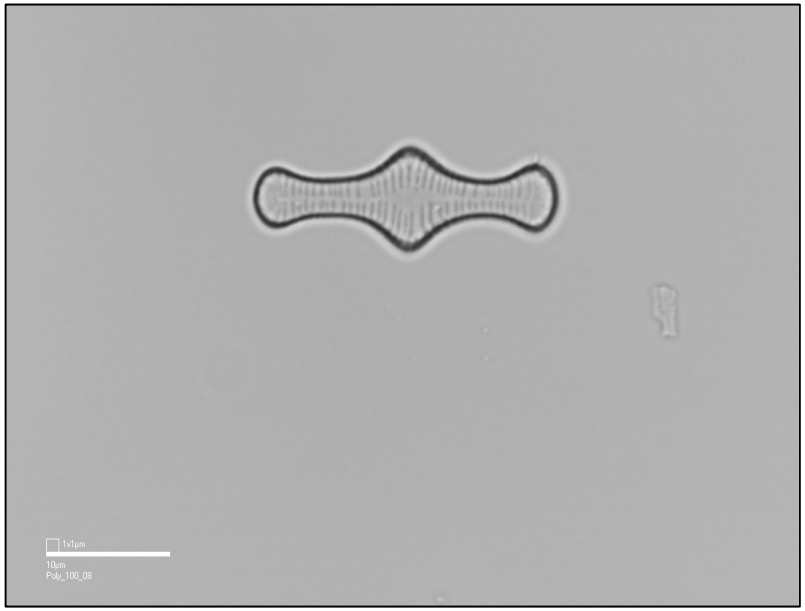


Meridion circulare* var. *constrictum

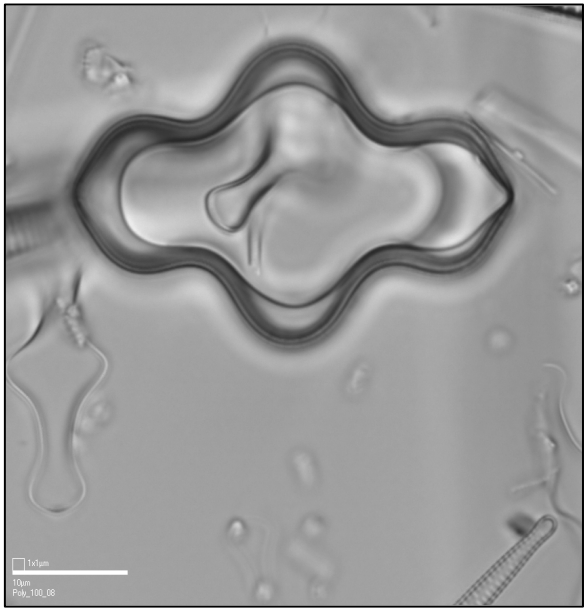


Meridion circulare

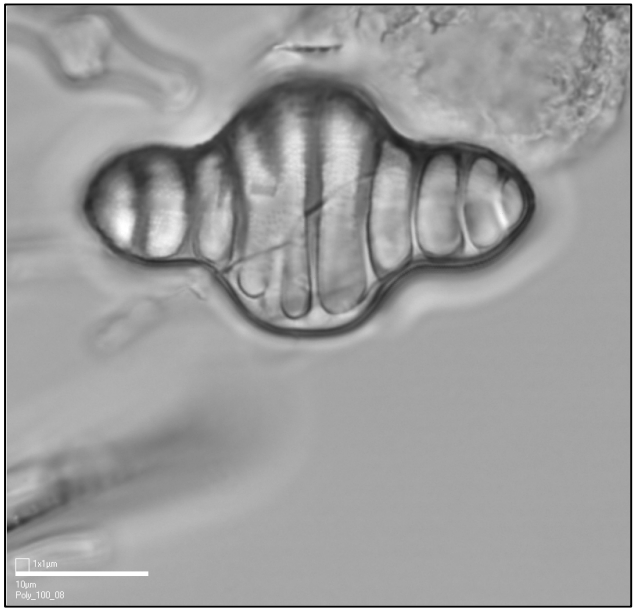
b) TABELLARIALES



Tabellaria flocculosa



Tetracyclus glans



Appendix 2. Correlation Tables

Variable 1	Variable 2	r	P_{value}
% Granite	pH	-0.698	P<0.001***
	log_e Conductivity	-0.840	P<0.001***
	log_e Alkalinity	-0.473	P<0.001***
	%Boulders	+0.298	P<0.001***
	%Small Stones	-0.251	P<0.001***
	Cl	-0.729	P<0.001***
	SO_4	+0.711	P<0.001***
	PO_4-P	+0.272	P<0.001***
	log_e Cd	+0.380	P<0.001***
	log_e Cr	+0.293	P<0.001***
	Cu	+0.255	P<0.001***
	log_e Pb	+0.845	P<0.001***
	log_e Ni	+0.183	P<0.001***
	log_e Zn	+0.700	P<0.001***
	Al	+0.586	P<0.001***
	log_e V	+0.662	P<0.001***
	Na	-0.347	P<0.001***
	log_e Kpot	-0.269	P<0.001***
	log_e Ca	-0.826	P<0.001***
	log_e Mg	-0.907	P<0.001***
	log_e Fe	+0.607	P<0.001***
	log_e Mn	+0.709	P<0.001***
% Mica Schist	pH	+0.218	P<0.01**
	log_e Conductivity	+0.284	P<0.01**
	log_e Alkalinity	+0.149	P<0.01**
	%Boulders	+0.288	P<0.001***
	%Large Stones	-0.185	P<0.001***
	%Small Stones	-0.143	P<0.01**
	Cl	-0.219	P<0.001***
	SO_4	-0.289	P<0.001***
	log_e Pb	-0.300	P<0.001***
	log_e Zn	-0.248	P<0.001***
	Al	-0.291	P<0.001***
	log_e V	-0.218	P<0.001***
	Na	+0.102	P<0.05*
	log_e Kpot	+0.190	P<0.001***
	log_e Ca	+0.212	P<0.001***
	log_e Mg	+0.240	P<0.001***
% Grandiorite	pH	-0.100	P<0.05*
	log_e Conductivity	-0.386	P<0.001***
	%Large Stones	+0.193	P<0.001***
	Cl	-0.511	P<0.001***
	PO_4-P	+0.232	P<0.001***
	log_e Cr	+0.248	P<0.001***
	log_e Pb	+0.117	P<0.05*

	$\log_e$ Zn	+0.123	P<0.05*
	$\log_e$ V	+0.204	P<0.001***
	Na	-0.412	P<0.001***
	$\log_e$ Kpot	+0.173	P<0.001***
	$\log_e$ Ca	-0.233	P<0.001***
	$\log_e$ Mg	-0.334	P<0.001***
	$\log_e$ Fe	+0.319	P<0.001***
	$\log_e$ Mn	+0.376	P<0.001***
% Diorite	pH	+0.128	P<0.05*
	$\log_e$ Conductivity	+0.100	P<0.05*
	% Large Stones	+0.200	P<0.001***
	Cl	-0.170	P<0.01**
	$\log_e$ Cd	-0.249	P<0.001***
	$\log_e$ Cr	-0.238	P<0.001***
	$\log_e$ Pb	-0.226	P<0.001***
	$\log_e$ Zn	-0.143	P<0.01**
	$\log_e$ V	-0.124	P<0.05*
	$\log_e$ Kpot	+0.281	P<0.001***
	$\log_e$ Ca	+0.223	P<0.001***
	$\log_e$ Mg	+0.277	P<0.001***
	$\log_e$ Fe	-0.181	P<0.001***
	$\log_e$ Mn	-0.195	P<0.001***
% Amphibolite	pH	+0.136	P<0.01**
	$\log_e$ Conductivity	+0.128	P<0.05*
	$\log_e$ Alkalinity	+0.111	P<0.05*
	%Boulders	-0.108	P<0.05*
	%Large Stones	+0.314	P<0.001***
	Cl	-0.323	P<0.001***
	$\log_e$ Cd	-0.124	P<0.05*
	$\log_e$ Pb	-0.221	P<0.001***
	$\log_e$ Zn	-0.141	P<0.01**
	Al	-0.121	P<0.05*
	Na	-0.167	P<0.01**
	$\log_e$ Kpot	+0.362	P<0.001***
	$\log_e$ Ca	+0.275	P<0.001***
	$\log_e$ Mg	+0.296	P<0.001***
	$\log_e$ Fe	-0.118	P<0.05*
	$\log_e$ Mn	-0.213	P<0.001***
% Serpentinite	pH	+0.135	P<0.01**
	$\log_e$ Conductivity	+0.129	P<0.05*
	$\log_e$ Alkalinity	+0.115	P<0.05*
	%Boulders	-0.142	P<0.01**
	%Large Stones	+0.349	P<0.001***
	Cl	-0.331	P<0.001***
	$\log_e$ Pb	-0.188	P<0.001***
	$\log_e$ Zn	-0.120	P<0.05*
	Al	-0.124	P<0.05*
	Na	-0.180	P<0.001***
	$\log_e$ Kpot	+0.339	P<0.001***

	log_e Ca	+0.210	P<0.001***
	log_e Mg	+0.260	P<0.001***
	log_e Mn	-0.188	P<0.001***
% QP	pH	+0.135	P<0.01**
	log_e Conductivity	+0.129	P<0.05*
	log_e Alkalinity	+0.115	P<0.05*
	%Boulders	-0.142	P<0.01**
	%Large Stones	+0.349	P<0.001***
	Cl	-0.331	P<0.001***
	log_e Pb	-0.188	P<0.001***
	log_e Zn	-0.120	P<0.05*
	Al	-0.124	P<0.05*
	Na	-0.186	P<0.001***
	log_e Kpot	+0.339	P<0.001***
	log_e Ca	+0.210	P<0.001***
	log_e Mg	+0.255	P<0.001***
	log_e Mn	-0.188	P<0.001***
% QPP	pH	+0.133	P<0.01**
	log_e Conductivity	+0.125	P<0.05*
	log_e Alkalinity	+0.113	P<0.05*
	%Large Stones	+0.287	P<0.001***
	Cl	-0.311	P<0.001***
	log_e Cd	-0.153	P<0.01**
	log_e Pb	-0.233	P<0.001***
	log_e Zn	-0.148	P<0.01**
	Al	-0.116	P<0.05*
	Na	-0.153	P<0.01**
	log_e Kpot	+0.365	P<0.001***
	log_e Ca	+0.206	P<0.001***
	log_e Mg	+0.245	P<0.001***
	log_e Fe	-0.135	P<0.01**
	log_e Mn	-0.220	P<0.001***
% DA	pH	+0.134	P<0.01**
	log_e Conductivity	+0.129	P<.05*
	log_e Alkalinity	+0.115	P<0.05*
	%Boulders	-0.143	P<0.01**
	%Large Stones	+0.349	P<0.001***
	Cl	-0.331	P<0.001***
	log_e Pb	-0.187	P<0.001***
	log_e Zn	-0.120	P<0.05*
	Al	-0.124	P<0.05*
	Na	-0.186	P<0.001***
	log_e Kpot	+0.338	P<0.001***
	log_e Ca	+0.210	P<0.001***
	log_e Mg	+0.255	P<0.001***
	log_e Mn	-0.188	P<0.001***
% Limestone	pH	+0.221	P<0.001*
	log_e Conductivity	+0.131	P<0.01**
	log_e Alkalinity	+0.120	P<0.05*

	%Large Stones	+0.221	P<0.001***
	Cl	-0.271	P<0.001***
	$\log_e$ Cd	-0.201	P<0.001***
	$\log_e$ Cr	-0.123	P<0.05*
	$\log_e$ Pb	-0.243	P<0.001***
	$\log_e$ Zn	-0.155	P<0.01**
	Al	-0.101	P<0.05*
	Na	-0.118	P<0.05*
	$\log_e$ Kpot	+0.352	P<0.001***
	$\log_e$ Ca	+0.435	P<0.001***
	$\log_e$ Mg	+0.447	P<0.001***
	$\log_e$ Fe	-0.161	P<0.01**
	$\log_e$ Mn	-0.222	P<0.001***
% Durness Limestone	pH	+0.489	P<0.001***
	$\log_e$ Conductivity	+0.702	P<0.001***
	$\log_e$ Alkalinity	+0.339	P<0.001***
	%Boulders	-0.244	P<0.001***
	%Small Stones	+0.175	P<0.001***
	%Gravel	+0.147	P<0.01**
	%Sand	+0.295	P<0.001***
	Cl	+0.742	P<0.001***
	SO_4	-0.588	P<0.001***
	$\log_e$ Cd	-0.225	P<0.001***
	$\log_e$ Cr	-0.254	P<0.001***
	Cu	-0.309	P<0.001***
	$\log_e$ Pb	-0.633	P<0.001***
	$\log_e$ Ni	-0.150	P<0.01**
	$\log_e$ Zn	-0.549	P<0.001***
	Al	-0.451	P<0.001***
	$\log_e$ V	-0.544	P<0.001***
	Na	+0.308	P<0.001***
	$\log_e$ Ca	+0.667	P<0.001***
	$\log_e$ Mg	+0.771	P<0.001***
	$\log_e$ Fe	-0.417	P<0.001***
	$\log_e$ Mn	-0.514	P<0.001***
% Eriboll Sandstone	pH	+0.511	P<0.001***
	$\log_e$ Conductivity	+0.751	P<0.001***
	$\log_e$ Alkalinity	+0.352	P<0.001***
	%Boulders	-0.171	P<0.01**
	%Large Stones	-0.184	P<0.001***
	%Small Stones	+0.269	P<0.001***
	%Gravel	+0.273	P<0.001***
	%Sand	+0.143	P<0.01**
	Cl	+0.603	P<0.001***
	SO_4	-0.408	P<0.001***
	$\log_e$ Cd	-0.168	P<0.01**
	$\log_e$ Cr	-0.188	P<0.001***
	Cu	-0.207	P<0.001***
	$\log_e$ Pb	-0.469	P<0.001***

	$\log_e$ Zn	-0.402	P<0.001***
	Al	-0.371	P<0.001***
	$\log_e$ V	-0.410	P<0.001***
	Na	+0.324	P<0.001***
	$\log_e$ Kpot	+0.118	P<0.05*
	$\log_e$ Ca	+0.689	P<0.001***
	$\log_e$ Mg	+0.744	P<0.001***
	$\log_e$ Fe	-0.372	P<0.001***
	$\log_e$ Mn	-0.326	P<0.001***
% Moine Schist	pH	+0.168	P<0.01**
	$\log_e$ Conductivity	+0.310	P<0.001***
	$\log_e$ Alkalinity	+0.166	P<0.01**
	%Boulders	-0.255	P<0.001***
	%Small Stones	-0.184	P<0.001***
	%Gravel	+0.209	P<0.001***
	%Sand	+0.360	P<0.001***
	Cl	+0.365	P<0.001***
	SO_4	-0.323	P<0.001***
	$\log_e$ Cd	-0.119	P<0.05*
	$\log_e$ Pb	-0.188	P<0.001***
	$\log_e$ V	-0.131	P<0.01**
	Na	+0.110	P<0.05*
	$\log_e$ Kpot	+0.100	P<0.05*
	$\log_e$ Ca	+0.303	P<0.001***
	$\log_e$ Mg	+0.381	P<0.001***
	$\log_e$ Fe	-0.183	P<0.001***
	$\log_e$ Mn	-0.426	P<0.001***
% Applecross Formation	pH	+0.458	P<0.001***
	$\log_e$ Conductivity	+0.641	P<0.001***
	$\log_e$ Alkalinity	+0.290	P<0.01**
	%Large Stones	-0.165	P<0.01**
	%Small Stones	+0.193	P<0.001***
	%Gravel	+0.185	P<0.001***
	Cl	+0.457	P<0.001***
	SO_4	-0.271	P<0.001***
	$\log_e$ Cd	-0.119	P<0.05*
	$\log_e$ Cr	-0.154	P<0.01**
	Cu	-0.256	P<0.001***
	$\log_e$ Pb	-0.403	P<0.001***
	$\log_e$ Zn	-0.410	P<0.001***
	Al	-0.344	P<0.001***
	$\log_e$ V	-0.369	P<0.001***
	Na	+0.288	P<0.001***
	$\log_e$ Ca	+0.579	P<0.001***
	$\log_e$ Mg	+0.598	P<0.001***
	$\log_e$ Fe	-0.303	P<0.001***
	$\log_e$ Mn	-0.133	P<0.01**
% An-t'Sron	pH	+0.458	P<0.001***
	$\log_e$ Conductivity	+0.641	P<0.001***

	$\log_e$ Alkalinity	+0.290	P<0.01**
	%Large Stones	-0.165	P<0.01**
	%Small Stones	+0.193	P<0.001***
	%Gravel	+0.185	P<0.001***
	Cl	+0.457	P<0.001***
	SO_4	-0.271	P<0.001***
	$\log_e$ Cd	-0.119	P<0.05*
	$\log_e$ Cr	-0.154	P<0.01**
	Cu	-0.256	P<0.001***
	$\log_e$ Pb	-0.403	P<0.001***
	$\log_e$ Zn	-0.410	P<0.001***
	Al	-0.344	P<0.001***
	$\log_e$ V	-0.369	P<0.001***
	Na	+0.288	P<0.001***
	$\log_e$ Ca	+0.579	P<0.001***
	$\log_e$ Mg	+0.598	P<0.001***
	$\log_e$ Fe	-0.303	P<0.001***
	$\log_e$ Mn	-0.133	P<0.01**
% Boulders	% Large Stones	-0.430	P<0.001***
	% Small Stones	-0.525	P<0.001***
	% Gravel	-0.397	P<0.001***
	% Sand	-0.158	P<0.01**
	$\log_e Z_{eu}$:$D^{1\%}$	-0.169	P<0.01**
	$\log_e Z_{eu}$:$D^{3\%}$	-0.156	P<0.01**
	pH	-0.190	P<0.001***
	$\log_e$ Conductivity	-0.177	P<0.001***
	√ Flow	+0.146	P<0.01**
	Substrate diversity (Simpson's D)	-0.338	P<0.001***
	Substrate dominance (Berger-Parker)	+0.364	P<0.001***
	Hydromorphological diversity (Simpson's D)	-0.332	P<0.001***
% Large Stones	% Small Stones	-0.114	P<0.05*
	% Gravel	-0.244	P<0.001***
	% Sand	-0.136	P<0.01**
	Substrate diversity (Simpson's D)	-0.205	P<0.001***
	Substrate dominance (Berger-Parker)	+0.213	P<0.001***
	Hydromorphological diversity (Simpson's D)	-0.205	P<0.001***
% Small Stones	% Gravel	+0.264	P<0.001***
	$\log_e Z_{eu}$:$D^{1\%}$	+0.168	P<0.01**
	$\log_e Z_{eu}$:$D^{3\%}$	+0.163	P<0.01**
	pH	+0.228	P<0.001***
	$\log_e$ Conductivity	+0.224	P<0.001***
	√ Flow	-0.107	P<0.01**
	Substrate diversity (Simpson's D)	+0.369	P<0.001***
	Substrate dominance (Berger-Parker)	-0.380	P<0.001***
	Hydromorphological diversity (Simpson's D)	+0.358	P<0.001***
% Gravel	% Sand	+0.149	P<0.01**
	pH	+0.230	P<0.001***
	$\log_e$ Conductivity	+0.240	P<0.001***
	√ Flow	-0.244	P<0.001***

	Substrate diversity (Simpson's D)	+0.340	P<0.001***
	Substrate dominance (Berger-Parker)	-0.328	P<0.001***
	Hydromorphological diversity (Simpson's D)	+0.320	P<0.001***
% Sand	pH	+0.165	P<0.01**
	$\log_e$ Conductivity	+0.222	P<0.001***
	√ Flow	-0.170	P<0.01**
	Substrate diversity (Simpson's D)	+0.114	P<0.05*
	Substrate dominance (Berger-Parker)	-0.118	P<0.05*
	Hydromorphological diversity (Simpson's D)	+0.111	P<0.05*
$\log_e$ D (m)	$\log_e Z_{eu}:D^{1\%}$	-0.643	P<0.001***
	$\log_e Z_{eu}:D^{3\%}$	-0.601	P<0.001***
	pH	-0.108	P<0.05*
	$\log_e$ Conductivity	-0.166	P<0.01**
	$\log_e$ Alkalinity	-0.305	P<0.001***
	√ Water Temperature	-0.468	P<0.001***
	PO_4-P	+0.319	P<0.001***
$\log_e$ K (m^{-1})	$\log_e Z_{eu}^{1\%}$	-0.763	P<0.001***
	$\log_e Z_{eu}^{3\%}$	-0.725	P<0.001***
	$\log_e Z_{eu}:D^{1\%}$	-0.312	P<0.001***
	$\log_e Z_{eu}:D^{3\%}$	-0.309	P<0.001***
	pH	-0.244	P<0.001***
	$\log_e$ Conductivity	-0.123	P<0.05*
	$\log_e$ Alkalinity	-0.139	P<0.01**
	√ Water Temperature	+0.173	P<0.001***
	Cl	-0.218	P<0.001***
	SO_4	+0.288	P<0.001***
	$\log_e$ Cd	+0.279	P<0.001***
	$\log_e$ Cr	+0.263	P<0.001***
	Cu	+0.190	P<0.001***
	$\log_e$ Pb	+0.291	P<0.001***
	$\log_e$ Ni	+0.455	P<0.001***
	$\log_e$ Zn	+0.233	P<0.001***
	Al	+0.367	P<0.001***
	$\log_e$ V	+0.359	P<0.001***
	$\log_e$ As	+0.274	P<0.001***
	Na	-0.205	P<0.001***
	$\log_e$ Fe	+0.439	P<0.001***
	$\log_e$ Mn	+0.307	P<0.001***
$\log_e Z_{eu}^{1\%}$ (m)	$\log_e Z_{eu}:D^{1\%}$	+0.559	P<0.001***
	pH	+0.264	P<0.001***
	$\log_e$ Conductivity	+0.200	P<0.001***
	$\log_e$ Alkalinity	+0.167	P<0.05*
	√ Water Temperature	-0.125	P<0.05*
	% Shade	-0.512	P<0.001***
	Cl	-0.323	P<0.001***
	SO_4	-0.412	P<0.001***
	$\log_e$ Cd	-0.124	P<0.05*
	Cu	-0.264	P<0.001***
	$\log_e$ Pb	-0.217	P<0.001***

	$\log_e$ Ni	-0.343	P<0.001***
	$\log_e$ Zn	-0.206	P<0.001***
	Al	-0.374	P<0.001***
	$\log_e$ V	-0.294	P<0.001***
	$\log_e$ Ca	+0.166	P<0.01**
	$\log_e$ Mg	+0.182	P<0.01**
	$\log_e$ Fe	-0.308	P<0.001***
	$\log_e$ Mn	-0.190	P<0.01**
	Height of Riparian Vegetation	-0.450	P<0.001***
$\log_e Z_{eu}^{3\%}$ (m)	$\log_e Z_{eu}$:$D^{3\%}$	+0.541	P<0.001***
	pH	+0.163	P<0.01**
	$\log_e$ Conductivity	+0.144	P<0.01**
	√ Water Temperature	-0.248	P<0.001***
	% Shade	-0.610	P<0.001***
	Cl	-0.268	P<0.001***
	SO_4	-0.426	P<0.001***
	$\log_e$ Cd	-0.123	P<0.05*
	Cu	-0.328	P<0.001***
	$\log_e$ Pb	-0.211	P<0.001***
	$\log_e$ Ni	-0.292	P<0.001***
	$\log_e$ Zn	-0.176	P<0.001***
	Al	-0.317	P<0.001***
	$\log_e$ V	-0.347	P<0.001***
	$\log_e$ Kpot	-0.125	P<0.05*
	$\log_e$ Mg	+0.140	P<0.01**
	$\log_e$ Fe	-0.334	P<0.001***
	$\log_e$ Mn	-0.152	P<0.01**
	%Shade	-0.470	P<0.001***
	Height of Riparian Vegetation	-0.470	P<0.001***
$\log_e Z_{eu}$:$D^{1\%}$	pH	+0.304	P<0.001***
	$\log_e$ Conductivity	+0.303	P<0.001***
	$\log_e$ Alkalinity	+0.397	P<0.001***
	√ Water Temperature	+0.304	P<0.001***
	% Shade	-0.320	P<0.001***
	Cl	+0.213	P<0.001***
	SO_4	-0.268	P<0.001***
	$\log_e$ Pb	-0.139	P<0.05*
	$\log_e$ Ni	-0.130	P<0.05*
	$\log_e$ Zn	-0.121	P<0.05*
	Al	-0.308	P<0.001***
	Na	+0.194	P<0.01**
	$\log_e$ Kpot	+0.263	P<0.001***
	$\log_e$ Ca	+0.334	P<0.001***
	$\log_e$ Mg	+0.289	P<0.001***
	Height of Riparian Vegetation	-0.419	P<0.001***
$\log_e Z_{eu}$:$D^{3\%}$	pH	+0.236	P<0.001***
	$\log_e$ Conductivity	+0.272	P<0.001***
	$\log_e$ Alkalinity	+0.280	P<0.001***
	√ Water Temperature	+0.209	P<0.001***

	% Shade	-0.427	P<0.001***
	Cl	+0.182	P<0.001***
	SO_4	-0.300	P<0.001***
	Cu	-0.146	P<0.01**
	$\log_e$ Pb	-0.145	P<0.01**
	$\log_e$ Ni	-0.100	P<0.05*
	$\log_e$ Zn	-0.104	P<0.05*
	Al	-0.280	P<0.001***
	Na	+0.112	P<0.05*
	$\log_e$ Kpot	+0.214	P<0.001***
	$\log_e$ Ca	+0.284	P<0.001***
	$\log_e$ Mg	+0.269	P<0.001***
	Height of Riparian Vegetation	-0.464	P<0.001***
pH	$\log_e$ Conductivity	+0.813	P<0.001***
	$\log_e$ Alkalinity	+0.831	P<0.001***
	√ Water Temperature	+0.244	P<0.001***
	% Boulders	-0.190	P<0.001***
	% Small Stones	+0.228	P<0.001***
	% Gravel	+0.230	P<0.001***
	% Sand	+0.125	P<0.05*
	Cl	+0.661	P<0.001***
	SO_4	-0.641	P<0.001***
	$\log_e$ Cd	-0.404	P<0.001***
	$\log_e$ Cr	-0.345	P<0.001***
	Cu	-0.187	P<0.001***
	$\log_e$ Pb	-0.755	P<0.001***
	$\log_e$ Ni	-0.343	P<0.01**
	$\log_e$ Zn	-0.687	P<0.001***
	Al	-0.765	P<0.001***
	$\log_e$ V	-0.469	P<0.001***
	Na	+0.502	P<0.001***
	$\log_e$ Kpot	+0.530	P<0.001***
	$\log_e$ Ca	+0.851	P<0.001***
	$\log_e$ Mg	+0.785	P<0.001***
	$\log_e$ Fe	-0.409	P<0.001***
	$\log_e$ Mn	-0.517	P<0.001***
$\log_e$ Conductivity ($\mu S\ cm^{-1}$)	$\log_e$ Alkalinity	+0.732	P<0.001***
	√ Water Temperature	+0.217	P<0.001***
	% Boulders	-0.177	P<0.001***
	% Small Stones	+0.224	P<0.001***
	% Gravel	+0.240	P<0.001***
	% Sand	+0.222	P<0.001***
	Cl	+0.798	P<0.001***
	SO_4	-0.563	P<0.001***
	PO_4-P	-0.303	P<0.001***
	$\log_e$ Cd	-0.314	P<0.001***
	$\log_e$ Cr	-0.382	P<0.001***
	Cu	-0.219	P<0.001***
	$\log_e$ Pb	-0.730	P<0.001***

	$\log_e$ Ni	-0.167	P<0.01**
	$\log_e$ Zn	-0.606	P<0.001***
	Al	-0.619	P<0.001***
	$\log_e$ V	-0.481	P<0.001***
	Na	+0.601	P<0.001***
	$\log_e$ Kpot	+0.504	P<0.001***
	$\log_e$ Ca	+0.964	P<0.001***
	$\log_e$ Mg	+0.974	P<0.001***
	$\log_e$ Fe	-0.405	P<0.001***
	$\log_e$ Mn	-0.525	P<0.001***
$\log_e$ Alkalinity (mg l^{-1})	√ Water Temperature	+0.622	P<0.001***
	√ Flow	-0.133	P<0.01**
	% Boulders	-0.140	P<0.01**
	% Small Stones	+0.171	P<0.01**
	% Gravel	+0.192	P<0.001***
	% Sand	+0.121	P<0.05*
	Cl	+0.516	P<0.001***
	SO_4	-0.417	P<0.001***
	PO_4-P	-0.390	P<0.001***
	$\log_e$ Cd	-0.341	P<0.001***
	$\log_e$ Cr	-0.240	P<0.001***
	Cu	-0.101	P<0.05*
	$\log_e$ Pb	-0.563	P<0.001***
	$\log_e$ Ni	-0.300	P<0.001***
	$\log_e$ Zn	-0.543	P<0.001***
	Al	-0.639	P<0.001***
	$\log_e$ V	-0.159	P<0.01**
	Na	+0.656	P<0.001***
	$\log_e$ Kpot	+0.607	P<0.001***
	$\log_e$ Ca	+0.825	P<0.001***
	$\log_e$ Mg	+0.686	P<0.001***
	$\log_e$ Fe	-0.113	P<0.05*
	$\log_e$ Mn	-0.403	P<0.001***
√ Water Temperature (^oC)	√ Flow	-0.317	P<0.001***
	%Shade	+0.181	P<0.001***
	PO_4-P	-0.602	P<0.001***
	$\log_e$ Kpot	+0.495	P<0.001***
	$\log_e$ Ca	+0.327	P<0.001**
	$\log_e$ Mg	+0.167	P<0.01**
√ Flow (m s^{-1})	% Boulders	+0.146	P<0.01**
	% Small stones	-0.107	P<0.05*
	% Gravel	-0.244	P<0.001***
	% Sand	-0.170	P<0.001***
% Shade	Height of Riparian Vegetation	+0.833	P<0.001***
Cl (mg l^{-1})	SO_4	-0.522	P<0.001***
	$\log_e$ Cd	-0.304	P<0.001***
	$\log_e$ Cr	-0.516	P<0.001***
	Cu	-0.395	P<0.001***
	$\log_e$ Pb	-0.621	P<0.001***

	$\log_e$ Ni	-0.396	P<0.001***
	$\log_e$ Zn	-0.535	P<0.01**
	Al	-0.605	P<0.001***
	$\log_e$ V	-0.603	P<0.001***
	Na	+0.701	P<0.001***
	$\log_e$ Kpot	+0.163	P<0.01**
	$\log_e$ Ca	+0.698	P<0.001***
	$\log_e$ Mg	+0.754	P<0.001***
	$\log_e$ Fe	-0.576	P<0.001***
	$\log_e$ Mn	-0.561	P<0.001***
SO_4 (mg l^{-1})	$\log_e$ Cd	+0.448	P<0.001***
	$\log_e$ Cr	+0.320	P<0.001***
	Cu	+0.388	P<0.001***
	$\log_e$ Pb	+0.724	P<0.01**
	$\log_e$ Ni	+0.356	P<0.001***
	$\log_e$ Zn	+0.568	P<0.001***
	Al	+0.728	P<0.001***
	$\log_e$ V	+0.620	P<0.001***
	$\log_e$ As	+0.273	P<0.001***
	Na	-0.102	P<0.05*
	$\log_e$ Kpot	-0.363	P<0.001***
	$\log_e$ Ca	-0.592	P<0.001***
	$\log_e$ Mg	-0.625	P<0.001***
	$\log_e$ Fe	+0.534	P<0.001***
	$\log_e$ Mn	+0.482	P<0.001***
$\log_e$ Cd (μg l^{-1})	$\log_e$ Cr	+0.651	P<0.001***
	Cu	+0.318	P<0.001***
	$\log_e$ Pb	+0.481	P<0.001***
	$\log_e$ Ni	+0.335	P<0.001***
	$\log_e$ Zn	+0.434	P<0.001***
	Al	+0.398	P<0.001***
	$\log_e$ V	+0.618	P<0.001***
	$\log_e$ As	+0.485	P<0.001***
	Na	-0.172	P<0.01**
	$\log_e$ Kpot	-0.284	P<0.001***
	$\log_e$ Ca	-0.367	P<0.001***
	$\log_e$ Mg	-0.338	P<0.001***
	$\log_e$ Fe	+0.379	P<0.001***
	$\log_e$ Mn	+0.459	P<0.001***
$\log_e$ Cr (μg l^{-1})	Cu	+0.388	P<0.001***
	$\log_e$ Pb	+0.373	P<0.001***
	$\log_e$ Ni	+0.397	P<0.001***
	$\log_e$ Zn	+0.280	P<0.001***
	Al	+0.455	P<0.001***
	$\log_e$ V	+0.614	P<0.001***
	$\log_e$ As	+0.386	P<0.001***
	Na	-0.317	P<0.001***
	$\log_e$ Kpot	-0.317	P<0.001***
	$\log_e$ Ca	-0.309	P<0.001***

	log_e Mg	-0.317	P<0.001***
	log_e Fe	+0.429	P<0.001***
	log_e Mn	+0.364	P<0.001***
Cu (µg l^{-1})	log_e Pb	+0.469	P<0.001***
	log_e Ni	+0.623	P<0.001***
	log_e Zn	+0.524	P<0.001***
	Al	+0.586	P<0.001***
	log_e V	+0.686	P<0.001***
	log_e As	+0.113	P<0.05*
	Na	-0.211	P<0.001***
	log_e Kpot	+0.177	P<0.001***
	log_e Ca	-0.148	P<0.01**
	log_e Mg	-0.168	P<0.01**
	log_e Fe	+0.368	P<0.001***
	log_e Mn	+0.147	P<0.01**
log_e Pb (µg l^{-1})	log_e Ni	+0.528	P<0.001***
	log_e Zn	+0.908	P<0.001***
	Al	+0.811	P<0.001***
	log_e V	+0.783	P<0.001***
	Na	-0.426	P<0.001***
	log_e Kpot	-0.345	P<0.001***
	log_e Ca	-0.749	P<0.001***
	log_e Mg	-0.758	P<0.001***
	log_e Fe	+0.724	P<0.001***
	log_e Mn	+0.746	P<0.001***
log_e Ni (µg l^{-1})	log_e Zn	+0.615	P<0.001***
	Al	+0.683	P<0.001***
	log_e V	+0.621	P<0.001***
	log_e As	+0.132	P<0.05*
	Na	-0.433	P<0.001***
	log_e Ca	-0.142	P<0.01**
	log_e Fe	+0.632	P<0.001***
	log_e Mn	+0.448	P<0.001***
log_e Zn (µg l^{-1})	Al	+0.789	P<0.001***
	log_e V	+0.730	P<0.001***
	Na	-0.426	P<0.001***
	log_e Ca	-0.635	P<0.001***
	log_e Mg	-0.615	P<0.001***
	log_e Fe	+0.623	P<0.001***
	log_e Mn	+0.686	P<0.001***
Al (µg l^{-1})	log_e V	+0.678	P<0.001***
	Na	-0.443	P<0.001***
	log_e Kpot	-0.340	P<0.001***
	log_e Ca	-0.624	P<0.001***
	log_e Mg	-0.576	P<0.001***
	log_e Fe	+0.592	P<0.001***
	log_e Mn	+0.542	P<0.001***
log_e V (µg l^{-1})	log_e As	+0.360	P<0.001***
	Na	-0.218	P<0.001***

	$\log_e$ Ca	-0.412	P<0.001***
	$\log_e$ Mg	-0.486	P<0.001***
	$\log_e$ Fe	+0.799	P<0.001***
	$\log_e$ Mn	+0.616	P<0.001***
Na (mg l^{-1})	$\log_e$ K	+0.212	P<0.001***
	$\log_e$ Ca	+0.542	P<0.001***
	$\log_e$ Mg	+0.489	P<0.001***
	$\log_e$ Fe	-0.384	P<0.001***
	$\log_e$ Mn	-0.514	P<0.001***
$\log_e$ Kpot (mg l^{-1})	$\log_e$ Ca	+0.578	P<0.001***
	$\log_e$ Mg	+0.496	P<0.001***
	$\log_e$ Mn	-0.126	P<0.05*
$\log_e$ Ca (mg l^{-1})	PO_4-P	-0.364	P<0.001***
	$\log_e$ Mg	+0.972	P<0.001***
	$\log_e$ Fe	-0.324	P<0.001***
	$\log_e$ Mn	-0.520	P<0.001***
$\log_e$ Mg (mg l^{-1})	PO_4-P	-0.323	P<0.001***
	$\log_e$ Fe	-0.405	P<0.001***
	$\log_e$ Mn	-0.558	P<0.001***
$\log_e$ Fe (mg l^{-1})	$\log_e$ Mn	+0.800	P<0.001***
Substrate diversity (Simpson's D)	Substrate dominance (Berger-Parker)	-0.918	P<0.001***
	Hydromorphological diversity (Simpson's D)	+0.998	P<0.001***
	pH	+0.239	P<0.001***
	$\log_e$ Conductivity	+0.298	P<0.001***
	$\log_e$ Alkalinity	+0.277	P<0.001***
	√ Flow	-0.170	P<0.01**
	$\log_e$ Ca	+0.321	P<0.001***
	$\log_e$ Mg	+0.302	P<0.001***
	% Granite	-0.243	P<0.001***
	% Eriboll Sandstone	+0.302	P<0.001***
Substrate dominance (Berger-Parker)	Hydromorphological diversity (Simpson's D)	-0.921	P<0.001***
	√ Flow	+0.156	P<0.01**
	% Eriboll Sandstone	-0.251	P<0.001***
Hydromorphological diversity (Simpson's D)	pH	+0.228	P<0.001***
	$\log_e$ Conductivity	+0.287	P<0.001***
	$\log_e$ Alkalinity	+0.274	P<0.001***
	√ Flow	-0.137	P<0.05*
	$\log_e$ Ca	+0.313	P<0.001***
	$\log_e$ Mg	+0.291	P<0.001***
	% Granite	-0.233	P<0.001***
	% Eriboll Sandstone	+0.290	P<0.001***

Appendix 2a. Significant (<0.05) Pearson product-moment correlation coefficients (r) and probability (P) values between normally distributed variables measured from all three sub-catchments streams (Water of Dye, River Girnock and Knockan Burn; n = 405). Note[1]: The term 'Kpot' is used here to distinguish the potassium ion (K^+) from the light attenuation coefficient, K.

Variable 1	Variable 2	r	P_{value}
log_e periphyton biomass (mg cm^{-2}) harvested from **short-term linoleum** substrates	log_e periphyton chlorophyll content (μg cm^{-2}) harvested from **short-term linoleum** substrates	+0.859	P<0.001***
	pH	+0.258	P<0.05*
	log_e Conductivity	+0.261	P<0.05*
	log_e Z_{eu}:D^1	+0.244	P<0.05*
	√ Water Temperature	+0.306	P<0.01**
	√ Flow	-0.218	P<0.05*
log_e periphyton chlorophyll content (μg cm^{-2}) harvested from **short-term linoleum** substrates	pH	+0.213	P<0.05*
	log_e Conductivity	+0.241	P<0.05*
	log_e Z_{eu}:D^1	+0.232	P<0.05*
	√ Water Temperature	+0.275	P<0.05*

Appendix 2b. Significant (<0.05) Pearson product-moment correlation coefficients (r) and probability (P) values between normally distributed variables: mean periphyton biomass per unit area, mean periphyton chlorophyll content per unit area, and mean environmental habitat conditions of short-term linoleum substrates for amalgamated sub-catchment data (Water of Dye, River Girnock and Knockan Burn; n = 50). Note[1]: relationships between environmental variables not shown (refer to Appendix 2a, for correlation of environmental variables).

Variable 1	Variable 2	r	P_{value}
log_e periphyton biomass (mg cm^{-2}) harvested from **all artificial** substrates sampled during surveys	log_e periphyton chlorophyll content (μg cm^{-2}) harvested from **all artificial** substrates sampled during surveys	+0.721	P<0.001***
	pH	+0.327	P<0.01**
	log_e Conductivity	+0.409	P<0.001***
	log_e Alkalinity	+0.317	P<0.01**
	log_e Z_{eu}:D^1	+0.227	P<0.05*
	√ Water Temperature	+0.250	P<0.05*
	√ Flow	-0.221	P<0.05*
	Cl	+0.393	P<0.01**
	SO_4	-0.342	P<0.01**
	log_e Cd	-0.298	P<0.05*
	log_e Pb	-0.392	P<0.01**
	log_e Zn	-0.433	P<0.001***
	Al	-0.509	P<0.001***
	log_e V	-0.434	P<0.001***
	Na	+0.245	P<0.05*
	log_e Ca	+0.432	P<0.001***
	log_e Mg	+0.404	P<0.001***
log_e periphyton chlorophyll content (μg cm^{-2}) harvested from **all artificial** substrates sampled during surveys	pH	+0.218	P<0.05*
	log_e Conductivity	+0.352	P<0.01**
	log_e Alkalinity	+0.320	P<0.01**
	log_e Z_{eu}:D^1	+0.215	P<0.05*
	√ Water Temperature	+0.223	P<0.05*
	SO_4	-0.306	P<0.01**
	log_e Cd	-0.345	P<0.01**
	log_e Pb	-0.296	P<0.05*
	log_e Zn	-0.284	P<0.05*
	Al	-0.405	P<0.001***
	log_e V	-0.361	P<0.01**
	log_e Ca	+0.388	P<0.01**
	log_e Mg	+0.357	P<0.01**

Appendix 2c. Significant (<0.05) Pearson product-moment correlation coefficients (r) and probability (P) values between normally distributed variables: mean periphyton biomass per unit area, mean periphyton chlorophyll content per unit area, and mean environmental habitat conditions of all artificial substrates sampled during survey dates: short-term linoleum, long-term linoleum, long-term Astroturf, and plastic aquarium plants for amalgamated sub-catchment data (Water of Dye, River Girnock and Knockan Burn; n = 93). Note1: relationships between environmental variables not shown (refer to Appendix 2a, for correlation of environmental variables).

Variable 1	Variable 2	r	P_{value}
log_e periphyton biomass (mg cm^{-2}) harvested from **all naturally-occurring** substrata during surveys	log_e periphyton chlorophyll content (μg cm^{-2}) harvested from **all naturally-occurring** substrata during surveys	+0.628	P<0.001***
	% Periphyton cover	+0.495	P<0.001***
	% Bare area	-0.298	P<0.05*
	log_e Z_{eu}:$D^{1\%}$	+0.303	P<0.01**
	pH	+0.597	P<0.001***
	log_e Conductivity	+0.447	P<0.001***
	log_e Alkalinity	+0.481	P<0.001***
	√ Water temperature	+0.228	P<0.05*
	√ Flow	-0.221	P<0.05*
	% Shade	-0.234	P<0.05*
	Height of riparian vegetation	-0.200	P<0.05*
	Cl	+0.515	P<0.001***
	SO_4	-0.442	P<0.001***
	log_e Cd	-0.428	P<0.001***
	log_e Pb	-0.402	P<0.001***
	log_e Zn	-0.408	P<0.001***
	Al	-0.468	P<0.001***
	log_e V	-0.454	P<0.001***
	Na	+0.216	P<0.05*
	log_e Ca	+0.462	P<0.001***
	log_e Mg	+0.426	P<0.001***
	% Gravel	+0.367	P<0.01**
	% Granite	-0.366	P<0.01**
	% Durness Limestone	+0.372	P<0.01**
	% Eriboll Sandstone	+0.267	P<0.05*
	% Applecross Formation	+0.249	P<0.05*
	% An-t'Sron	+0.249	P<0.05*
	Substrate diversity (Simpson's D)	+0.288	P<0.05*
	Substrate dominance (Berger-Parker)	-0.209	P<0.05*
	Hydromorphological diversity (Simpson's D)	+0.273	P<0.05*
log_e periphyton chlorophyll content (μg cm^{-2}) harvested from **all naturally-occurring** substrata	% Periphyton cover	+0.345	P<0.01**
	% Bare area	-0.198	P<0.05*
	log_e Z_{eu}:$D^{1\%}$	+0.227	P<0.05*
	pH	+0.324	P<0.01**
	log_e Conductivity	+0.247	P<0.05*
	log_e Alkalinity	+0.267	P<0.05*
	% Shade	-0.236	P<0.05*
	Height of riparian vegetation	-0.217	P<0.05*
	SO_4	-0.360	P<0.01**
	log_e Cd	-0.206	P<0.05*
	log_e Pb	-0.278	P<0.05*
	log_e Zn	-0.262	P<0.05*
	Al	-0.259	P<0.05*
	log_e V	-0.215	P<0.05*
	log_e Ca	+0.313	P<0.01**
	log_e Mg	+0.311	P<0.01**
	% Granite	-0.317	P<0.01**

	% Durness Limestone	+0.350	P<0.01**
% Periphyton cover on **all naturally-occurring** substrata	% Bare area	-0.552	P<0.001***
	$\log_e Z_{eu}:D^{1\%}$	+0.208	P<0.05*
	√ Water temperature	+0.288	P<0.001***
	√ Flow	-0.252	P<0.05*
	% Large Stones	+0.306	P<0.01**
	% Gravel	+0.286	P<0.05*
	% Granodiorite	+0.676	P<0.001***
	% Amphibolite	+0.430	P<0.001***
	% Serpentinite	+0.474	P<0.001***
	% QP	+0.474	P<0.001***
	% DA	+0.474	P<0.001***
	% QPP	+0.396	P<0.01**
	% Limestone	+0.307	P<0.01**
	% Durness Limestone	-0.348	P<0.01**
	% Eriboll Sandstone	-0.280	P<0.05*
% Bare area on **all naturally-occurring** substrata	√ Flow	+0.233	P<0.05*
	PO_4-P	-0.225	P<0.05*
	Cl	+0.346	P<0.01**
	SO_4	-0.213	P<0.05*
	$\log_e$ Pb	-0.227	P<0.05*
	$\log_e$ Zn	-0.218	P<0.05*
	Al	-0.291	P<0.05*
	% Boulders	-0.271	P<0.05*
	% Large Stones	-0.296	P<0.05*
	% Small stones	+0.313	P<0.01**
	% Gravel	+0.364	P<0.01**
	% Granite	-0.248	P<0.05*
	% Granodiorite	-0.479	P<0.001***
	% Mica Schist	+0.266	P<0.05*
	% Amphibolite	-0.334	P<0.01**
	% Serpentinite	-0.346	P<0.01**
	% QP	-0.346	P<0.01**
	% DA	-0.346	P<0.01**
	% QPP	-0.319	P<0.01**
	% Limestone	-0.272	P<0.05*
	% Durness Limestone	+0.348	P<0.01**

Appendix 2d. Significant (<0.05) Pearson product-moment correlation coefficients (r) and probability (P) values between normally distributed variables: mean periphyton biomass per unit area, mean periphyton chlorophyll content per unit area, mean periphyton abundance, mean bare area, and mean environmental habitat conditions of all naturally-occurring substrata: mineral particles, aquatic bryophytes and vascular submerged macrophytes for amalgamated sub-catchment data (Water of Dye, River Girnock and Knockan Burn; n = 163). Note[1]: relationships between environmental variables not shown (refer to Appendix 2a, for correlation of environmental variables).

Variable 1	Variable 2	r	P_{value}
log_e **aquatic bryophyte** biomass (mg cm^{-2})	log_e **aquatic bryophyte** chlorophyll content (µg cm^{-2})	+0.795	P<0.001***
	% **aquatic bryophyte** cover	+0.715	P<0.001***
	% Bare area	-0.353	P<0.01**
	√ Water Temperature	-0.318	P<0.01**
	√ Flow	+0.322	P<0.01**
	% Shade	-0.256	P<0.05*
	Height of riparian vegetation	-0.235	P<0.05*
	Cl	+0.472	P<0.001***
	log_e Ca	+0.323	P<0.01**
	log_e Mg	+0.317	P<0.01**
	% Boulders	+0.363	P<0.01**
	% Small Stones	-0.258	P<0.05*
	% Gravel	-0.267	P<0.05*
	% Granite	+0.383	P<0.01**
	% Granodiorite	+0.247	P<0.05*
	% Amphibolite	-0.520	P<0.001***
	% Serpentinite	-0.532	P<0.001***
	% QP	-0.532	P<0.001***
	% QPP	-0.532	P<0.001***
	%DA	-0.501	P<0.001***
	% Limestone	-0.431	P<0.001***
	% Durness Limestone	+0.593	P<0.001***
	% Moine Schist	+0.466	P<0.001***
	% Eriboll Sandstone	+0.367	P<0.01**
log_e **aquatic bryophyte** chlorophyll content (µg cm^{-2})	% **Aquatic bryophyte** cover	+0.635	P<0.001***
	% Bare area	-0.277	P<0.05*
	√ Water Temperature	-0.358	P<0.01**
	√ Flow	+0.233	P<0.05*
	% Shade	-0.240	P<0.05*
	Height of riparian vegetation	-0.222	P<0.05*
	% Boulders	+0.316	P<0.01**
	% Small Stones	-0.238	P<0.05*
	% Gravel	-0.244	P<0.05*
	% Granite	+0.347	P<0.01**
	% Granodiorite	+0.213	P<0.05*
	% Amphibolite	-0.534	P<0.001***
	% Serpentinite	-0.527	P<0.001***
	% QP	-0.527	P<0.001***
	% QPP	-0.527	P<0.001***
	%DA	-0.524	P<0.001***
	% Limestone	+0.473	P<0.001***
	% Durness Limestone	+0.325	P<0.01**
	% Eriboll Sandstone	+0.271	P<0.05*
% **Aquatic bryophyte** cover	% Bare area	-0.458	P<0.001***
	√ Water Temperature	-0.372	P<0.01**
	√ Flow	+0.386	P<0.01**
	% Shade	-0.289	P<0.05*
	Height of riparian vegetation	-0.260	P<0.05*

	% Boulders	+0.334	P<0.01**
	% Small Stones	-0.317	P<0.01**
	% Gravel	-0.370	P<0.01**
	% Granite	+0.459	P<0.001***
	% Grandiorite	+0.255	P<0.05*
	% Amphibolite	-0.550	P<0.001***
	% Serpentinite	-0.530	P<0.001***
	% QP	-0.530	P<0.001***
	% QPP	-0.530	P<0.001***
	%DA	-0.546	P<0.001***
	% Limestone	-0.511	P<0.001***

Appendix 2e. Significant (<0.05) Pearson product-moment correlation coefficients (r) and probability (P) values between normally distributed variables: mean aquatic bryophyte biomass per unit area, mean aquatic bryophyte chlorophyll content per unit area, mean aquatic bryophyte abundance, mean bare area, and mean environmental habitat conditions for amalgamated sub-catchment data (Water of Dye, River Girnock and Knockan Burn; n = 79). Note1: relationships between environmental variables not shown (refer to Appendix 2a, for correlation of environmental variables).

Variable 1	Variable 2	r	P_{value}
Vascular submerged macrophyte biomass (mg cm^{-2})	**Vascular submerged macrophyte** chlorophyll content (μg cm^{-2})	+0.835	P<0.001***
	Vascular submerged macrophyte cover	+0.913	P<0.001***
	Bare area	-0.289	P<0.05*
	Z_{eu} [3]	+0.233	P<0.05*
	pH	+0.375	P<0.01**
	Conductivity	+0.492	P<0.001***
	Alkalinity	+0.233	P<0.05*
	Cl	+0.430	P<0.001***
	SO_4	-0.353	P<0.01**
	Pb	-0.356	P<0.01**
	Zn	-0.273	P<0.05*
	Al	-0.289	P<0.05*
	Ca	+0.448	P<0.001***
	Mg	+0.472	P<0.001***
	Mn	-0.345	P<0.01**
	Boulders	-0.260	P<0.05*
	Large Stones	-0.247	P<0.05*
	Small Stones	+0.293	P<0.05*
	Gravel	+0.311	P<0.01**
	Sand	+0.642	P<0.001***
	Granite	-0.459	P<0.001***
	Durness Limestone	+0.567	P<0.001***
	Eriboll Sandstone	+0.735	P<0.001***
	Moine Schist	+0.360	P<0.01**
	Applecross Formation	+0.450	P<0.001***
	An-t'Sron	+0.450	P<0.001***
	Substrate diversity (Simpson's D)	+0.389	P<0.01**
	Substrate dominance (Berger-Parker)	-0.378	P<0.01**
	Hydromorphological diversity (Simpson's D)	+0.376	P<0.01**
V**ascular submerged macrophyte** chlorophyll content (μg cm^{-2})	**Vascular submerged macrophyte** cover	+0.780	P<0.001***
	Z_{eu} [3]	+0.228	P<0.05*
	pH	+0.373	P<0.01**
	Conductivity	+0.495	P<0.001***
	Alkalinity	+0.233	P<0.05*
	Cl	+0.430	P<0.001***
	SO_4	-0.353	P<0.01**
	Pb	-0.345	P<0.01**
	Zn	-0.259	P<0.05*
	Al	-0.265	P<0.05*
	Ca	+0.450	P<0.001***
	Mg	+0.475	P<0.001***
	Mn	-0.345	P<0.01**
	Boulders	-0.223	P<0.05*
	Large Stones	-0.219	P<0.05*
	Small Stones	+0.287	P<0.05*
	Gravel	+0.303	P<0.01**
	Sand	+0.524	P<0.001***
	Granite	-0.450	P<0.001***

	Durness Limestone	+0.562	P<0.001***
	Eriboll Sandstone	+0.731	P<0.001***
	Moine Schist	+0.349	P<0.01**
	Applecross Formation	+0.435	P<0.001***
	An-t'Sron	+0.435	P<0.001***
	Substrate diversity (Simpson's D)	+0.392	P<0.01**
	Substrate dominance (Berger-Parker)	-0.386	P<0.01**
	Hydromorphological diversity (Simpson's D)	+0.378	P<0.01**
Vascular submerged macrophyte cover	Bare area	-0.224	P<0.05*
	Z_{eu}[3]	+0.230	P<0.05*
	pH	+0.373	P<0.01**
	Conductivity	+0.495	P<0.001***
	Alkalinity	+0.233	P<0.01**
	Cl	+0.430	P<0.001***
	SO_4	-0.353	P<0.01**
	Pb	-0.356	P<0.01**
	Zn	-0.273	P<0.05*
	Al	-0.289	P<0.05*
	Ca	+0.448	P<0.001***
	Mg	+0.472	P<0.001***
	Mn	-0.345	P<0.01**
	Boulders	-0.247	P<0.05*
	Large Stones	-0.266	P<0.05*
	Small Stones	+0.290	P<0.05*
	Gravel	+0.306	P<0.001***
	Sand	+0.620	P<0.001***
	Granite	-0.459	P<0.001***
	Durness Limestone	+0.567	P<0.001***
	Eriboll Sandstone	+0.735	P<0.001***
	Moine Schist	+0.360	P<0.01**
	Applecross Formation	+0.450	P<0.001***
	An-t'Sron	+0.450	P<0.001***
	Substrate diversity (Simpson's D)	+0.390	P<0.01**
	Substrate dominance (Berger-Parker)	-0.382	P<0.01**
	Hydromorphological diversity (Simpson's D)	+0.378	P<0.01**

Appendix 2f. Significant (<0.05) Spearman rank-order correlation coefficients (r) and probability (P) values between ranked variables: median vascular submerged macrophyte biomass per unit area, median vascular submerged macrophyte chlorophyll content per unit area, median vascular submerged macrophyte abundance, median bare area, and median environmental habitat conditions for amalgamated sub-catchment data (Water of Dye, River Girnock and Knockan Burn; n = 79). Note1: relationships between environmental variables not shown (refer to Appendix 2a, for correlation of environmental variables).

Variable 1	Variable 2	r	P_{value}
log_e Freshwater vegetation biomass (mg cm^{-2})	log_e Freshwater vegetation chlorophyll content (μg cm^{-2})	+0.723	P<0.001***
	% Freshwater vegetation cover	+0.390	P<0.01**
	log_e Z_{eu}[1]	+0.323	P<0.01**
	log_e Z_{eu}[3]	+0.388	P<0.01**
	% Shade	-0.452	P<0.001***
	Height of riparian vegetation	-0.443	P<0.001***
	Cl	+0.411	P<0.001***
	% Diorite	-0.485	P<0.001***
	% Amphibolite	-0.568	P<0.001***
	% Serpentinite	-0.515	P<0.001***
	% QP	-0.515	P<0.001***
	% DA	-0.510	P<0.001***
	% QPP	-0.580	P<0.001***
	% Limestone	-0.575	P<0.001***
	% Durness Limestone	+0.537	P<0.001***
log_e Freshwater vegetation chlorophyll content (μg cm^{-2})	% Freshwater vegetation cover	+0.385	P<0.01**
	log_e Z_{eu}[1]	+0.338	P<0.01**
	log_e Z_{eu}[3]	+0.385	P<0.01**
	% Shade	-0.484	P<0.001***
	Height of riparian vegetation	-0.438	P<0.001***
	% Diorite	-0.568	P<0.001***
	% Amphibolite	-0.555	P<0.001***
	% Serpentinite	-0.479	P<0.001***
	% QP	-0.479	P<0.001***
	% DA	-0.467	P<0.001***
	% QPP	-0.583	P<0.001***
	% Limestone	-0.610	P<0.001***
% Freshwater vegetation cover	% Unvegtated area	-0.551	P<0.001***
	log_e Z_{eu}[1]	+0.275	P<0.05*
	log_e Z_{eu}[3]	+0.292	P<0.05*
	% Shade	-0.375	P<0.01**
	Height of riparian vegetation	-0.323	P<0.01**

Appendix 2g. Significant (<0.05) Pearson product-moment correlation coefficients (r) and probability (P) values between normally distributed variables: mean freshwater plant biomass per unit area, mean freshwater plant chlorophyll content per unit area, mean freshwater plant abundance, and mean environmental habitat conditions for amalgamated sub-catchment data (Water of Dye, River Girnock and Knockan Burn; n = 79). Note[1]: relationships between environmental variables not shown (refer to Appendix 2a, for correlation of environmental variables).

Variable 1	Variable 2	r	P_{value}
Periphyton species richness: **S** harvested from **short-term linoleum** substrates	Periphyton species diversity: **H**	+0.891	P<0.001***
	Periphyton species dominance	-0.715	P<0.001***
	$\log_e$ D	-0.577	P<0.001***
	$\log_e$ Z_{eu}:D^1	+0.248	P<0.05*
	pH	+0.403	P<0.001***
	$\log_e$ Conductivity	+0.289	P<0.001***
	√ Water Temperature	+0.693	P<0.001***
	√ Flow	-0.332	P<0.01**
	$\log_e$ **periphyton** biomass (mg cm^{-2})	+0.294	P<0.05*
	$\log_e$ **periphyton** chlorophyll content (μg cm^{-2})	+0.253	P<0.05*
Periphyton species diversity: **H** harvested from **short-term linoleum** substrates	Periphyton species dominance	-0.831	P<0.001***
	$\log_e$ D	-0.514	P<0.001***
	$\log_e$ Z_{eu}:D^1	+0.289	P<0.05*
	pH	+0.511	P<0.001***
	$\log_e$ Conductivity	+0.465	P<0.001***
	√ Water Temperature	+0.555	P<0.001***
	√ Flow	-0.290	P<0.05*
	$\log_e$ **periphyton** biomass (mg cm^{-2})	+0.322	P<0.01**
	$\log_e$ **periphyton** chlorophyll content (μg cm^{-2})	+0.285	P<0.05*
Periphyton species dominance harvested from **short-term linoleum** substrates	$\log_e$ D	+0.367	P<0.01**
	$\log_e$ Z_{eu}:D^1	-0.256	P<0.05*
	pH	-0.443	P<0.001***
	$\log_e$ Conductivity	-0.394	P<0.01**
	√ Water Temperature	-0.493	P<0.001***
	√ Flow	+0.320	P<0.01**
	$\log_e$ **periphyton** biomass (mg cm^{-2})	-0.436	P<0.001***
	$\log_e$ **periphyton** chlorophyll content (μg cm^{-2})	-0.360	P<0.01**

Appendix 2h. Significant (<0.05) Pearson product-moment correlation coefficients (r) and probability (P) values between normally distributed variables: mean periphyton species richness per unit area, mean periphyton species diversity per unit area, mean periphyton species dominance per unit area, mean periphyton production attributes (biomass, chlorophyll content and % cover) per unit area, and mean environmental habitat conditions of short-term linoleum substrates for amalgamated sub-catchment data (Water of Dye, River Girnock and Knockan Burn; n = 50). Note1: relationships between environmental variables not shown (refer to Appendix 2a, for correlation of environmental variables).

Variable 1	Variable 2	r	P_{value}
Periphyton species richness: **S** harvested from **all artificial** substrates sampled during surveys	Periphyton species diversity: **H**	+0.839	P<0.001***
	Periphyton species dominance	-0.564	P<0.001***
	$\log_e$ D	-0.439	P<0.001***
	$\log_e$ Z_{eu}:D[1]	+0.431	P<0.001***
	pH	+0.321	P<0.001***
	$\log_e$ Alkalinity	+0.431	P<0.001***
	√ Water Temperature	+0.549	P<0.001***
	√ Flow	-0.285	P<0.05*
	SO_4	-0.274	P<0.05*
	$\log_e$ Pb	-0.272	P<0.05*
	$\log_e$ Zn	-0.264	P<0.05*
	Al	-0.332	P<0.01**
	$\log_e$ Kpot	+0.496	P<0.001***
	$\log_e$ Ca	+0.332	P<0.01**
	$\log_e$ Mg	+0.298	P<0.05*
	$\log_e$ **periphyton** biomass (mg cm^{-2})	+0.296	P<0.05*
	$\log_e$ **periphyton** chlorophyll content (μg cm^{-2})	+0.242	P<0.05*
Periphyton species diversity: **H** harvested from **all artificial** substrates sampled during surveys	Periphyton species dominance	-0.779	P<0.001***
	$\log_e$ D	-0.450	P<0.001***
	$\log_e$ Z_{eu}:D[1]	+0.532	P<0.001***
	pH	+0.481	P<0.001***
	$\log_e$ Conductivity	+0.437	P<0.001***
	$\log_e$ Alkalinity	+0.582	P<0.001***
	√ Water Temperature	+0.476	P<0.001***
	√ Flow	-0.270	P<0.05*
	SO_4	-0.356	P<0.01**
	$\log_e$ Pb	-0.348	P<0.01**
	$\log_e$ Zn	-0.274	P<0.05*
	Al	-0.402	P<0.001***
	$\log_e$ Kpot	+0.620	P<0.001***
	$\log_e$ Ca	+0.465	P<0.001***
	$\log_e$ Mg	+0.372	P<0.01**
	$\log_e$ **periphyton** biomass (mg cm^{-2})	+0.298	P<0.05*
	$\log_e$ **periphyton** chlorophyll content (μg cm^{-2})	+0.260	P<0.05*
Periphyton species dominance harvested from **all artificial** substrates sampled during surveys	$\log_e$ D	+0.472	P<0.001***
	$\log_e$ Z_{eu}:D[1]	-0.501	P<0.001***
	pH	-0.473	P<0.001***
	$\log_e$ Conductivity	-0.505	P<0.001***
	$\log_e$ Alkalinity	-0.645	P<0.001***
	√ Water Temperature	-0.465	P<0.001***
	√ Flow	+0.287	P<0.001***
	SO_4	+0.396	P<0.01**
	$\log_e$ Pb	+0.363	P<0.01**
	$\log_e$ Zn	+0.300	P<0.01**
	Al	+0.434	P<0.001***
	$\log_e$ Kpot	-0.522	P<0.001***
	$\log_e$ Ca	-0.568	P<0.001***
	$\log_e$ Mg	-0.489	P<0.001***

	$\log_e$ **periphyton** biomass (mg cm^{-2})	-0.443	P<0.001***
	$\log_e$ **periphyton** chlorophyll content (μg cm^{-2})	-0.475	P<0.001***

Appendix 2i. Significant (<0.05) Pearson product-moment correlation coefficients (r) and probability (P) values between normally distributed variables: mean periphyton species richness per unit area, mean periphyton species diversity per unit area, mean periphyton species dominance per unit area, mean periphyton production attributes (biomass, chlorophyll content and % cover) per unit area, and mean environmental habitat conditions of all artificial substrates sampled during survey dates: short-term linoleum, long-term linoleum, long-term Astroturf, and plastic aquarium plants for amalgamated sub-catchment data (Water of Dye, River Girnock and Knockan Burn; n = 93). Note1: relationships between environmental variables not shown (refer to Appendix 2a, for correlation of environmental variables).

Variable 1	Variable 2	r	P_{value}
Periphyton species richness: **S** harvested from **all naturally-occurring** substrata during surveys	Periphyton species diversity: **H**	+0.837	P<0.001***
	Periphyton species dominance	-0.581	P<0.001***
	$\log_e$ D	-0.297	P<0.05*
	$\log_e$ Z_{eu}:D[1]	+0.286	P<0.05*
	pH	+0.321	P<0.01**
	$\log_e$ Alkalinity	+0.386	P<0.01**
	√ Water Temperature	+0.373	P<0.01**
	√ Flow	-0.245	P<0.05*
	$\log_e$ Pb	-0.227	P<0.05*
	$\log_e$ Zn	-0.243	P<0.05*
	Al	-0.286	P<0.05*
	$\log_e$ Kpot	+0.546	P<0.001***
	$\log_e$ Ca	+0.332	P<0.01**
	$\log_e$ Mg	+0.317	P<0.01**
	% Granodiorite	+0.588	P<0.001***
	% Amphibolite	+0.373	P<0.01**
	% Serpentinite	+0.407	P<0.001***
	% QP	+0.407	P<0.001***
	% DA	+0.407	P<0.001***
	% QPP	+0.347	P<0.01**
	% Limestone	+0.273	P<0.05*
	$\log_e$ **periphyton** biomass (mg cm^{-2})	+0.292	P<0.05*
	$\log_e$ **periphyton** chlorophyll content (μg cm^{-2})	+0.265	P<0.05*
	Periphyton cover (%)	+0.619	P<0.001***
	Bare area	-0.318	P<0.01**
Periphyton species diversity: **H** harvested from **all naturally-occurring** substrata during surveys	Periphyton species dominance	-0.815	P<0.001***
	$\log_e$ D	-0.277	P<0.05*
	$\log_e$ Z_{eu}:D[1]	+0.404	P<0.001***
	pH	+0.545	P<0.001***
	$\log_e$ Conductivity	+0.419	P<0.001***
	$\log_e$ Alkalinity	+0.563	P<0.001***
	√ Water Temperature	+0.293	P<0.05*
	√ Flow	-0.279	P<0.05*
	SO_4	-0.423	P<0.001***
	$\log_e$ Pb	-0.471	P<0.001***
	$\log_e$ Zn	-0.389	P<0.01**
	Al	-0.488	P<0.001***
	$\log_e$ Kpot	+0.551	P<0.001***
	$\log_e$ Ca	+0.517	P<0.001***
	$\log_e$ Mg	+0.440	P<0.01**
	% Granite	-0.323	P<0.01**
	% Granodiorite	+0.439	P<0.001***
	% Amphibolite	+0.274	P<0.05*
	% Serpentinite	+0.299	P<0.05*
	% QP	+0.299	P<0.05*
	% DA	+0.299	P<0.05*
	% QPP	+0.257	P<0.05*
	% Limestone	+0.214	P<0.05*

	% Durness Limestone	+0.218	P<0.05*
	% Eriboll Sandstone	+0.229	P<0.05*
	% Applecross Formation	+0.256	P<0.05*
	% An-t'Sron	+0.256	P<0.05*
	$\log_e$ **periphyton** biomass (mg cm^{-2})	+0.379	P<0.05*
	$\log_e$ **periphyton** chlorophyll content (μg cm^{-2})	+0.435	P<0.05*
	Periphyton cover (%)	+0.449	P<0.001***
Periphyton species dominance harvested from **all naturally-occurring** substrata during surveys	$\log_e$ D	+0.239	P<0.05*
	$\log_e$ Z_{eu}:D[1]	-0.369	P<0.01**
	pH	-0.683	P<0.001***
	$\log_e$ Conductivity	-0.566	P<0.001***
	$\log_e$ Alkalinity	-0.598	P<0.001***
	√ Water Temperature	-0.245	P<0.05*
	√ Flow	+0.204	P<0.05*
	SO_4	+0.575	P<0.001***
	$\log_e$ Pb	+0.577	P<0.001***
	$\log_e$ Zn	+0.524	P<0.001***
	Al	+0.618	P<0.001***
	$\log_e$ Kpot	-0.416	P<0.001***
	$\log_e$ Ca	-0.626	P<0.001***
	$\log_e$ Mg	-0.582	P<0.001***
	% Granite	+0.516	P<0.001***
	% Durness Limestone	-0.415	P<0.001***
	% Eriboll Sandstone	-0.371	P<0.01**
	% Applecross Formation	-0.368	P<0.01**
	% An-t'Sron	-0.368	P<0.01**
	$\log_e$ **periphyton** biomass (mg cm^{-2})	-0.383	P<0.01**
	$\log_e$ **periphyton** chlorophyll content (μg cm^{-2})	-0.590	P<0.001***
	Periphyton cover (%)	-0.278	P<0.05*

Appendix 2j. Significant (<0.05) Pearson product-moment correlation coefficients (r) and probability (P) values between normally distributed variables: mean periphyton species richness per unit area, mean periphyton species diversity per unit area, mean periphyton species dominance per unit area, mean periphyton production attributes (biomass, chlorophyll content and % cover) per unit area, and mean environmental habitat conditions of all naturally-occurring substrata: mineral particles, aquatic bryophytes and vascular submerged macrophytes for amalgamated sub-catchment data (Water of Dye, River Girnock and Knockan Burn; n = 163). Note1: relationships between environmental variables not shown (refer to Appendix 2a, for correlation of environmental variables).

Variable 1	Variable 2	r	P_{value}
Aquatic bryophyte species richness: **S**	Aquatic bryophyte species diversity: **H**	+0.956	P<0.001***
	Aquatic bryophyte species dominance	-0.340	P<0.01**
	% Boulders	+0.346	P<0.01**
	% Small Stones	-0.318	P<0.01**
	% Gravel	-0.358	P<0.01**
	pH	-0.348	P<0.01**
	$\log_e$ Alkalinity	-0.275	P<0.05*
	√ Flow	+0.306	P<0.01**
	SO_4	+0.241	P<0.05*
	$\log_e$ Cd	+0.238	P<0.05*
	$\log_e$ Pb	+0.222	P<0.05*
	Al	+0.256	P<0.05*
	% Diorite	-0.253	P<0.05*
	% Amphibolite	-0.361	P<0.01**
	% Serpentinite	-0.347	P<0.01**
	% QP	-0.347	P<0.01**
	% DA	-0.347	P<0.01**
	% QPP	-0.359	P<0.01**
	% Limestone	-0.336	P<0.01**
	% Moine Schist	-0.348	P<0.01**
	$\log_e$ **aquatic bryophyte** biomass (mg cm^{-2})	+0.498	P<0.001***
	$\log_e$ **aquatic bryophyte** chlorophyll content (μg cm^{-2})	+0.448	P<0.001***
	% **aquatic bryophyte** cover	+0.489	P<0.001***
Aquatic bryophyte species diversity: **H**	Aquatic bryophyte species dominance	-0.526	P<0.001***
	% Boulders	+0.354	P<0.01**
	% Small Stones	-0.298	P<0.05*
	% Gravel	-0.324	P<0.01**
	pH	-0.335	P<0.01**
	$\log_e$ Alkalinity	-0.272	P<0.05*
	√ Flow	+0.275	P<0.05*
	SO_4	+0.241	P<0.05*
	$\log_e$ Cd	+0.229	P<0.05*
	$\log_e$ Pb	+0.251	P<0.05*
	Al	+0.291	P<0.01**
	% Amphibolite	-0.351	P<0.01**
	% Serpentinite	-0.352	P<0.01**
	% QP	-0.352	P<0.01**
	% DA	-0.351	P<0.01**
	% QPP	-0.342	P<0.01**
	% Limestone	-0.306	P<0.01**
	% Moine Schist	-0.416	P<0.001***
	$\log_e$ **aquatic bryophyte** biomass (mg cm^{-2})	+0.300	P<0.001***
	$\log_e$ **aquatic bryophyte** chlorophyll content (μg cm^{-2})	+0.440	P<0.001***
	% **aquatic bryophyte** cover	+0.461	P<0.001***
Aquatic bryophyte species dominance	% Serpentinite	+0.223	P<0.05*
	% QP	+0.223	P<0.05*
	% DA	+0.225	P<0.05*
	% Moine Schist	+0.285	P<0.05*

Appendix 2k. Significant (<0.05) Pearson product-moment correlation coefficients (r) and probability (P) values between normally distributed variables: mean aquatic bryophyte species richness per unit area, mean aquatic bryophyte species diversity per unit area, mean aquatic bryophyte species dominance per unit area, mean aquatic bryophyte production attributes (biomass, chlorophyll content and % cover) per unit area, and mean environmental habitat conditions for amalgamated sub-catchment data (Water of Dye, River Girnock and Knockan Burn; n = 79). Note1: relationships between environmental variables not shown (refer to Appendix 2a, for correlation of environmental variables).

Variable 1	Variable 2	r	P_{value}
Vascular submerged macrophyte species richness: **S**	Vascular submerged macrophyte species diversity: **H**	+0.953	P<0.001***
	% Boulders	-0.279	P<0.05*
	% Large Stones	-0.238	P<0.05*
	% Small Stones	+0.282	P<0.05*
	% Gravel	+0.304	P<0.01**
	% Sand	+0.627	P<0.001***
	pH	+0.365	P<0.01**
	log_e Conductivity	+0.489	P<0.001***
	log_e Alkalinity	+0.237	P<0.05*
	SO_4	-0.356	P<0.01**
	log_e Pb	-0.353	P<0.01**
	log_e Zn	-0.267	P<0.05*
	Al	-0.256	P<0.05*
	log_e Ca	+0.442	P<0.001***
	log_e Mg	+0.467	P<0.001***
	log_e Mn	-0.342	P<0.01**
	% Granite	-0.459	P<0.001***
	% Durness Limestone	+0.592	P<0.001***
	% Moine Schist	+0.478	P<0.001***
	log_e **vascular submerged macrophyte** biomass (mg cm^{-2})	+0.918	P<0.001***
	log_e **vascular submerged macrophyte** chlorophyll content (μg cm^2)	+0.798	P<0.001***
	% **Vascular submerged macrophyte** cover	+0.864	P<0.001***
	Substrate diversity (Simpson's D)	+0.280	P<0.05*
	Substrate dominance (Berger-Parker)	-0.251	P<0.05*
	Hydromorphological diversity (Simpson's D)	+0.271	P<0.05*
Vascular submerged macrophyte species diversity: **H**	% Boulders	-0.336	P<0.01**
	% Large Stones	-0.267	P<0.05*
	% Small Stones	+0.228	P<0.05*
	% Gravel	+0.339	P<0.01**
	% Sand	+0.518	P<0.001***
	% Granite	-0.324	P<0.01**
	% Durness Limestone	+0.439	P<0.001***
	% Moine Schist	+0.404	P<0.001***
	log_e **vascular submerged macrophyte** biomass (mg cm^{-2})	+0.805	P<0.001***
	log_e **vascular submerged macrophyte** chlorophyll content (μg cm^{-2})	+0.677	P<0.001***
	% **Vascular submerged macrophyte** cover	+0.780	P<0.001***
	Substrate diversity (Simpson's D)	+0.214	P<0.05*
	Substrate dominance (Berger-Parker)	-0.210	P<0.05*
	Hydromorphological diversity (Simpson's D)	+0.211	P<0.05*

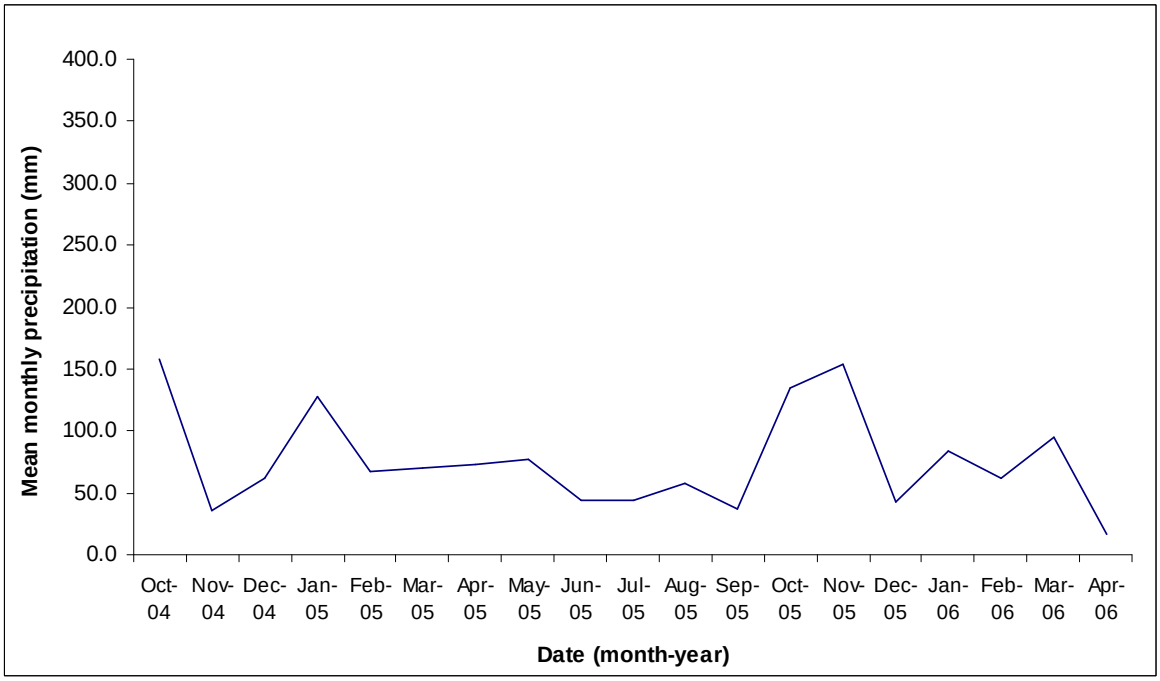
Appendix 2l. Significant (<0.05) Spearman rank-order correlation coefficients (r) and probability (P) values between ranked variables: median vascular submerged macrophyte species richness per unit area, median vascular submerged macrophyte species diversity per unit area, median vascular submerged macrophyte production attributes (biomass, chlorophyll content, % cover) per unit area, and median environmental habitat conditions for amalgamated sub-catchment data (Water of Dye, River Girnock and Knockan Burn; n = 79). Note1: relationships between environmental variables not shown (refer to Appendix 2a, for correlation of environmental variables).

Variable 1	Variable 2	r	P_{value}
Freshwater vegetation species richness: **S**	Freshwater vegetation species diversity: **H**	+0.860	P<0.001***
	Freshwater vegetation species dominance	-0.654	P<0.001***
	pH	+0.268	P<0.05*
	$\log_e$ Alkalinity	+0.339	P<0.01**
	√ Water Temperature	+0.308	P<0.01**
	$\log_e$ Pb	-0.252	P<0.05*
	$\log_e$ Zn	-0.248	P<0.05*
	Al	-0.265	P<0.05*
	$\log_e$ Kpot	+0.503	P<0.001***
	$\log_e$ Ca	+0.327	P<0.01**
	$\log_e$ Mg	+0.312	P<0.01**
	% Granodiorite	+0.594	P<0.001***
	% Amphibolite	+0.367	P<0.01**
	% Serpentinite	+0.385	P<0.01**
	% QP	+0.385	P<0.01**
	% DA	+0.385	P<0.01**
	% QPP	+0.348	P<0.01**
	% Limestone	+0.293	P<0.05*
	% Freshwater vegetation cover	+0.292	P<0.01**
	% Bare Area	-0.358	P<0.01**
	Substrate diversity (Simpson's D)	+0.278	P<0.05*
	Substrate dominance (Berger-Parker)	-0.265	P<0.05*
	Hydromorphological diversity (Simpson's D)	+0.275	P<0.05*
Freshwater vegetation species diversity: **H**	Freshwater vegetation species dominance	-0.854	P<0.001***
	pH	+0.393	P<0.01**
	$\log_e$ Conductivity	+0.365	P<0.01**
	$\log_e$ Alkalinity	+0.428	P<0.001***
	√ Water Temperature	+0.255	P<0.05*
	SO_4	-0.345	P<0.01**
	$\log_e$ Pb	-0.450	P<0.001***
	$\log_e$ Zn	-0.383	P<0.01**
	Al	-0.375	P<0.01**
	$\log_e$ Kpot	+0.432	P<0.001***
	$\log_e$ Ca	+0.459	P<0.001***
	$\log_e$ Mg	+0.403	P<0.001***
	% Granite	-0.315	P<0.01**
	% Granodiorite	+0.426	P<0.001***
	% Schist	-0.473	P<0.001***
	% Durness Limestone	+0.250	P<0.05*
	% Eriboll Sandstone	+0.237	P<0.05*
	% Applecross Formation	+0.276	P<0.05*
	% An-t'Sron	+0.276	P<0.05*
	% Freshwater vegetation cover	+0.235	P<0.05*
	Substrate diversity (Simpson's D)	+0.373	P<0.01**
	Substrate dominance (Berger-Parker)	-0.323	P<0.01**
	Hydromorphological diversity (Simpson's D)	+0.368	P<0.01**
Freshwater vegetation species dominance	pH	-0.343	P<0.01**
	$\log_e$ Conductivity	-0.317	P<0.01**

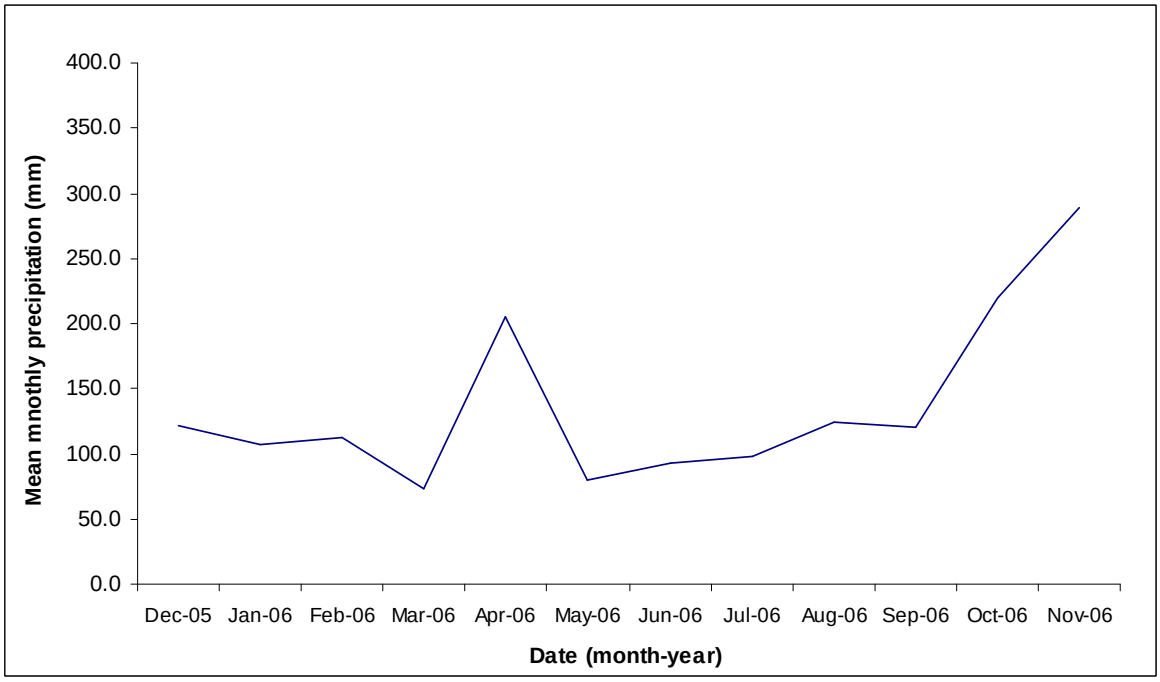
	$\log_e$ Alkalinity	-0.289	P<0.05*
	√ Water Temperature	+0.255	P<0.05*
	SO_4	+0.379	P<0.01**
	$\log_e$ Pb	+0.388	P<0.01**
	$\log_e$ Zn	+0.340	P<0.01**
	Al	+0.338	P<0.01**
	$\log_e$ Kpot	-0.330	P<0.01**
	$\log_e$ Ca	-0.373	P<0.01**
	$\log_e$ Mg	-0.356	P<0.01**
	% Granite	+0.333	P<0.01**
	% Granodiorite	-0.260	P<0.05*
	% Schist	+0.461	P<0.001***
	% Durness Limestone	-0.272	P<0.05*
	% Eriboll Sandstone	-0.225	P<0.05*
	% Applecross Formation	-0.257	P<0.05*
	% An-t'Sron	-0.257	P<0.05*
	% Freshwater vegetation community cover	-0.223	P<0.05*
	Substrate diversity (Simpson's D)	-0.325	P<0.01**
	Substrate dominance (Berger-Parker)	+0.288	P<0.05*
	Hydromorphological diversity (Simpson's D)	-0.320	P<0.01**

Appendix 2m. Significant (<0.05) Pearson product-moment correlation coefficients (r) and probability (P) values between normally distributed variables: mean freshwater plant species richness per unit area, mean freshwater plant species diversity content per unit area, mean freshwater plant species dominance per unit area, mean production attributes (biomass, chlorophyll content and % cover) per unit area, and mean environmental habitat conditions for amalgamated sub-catchment data (Water of Dye, River Girnock and Knockan Burn; n = 79). Note1: relationships between environmental variables not shown (refer to Appendix 2a, for correlation of environmental variables).

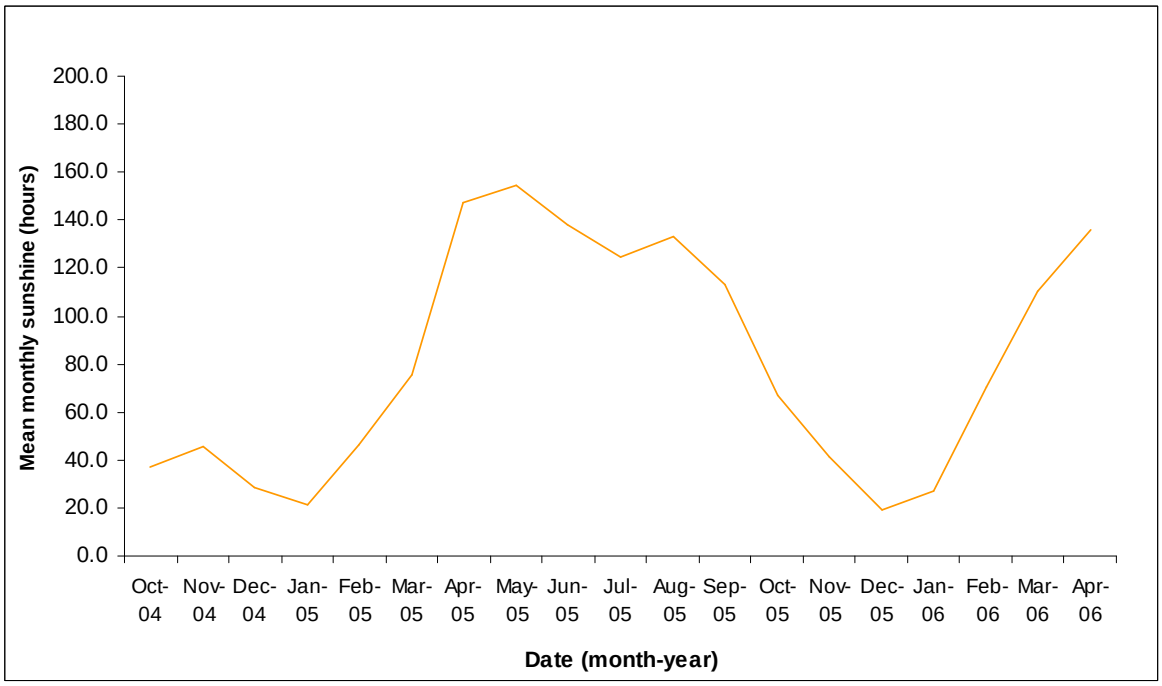
Appendix 3. MET Office Data



Appendix 3a. Mean monthly precipitation records for Braemar, near the Water of Dye and River Girnock (R. Dee catchment) from October 2004 to April 2006 (data provided by the Met Office).

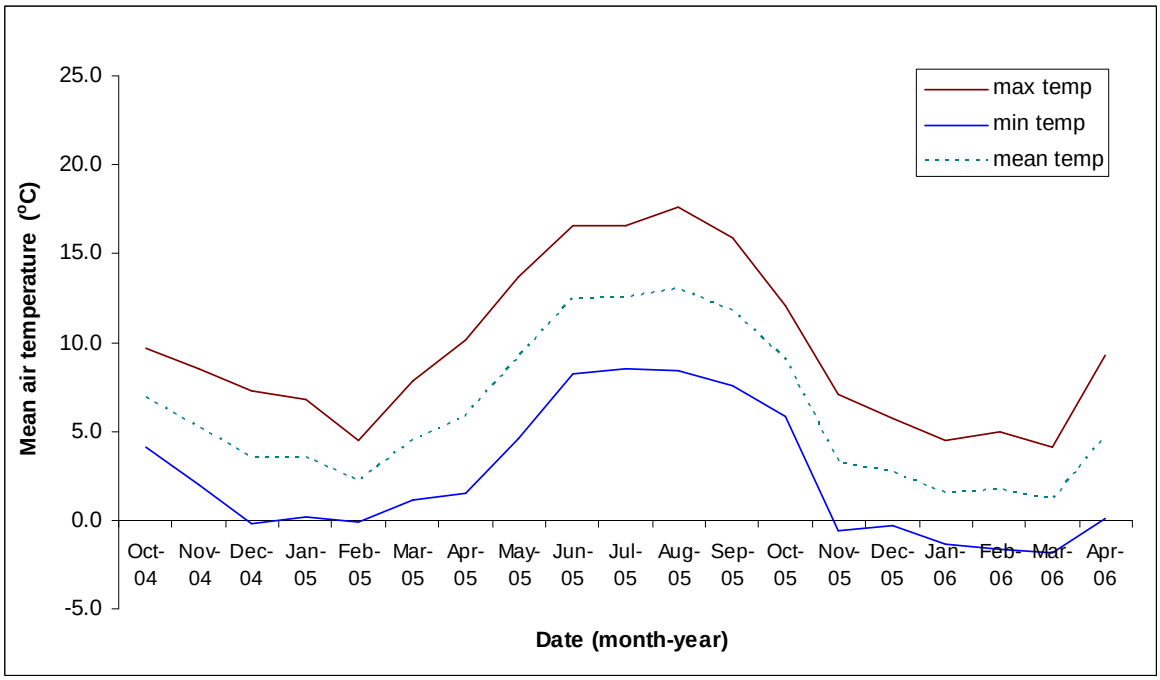


Appendix 3b. Mean monthly precipitation records for Ledmore, near Knockan Burn (R. Kirkaig catchment) from December 2005 to November 2006 (data provided by the Met Office).

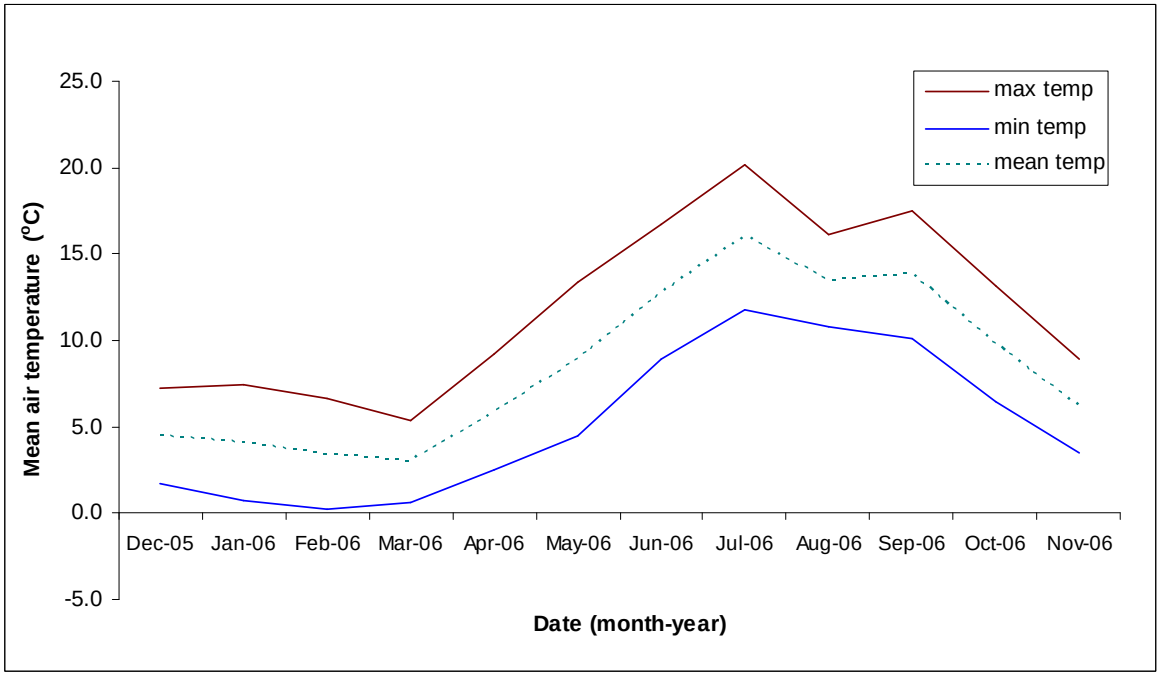


Appendix 3c. Mean monthly sunshine records for Braemar, near the Water of Dye and River Girnock (R. Dee catchment) from October 2004 to April 2006 (data provided by the Met Office).

Appendix 3d. Mean monthly sunshine records for Ledmore, near Knockan Burn (R. Kirkaig catchment) from December 2005 to November 2006 (data provided by the Met Office).

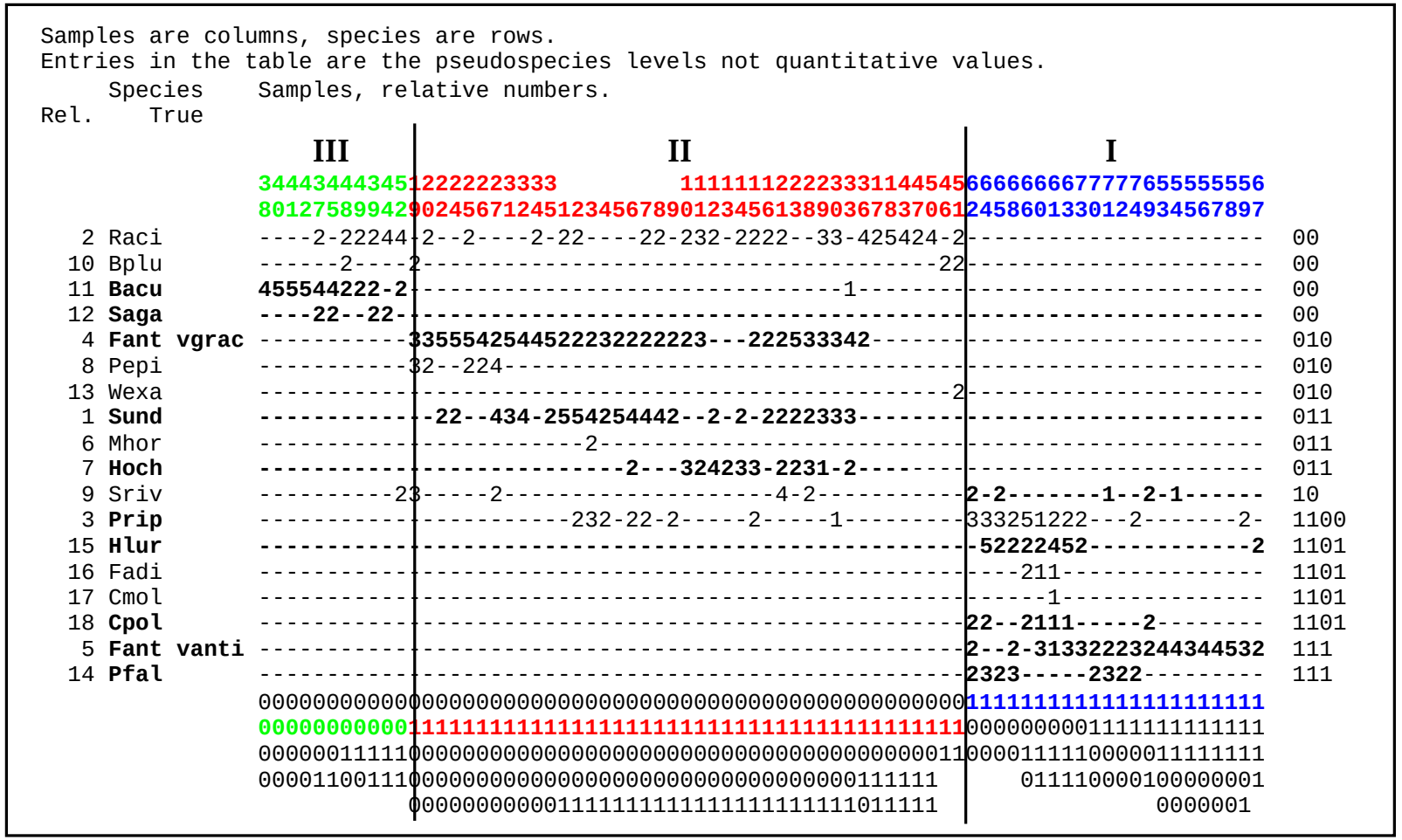


Appendix 3e. Mean monthly air temperature records for Braemar, near the Water of Dye and River Girnock (R. Dee catchment) from October 2004 to April 2006 (data provided by the Met Office).

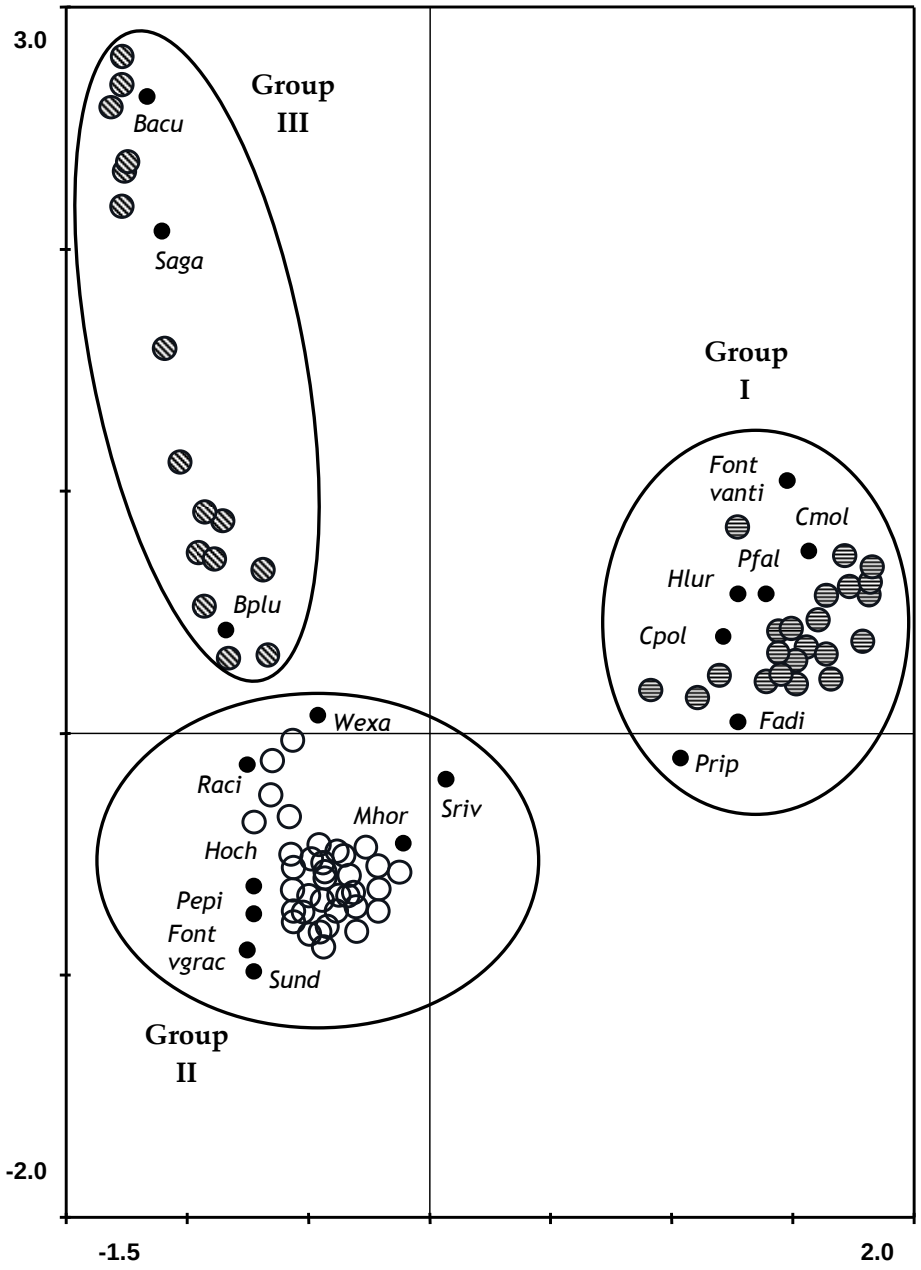


Appendix 3f. Mean monthly air temperature records for Ledmore, near Knockan Burn (R. Kirkaig catchment) from December 2005 to November 2006 (data provided by the Met Office).

Appendix 4. Additional Data



Appendix 4a. TWINSPAN output depicting 79 samples and 4 aquatic bryophyte species assemblages, with indicator species highlighted in bold font and colour-coding as appropriate for TWINSPAN sample-groups I (blue), II (red), and III (green). For aquatic bryophyte species codes refer to Figure 4.28.



Appendix 4b. CCA ordination of 18 aquatic bryophyte species and 74 samples, with TWINSPAN sample-group boundaries overlaid. TWINSPAN sample-group identifiers as follows: Group I (n=22: UKAPP06, UKAPG06, UKAPR06, UKSMP06, UKNVP06, UKNVG06, MKAPP06, MKAPG06, MKAPR06, MKSMP06, MKSMG06, MKSMR06, MKNVG06, MKNVR06, LKAPP06, LKAPG06, LKAPR06, LKSMP06, LKSMG06, LKSMR06, LKNVP06, LKNVG06): diagonally striped circles ; Group II (n=41: BBMYP05, BBMYG05, BBMYR05, BBAUP05, BBAUG05, BBAUR05, BBAPP06, BBAPG06, BBAPR06, CFMYP05, CFMYG05, CFMYR05, CFAUP05, CFAUG05, CFAUR05, CFAPP06, CFAPG06, CFAPR06, BDMYP05, BDMYG05, BDMYR05, BDAUP05, BDAUG05, BDAUR05, BDAPP06, BDAPG06, BDAPR06, IBMYP05, IBMYG05, IBMYR05, IBAUP05, IBAUG05, IBAUR05, IBAPP06, IBAPG06, IBAPR06, HBAPP06, LMMYP05, LMMYG05, LMAPP06, LMAPG06): open circles ; Group III (n=11: HBMYP05, HBMYG05, HBMYR05, HBAUP05, HBAUG05, HBAUR05, HBAPG05, HBAPR05, LMMYR05, LMAUR05, LMAPR06): horizontally striped circles . For sample site-codes: Water of Dye sites: Brocky Burn (BB), Charr Flume (CF) and Bogendreip (BD); River Girnock sites: Iron Bridge (IB), Hampshire's Bridge (HB) and Littlemill (LM); Knockan Burn sites: Upper Knockan (UK), Mid-Knockan (MK) and Lower Knockan (LK). Each site code is completed using code letters for survey date (AP: April; MY: May; AU: August; SM: September; NV: November), flow regime (P: Pool; G: Glide; R: Riffle) and year sampled (05: 2005; 06: 2006). Example: BBMYR05 = Brocky Burn May Riffle 2005. For aquatic bryophyte species codes: *Blindia acuta* (Bacu), *Brachythecium plumosum* (Bplu), *Ctenidium molluscum* (Cmol), *Fissidens adianthoides* (Fadi), *Fontinalis antipyretica* var. *gracilis* (Fant vgrac), *Fontinalis antipyretica* var. *antipyretica* (Fant vanti), *Hygrohypnum luridum* (Hlur), *Hygrohypnum ochraceum* (Hoch), *Mnium hornum* (Mhor), *Palustriella falcata* (Pfal), *Platyhypnidium riparioides* (Prip), *Racomitrium aciculare* (Raci), *Schistidium agassizii* (Saga), *Schistidium rivulare* (Sriv), *Warnstorfia exannulata* (Wexa), *Chiloscyphus polyanthus* (Cpol), *Pellia epiphylla* (Pepi), and *Scapania undulata* (Sund). Monte Carlo significance test: Axis 1: P<0.005; all canonical axes: P<0.005. Eigenvalues: Axis 1: 0.901; Axis 2: 0.799.

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