THE EFFICACY AND ECOLOGICAL IMPACTS OF THE MANAGEMENT OF SUBMERGED VEGETATION IN FLOWING WATER

by

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SUMMARY

Freshwater macrophytes are an essential component of a lotic ecosystem. In large quantities they can cause problems in watercourses, by increasing the risks of flooding and obstructing fishing.

The rapidly-acting, contact herbicide diquat has been formulated with sodium alginate to produce a viscous aquatic herbicide which may be used for specific placement in static and flowing water.

Factors which influence the efficacy of diquat-alginate and the ecological consequences of its use, have been studied using three approaches.

1) Small-scale laboratory experiments were used to investigate the effects of:

Herbicide exposure period
Calcium concentration
Water temperature

on the efficacy of the herbicide.

Increased concentrations of calcium (100-300mg l^{-1}) reduced the activity of the herbicide. This antagonism was removed if the exposure period was long enough (24 hours). It was suggested that the calcium and diquat ions may compete for uptake into the plants. A mechanism explaining the uptake of cations, linked to the photosynthetic utilisation of bicarbonate ions, may also explain the uptake of diquat.

The influence of temperature on the herbicidal activity is likely to be related to the control of the rate of metabolic processes at the point of diquat's uptake, or mode of action, in the plant tissues.

2) Field trials to compare watercourse management by use of diquat-alginate and weed cutting, were carried out in four rivers and a canal.

The herbicide was very effective in removing Ranunculus in the swiftly-flowing, moderately calcareous (60-80mg l^{-1}) and shallow R.Petteril and Mouse Water. Plants were removed,or damaged, for considerable distances downstream of the point of herbicide application. Less susceptible species, such as filamentous algae,

Potamogeton natans and Sparganium emersum, only showed localised damage. The concentrations and availance of diquat residues in these rivers were high.

Despite a late application, diquat-alginate appeared to remove a large proportion of the Ranunculus in the R.Coln, but this reduction in biomass was not significant because of the great variability of the data. In the similarly sized and calcareous (100-120mg l^{-1}) river, the R.Windrush, the herbicide appeared to have no effect at all. A combination of conditions less favourable in the R.Windrush than in the R.Coln, would have reduced the effect of the herbicide. The principal factor would have been the high turbidity of the water in the R.Windrush, resulting in the adsorption and inactivation of the diquat prior to entering the plants.

As in the R.Windrush, a predominance of moderately susceptible species in the Union Canal, may have limited the overall reduction of macrophyte biomass caused by the herbicide.

The cutting treatments in the R.Coln and R.Petteril were very specific. In the latter case, the Ranunculus was cut sufficiently late to prevent regrowth. A central channel was cleared of weeds in the R.Windrush but regrowth was evident after six weeks. The regrowth of Elodea canadensis in the Union Canal was greater than the plant growth in the untreated sections.

None of the water chemistry parameters measured appeared to be directly influenced by the management regimes, or the loss of macrophytes. None of the taxa of macroinvertebrates appeared to be directly affected either. The reduction of plant biomass in the R.Petteril and R.Windrush may have indirectly resulted in a reduction in the populations of some taxa, (e.g. chironomids and Trichoptera). The areas of untreated or unaffected vegetation in the rivers will have buffered the ecological impacts of successful management.

Multivariate comparisons of the vegetation in the river samples indicated that there were greater differences between the plant communities in the four rivers than between the sections subject to different management regimes.

3) Beds of Ranunculus were established and grown over-winter in large-scale recirculating channels. Two herbicide trials were carried out in the summer, with diquat-alginate directly applied to half of one channel.

In the first trial the loss of Ranunculus from the untreated channel was almost as great as from the one treated with diquat-alginate at 0.5l $100m^{-2}$, but the percentage of decayed tissue was significantly less. Blooms of filamentous and blue-green algae in the untreated channel probably caused mechanical damage to the Ranunculus.

The algae were regularly removed in the second trial and the herbicide (1.0l $100m^{-2}$) was very effective in reducing the biomass of Ranunculus. Directly treated plants were damaged more quickly than those receiving an indirect dose of diquat residues.

The removal of an inert solute from the channel, by dilution, was modelled and tested. Diquat residues showed an initially greater loss from the channel than would have been predicted by dilution alone. This would have been due to uptake and adsorption by plants and other substrates, and the incomplete release of diquat ions from the alginate. After four hours the delayed release of diquat resulted in a slower loss rate than predicted by dilution.

The macroinvertebrates on the Ranunculus were not directly affected by the herbicide. There was no evidence that the diurnal fluctuations of chemical parameters, such as pH and dissolved oxygen concentrations, were influenced by the decay of herbicide treated Ranunculus plants. The remaining algae probably buffered any effects that decaying macrophytes might have been expected to have on the water chemistry.

PROJECT AIMS AND APPROACHES

This project had two principal aims:

1) To compare the efficacies of cutting and herbicide use as methods of controlling submerged vegetation in flowing water, and to investigate the environmental factors which may influence the efficiency of either method.

2) To investigate the ecological effects of aquatic management by these two methods, on non-target components of flowing water ecosystems.

Four approaches were taken to provide the information necessary to fulfil these aims:

1) A survey of the literature relevant to the uses of cutting and herbicides as management techniques, and to the ecological consequences of their uses. Chapter Two

2) Laboratory studies of the influences of certain environmental factors on herbicide efficacy. Chapter Three

3) Field trials to compare the efficacies and ecological effects of the two methods in rivers and canals. Chapters Four-Six

4) Use of large-scale recirculating channels to provide an experimental medium intermediate between the field and small-scale laboratory experiments. Chapter Seven

CHAPTER ONE

INTRODUCTION

1.1 The Roles of Vegetation in Freshwater

1.1.1 Submerged macrophytes in watercourses

The presence of submerged macrophytes in watercourses may be regarded as a benefit, or a problem, depending upon the extent of the vegetation, the type of watercourse, and the viewpoint of the observer. Unlike the marine algae, all of which are called sea-weeds, regardless of their influence, or lack of it, on man's activities, freshwater vegetation may be interchangeably termed aquatic macrophytes or water weeds. The choice of terminology does not necessarily reveal the attitude of the user.

There are estimated to be about 162,509km of waterway in the U.K. (Newbold 1975), providing a large area of lotic habitat for aquatic vegetation. A sizable proportion of these watercourses are managed.

	Approx. % of total length of watercourses in the U.K.	% Managed
Main rivers	20	32
Intermediate rivers and large drains	20	almost 100
Canals	2	16
Private ditches and dykes	58	?

The literature concerning the roles of aquatic macrophytes has expanded rapidly over the last twenty years (Marshall and Westlake 1978). This is partly because of the realisation that management, or preservation, of any biological system can only develop if there is a good understanding of the biology and interrelationships of the components of the system.

1.1.2 Beneficial roles of submerged macrophytes

The beneficial, and often essential, roles of submerged macrophytes in aquatic systems have been discussed or reviewed by many authors (Sculthorpe 1967, Gaudet 1974, Hartog 1978, Haslam 1978, Marshall and Westlake 1978). Seven functions of submerged macrophytes in the aquatic habitat, can be identified. Examples of references specifically concerned with flowing water are given.

1) Primary production, food source for herbivores and detritivores (Odum 1956, Westlake et al. 1972)

2) Nutrient recycling

3) Photosynthetic oxygen production (Edwards 1968)

4) Substrate for algae (O'Neill Morin and Kimball 1983), macro-invertebrates (Whitcombe 1968) and fish eggs (Mills 1981)

5) Shelter for invertebrates and fish from water currents and predators (Swales 1982)

6) Stabilisation of river beds and banks, and silt accumulation (Dawson 1978a)

7) Aesthetic value (Haslam 1978)

The combined effect of all these roles is the provision of a diversity of habitats for many other members of the lotic systems. For example, larger and more varied macroinvertebrate populations occur in macrophyte-rich compared to macrophyte-poor areas of rivers (Westlake 1975).

1.1.3 Detrimental roles of submerged weeds

There are five main ways in which submerged vegetation can cause problems in rivers. These are discussed by Mitchell (1974), Barrett (1978a), Haslam (1978) and Kelsall (1981).

1) Flooding

2) Interference with sport fisheries

3) Imbalance of dissolved oxygen in hot weather

4) Blockage or reduction of flow and water supply

5) Hindrance of recreational activities e.g. boating, swimming

The first two problems are the most important in rivers, in the U.K. Extreme concentrations of dissolved oxygen, (usually anoxia at night) can cause fish-kills in static water, but are rarely a problem in temperate rivers. However, in exceptional conditions of low water levels and high temperatures, such events can occur (Brooker et al. 1977).

Interference with water supply and recreational activities rarely occurs in flowing water in the U.K., but can be important in warmer countries, dependent upon irrigation. Most other problems that are encountered in warmer climates are caused by floating vegetation which can impede navigation, harbour insect pests, and increase water loss through transpiration (Austin 1963).

Flooding

If large quantities of emergent or submerged weeds accumulate in a river channel, there are risks of ponding (raising of water levels in a channel) and flooding (inability of run-off after heavy rain to move freely along a channel) (Haslam 1976). Such risks are particularly high in lowland rivers, which may be surrounded by valuable agricultural or urban land. For example, in 1968 severe summer floods in the Fens destroyed over 24,000ha of arable crops, worth over £4.5m at 1986 prices (Miles 1976, Spencer-Jones 1986).

As the biomass of macrophytes in a channel increases, so does the drag (or hydraulic roughness, Manning's "n"), causing the mean water velocity to decrease, with an increase in water depth (Dawson 1978). Large quantities of plants, such as Ranunculus spp. can commonly cause rises in water levels of 30-50cm, or exceptionally up to two metres, if the resistance to flow is sufficient to cause storm water to back-up rather than to flow away. Increases in water depth of this order would often cause flooding (Haslam 1978).

Although, the overall plant-flow relationships are complex, the mean water velocity within macrophyte beds is significantly lower than outside the beds (Marshall 1978). The reduction of water velocities in and outside macrophyte beds, allows an increased rate of accumulation of silt. This reduces the effective cross-section of the channel available for discharge. Thus, the beds of vegetation, with little through-flow, are surrounded by deeper and faster flowing channels of water. Under spate conditions these channels cannot carry all the additional water, causing flooding.

1.2 The Management of Aquatic Habitats

1.2.1 The balance of vegetation in aquatic habitats

It is evident that aquatic vegetation can be both an essential part of a river habitat and a major problem. Below certain levels of biomass or cover, plants are desirable, but above these levels, the same species are a nuisance, and may be termed a weed problem. This dilemma, no more keenly felt than by those aiming to maintain productive fisheries, was recognised over half a century ago by Butcher (1933). Butcher promoted the importance of a good scientific knowledge of the weed species useful to fishermen, and criticized the wholesale eradication of weeds. Instead he propounded the idea of selective control of nuisance species, coupled with the encouragement of plants which provide particularly suitable habitats for fish. These attitudes are still shared by fisheries managers (Kelsall 1981).

Aquatic plants only become a nuisance, by definition "weeds", if they adversely affect man's activities. The question that is difficult to resolve is, at what level of abundance does a useful plant become a weed? The answer to that will depend upon the viewpoint of the observer. Scientists may attempt to provide formulae for determining desirable and nuisance levels of vegetation, but this approach still begs the question of what criteria should be used to decide where the dividing line, between these extremes, should be drawn (Wong and Clark 1979).

A 25% cover of vegetation in a channel has been recommended for sport fisheries (Bouquet 1978, Haslam 1978). This amount of vegetation will provide sufficient shelter for fish and their prey, without the risks of deoxygenation in the summer, or of obstructing the water surface for the casting of fishing lines. The optimum levels of vegetation cover that need to be maintained for conservation, will depend upon the requirements of the rare species, or community, that is to be protected. A high degree of management may be necessary if communities associated with an early stage of hydroseral succession are to be conserved, or if the invasion of a native habitat, by an undesirable exotic, is to be prevented (Cooke 1978).

The main difficulties arise in the cases of water resources which have to satisfy a variety of needs. These conflicting interests may perceive the optimum balance of vegetation at quite different

levels (Barko et al. 1986). A drainage engineer may have some justification in claiming that artificial channels can best serve their primary function, for which they were built, in the complete absence of plants (Cave 1981). Lowland farmers may have sympathy for this attitude if also applied to natural watercourses. There would be many anglers and other interested parties opposed to such ideas.

Guiver (1981) has pointed out that even within organisations, such as the Water Authorities (England and Wales) and the River Purification Boards (Scotland), groups, such as river engineers and fisheries biologists, may have conflicting interests.

There are encouraging signs that efforts are being increased to develop management policies for natural and artificial waterways, which involve co-operation between all interested parties, e.g., co-operation between engineers and conservationists (Robinson 1986). Such co-operation is best encouraged by the open discussion of objectives, and the exchange of new information. National and international meetings of scientists and engineers (such as the annual Robson Meeting on Aquatic Weed Research, held in the U.K. and the 4-yearly European Weed Research Society's International Symposium on Aquatic Weeds) serve great purpose in providing such opportunities.

1.2.2 Aquatic habitat management

The management of freshwater habitats has in the past, like many other human activities, followed trends and fashions which have avidly pursued each technological advance. The swing from mechanical to chemical weed control methods in the 1950's, in the developed countries at least, was an example of the assumption that technology could provide a single and cheaper, general solution to a diverse set of problems. The errors of this assumption were noted by Robson (1972), who also stressed the need for the development of ecosystem management, as opposed to the control of isolated components (Robson 1973a).

This concept, of an holistic approach to the aims and methods of managing aquatic habitats, is gathering momentum, (Anderson 1986, Mitchell 1986, Robson 1986) and there are three principal reasons for this:

1) The expanding literature on the biology of potential aquatic weed species, is providing a firm foundation for an increased understanding of the ecological interactions and functioning of aquatic systems. Autecological research on particular problem species (e.g. Whitton 1970, Dawson 1976), and more general studies of the common characteristics of weeds (e.g. Westlake 1968,1981), are important for predicting the response of aquatic habitats to perturbations, such as, dredging or herbicide applications.
The study of interactions within ecosystems, is particularly benefiting from the technological developments in computing, that have provided the capacity to handle larger and more complex data inputs and modelling techniques (e.g. Best et al. 1986).

2) The perception that the problems caused by submerged weeds are increasing (e.g. Steward et al. 1984). This may be due to a physical increase in the biomass of vegetation caused by factors, such as;

   Changes in land use and river discharge
   Increases in nutrient supply from agricultural run-off and domestic sources (Thomas 1978)
   Unintentional introduction, or spread, of exotic species (e.g. Elodea canadensis in Europe, Sculthorpe 1967)

   Alternatively, it may only be the perception of the problem that has increased, as there are greater pressures on the diverse use of water resources, from the expanding population. A combination of these two explanations is most likely.

3) In Western societies, in particular, there is an increasing awareness of environmental issues. Many human activities that influence the natural world, are coming under intensified scrutiny from pressure groups and politicians. The "Greening" of European politics is a testimony to this change in attitude.

Whichever approach to freshwater management is employed, whether by the control of one particular problem species, such as a dominating exotic, or the manipulation of the whole ecosystem, a good understanding of the range of available methods and their limitations is necessary.

Four groups of management techniques can be recognised:

1) Physical
2) Chemical
3) Biological
4) Environmental

Before expanding upon these topics, it is worth putting them into an historical perspective.

1.2.3 Watercourse management - an historical perspective

Attitudes and approaches may be constantly evolving, but the management of watercourses has been practised for centuries. Anderson (1986) provides an interesting view of the history of aquatic weed impacts and control methods. This view passes from the manual labour of cleaning irrigation and flood control channels, in the ancient Chinese dynasties through the development of mechanical removal methods at the beginning of the twentieth century. The boom in chemical control techniques that occurred in the 1950-70's, is now being complemented by the use of biological control or environmental manipulation.

The role of long-term management in creating certain floras and habitats, must not be ignored. For example, the composition of the plant and animal communities in regularly maintained artificial channels, has tended to evolve in direct response to human activities. Management is an integral part of the seasonal cycle in such systems (Robinson 1986).

Perhaps more surprising, is the observation that the submerged plants of most chalk streams, owe their existence in that habitat to man's activities, in changing the catchments from forest to intensive agriculture. The ill-defined channels would have been dominated by emergent vegetation and surrounded by alder and willow carr, with little submerged vegetation at all. Most chalk streams have been subjected to regular management, probably for centuries, so that these 'natural' rivers have been artificially evolved and maintained by human pressures (Westlake 1979).

1.2.4 General methods of submerged weed control in watercourses

The general strategies for controlling submerged weed problems have not changed much in the last twenty years (Austin 1963, Little 1968), but the specific weapons and tactics have been continuously developing (Robson 1973b, Mitchell 1974, Barrett 1978a,Anderson 1986).

1) Physical methods

This group includes manual and mechanical removal of vegetation. This may range from the selective removal of specific plants by hand, scythe-cutting or raking, to the use of weed cutting launches or the less selective removal of vegetation and substrate by weed-dredging boats or bank-based excavators (Price 1981).

2) Chemical methods

Inorganic chemicals, or more commonly, organic herbicides are applied to the plants in the water, and the vegetation is allowed to die and decay in situ (Robson and Barrett 1977).

3) Biological methods

One approach is the exploitation of biological competition between plant species so that undesirable species may be replaced by desirable ones (Yeo 1980).

Another, promising method, being investigated in the U.K. is the use of herbivorous fish, such as, the Chinese Grass Carp, Ctenopharyngodon idella (Stott 1981). Although herbivorous fish may have some food preferences, they do usually remove more than one plant species.

Parasites and insects that destroy specific plants are proving to be of great value in the tropics, particularly against floating species (e.g. Room 1986). They are currently considered to be of little value in temperate climates.

3) Environmental methods

Weed control by environmental manipulation tends not to be species selective, but involves the management of a whole, or a major part of, the habitat.

A wide range of environmental manipulation techniques, that may be employed in weed management, are being revealed,as the ecological interactions within aquatic systems, are better understood (Barko et al. 1986).

Since solar radiation is essential for primary production, the reduction of light, by various means, has been investigated (Dawson 1981). Natural shading by marginal vegetation may be encouraged, e.g. by tree planting along banks (Krause 1977, Dawson and Kern-Hansen 1978). Macrophytes with floating leaves, which do not obstruct water movement through a channel, may be encouraged, to shade the submerged vegetation (Pitlo 1978).

Artificial shading may include the use of dyes (of little value in flowing water) or the covering of substrates with opaque sheeting (Dawson and Hallows 1983). The mechanical disturbance of sediments, increasing water turbidity, caused by boat traffic, may provide weed control in navigable waterways, but in frequently used channels, this may impoverish the habitat (Murphy and Eaton 1983).

Suggestions that excessive weed growth is the result of eutrophication (Thomas 1978), have prompted ideas of environmental control of weeds by reducing the concentrations of essential nutrients to limiting levels. This might be of value in large, static water bodies where nutrients may be in low concentrations in the water. However, most rooted macrophytes can fulfil their nutrient requirements from the sediments (Barko et al. 1986). In many lowland watercourses the naturally occurring concentrations of nitrates and phosphates, are usually well above limiting concentrations (Ladle and Casey 1971, Casey and Westlake 1974, Dawson 1978). Despite this, efforts should still be made to prevent further increases in nutrient loading because eutrophication of static waters, may result in the replacement of rooted macrophytes with blooms of phytoplankton, which may be less desirable (Philips et al. 1978). Problems with algae may also occur in streams, if nutrient concentrations are artificially increased (Kern-Hansen and Dawson 1978).

Another type of management, which might be given its own grouping, is that of vegetation utilisation. Here, the economic losses caused by excessive aquatic vegetation, are turned to gains by the use of the vegetation for food, stock-feeding, fertilizers, fuel, or fibre. Such utilisation is likely to be of most importance in developing countries where labour costs are low.

CHAPTER TWO

A REVIEW OF THE USE OF WEED CUTTING AND AQUATIC HERBICIDES AS WATERCOURSE MANAGEMENT POLICIES

2.1 The Management of Submerged Macrophytes by Cutting and Removal

2.1.1 Methods of cutting weeds

Despite the fact that the physical removal of aquatic weeds has been practised in watercourses for centuries, there is surprisingly little literature on the subject. It is probably because it has been practised for so long and is accepted as a traditional, almost natural, annual course of events in many lowland rivers, that research on the subject has seemed unnecessary.

There is also the problem that there are few similar enough rivers that have not been managed, for comparison with those that are regularly maintained. A site would have to be left unmanaged for several seasons before comparisons could be made with an annually cut site, to ensure that a recovery period was not being sampled.

Much of the literature that does exist on the cutting of macrophytes has concentrated on lowland chalk streams in southern England. This is because these are the types of rivers in which excessive plant growth most frequently occurs and causes problems in the U.K. Also many of the authors have been based at the Freshwater Biological Association's (F.B.A.) River Laboratory in Dorset, which is sited near a large chalk river, the River Frome. Much of the rest of the research in the U.K. has been concerned with artifical waterways, canals and drainage ditches.

The specific method of cutting chosen in any instance will be determined by factors such as the cost of equipment or labour, the type of management required and the accessibility of a site. Labour costs are so high in the U.K. that manual cutting and removal are usually only employed in watercourses that are too small or inaccessible to machinery, or where very selective removal is required, such as removing specific weed beds from a trout fishery.

Excavators with weed cutting buckets are sometimes used in rivers but they tend to be limited by their reach, usually about 12m, and the need for clear access along the banks. These machines, either hydraulic or dragline excavators, are commonly used for drains and ditches where a thorough clearance of vegetation is needed. This equipment has the advantage of cutting and removing material in one process.

Probably the most important method of weed cutting and removal regularly carried out in rivers and canals is the use of cutting boats. Summaries of the types of boats available are given by Price (1981) and Robinson (1986). An essential feature must be a means of propulsion that will cope with weed-filled waters, such as paddles or an Archimedian screw. Cutting and removal are usually two separate processes. In waters with slow flow the same boat may be used for weed removal with a rake attachment, fitted after the cutting is finished. In rivers where cut material is immediately washed downstream, booms are placed across the channel so that the vegetation is caught and can be removed by an excavator from the bank.

2.1.2 The timing and periodicity of cutting

The vegetation in many lowland rivers in southern England is cut twice a year, in the spring and summer. The timing of the first cut may be influenced by a variety of factors but is chiefly determined by an estimation of flood risk. This judgement is based upon observations of plant cover and the water discharge during the spring. In popular trout fisheries, such as the River Frome, there is the additional requirement to avoid, usually by preceding, the Mayfly (*Ephemera danica*) hatch which occurs in late May.

The timing of the summer cut is also based on visual estimations of plant cover and the expectation of storm discharges. The former consideration will mean that the time of the second cut is related to the timing and efficacy of the first one, and to the intervening growth conditions. The timing and efficiency of weed cuts in the River Frome carried out over thirty years have been studied by Westlake and Dawson (1982), with the objective of discovering what factors influence the execution and success of this annual management.

A consequence of this research, when combined with autecological studies of the dominant species, *Ranunculus penicillatus* var. *calcareus*, has been the proposal of a modified management regime.

Close autumn cuts reduce the cutting effort needed in the following spring and may, in favourable conditions, result in a need for only one limited cut a year, giving a considerable financial saving (Westlake and Dawson 1986).

There is also evidence to suggest that in many cases cutting may actually increase the total plant biomass and productivity, so that plants are being pruned back and are stimulated to regrow more vigorously. Dawson (1976) suggested that cutting, in the River Frome, only reduced the total macrophyte biomass by two-thirds and the consequent reduction in water level increased the light reaching the remaining vegetation so that submerged regrowth was very rapid. In the smaller Bere stream (a tributary of the River Piddle) he observed that when a traditionally-cut site was left unmanaged for four years, the plant growth was reduced to half the previous maximum biomass so that cutting was no longer necessary. The percentage cover of the stream bed was not significantly reduced over the same period, indicating that changes in plant density within weed beds had occurred and that cover is not necessarily an accurate indicator of standing crop.

A critical factor in the effects of cutting on regrowth and annual biomass is the timing of the cut in the growth cycle of the plants. Experiments in which macrophytes were cut and removed from 5-10m wide bands in a Ranunculus dominated chalk stream, in April, May, and June, showed the important relationship between the time of the cutting and of flowering. Cuts prior to flowering did not control growth but stimulated an extensive regrowth at the time that uncut plants would have been washed-out after flowering. Cutting at the time of flowering was recommended as the most effective way to control summer growth. However if the plants were left to flower in May-June, the natural die-back, as the weaker flowering stems were lost, removed the problem so that no cut was needed at all (Ham et al. 1982).

Unfortunately, in most rivers the plant biomass considered to present a risk of flooding or disruption of fishing, is reached well before flowering, and consequently pre-flowering cuts are made. The regrowth that this stimulates may then require cutting later in the summer, resulting in the costly double cut per season. It is hoped that alterations in this cutting pattern, with autumn cuts, may remove enough of the over-wintering inoculum to reduce the plant biomass and

flooding risks. This would allow the natural post-flowering die-back to replace the two cuts.

Annual river cuts may have been regarded as inevitable for a long time, but it is evident that management regimes can be improved with the application of knowledge gained from both autecological studies and manipulative field trials.

2.2 The Management of Submerged Macrophytes Using Aquatic Herbicides

2.2.1 The regulation of aquatic herbicide use in the U.K.

The use of organic herbicides in agriculture has rapidly increased since the development, for military use, of the phenoxy-acetic acids, at the end of the Second World War (Murphy 1983). The deliberate use of organic herbicides in aquatic systems has been a more recent development.

Some chemical control of nuisance plants in freshwater was carried out with copper sulphate as early as 1905 and other inorganic compounds such as sodium arsenite were used, often as quite efficient herbicides, but with devastating side-effects on non-target organisms such as fish.

A rapid expansion of the use of organic chemicals in or near water occurred in the 1950's but only two compounds, glyphosate and fluridone, have come onto the market since 1976 (fluridone,only in the U.S.A.) (Anderson 1986). There is little prospect of any new aquatic herbicides coming onto the market in the foreseeable future (Robson 1978, Spencer-Jones 1986).

Due to the potential damage that misused herbicides might cause, not only in the aquatic environment to which they are applied, but also to other areas to which treated water is transported (e.g., irrigated crops, domestic water supply), the regulations concerning the use of aquatic herbicides in the U.K. are strict compared with those for terrestrial herbicides.

Pesticides have had an unfavourable public image since the alarming stories, and genuine mistakes, of the early development and distribution of various pesticides (principally insecticides) was high-lighted, over twenty years ago, in Rachel Carson's book "Silent Spring" (Carson 1962). The current concern about the application of these artificial chemicals to the aquatic environment has resulted in the banning of their use in some European countries (e.g., Denmark). In the U.K. this concern has ensured that all formulations of chemicals for aquatic use do not reach the public market until certain standards of safety have been proven.

A pesticide cannot be sold in the U.K. until it is cleared under the Pesticides Safety Precaution Scheme (PSPS). For this, data are required concerning:

1) Specifications of the compound
2) Mammalian toxicity, especially risks to users and consumers of treated material
3) Fates of residues
4) Effects on wildlife and the environment.

Prior to 1986 PSPS was a voluntary system of registration which was only formally recommended, and there was no "Pesticide Law" as such (Makepeace 1971). There were four levels of clearance depending upon the progress that had been made in fulfilling the provision of data for the requirements above:

a) Trials
b) Limited
c) Provisional
d) Full commercial (Thomas 1981).

In addition to PSPS most herbicides were also subject to the voluntary Agricultural Chemicals Approvals Scheme (ACAS). ACAS certified that field trials had been carried out that showed that the product acts efficiently against targets weeds, if used correctly (Makepeace 1971).

In October 1986 the Control of Pesticides Regulations 1986 were brought into operation under Governmental Statutory Control. The PSPS clearance and ACAS approval have been replaced by a single 'Approval' operation, implemented as part of the Food and Environment Protection Act 1985.

Three levels of approval have been defined:

a) Trials clearance

b) Limited approval

c) Full approval

Limited approval would have to be obtained before a product could be sold (Tooby 1986, Control of Pesticides Regulations 1986)

In addition to these regulations, which cover many aspects of the sale, storage and use of any pesticides, there are various laws which may be broken if aquatic herbicides are misused. These Acts are listed by MAFF (1985). Laws covered by the Salmon & Freshwater Fisheries Act 1975 and the Rivers (Prevention of Pollution) Acts 1951-1961 might, for example, be broken if water was deemed to have been poisoned or polluted by the application of un-approved chemicals or if manufacturers' recommendations were not followed.

The requirements for approval do not include the long-term and full-scale field trials necessary to evaluate many of the ecological effects of herbicides. There is also much debate about the validity of the toxicological studies that were specified under PSPS. Various suggestions have been made for improved schemes to provide a better assessment of such potential environmental hazards (Price 1976, Tooby 1978,1981).

In the U.K., in 1986, nine herbicides and one growth retardant (maleic hydrazide) had been cleared for use in or near water. There is at least one approved formulation of each compound (MAFF 1985).

Asulam
2,4-D amine
Dalapon
Fosamine ammonium
Glyphosate
Maleic hydrazide

Chlorthiamid
Dichlobenil
Diquat
Terbutryne.

The latter four are suitable for controlling submerged weeds and may be applied directly to static water. By comparison 8 chemicals are approved for use in water in the U.S.A. (Thayer et al. 1986).

The most appropriate chemical for a particular problem can be selected by reference to Hellawell and Bryan (1982) and the MAFF Guidelines (1985).

Until 1982 none of the four aquatic herbicides was recommended for use in water flowing at more than 90m per hour because liquid applications of even the fastest acting herbicide, diquat, were removed by the flow from the target sites, before achieving a phytotoxic effect on the plants (Barrett 1978b).

2.2.2 Herbicide formulations for slow-release, localised placement or use in flowing water.

Even in static water some of these aquatic herbicides (e.g., terbutryne) have to be applied in slow-release formulations. In the case of terbutryne,this is because at the low doses which are acceptable, under its approval, the chemical acts very slowly and has to be maintained in the water at the recommended concentration of 0.1mg l^{-1} for several days. A variety of slow-release formulations have been developed, including granules, pellets, and impregnated rubber granules (Robson and Barrett 1980, Terry et al. 1981, Murphy 1982).

These formulations will provide some localisation of effect, but over the seven day periods for which the doses of chlorthiamid, dichlobenil, and terbutryne must be maintained, it is inevitable that some dispersal of water by wind, convection currents or in/out flow will occur. In view of the lack of new chemicals being brought onto the market, the development of formulations that will improve the application of existing herbicides has been, and will be, important (Robson 1978, Barrett 1981a).

For localised control in static water, and certainly for use in flowing waters, a fast-acting chemical is required. The recommended exposure period for diquat is 24 hours so that a reasonable localisation of control in static water might be expected. Diquat has a short persistence time and is readily adsorbed and biologically inactivated on many substrates,or suspended solids in the water (Simsiman and Chesters 1976). A slow-release formulation of diquat is not required, but a formulation which would place the chemical onto the plants.

Invert oil emulsions (e.g., hydrophobic carriers, diesel oils, treated citrus oils) have been regularly used for the application of

diquat to waters (often with copper sulphate), in the southern states of America (e.g., Haller et al. 1983). However, the preparation of invert mixtures requires bulky equipment and considerable skill by the operators.

An alternative carrier of diquat, for localised placement in static water, was developed by Barrett (1978b). It also became evident that this formulation could be used in flowing water. The carrier was sodium alginate, which is obtained from brown seaweeds (Phaeophyceae).

Sodium alginate is a soluble salt, but several of the other salts, such as calcium alginate, are insoluble. The viscosity of a solution of sodium alginate will depend upon its concentration, the temperature, the degree of polymerisation of the alginate, and the ratios of the polysaccharide acid residues, mannuronic and guluronic acids. The addition of a small proportion of a base, such as calcium, that forms an insoluble alginate, can cause a marked increase in the viscosity of the alginate (McDowell 1973).

Barrett (1978b) described how the calcium in natural waters can react with aqueous sodium alginate to form a gel of calcium alginate. This gel becomes entangled amongst, and sticks to, weeds so that little drift in currents or downstream flow will occur. Diquat behaves as a contact herbicide, with little translocation, in aquatic plants (Funderburk and Lawrence 1963a, Davies and Seaman 1968). The adherence of the diquat to the plants should ensure not only a localised, but also a more efficient control.

Development and trials carried out over several years (Barrett 1978, 1981a, 1981b, W.R.O. 1980, 1981, Barrett and Murphy 1982), resulted in 1982 in a formulation that was cleared and approved as a commercial product, Midstream. Midstream is produced by Imperial Chemical Industries (I.C.I.) and is marketed in the U.K. by Midox Ltd. Diquat-alginate is available in New Zealand under the Trade name 'Torpedo'.

2.2.3 The behaviour of diquat-alginate in solution

The formulation of diquat-alginate used in Midstream is a dark brown liquid with the viscosity of thick syrup. If applied in strings to the surface of static water the gel, formed on reaction with calcium in the water, will sink down through the water column. Strings that land on the surface of weed beds will then drip down between the plants. The rate of this dripping will depend upon the viscosity of the gel, which is determined by the proportion of calcium alginate that forms.

In flowing water the alginate strings will be broken up by the flow but the resulting blobs, or 'tadpoles' of gel will still sink through the water and entangle on the plants. The velocity and depth of the water will determine how far the tadpoles will be carried downstream before landing on the target plants.

In unpublished laboratory experiments, it was shown that if diquat and alginic acid were combined in known quantities and placed in dialysis bags, the diquat could not be washed out with distilled water. Diquat is either held within the alginate matrix by strong physical forces or, more likely, a chemical combination of the diquat cation with the alginic acid forms the diquat-alginate salt. It is not known whether, or under what conditions, this salt may ionise in solution. When the diquat-alginate mixture was washed with a calcium solution, all of the diquat was recovered, probably displaced by cation exchange (Barrett pers.comm.)

The rate of release of diquat from a diquat-alginate gel is proportional to the calcium ion concentration of the water around, or flowing over, the string (Barrett 1978b). Thus, the calcium concentration has two important roles in influencing the behaviour of diquat-alginate:

1) Determining the viscosity of the alginate gel, and hence its resistance to dispersal
2) Determining the rate of displacement of the diquat from the alginate

There are several factors, other than the calcium ion concentration, which influence the displacement of diquat from the alginate. One of these factors is the surface area to volume ratio of the strings or tadpoles of gel (Barrett 1981b, 1981c). This ratio will be

determined by the structure of the macrophyte substrate and the force of the water stretching or flattening the gel. These considerations mean that the rate of release of diquat, from the alginate gel, is very difficult to predict.

The alginate carrier has two functions:

1) To hold the diquat against the target plant, ensuring a close physical contact
2) Prolonging the release of the diquat after the moment of application, so that the whole dose is not available in solution, for immediate dilution and dispersal.

2.2.4 Comparisons of the activities of diquat-alginate and diquat solutions

A variety of experiments have been conducted in the field and laboratory, in static and flowing water, to compare the efficacy of diquat-alginate with the standard liquid formulation of diquat, Reglone.

In a series of unpublished experiments in small flowing water channels, Barrett and Newman (pers.comm.) compared the loss of diquat from applications of Reglone and pure diquat-alginate, in the presence and absence of plants. Pure diquat-alginate contains no unbound diquat so that the exact amount of diquat applied is known. The alginate formulation was diluted to the same viscosity as water, and calcium-free, deionised water was used throughout.

All of the diquat applied to the empty channels as Reglone was recovered, but some of the herbicide was lost from the alginate formulation. Some of the polymers of the diquat-alginate were assumed to have become tangled and caught on the surface of the channel. The 'lost' diquat could be recovered by flushing the channel with a calcium solution.

In the presence of plants there was a reduction in the amount of diquat recovered after an application of Reglone, indicating that some of the herbicide had been adsorbed by the plants. There was a significantly greater reduction in the recovery of diquat after the application of diquat-alginate, in the presence of plants.

Reglone applied over a two hour period to Ranunculus beds in the River Eden, produced few phytotoxic symptoms. Diquat-alginate, diluted to the same viscosity as the Reglone, applied under the same conditions, was almost as effective in damaging the Ranunculus, as the

undiluted diquat-alginate, which completely removed the target beds (Barrett 1981b,1981c). The improved herbicidal effects of formulating diquat with alginate are not just the result of the physical increase in viscosity. Even at the same viscosity as Reglone, there is a greater retention of diquat-alginate molecules, by plants, than of the much smaller diquat dibromide molecules found in Reglone.

The fact that Reglone only started to cause phytotoxic symptoms if it was applied over a period of two hours but not if only over forty minutes, suggests that the improved performance of diquat formulated in alginate may, also, be related to a delayed release of the herbicide.

Further comparisons of different formulations of diquat, in flowing water, have shown that alginate formulations are always more effective than the diquat dibromide solution (W.R.O. 1980). Similar comparisons were made in static water. These experiments showed that although Reglone often caused visible phytotoxic effects, the weed control provided by the diquat-alginate was usually more localised and effective (W.R.O. 1980, Clayton and Tanner 1982).

2.2.5 The application of diquat-alginate and its use since receiving PSPS clearance

In all the experiments discussed so far, the diquat-alginate was applied by being poured directly into the water or by injection from hand-held syringes. This slow and labour intensive method was obviously not suitable for large-scale applications. In New Zealand, for example, Reglone had been applied to large areas of water from boats or helicopters (Clayton and Tanner 1983).

Field trials were conducted in flowing and static waters in which diquat-alginate of various viscosities was applied from a modified knapsack sprayer, using nozzles of a range of sizes. These trials indicated that there was little difference in herbicidal efficacy between the formulations with high or low proportions of alginate. The size of alginate strings produced by the different nozzles, did not appear to influence the efficacy of the herbicide either. The use of the low alginate formulation (1.0% alginate) and a 2mm nozzle was found to be operationally most convenient. The 2mm nozzle allowed a delivery range of up to 10m at a reasonable delivery rate (W.R.O. 1981, Barrett and Murphy 1982). Motorised pumping equipment, suitable for large-scale applications, such as from boats

or helicopters, is described by Clayton and Tanner (1983).

It was concluded from these, and other experiments, that the dose rates for diquat-alginate could be calculated per unit area of water surface to be treated. Although water depth did not appear to influence the efficacy of the herbicide directly, the volume of water treated will affect the final concentration of diquat (Barrett and Logan 1982). The maximum concentration of diquat permitted under PSPS is 2.0mg l^{-1}.

One litre of Midstream contains 100g of diquat. The application rates recommended by the manufacturer are:

0.5 litre per 100m^2 water surface for water less than 30cm deep

1.0 litre per 100m^2 water surface for water more than 30cm deep

Since the release of Midstream onto the commercial market, most will have been used by, Water Authorities, River Purification Boards, Internal Drainage Boards and the British Waterways Board, in field trials or management programmes. Only a few of these applications will have been reported (e.g. Logan 1984). Barrett collected questionnaires from 39 trials carried out by these organisations (W.R.O. 1980, 1981). The success of these trials, carried out in static and flowing waters, under a wide range of environmental conditions, was variable. It was one of the aims of this project to try to ascertain which environmental factors might be important in influencing the efficacy of Midstream.

2.3 Watercourse Management Policies

2.3.1 Factors determining the management methods used at specific sites

The choice of management method for a specific site is principally based upon four criteria:

1) Extent and type of the weed problem

   e.g. Is the extent of the problem too large to employ manual removal methods?
   Are the problem species susceptible to herbivory, or to a particular herbicide?

2) Physical charactersitics of the site

   e.g. Size, could a cutting boat manoeuvre in the channel?
   Accessibility, could a bank-based excavator get to the whole site?
   Flow, is the flow too swift for a slow-acting herbicide?

3) Uses of the watercourse

   e.g. Does some vegetation need to be left for fish?
   Will water need to be extracted for irrigation when there might be herbicide residues present?

4) General management policies of the organisation with overall responsibility for the site

   e.g. One Internal Drainage Board and some Water Authorities do not use herbicides if alternative methods are possible (Spencer-Jones 1986)

2.3.2 Factors determining general management policies

There are two main issues to be considered for sites where there is more than one option for a method of weed control, or if a general management policy to cover a range of sites has to be chosen:

1) Economics

2) Safety to operators and the environment

The question of which of these issues should be, or is, most important, may immediately depend upon available resources. Ultimately, however, resource allocation and the decisions about priority, are matters of local, national, or possibly international, politics.

1) Economics

There must be a balance, in assessing the economic issues of aquatic weed management, between the estimated cost of a problem, in terms of the consequences of failing to carry out the necessary weed control, and the cost of the method involved. These 'costs' cannot all be given monetary values, e.g., maintaining the aesthetic value of a site, conservation of a native habitat threatened by an exotic species.

The costs of failing to provide adequate weed control may be realised very quickly, e.g., the loss of £4.5m worth of crops in the Fen floods of 1968 (Miles 1976). The cost of management may be dependent upon longer-term calculations. For example, manual weed control methods are cheap on equipment but labour costs,in the U.K., are becoming increasingly expensive. One Inland Drainage Board employed over 70 people to carry out weed control, 35 years ago, but in 1981 only 25 staff were employed (Price 1981). Mechanical methods require large capital expenditure but tend to have a low manpower requirement, with fairly low running costs. Chemical methods have low capital and manpower costs but the herbicides are expensive 'consumables'.

The efficiency of a weed control method has to be included in the economic assessments e.g., whether a single herbicide application will control the vegetation for one, or more, season, or whether more than one weed cut per year is necessary. This factor of efficiency, can be highly site-specific and difficult to predict. Confidence in the efficacy of a method may be important in deteriming the risks vs.

cost issue. In this respect, tradition may have a major advantage over innovation.

Economic comparisons of different weed control methods need to be related to particular sites or, at least, to certain types of site. Comparisons made at specific sites cannot be used to provide general principles for weed management, but may indicate the relative costs and effects of the methods if used under similar environmental conditions.

In 1972 Brooker and Baird (1974) made a direct comparison of the use of chemical and manual methods of controlling emergent vegetation in 1345km of watercourse in Essex. They estimated that the annual cost of the herbicides was 50-75% of the cost of the manual method, assuming that the herbicide was effective for four years. In 1975, although the actual management costs had doubled, there was still a net saving of 25-50% per year using herbicides. Some of the herbicides had been effective for up to seven years and so had reduced the annual costs (Brooker 1975).

In land drains the suitability and costs of weed control methods depend upon the drain width and depth. In a range of these conditions manual weed control costs 2-4 times as much as the use of herbicides, and ten times as much if the problem is caused by filamentous algae. The use of herbicides in these drains costs 3-7 times as much as boat cutting and 1½ times the cost of cutting bucket excavation. These comparisons were calculated for the expenditure per 100m length of drain and do not compare how effective the methods are over several seasons (Cave 1981).

Some examples of the estimated costs of aquatic weed control using cutting or chemical methods, are presented in Table 1.

The cost-effectiveness of any management regime will depend upon how carefully the weed control methods are applied. Applying more herbicide than is needed to achieve the required weed control, is an obvious wastage. However,the costs of more traditional methods may also be reduced. It may be possible to halve the normal annual costs of weed cutting if the timing of the cuts is improved. If it costs £30,000 a year to cut one river, River Frome, there would be a considerable saving if only half the number of annual cuts were needed (Westlake and Dawson 1986).

Table 1

Some examples of the estimated costs of aquatic weed control in watercourses in the U.K.

Year	Cost	Method	Sites	Reference
1972	£2.5m p.a. £6.0m p.a.	Herbicides Weed cutting	55,000-60,000km main rivers and tributaries in U.K.	Robson (1972)
1982	£350 km^{-1}	Boat cut	R.Frome, Dorset	Westlake and Dawson (1982)
1983	£400 ha^{-1} £500 ha^{-1} £250-£11,000 ha^{-1}	Single cut Herbicide Typar screen (in water shading)	Chalk stream	Dawson and Hallows (1983)
1986	£100-£600 km^{-1} £750-£1000 km^{-1}	General weed control Dredging	Regularly maintained drainage channels	Robinson (1986)

Unless new biological or environmental management methods are developed, it is likely that most reductions in the costs of aquatic weed control will only result from the more efficient application of current methods. New aquatic herbicides are unlikely to appear on the small British markets, when it is estimated to cost £10m-£16m to develop a new chemical. This is a worrying situation for the engineers anxious for an efficient algicide (Cave 1981). However, it may be possible to improve the range of applications of familiar compounds e.g., hydrogen peroxide (Fowler and Barrett 1986).

2) Safety

a) To operators. The execution of any weed control method is covered, in the U.K., by the Health and Safety at Work Act 1974. The operational instructions for machinery and chemicals must be followed, with no short-cuts taken in the recommended procedures, in order to save time or money. Under PSPS chemical manufacturers were obliged to provide adequate instructions for the safe use of any herbicide. Instructions for remedial action to be taken in cases of accidental spillage or contamination, also had to be provided on the product label. These requirements still have to be met under the Control of Pesticides Regulations, 1986 but the product label has become a 'legal document' (Tooby 1986).

The potential risks of most aquatic weed control methods have been discussed by Leafe (1981).

b) To the environment. The increasing public awareness of environmental issues (mentioned in section 1.2.2) means that the ecological consequences of aquatic management are no longer just the concern of those in the water industry, anglers or naturalists.

The following section is intended to provide a summary of some of the ecological effects that may result from aquatic management by weed cutting or herbicide application. Most of these topics will be discussed in greater detail in later chapters. Example references have been provided where appropriate.

2.4 The Ecological Effects of Watercourse Management

The ecological effects of any management regime can be divided into two categories:

1) Direct effects of the management (including its efficacy on the target plants), specific to the control method. These tend to be short-term effects.

2) Indirect effects resulting from the removal or death of the target plants. These effects may be specific to the control method or may be the general consequence of a common result. Some of these effects may be long-term.

2.4.1 The ecological effects of aquatic management by weed cutting

1) Direct effects.

Effects of the physical disturbance caused by the cutting process.

e.g. Disturbance of sediments causing an increase in water turbidity (Carpenter and Gasith 1978).

Disturbance of invertebrates within, or emerging from, weed beds, causing increased dispersal by drift (Kern-Hansen 1978).

Loss of organisms or chemicals from the habitat.

e.g. Loss of macrophyte-associated invertebrates when cut vegetation is removed to the river bank (Dawson 1984).

Loss of fish harvested by weed cutting and removal machines (Haller et al. 1980).

Exudation of organic and inorganic nutrients from cut stem stumps (Carpenter and Gasith 1978).

2) Indirect effects.

The indirect ecological effects specific to weed cutting methods are related to the sudden removal of the plant biomass, rather than a slow decay or wash-out.

a) Sudden loss of substrate for macrophyte-associated invertebrates which may be repeated if cutting progresses downstream, following the drift. This may lead to an overall loss of some invertebrates from a cut area e.g. Brachycentrus subnubilus (Trichoptera) (Gunn 1985). Other species may be able to migrate from the plants to the substrate, with an eventual recolonisation of any plant regrowth (Pearson and Jones 1978)

b) Sudden loss of shelter for fish and fish-food may result in changes in fish behaviour, or the loss of fish normally associated with macrophytes, from the cut area (Swales 1982).

c) Sudden drop in water levels, which may expose fish eggs laid near the water surface. e.g. Roach eggs on upper stems of the moss Fontinalis antipyretica (Mills 1981).

d) Sudden removal of dominant plant species at specific times in their life-cycles, may alter the subsequent species succession and dominance patterns (Soulsby 1974, Dawson et al. 1978).

2.4.2 Ecological effects of aquatic management using herbicides

Several reviews of the ecological effects resulting from the application of aquatic herbicides, have been written (Brooker and Edwards 1975, Newbold 1975, 1976, Robson and Barrett 1977). The effects of the bipyridinium herbicides (i.e. diquat and paraquat) have been specifically reviewed by Calderbank (1972) and Summers (1980).

1) Direct effects.

The direct ecological effects of herbicide treatments largely depend upon the concentrations of residues available to non-target organisms. This is particularly important for persistent herbicides, such as dichlobenil. Diquat has a short persistence in water, being rapidly removed by adsorption onto plants and sediments. Once adsorbed, diquat is biologically inactive and desorption back into solution is unlikely (Simsiman and Chesters 1976).

The toxicity of the bipyridinium herbicides to non-target organisms has been reviewed by Summers (1980). Because of the short persistence and reasonably low concentrations of diquat applied to water, there are few non-target organisms that are likely to suffer direct toxicity effects from the correct use of diquat.

There are four groups of organisms that might be directly affected by the use of aquatic herbicides:

a) Aquatic invertebrate fauna, which may be important links in the aquatic food chain, e.g., Daphnia magna, Cladocera (Crosby and Tucker 1966, Gilderhus 1967, Wilson and Bond 1969).

b) Fish may suffer from acute toxicity effects of the immediate herbicide application (Earnest 1971, Tooby 1971), or from chronic, possibly sub-lethal, effects that may influence behaviour or the survival of different stages of the life-cycle, e.g., eggs or fry (Tooby 1976).

c) Consumers of treated water supplies, e.g., livestock, humans (Goulding 1981). The toxicity of diquat and paraquat to a variety of mammals, including man, is reviewed by Summers (1980). In all cases, the toxic concentrations far exceed the concentrations that would occur as a result of aquatic weed control.

d) Crops irrigated with treated water. The minimum time interval between the herbicide application and use of water for irrigation, has to be specified on the product label. For diquat this period is 10 days, or when concentrations are less than 0.02mg l^{-1} (MAFF 1985).

2) Indirect effects.

There are several, potential, indirect ecological effects of aquatic herbicide use, some of which are related to the changes in water chemistry which may result from the in situ decay of treated vegetation.

a) Reduction in the concentrations of dissolved oxygen.
Herbicides which inhibit photosynthesis but not respiration e.g. terbutryne (Murphy 1982), can cause significant reductions in the dissolved oxygen concentrations of static waters. Fast-acting herbicides,which cause a rapid kill and in situ decay, e.g. diquat, can also reduce dissolved oxygen concentrations in static water. If the oxygen consumption,by the microorganisms causing the plant decay, is sufficient to reduce the concentrations below the tolerance levels for the fish, then fish-kills may result (Earnest 1971,Brooker 1974). The problem of deoxygenation is less acute in flowing waters, which receive a constant input of fresh water from upstream, and in which reaeration is greater than in static water (Gameson and Truesdale 1959).

b) It might be anticipated that the decay of vegetation in situ would result in the release of plant nutrients (e.g. nitrates and phosphates) into the water, causing eutrophication. The evidence for this in static water, from laboratory (Nichols and Keeney 1973) and field experiments (Walker 1963, Strange 1976), is limited and contradictory. The nutrient balance achieved after a large amount of in situ decay, is likely to be affected by many factors, such as the ambient dissolved nutrient concentrations, the nutrient sinks and the sediment types.

c) The plant species removed by the herbicide may be replaced by a succession of species, less susceptible to the herbicide. This succession is usually by rapidly colonising, opportunistic species, such as filamentous algae (Robson et al. 1978), or by moderately susceptible species, such as Chara spp., Nitella spp. (Fish 1966, Brooker and Edwards 1973a). Herbicide resistant biotypes of some weeds,occurring in terrestrial environments which are repeatedly treated with herbicides, (e.g. croplands) have developed (Murphy 1983). This problem, of resistance, has not yet become evident with the herbicides and treatment regimes used in freshwater.

2.4.3 General indirect ecological effects of watercourse management

There are a variety of complex changes that may occur in an aquatic system if all, or part, of the vegetation is removed.

1) Reduction in primary production and the photosynthetic evolution of dissolved oxygen.
2) Loss of habitat for epiphytic organisms, e.g., macrophyte associated macroinvertebrates (Hilsenhoff 1966). Loss of shelter for fish, e.g., the reduction in physical complexity of a stream habitat can result in increased territorial behaviour and mortality in trout fry (Mortensen 1977).
3) Changes in physical characteristics of a water body, e.g., lowering of water levels, increased mixing of water and sediments, wash-out of silt accumulated in weed beds.
4) Changes in water chemistry associated with alterations in the photosynthetic balance of dissolved gases, removal of the macrophyte nutrient sink, and the disruption of chemical cycles associated with the sediments (e.g. Small et al.1985).

Whichever method of macrophyte removal is used, the severity and importance of the direct, and indirect, ecological effects can be reduced by sensible management procedures. For example, treating only parts of a water body at one time, so that sufficient plants remain to prevent anoxic conditions from developing (Brooker 1974), and to provide an untreated refuge for the fauna (Kern-Hansen 1978).

Careful timing of treatments, when the reduction in biomass will not be too severe, is also important. Management should be timed to avoid the most susceptible stages of invertebrate and fish life-cycles (e.g. periods of insect emergence or fish breeding). Herbicide treatments that might cause reductions in dissolved oxygen should be avoided in periods of high temperatures when decay rates and oxygen demand will be greatest (Newman 1967).

2.4.4 Long-term ecological effects of watercourse management

Long-term ecological effects of watercourse management have been studied at three levels:

1) Studies, carried out over several decades, of changes in aquatic biology, not related to specific management regimes.
   e.g. Changes in the river flora of the U.K. in relation to changes in water use and management (Haslam 1981,1986).
   National impoverishment of the aquatic flora, by the reduction in distribution of common species and the loss of rare species, due to the losses and changes of aquatic habitats (Newbold 1981).

2) Studies of particular habitats that have been subjected to specific, repeated management regimes.
   e.g. Re-initiation and maintenance of hydroseral succession by regular excavation of drainage channels (Wade 1978).
   Influence of repeated weed cutting in determining the character of chalk streams in the south of England (Westlake 1979).
   Annual cuts or herbicide treatments of a lake causing little change in species richness but major changes in the species predominating and their relative abundances (Nicholson 1981).

3) Studies of the recovery of aquatic communities after single, or infrequently applied, management regimes.

e.g. Lake community recovery over one year, after a single application of paraquat (Way et al. 1971, Brooker and Edwards 1973a, 1973b, 1974).

Recovery of macrophyte communities over 2-3 years after one or two herbicide applications (Wade 1982).

Studies of the recovery of aquatic communities are important so that the effects of repeated treatments can be estimated. This information would be useful in assessing the frequency with which weed control methods can be efficiently and safely used (Wade 1981).

In assessing the potential damage to a habitat caused by repeated management, it is necessary to establish which characteristics of a flora are most desirable. In some situations the preservation of a particularly rare species or community will be most important, in other cases a maintenance of floral diversity will have priority. Management may be necessary to maintain certain, especially early, stages of hydroseral succession. Floral diversity may also be increased if the management suppresses the dominant species and increases the niches for the less competitive species (Wade 1982, Cooke 1986).

Repeated applications of the same management regime may have deleterious long-term effects if species which are rapid recolonisers or are less susceptible to the weed control method, are selected (Nicholson 1981). This may be avoided if a combination of management methods are used or if the regime is modified to selectively remove the advantaged species. For example, in Egypt, regular canal clearance promoted the growth of Potamogeton pectinatus from tubers. If the cutting was correctly timed, prior to tuber formation, the plant species recovery pattern could be changed (Bliek et al 1982).

The recovery of aquatic communities, after management, has been mostly concentrated on the target organisms, the macrophytes, but some studies have considered other organisms. The rate, and magnitude, of the recovery of macroinvertebrates and fish after management by use of herbicides or cutting, are dependent upon the rate and extent of the recovery of the flora. Recolonisation will usually be complete if untreated refuges, suitable for the fauna, are available adjacent to the treated areas (Harbott and Rey 1981, Swales 1982).

CHAPTER THREE

A LABORATORY INVESTIGATION OF ENVIRONMENTAL FACTORS WHICH INFLUENCE THE ACTIVITY OF DIQUAT-ALGINATE

3.1 Introduction

In assessing the most suitable management regime for a particular site, it is necessary to have an idea of the efficacy of the methods available. This knowledge is not only important in terms of economics, but also for estimating the ecological effects, specifically the indirect effects, that removal of the macrophytes will have. A very efficient method, removing all the vegetation, is likely to have severe ecological effects. An inefficient method will cause less disruption to the ecosystem, but by not achieving the intended level of control, will have wasted resources.

Apart from direct toxicity tests and studies of the effects of herbicides on micro-organisms (e.g., Kersting 1978, Cragg and Fry 1984), studies of the ecological effects of management methods are usually carried out in the field. Efficacy experiments, however, may be conveniently conducted in laboratory conditions, where influences of individual environmental factors, can be controlled and examined.

Assessments of the efficacy of mechanical cutting methods are usually, because of the size of the machinery, carried out in the field. Studies comparing the severity and timing of cuts, and the effects on different plant species,have been discussed in Section 2.1. One of the most important factors determining the efficiency of a cutting method, is the subsequent rate of plant regrowth. The regrowth of various submerged macrophytes under a range of environmental conditions has been studied in the laboratory (Agami and Waisel 1986).

The efficacies of herbicide treatments are not only determined by the susceptibility of the target species (e.g. White 1963), and the timing of the applications (e.g. Barrett and Murphy 1982), but a variety of environmental influences may be involved.

Despite being an ACAS Approved product (see Section 2.2.1), cases have been reported in which the use of Midstream has resulted in poor weed control, even though applied to susceptible species (W.R.O. 1980, 1981). Some of the environmental factors known to influence the activity of the bipyridinium herbicides are listed in Table 2. Example references have been included if available.

Three of these factors were chosen for laboratory study because they were likely to be important in the field trials.

Calcium. Calcium has been shown to be antagonistic to the herbicidal activity of paraquat (Parker 1966). The activity of diquat appears to be reduced in a similar way, regardless of whether it is formulated with alginate or in solution (Barrett unpublished data).

There is a wide range of calcium concentrations in freshwaters throughout the U.K., principally determined by the geology of the catchments. Many of the rivers in the south of England which have major weed problems, are chalk streams with high calcium concentrations, in the range 60-120mg l^{-1} calcium (Westlake et al. 1972). Thus, it is important to assess how much the herbicide's performance might be reduced in such calcium concentrations.

Temperature. Laboratory experiments have shown that water temperature may also influence the rapidity and severity of diquat's action (Mackenzie et al. 1971). The influence of this factor, in the field, may be difficult to distinguish from the influence of the age and biomass of the plants. Water temperatures in late spring are likely to differ throughout the country, not only on a north-south gradient, but also depending upon the water source (Hynes 1970). For example, chalk aquifers tend to have fairly stable annual temperatures (e.g. Westlake et al. 1972) but water flowing off resistant rock is likely to be more influenced by the ambient air temperature (e.g. Crisp and Howson 1982).

Exposure period. The period of exposure to diquat required for its efficient action, may be highly dependent upon the influence of factors, such as, calcium concentration and temperature. In flowing water the diquat, released from the alginate into solution, may be rapidly removed from the target plant and the potential period of exposure is very brief. The situation is further complicated by the influence that the calcium concentration has on the rate of release of diquat from the alginate (Barrett 1978b).

Table 2

Some of the environmental factors known to influence the efficacy of the bipyridinium herbicides

Water temperature	Mackenzie et al. (1971) Clayton and Tanner (1982)
Calcium concentration	Parker (1966)
Water turbidity	W.R.O. (1981) Bowmer (1982a)
Presence of epiphytes (e.g. algae and bacteria) or encrustations on target-plant surfaces	Bowmer (1982b)
Target-plant susceptibility	White (1963)
Age and physiological state of target plants	W.R.O. (1981) Clayton and Tanner (1982)
Period of exposure and concentration of herbicide at the plant surface	Mackenzie et al. (1971)
Water velocity and dilution rate of the herbicide	O'Loughlin and Bowmer (1975)

Some laboratory experiments were devised to investigate the effects of temperature, calcium concentration and exposure period, on the efficacy of diquat-alginate. These experiments were conducted in static cultures, using Ranunculus penicillatus var. calcareus. These experiments were based upon a methodology employed by Prescott (unpublished data, Reading University).

3.2 Preparation of the Laboratory Experiments

3.2.1 Materials

Fresh samples of Ranunculus penicillatus var. calcareus were collected for each experiment, from the River Alre at New Alresford, Hampshire. An experimental unit consisted of an apical stem of 4½ internodes, with roots on at least two of the nodes. Each stem was kept fully submerged in a 250ml beaker containing 200ml of a culture medium, which excluded calcium, prepared from the recipe in Table A-1, Appendix 1. This medium, based on a modified Hoagland's Solution, was chosen because samples of Ranunculus had been successfully maintained in it for 2-3 weeks, prior to starting the experiments. The required calcium concentrations were obtained by additions of calcium chloride crystals ($CaCl_2 .6H_2 O$).

The volumes of herbicide required to produce a concentration of 1mg l^{-1} active ingredient, were too small to use Midstream. Instead a solution was prepared from diquat-alginate powder. The 5ml doses of diquat-alginate were applied to each beaker using a syringe. The solution was too dilute to be seen to gel, even at the highest calcium concentration. Five millilitres of distilled water were added to the control replicates which were not treated with herbicide.

3.2.2 Methods

The conditions for the two experiments are summarised in Table 3. Factorial, random-block experimental designs were used with three replicates of each treatment. Each beaker was identified by a system of random numbers, to reduce bias in subsequent assessments.

The beakers stood in two water-baths kept at a constant temperature ($\pm$ 0.5°C), under daylight lamps in a glasshouse. In a preliminary experiment using Elodea canadensis, there had been problems with algal blooms developing in the beakers, due to the high

Table 3

Conditions for the two laboratory experiments

Calcium ion concentrations (mg l^{-1})	0, 100, 200, 300	10, 50, 100, 200
Temperature (°C)	22	9, 18
Herbicide exposure period (hours) (1mg l^{-1} diquat)	0, 0.33, 2, 24	0.33
Light intensity (microeinsteins m^{-2} sec^{-1}) (daylight lamps, daylength 14:10)	Block 1 and half of block 2 167 Block 3 and half of block 2 282	All blocks 123

light intensity and temperature. A coarse nylon mesh was placed just below the lamps to reduce the light intensity. Measurements of the light intensity were made with a meter which measured photosynthetically active radiation (PAR).

After the required period of exposure to the diquat, the culture medium in each beaker was changed. The plants were rinsed to remove the diquat residues and the beakers were thoroughly wiped to remove any diatoms. The control plants, without herbicide, were subject to the same cleaning procedure. In the preliminary experiment the culture medium had been completely changed regularly. This was found to cause too much disturbance to the plants, so in these experiments, the medium was just topped-up every 2-3 days.

Damage assessment

The condition of the plants was assessed at the following intervals:

1) Three days prior to the herbicide treatments
2) Immediately prior to the treatment
3) At regular, often daily, intervals after treatment

The following parameters were measured:

a) Dissolved oxygen - measured using a membrane oxygen electrode with a portable Data Scientific digital meter.

b) pH - measured using a glass pH electrode and a W.P.A. digital meter.

c) Growth - assessed by measuring the stem lengths twice before and once after treatment. The linear growth increments calculated per day, were compared before and after the herbicide treatment, to give a relative growth rate.

d) Visual damage assessment - based on observations of the material, these were scored using the following scale;

0 = no visible damage
1 = some browning of stem, node or broken surface
2 = browning of leaf bases and some roots, or a slight bleaching of apical leaves
3 = browning all over the plant and some leaf-bleaching
4 = obvious leaf-bleaching and tissues all very brown
5 = disintegration

Dissolved oxygen and pH were measured at the same time each day. The electrodes were gently stirred in the media so as to minimise the disturbance caused to the plant material.

3.2.3 Statistical analyses of the data

The results were analysed using an appropriate Analysis of Variance (ANOVA), with the GENSTAT statistical package (Alvey et al. 1982) on the Reading University main-frame computer.

For each parameter measured, the data were analysed separately, for each time of recording, and together for all the readings from a given experiment. In analyses carried out with several sampling times, time was not treated as a simple factor. Following the example of Little and Hills (1978), a split-plot design was used. In this design, treatments are assigned to main-plots and the sampling times are analysed as sub-plots. These sub-plots differ from those usually found in agricultural experiments (e.g. Mead and Curnow 1983) in that they consist of data taken from the entire main plot rather than from just a specific proportion of the data set.

To illustrate which comparisons between means were significant, tests of Least Significant Difference (LSD) were applied. Comparisons of results within treatments and between sampling times, or between treatments within a sampling time, could be made. All the comparisons of individual means were orthogonal so the orthogonal LSD test was used:

$$t = \frac{\text{difference between means to be compared}}{\text{standard error of the comparison}}$$

t is tested with the Student's 't' distribution at the 5% level using the number of degrees of freedom associated with the mean square, from the ANOVA. The methods for calculating the standard errors of the comparisons for a split-plot design, are described by Little and Hills (1978). Using GENSTAT the standard errors of differences of means and the degrees of freedom of the factors are given with the ANOVA. An example of an example of some of the GENSTAT output is given in Appendix 2.

If the data have not been transformed before analysis, the LSD for the appropriate factor, or interaction, has been indicated on the plot of the data. If the plot of residuals (part of the GENSTAT output) showed a random scatter of points, the data were left untransformed. If there was a clear pattern to the plot of residuals, this

indicated that the data were not normally distributed. A normal distribution of the data is one of the conditions that has to be satisfied for the valid use of ANOVA (Finney 1973). In these experiments, only the length increment data had to be transformed. The data were normalised using a $\log_e$ (X x 100 + 1) transformation.

The original, rather than transformed, data have been plotted because they provide a more easily understood illustration of the results. The LSD values calculated on transformed data cannot be back-transformed to be shown on the plot of the original data. Instead, comparisons between means have been tested on the transformed data, and letters have been used on the plot of the original data, to distinguish means that are significantly different. For simplicity, only significant differences between treatments per time, or between times per treatment, have been indicated. It should be noted that differences between means may appear to be lesser, or greater, on the plotted untransformed data than on the analysed, transformed data.

In all the following presentations and discussions of results, the word 'significant' will only be used when a statistical test has been applied to the data.

The variance ratio for a factor or interaction will be indicated by:

$$F_{x,y} = z$$

F = variance ratio

x,y = degrees of freedom associated with F

3.3 Results of the Laboratory Experiments

3.3.1 Results of Experiment 1

Testing the effects of the calcium concentration and the diquat exposure period on the efficacy of the herbicide

The experiment was monitored every day, for four days after the herbicide treatment. By seven days after treatment, significant differences between blocks, were appearing for some parameters.

Damage score

The damage scores for all treatment combinations (means of the three replicates), recorded over four days, are presented in Fig. 1. All factors and treatment interactions, except blocks, were significant in the ANOVA. Damage scores from a single sampling time, four days after treatment, are presented in Fig. 2. The blocks factor was the only factor that was not significant in the ANOVA.

The calcium concentration had no significant effect on the damage score of plants which were not treated with diquat. There was a significant interaction between the calcium concentration and the herbicide exposure period. If there was a short period of exposure to the herbicide, twenty minutes, the calcium concentration was highly influential. With 200mg l^{-1} of calcium the herbicide appeared to be ineffectual.

The inhibitory effect of the calcium was reduced as the period of exposure to the herbicide increased. After 24 hours of exposure to diquat, the calcium concentration had no influence on the herbicide induced damage.

Dissolved oxygen

Dissolved oxygen (and pH) were measured at midday, when the dissolved oxygen concentrations would be increasing, due to photosynthesis. Except for the cultures lacking calcium, the dissolved oxygen concentrations in the controls, without herbicide, were fairly high, about 114% saturation. After four days there was significantly less dissolved oxygen in the controls without calcium, with only 85% saturation.

Cultures in which photosynthesis has been disrupted and in which decay processes with increased respiration were occurring, would be expected to have depleted dissolved oxygen concentrations. This

Fig. 1

Damage scores for four days after herbicide application

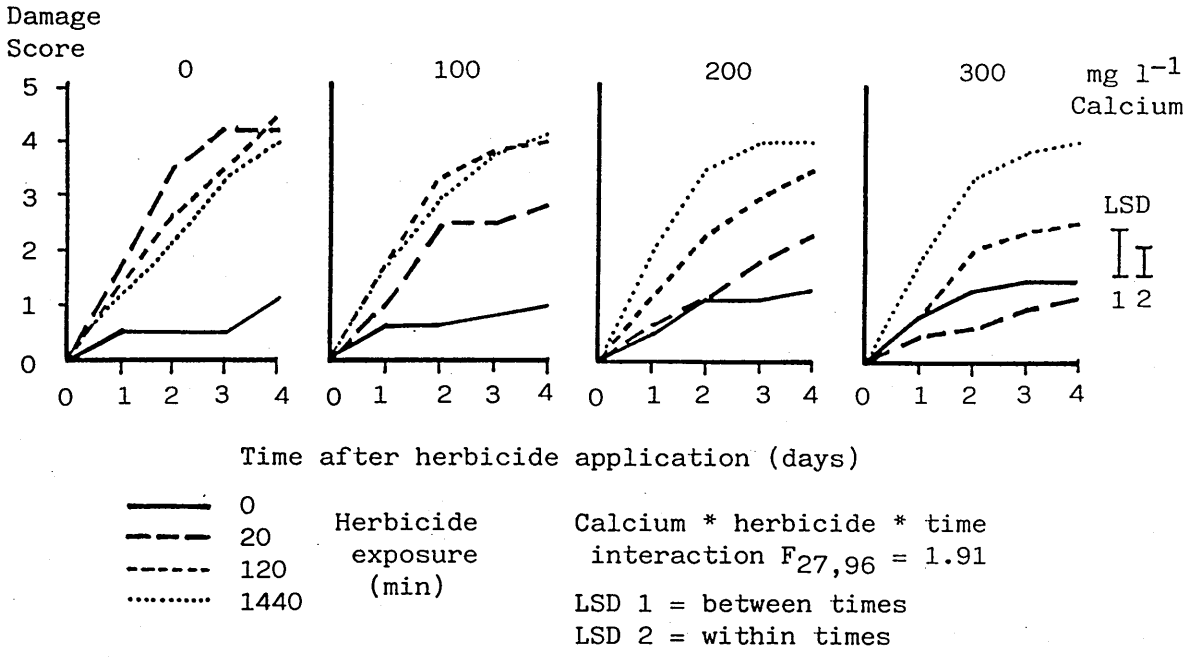
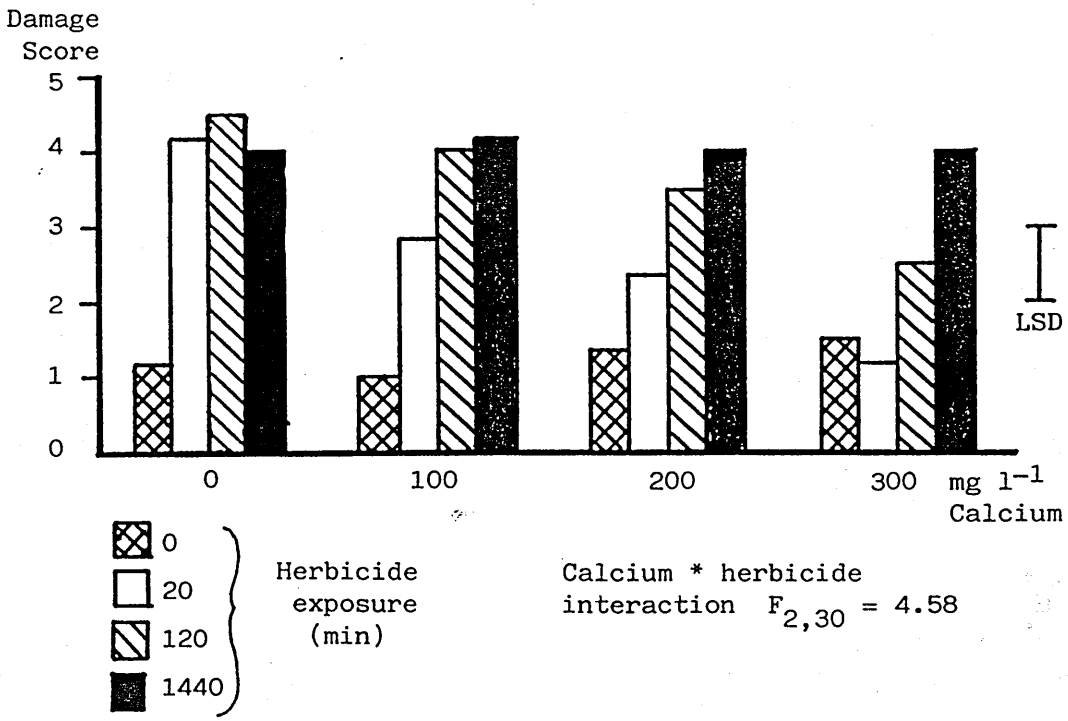


Fig. 2

Damage score on fourth day after herbicide application



reduction was evident in all the herbicide treated samples free of calcium. In static cultures of small volume, there would not need to be a very great imbalance of photosynthesis and respiration to create a deficit from 100% saturation of dissolved oxygen. The reduction in dissolved oxygen concentrations was evident after 24 hours, indicating that the action of the herbicide was very rapid.

There were no significant differences in dissolved oxygen concentrations between the cultures, prior to treatment. The speed and severity of the oxygen depletion were related to the exposure period and the calcium concentration, with the same interactions as shown by the damage score. The same factors and interactions were significant in the ANOVA, as for the damage score. The dissolved oxygen concentrations recorded over four days, after treatment are presented in Fig. 3, and data from the fourth day after treatment are presented in Fig.4.

By seven days after treatment the herbicide exposure * calcium concentration interaction was no longer significant. The dissolved oxygen concentrations were significantly higher, by this time, in block 3. The light intensity over the two water-baths was found to be unequal, with block 3 and half of block 2 in the brighter bath. After the fourth day after treatment, a conspicuous algal bloom had developed in the cultures in the brighter water-bath. The photo-synthetic oxygen production of these algae caused the dissolved oxygen concentrations in the cultures to increase. For this reason, the data from seven days after treatment were not included in the analysis of the results.

pH

The pH of the culture media did not significantly differ between treatment prior to the application of the herbicide. As with the damage scores and dissolved oxygen concentrations, there were significant interactions between the herbicide exposure period, the calcium concentration and time. The pH values recorded over the four days following the herbicide treatment, are presented in Fig. 5. Data from the fourth day after treatment are presented in Fig. 6.

As with the other parameters, the pH of the herbicide-free control samples without calcium, significantly differed from the other controls. The pH of the calcium-free cultures which had a twenty minute exposure to diquat, rose suddenly after three days, for no

Fig. 3

Dissolved oxygen concentrations for four days after herbicide application

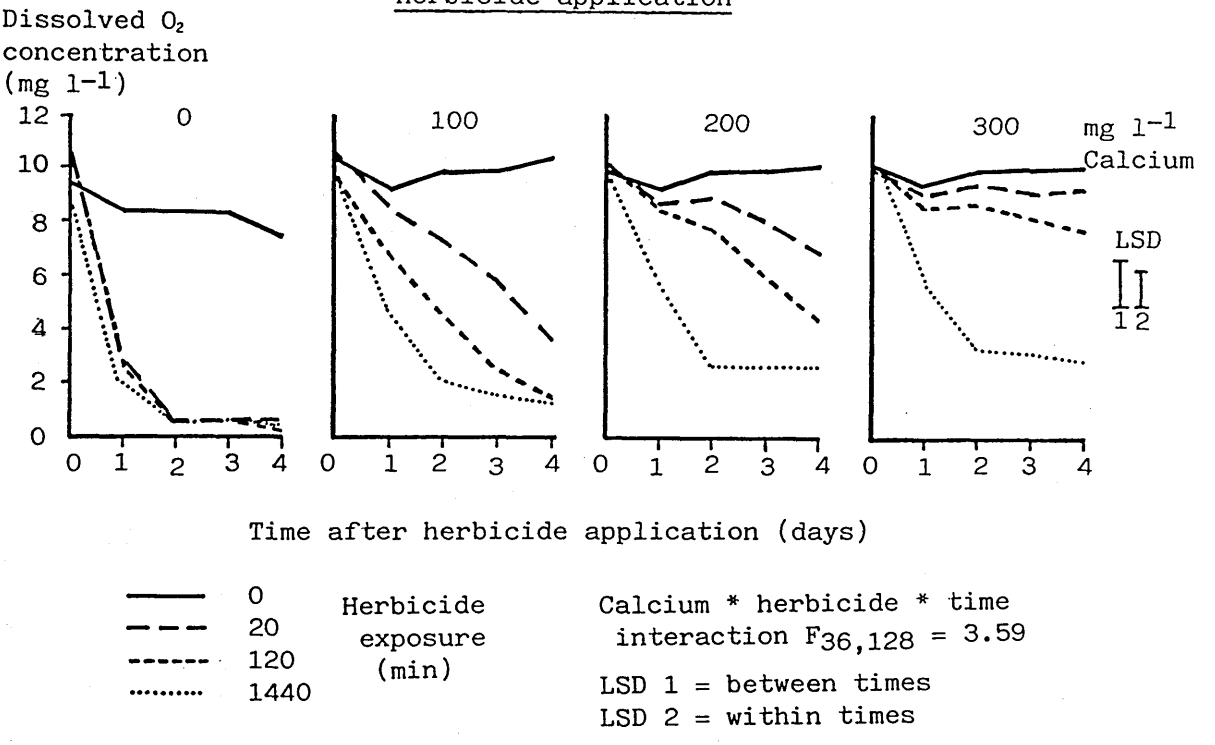


Fig. 4

Dissolved oxygen concentrations on the fourth day after herbicide application

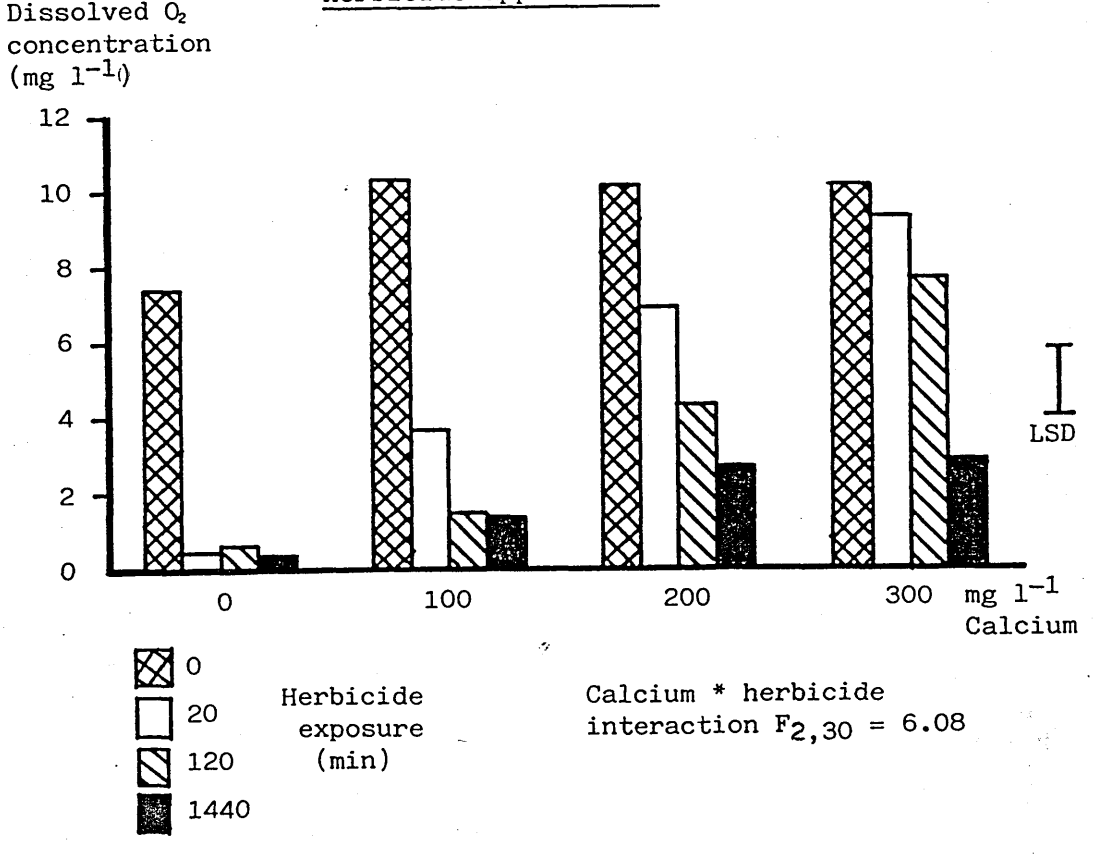


Fig. 5

pH for four days after herbicide application

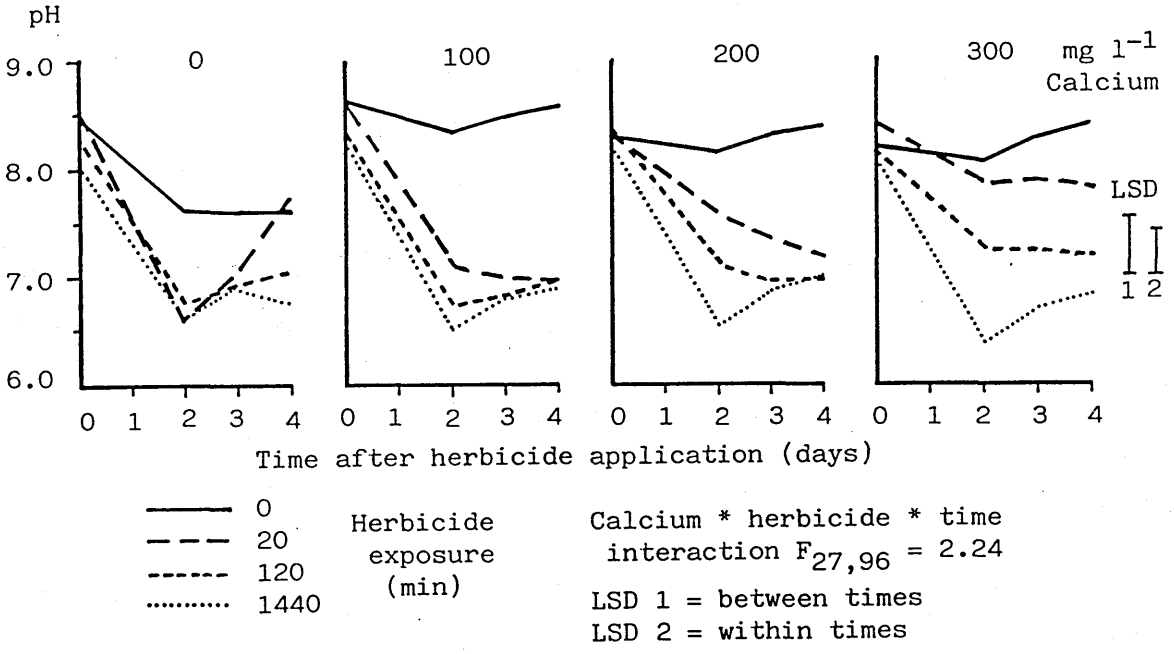
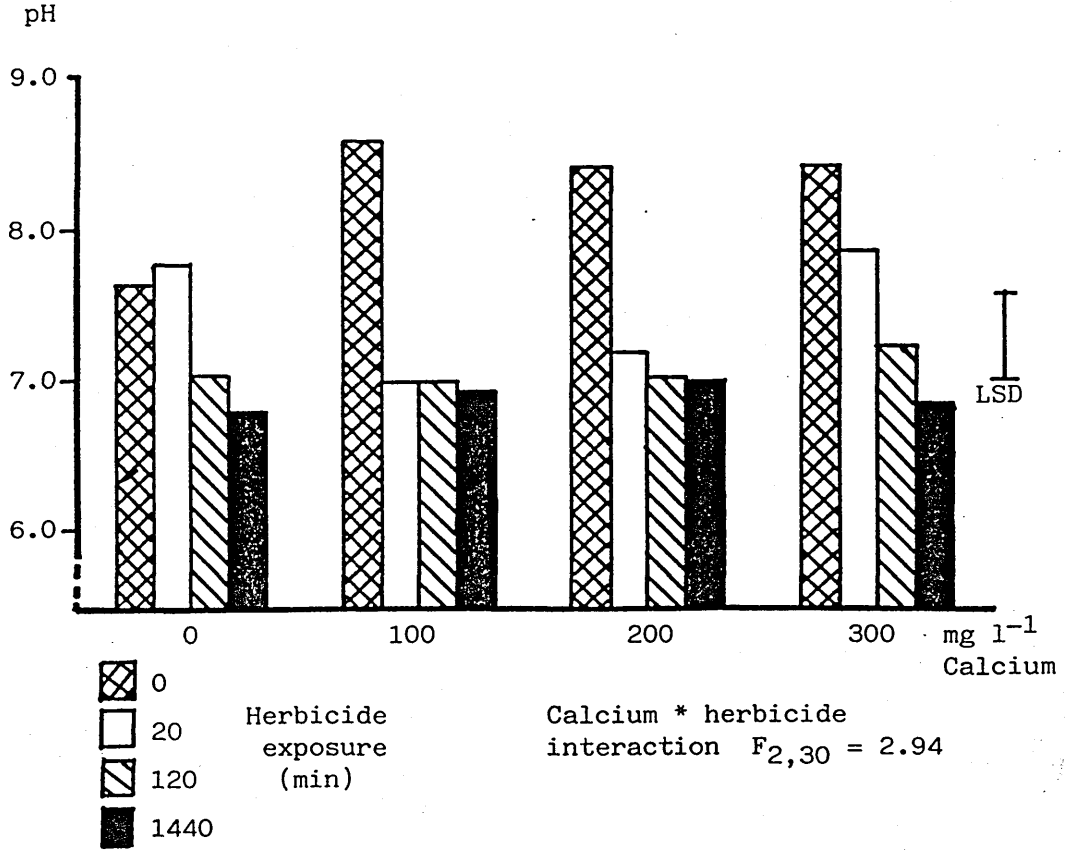


Fig. 6

pH on the fourth day after herbicide application



apparent reason. This created a misleading anomaly in the result from the fourth day after treatment. Until that time the pH from these cultures had matched the pH in the cultures which had been exposed to the herbicide for longer.

Stem length

The stems were not exactly the same length initially, and because this variation would be greater than the growth expected over a few days, the absolute lengths were not compared.

Growth, measured as the linear increment per day, was analysed in three ways.

1) A comparison of growth before and after the herbicide application was made, in which the data were normalised using a $\log_e$ (X x 100 +1) transformation. This ANOVA showed a significant interaction between the calcium concentration, herbicide exposure and time, Fig. 7. The pre-herbicide treatment growth, did not significantly differ between all, but one, cultures.

2) The growth, after the herbicide application, of the herbicide treated plants, was calculated as a percentage of the growth of the herbicide-free controls in the same calcium concentration. Individual plants are not related, only the means of the three replicates can be compared. Parametric tests requiring replicates, such as ANOVA, cannot be used on these results, Fig. 8.

In both of these types of analysis, the effect of the herbicide in inhibiting growth was seen to be affected by the exposure period and the calcium concentration, with the same interaction as observed in the other parameters.

3) Relative growth rates were calculated for each plant:

$$RGR = \frac{\text{length increment per day after herbicide treatment}}{\text{length increment per day before herbicide treatment}}$$

Ideally, the relative growth rates of the controls, without herbicide, were all expected to be about 1, and significantly less than 1 if the growth had been inhibited by the herbicide. These data (Fig. 9), were transformed for the ANOVA by $\log_e$ (X x 100 + 1). The calcium * herbicide interaction was not significant. Only the herbicide exposure factor was significant. The variability in the pre-treatment growth and the small changes in length occurring over three days, were

Fig.7

Growth increments before and after herbicide application

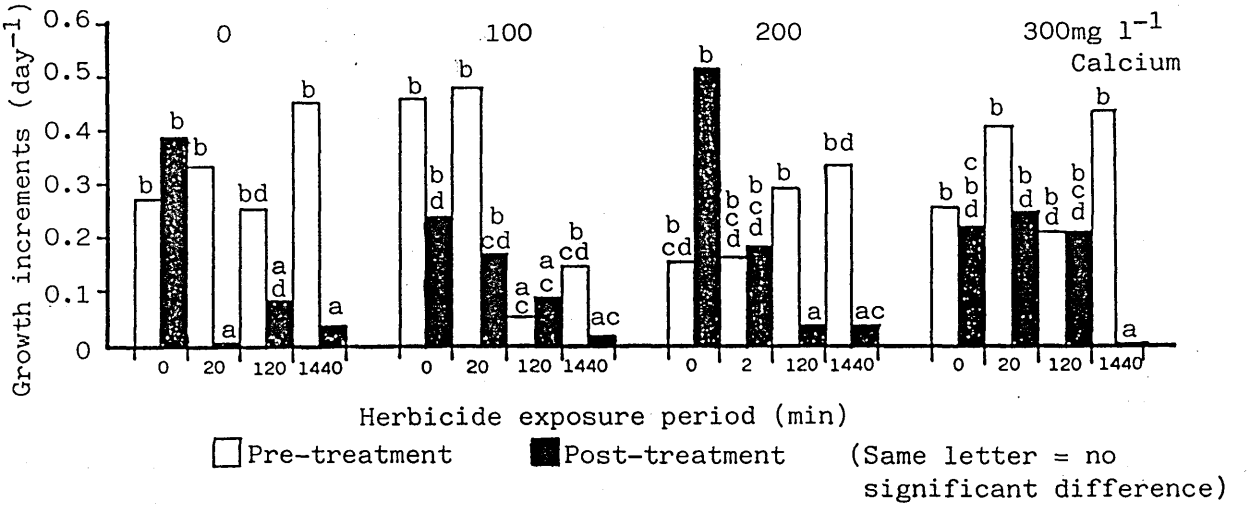


Fig.8

Percentage of control growth after treatment

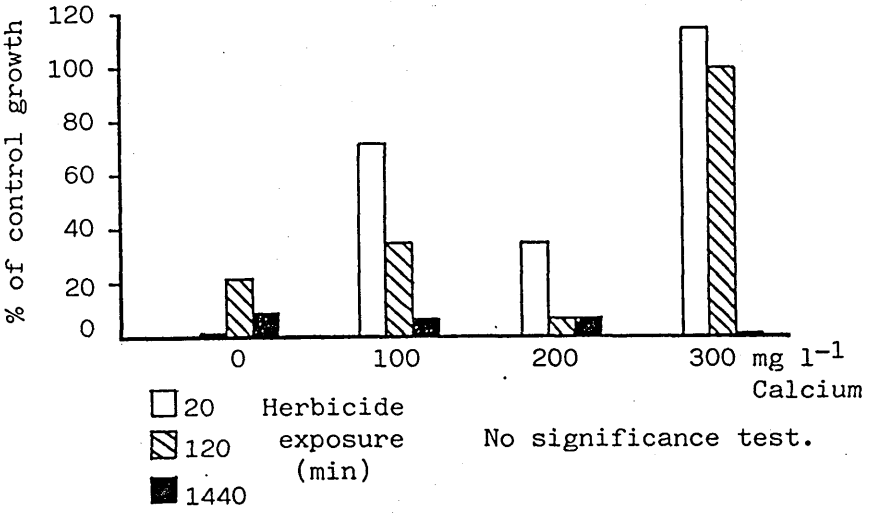
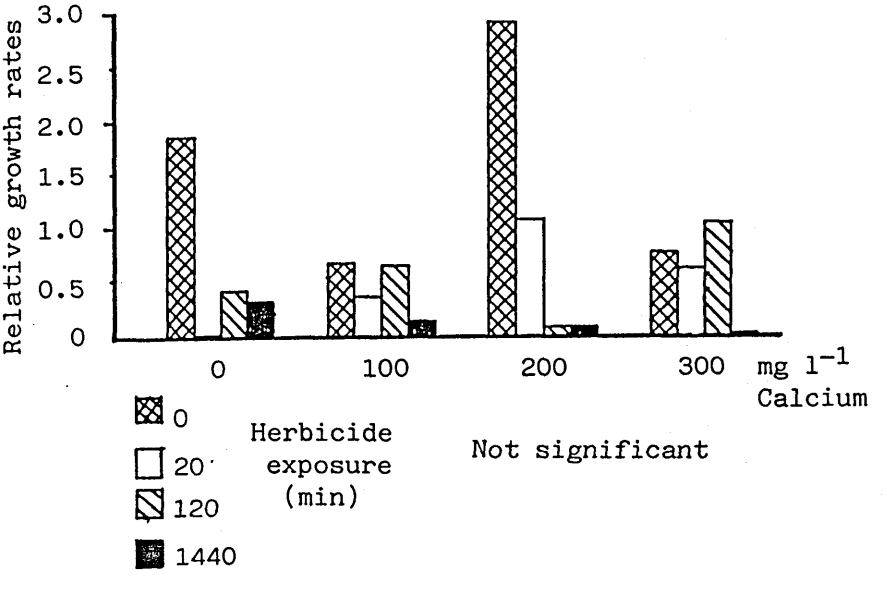


Fig. 9

Relative growth rates (as defined on P.47)



responsible for this analysis being only sensitive to gross effects, such as herbicide presence, rather than to the subtler, interaction effects.

3.3.2 Results of Experiment 2

Testing the effects of calcium concentration and temperature on the efficacy of the herbicide

Unlike Experiment 1, there were never any significant differences between the blocks, for any of the parameters measured. The data from each sampling time, up to ten days after the herbicide treatment, were analysed.

When analyses of the complete data set, with all three factors (herbicide presence, temperature, calcium concentration) and time, were carried out using the split-plot ANOVA on GENSTAT, no term for the interactions of all four factors was given.

Damage score

The effect of temperature on the rate of plant decay is shown in Fig. 10. The temperature * herbicide * time interaction was significant. For each herbicide/temperature combination, there was a constant rate of increase of damaged tissue. The rate of damage to plants at 9°C with herbicide, was less than of plants at 18°C, even without herbicide.

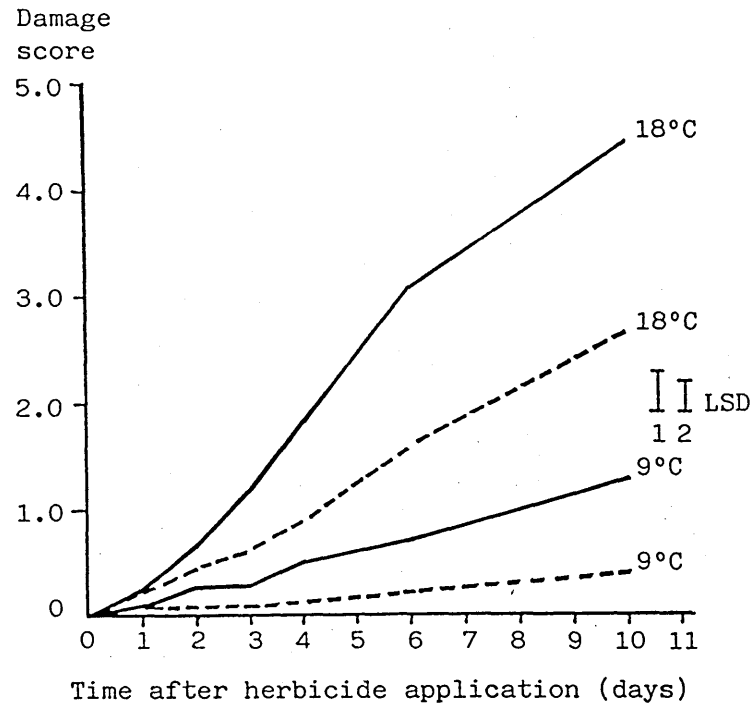
The herbicide * calcium * time interaction (Fig. 12) was significant, confirming the results of Experiment 1. The calcium * herbicide interaction was apparent, four days after the herbicide application, at both temperatures (Fig. 11). The herbicide-induced damage at 9°C was never significantly more (sometimes significantly less) than the damage in the herbicide-free controls at 18°C. The herbicide did cause significantly more damage, at the lowest calcium concentration, at 9°C, than occurred in the herbicide-free control.

Dissolved oxygen

The concentration of a gas that can be dissolved in a liquid, is dependent upon temperature. If the data from this experiment were analysed using the concentration of dissolved oxygen (mg l^{-1}) there would be very significant differences ($F_{1,30} = 183.5$) between the temperature conditions, prior to the herbicide application.

Fig. 10

Damage scores for ten days after herbicide application



—— + Herbicide

----- - Herbicide

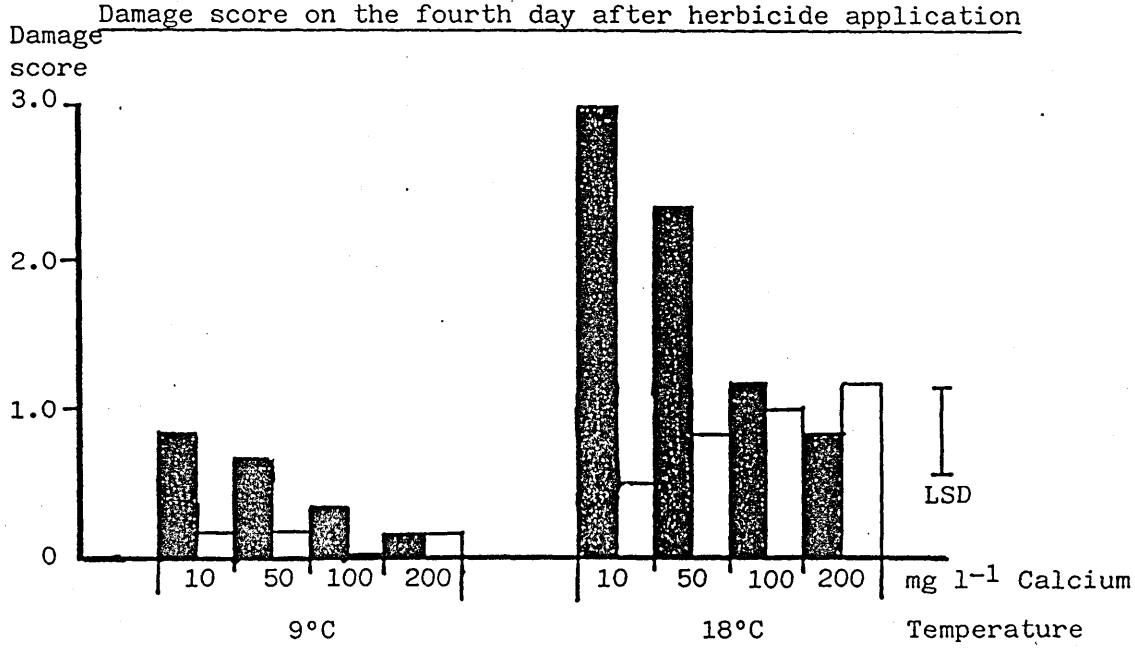
Temperature * time * herbicide interaction $F_{5,175} = 4.33$

LSD 1 = between times

LSD 2 = within times

Fig. 11

Damage score on the fourth day after herbicide application



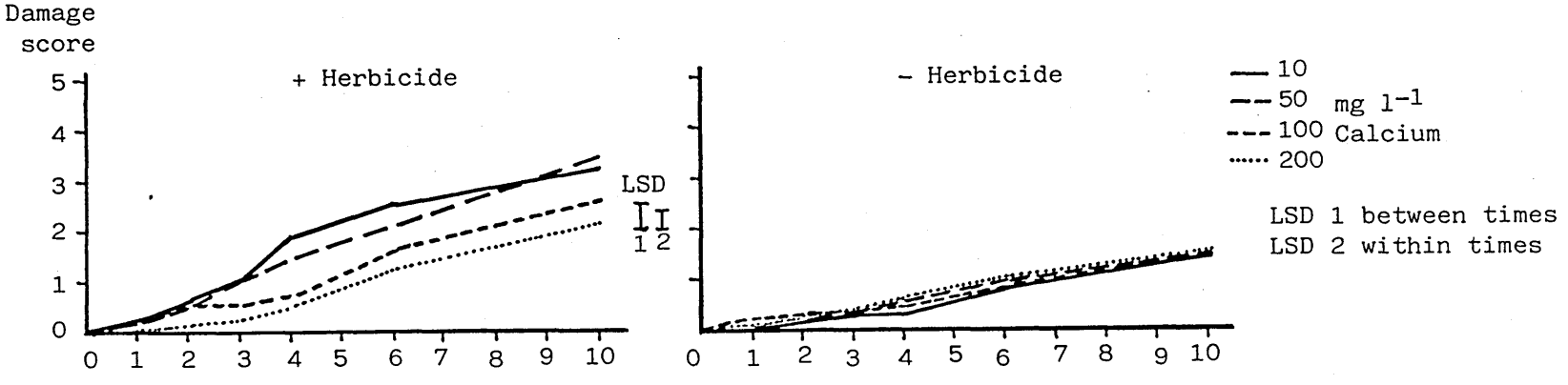
+ Herbicide

- Herbicide

Calcium * herbicide * temperature interaction $F_{3,30} = 6.29$

Fig.12

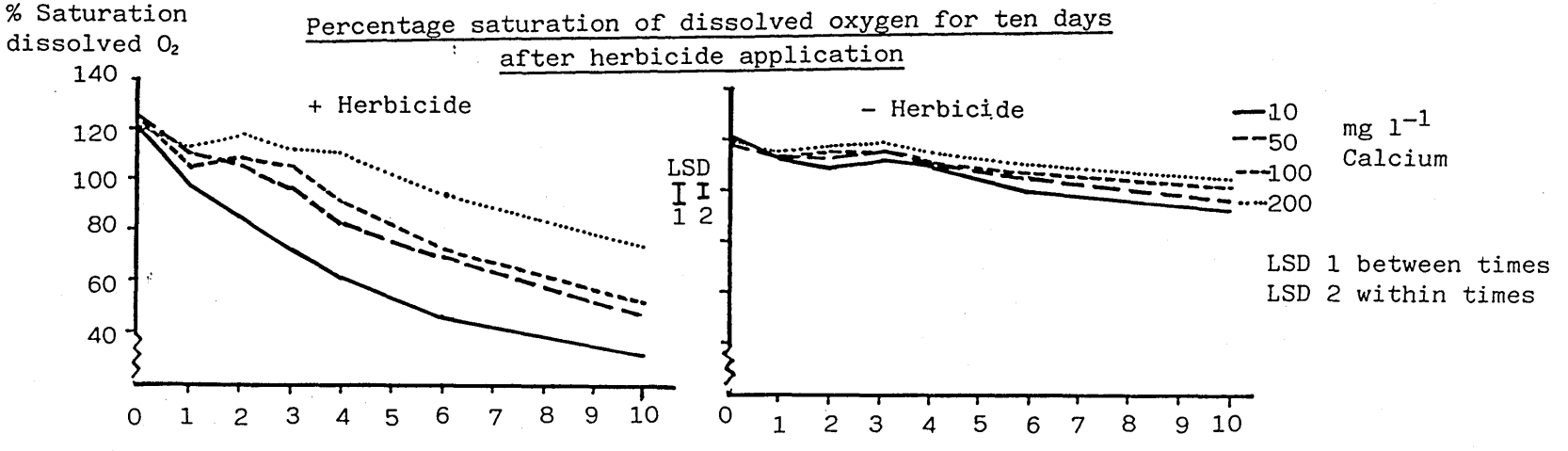
Damage scores for ten days after herbicide application



Calcium * herbicide * time interaction $F_{15,175} = 1.764$

Fig. 13

Percentage saturation of dissolved oxygen for ten days after herbicide application



Calcium * herbicide * time interaction $F_{18,210} = 2.929$

Conversion of the dissolved oxygen concentrations, at their respective temperatures, to a percentage of air saturation, allows a comparison to be made between all experimental units, (11.6 mg l^{-1}= 100% sat.at 9°C, 9.45 mg l^{-1}= 100% sat. at 18°C).

Despite this correction, there was still a significant difference between the two temperature treatments prior to the herbicide application. This may be due to a greater reduction of photosynthesis at 9°C, than of respiration. However, the mean percentage saturation of dissolved oxygen at 9°C (119%) was higher than the value (117%) observed at 22°C, prior to herbicide application, in Experiment 1.

If the data from all sampling times were analysed together, the same factors and interactions were significant for dissolved oxygen, as for damage score. Considering the temperature * herbicide * time interaction (Fig. 14), the percentage saturation of dissolved oxygen did not significantly differ between the herbicide-free controls at either temperature. The herbicide treatments reduced the dissolved oxygen, to less than in the controls, the effect being greatest at 18°C.

The significance of the herbicide * calcium * time interaction (Fig. 13) confirms the results of Experiment 1.

As with the damage score, the percentage saturation of dissolved oxygen, four days after the herbicide application, did not significantly differ between the controls. At 9°C the herbicide only caused a significant reduction in dissolved oxygen, in the cultures without calcium. At 18°C, the herbicide caused a significant reduction in dissolved oxygen in all but the highest calcium concentration (Fig. 15).

pH

As in Experiment 1, the changes in pH were less well defined than for the damage score and dissolved oxygen parameters. Over all sampling times,the temperature * herbicide * time interaction (Fig.16) and the herbicide * calcium * time interaction (Fig. 18), were significant. If each sampling time was analysed separately, (e.g. four days after herbicide application, Fig. 17), the herbicide * calcium * temperature interaction was never significant.

As in Experiment 1, there was a noticeable reduction in pH in all cultures, immediately after the herbicide application. This change in pH was probably due to the change of culture medium.

Fig. 14

Percentage saturation of dissolved oxygen for ten days after herbicide application

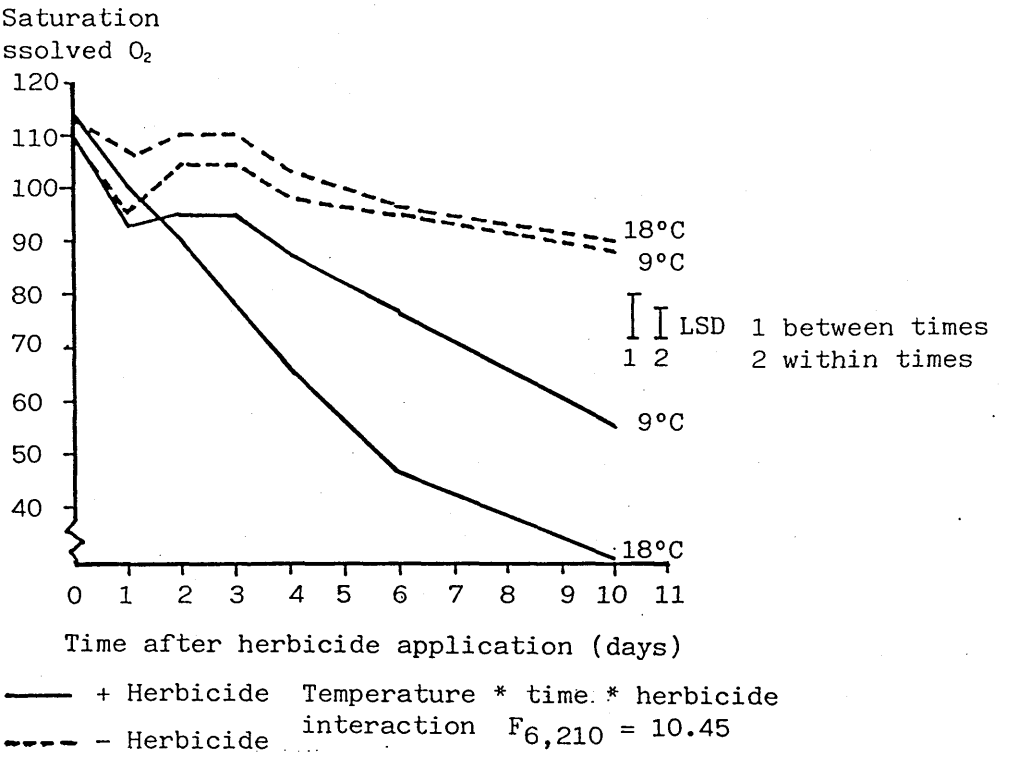


Fig. 15

Percentage saturation of dissolved oxygen on the fourth day after herbicide application

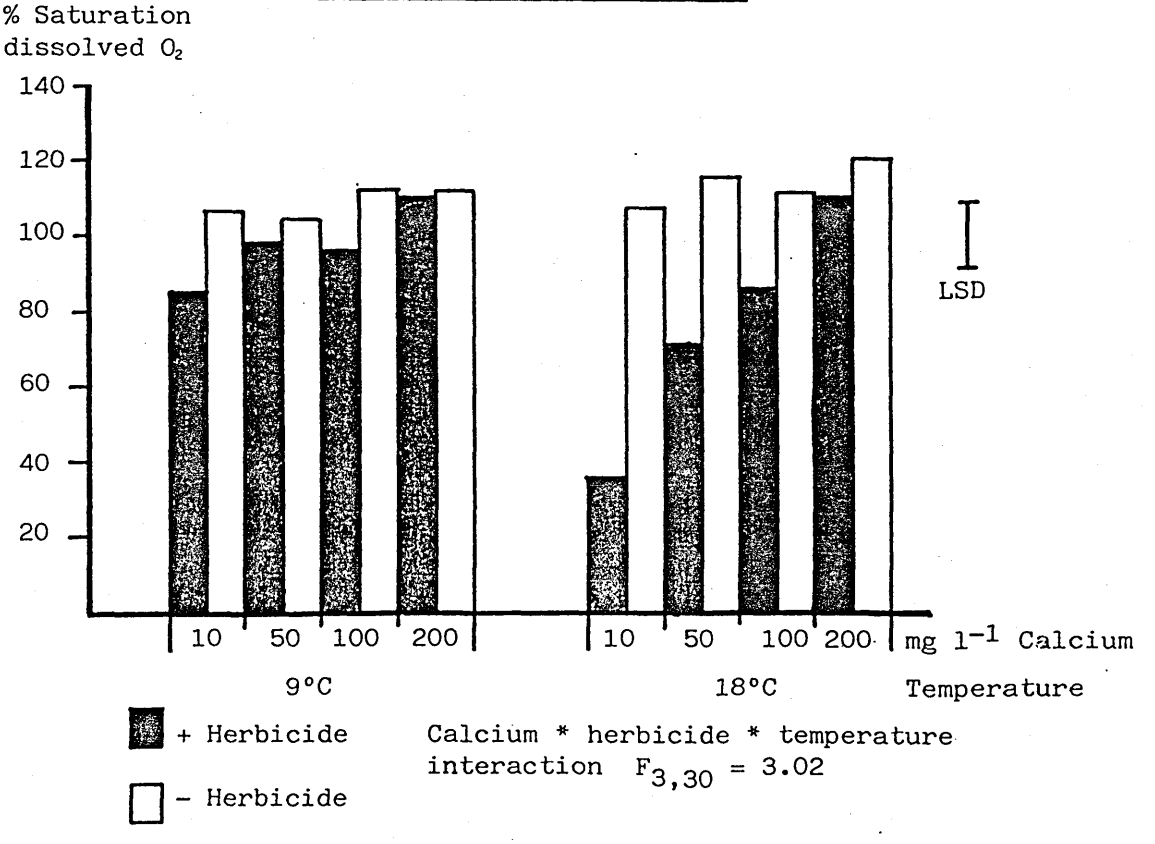
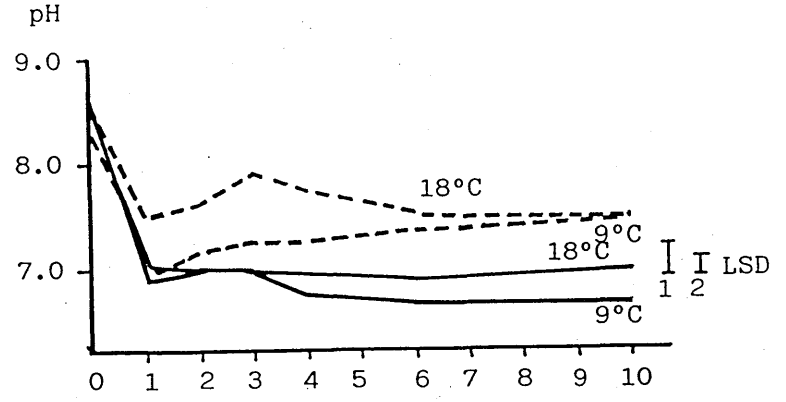


Fig. 16

pH for ten days after herbicide application



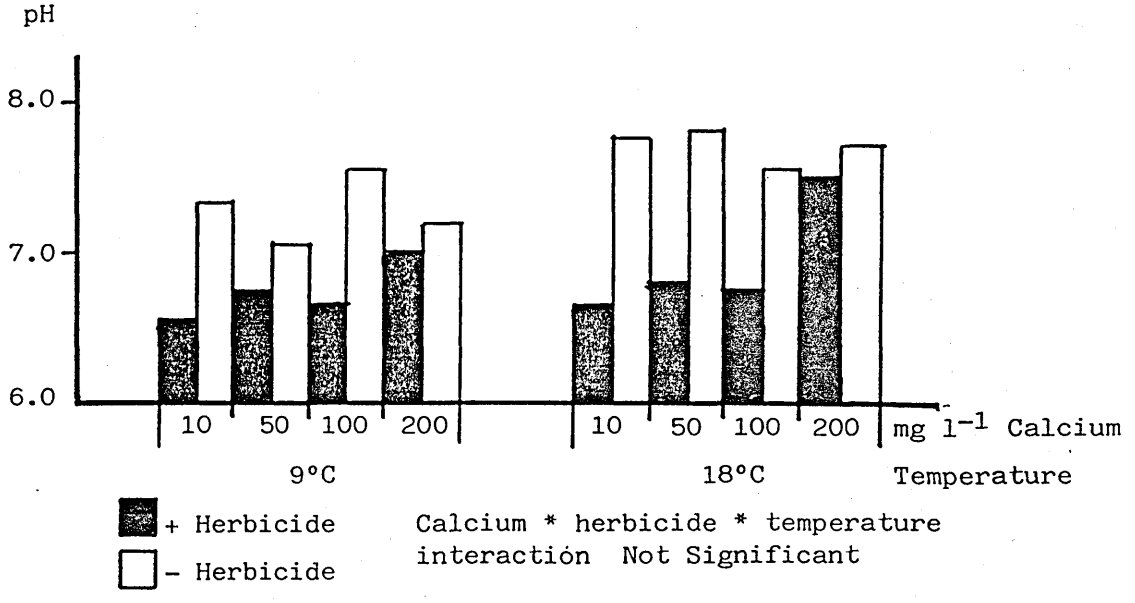
——— + Herbicide
- - - - - - Herbicide

Temperature * time * herbicide interaction $F_{6,210} = 6.611$

LSD 1 between times
LSD 2 within times

Fig. 17

pH on the fourth day after herbicide application



Calcium * herbicide * temperature interaction Not Significant

Stem length

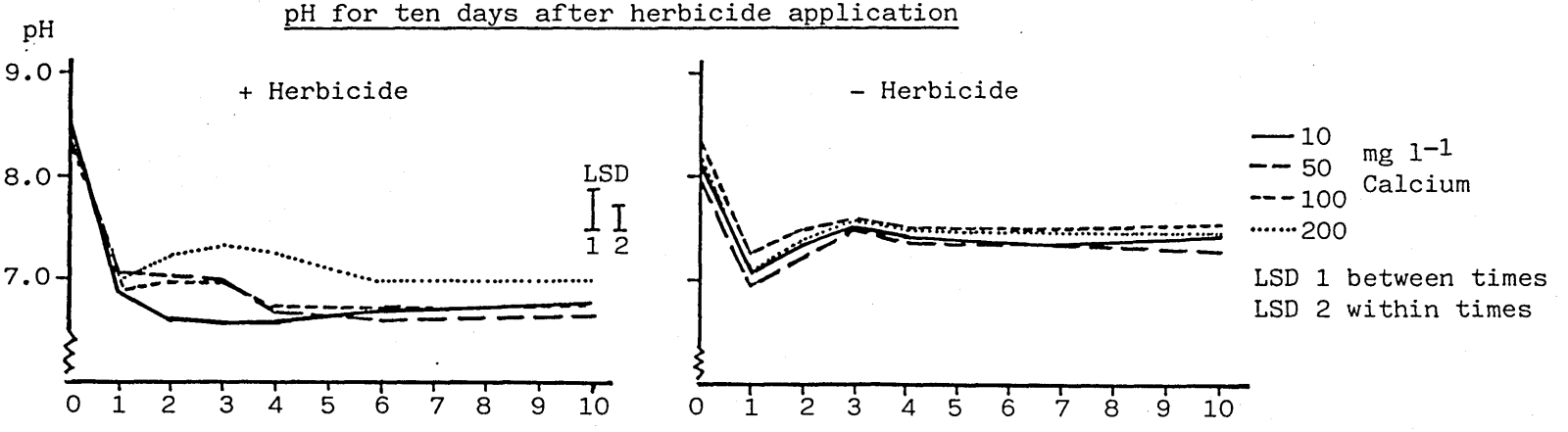
1) A $\log_e$ (X x 100 + 1) transformation of the linear growth increments, was used for the ANOVA comparing the pre- and post-herbicide application data. The only significant interaction was of temperature * time. It was apparent from the original data (Fig. 19) that there was considerable variability in the growth of plants prior to the herbicide application, and between the plants which were not treated with herbicide. The herbicide and temperature factors were significant.

2) The results for the percentage growth of the herbicide treated plants (compared to the herbicide-free controls) were not as straightforward as those results from Experiment 1 (Fig. 20). In some conditions, the trends of these results agree with the other parameters, with the herbicide causing the greatest inhibition of growth at low calcium concentrations and high temperatures. There appears to have been an unexpected reduction in growth of plants at the highest calcium concentrations. At 18°C the herbicide treated plants, in a solution of 50 mg l^{-1} calcium, grew better than the controls.

3) The analysis of relative growth rates, showed no significant interactions, as was found in Experiment 1. The herbicide factor was not even significant, and the only factor which was significant was temperature, with an overall reduced growth rate at 18°C (Fig. 21).

Fig. 18

pH for ten days after herbicide application



Time after herbicide application (days)

Calcium * herbicide * time interaction $F_{18,210} = 1.991$

Fig. 19

Growth increments before and after herbicide application

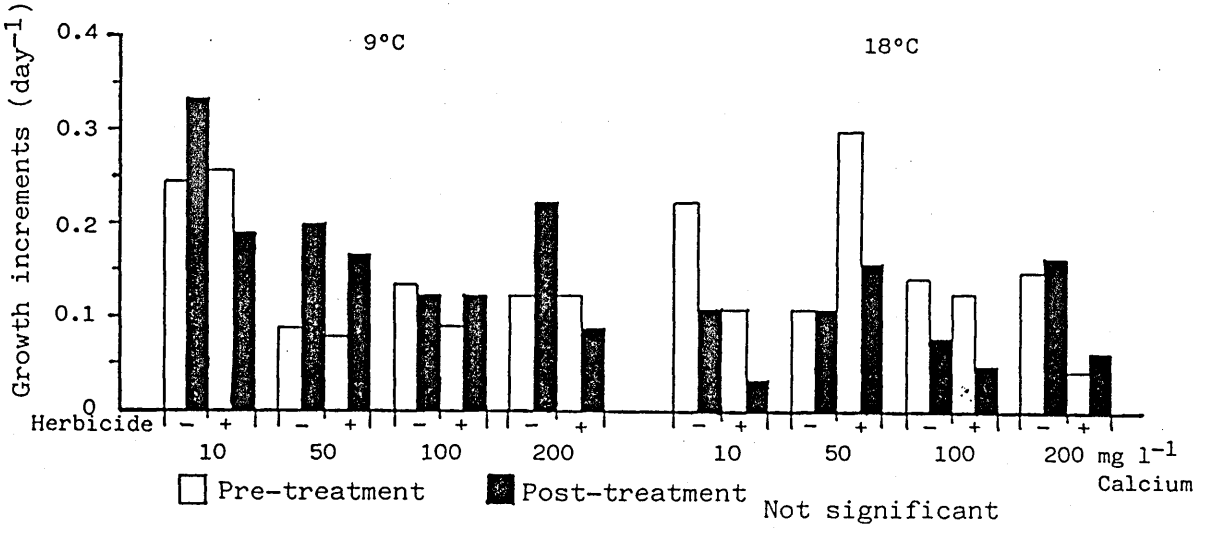


Fig. 20

Percentage growth of herbicide treated plants compared to their controls

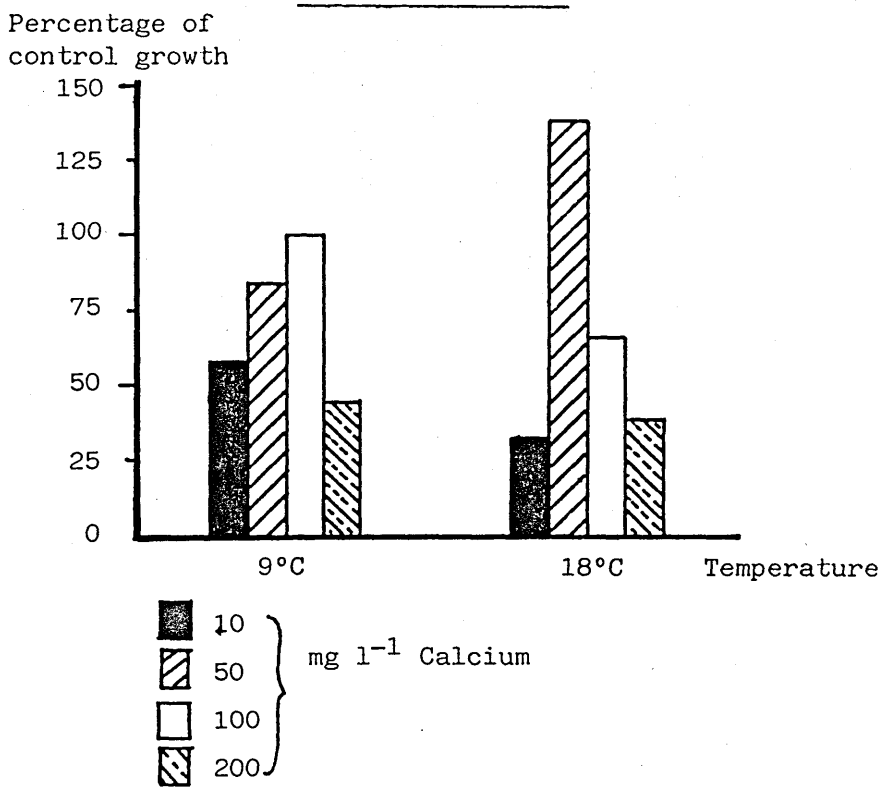
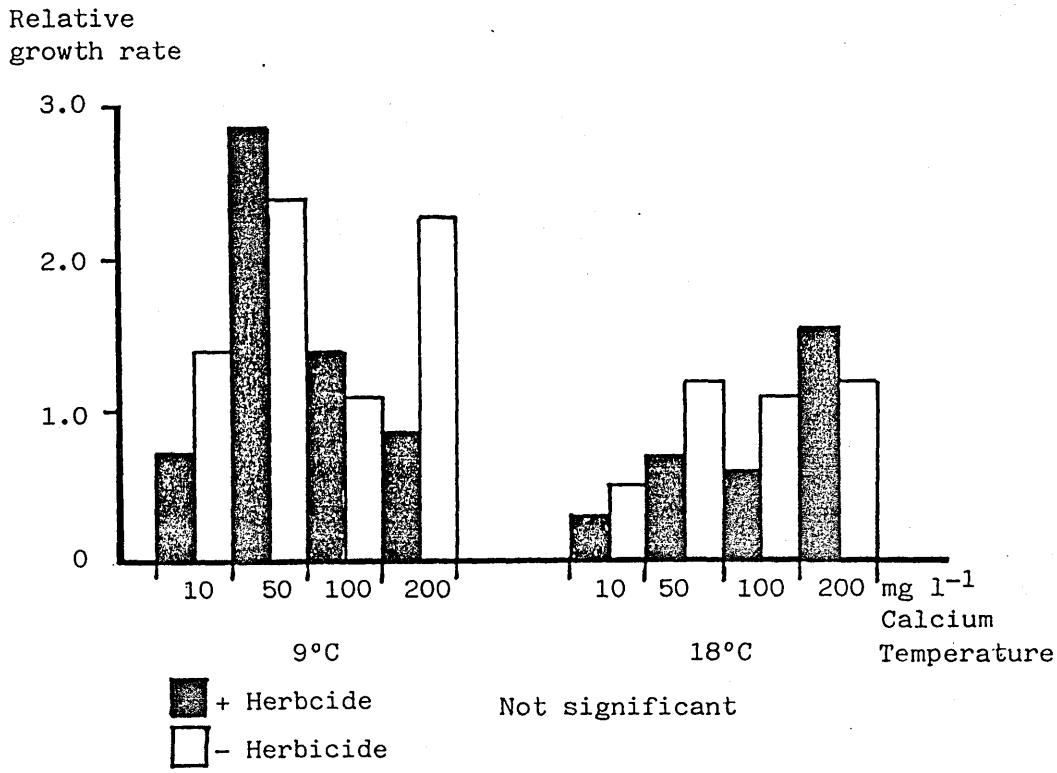


Fig. 21

Relative growth rates of herbicide treated and untreated plants



3.4 Discussion of Laboratory Experiments

3.4.1 Assessment of the methods used to determine the efficacy of the herbicide

The scoring of visible damage and the measurement of dissolved oxygen, in the culture medium, both appeared to provide useful methods of assessing the type and severity of the influence that the herbicide was having on the Ranunculus. These responses developed rapidly and were easily measured, allowing a large amount of information to be gathered in a short time. The long-term maintenance of the cultures was also unnecessary.

Damage score

It was assumed that the damage score at the time of herbicide application was zero. Had any plants appeared damaged at that stage, they would have been replaced by spare material which had been kept under similar conditions. Throughout the duration of these experiments the control material remained reasonably healthy, but the damage did increase gradually. This gradual deterioration was particularly noticeable over ten days at 18°C, in Experiment 2, and would limit the period over which such experiments could be run, unless more favourable conditions could be provided.

In these experiments, using a fast-acting herbicide, the long-term maintenance of the cultures was not necessary. For experiments with slow-acting herbicides (e.g. terbutryne), or if long-term responses, such as recovery, were to be investigated, this problem of culture maintenance would have to be addressed.

Although the damage score was increasing under all conditions and the end result, of total decay, would be reached irrespective of the herbicide effect, the important distinction was between the rates of deterioration.

Dissolved oxygen

The importance of the light conditions in influencing the dissolved oxygen concentrations, was illustrated by the divergence of conditions in the blocks in Experiment 1. This influence may have been a direct effect on the plants' rate of photosynthesis (unless above the photosynthetic saturation light intensity) or, more likely, the indirect effect on the photosynthesis and growth of the algae, which appeared to thrive in the high light intensities.

The diurnal fluctuations in dissolved oxygen concentration would have been exaggerated in the small-volume, static system. High rates of photosynthesis would have caused the supersaturation of oxygen, during the day. It was likely that many of the cultures would have become anoxic at night, when oxygen consumption by the respiration of the Ranunculus and the organisms causing decay, would not have been balanced by the photosynthetic evolution of oxygen. It is because of these diurnal fluctuations that dissolved oxygen and pH measurements need to be taken at the same time each day.

pH

The changes in pH of the culture media generally reflected the same responses to the treatments as the damage score and dissolved oxygen concentrations. The pattern of changes in pH was less obvious because this parameter is determined by a complex interaction of many factors.

The major influence on pH which would have been detected in these experiments, would have been the exchanges of dissolved carbon dioxide resulting from photosynthesis and respiration. The pH of the controls was relatively high because carbon dioxide was being removed from the water by photosynthesis. If photosynthesis was limited, by the herbicide's action, or if respiration in the cultures was increased, by the decay processes, the increase in dissolved carbon dioxide would result in a drop in pH (Hutchinson 1957).

Stem growth

During the few days' duration of these experiments, the increases in stem length were small in comparison to the total lengths (about 1%). Combined with the potential errors in trying to straighten and measure the stems, without damaging them, it was not surprising that significant differences between the treatments, were difficult to detect. The variation in growth between plants, prior to the herbicide application, was almost as great as that occurring afterwards.

The measurements might have been simplified if each stem had been cut to the same length initially, but this would not have prevented differences occurring between the pre-herbicide growth rates. The selection of stems which did show similar initial growth rates, would be a very time and resource-consuming process.

Measuring length increments is a useful method to provide a quantitative assessment of the plant growth. Alternative methods would require destructive sampling, to assess dry biomass, and that would mean that the same plant could not be remeasured before and after the herbicide application. The changes in biomass would need to be large to be significantly greater than the variation between plants. Fresh weight determinations would not be accurate enough on this scale.

For these short-term experiments, length increment is not a particularly useful parameter, especially since the post-herbicide application measurement must be made before the material is too damaged to handle. For experiments on growth retardants or slow-acting herbicides growth rates may be important, but with fast-acting chemicals like diquat the objective is to kill and remove the vegetation, so that the growth rates are not as relevant.

3.4.2 Relating the results of the experiments to the mode of action of the herbicide

The reduction in the concentration of dissolved oxygen in some of the herbicide treated cultures, was the net result of a disruption of the ratio of photosynthesis / respiration. These data do not indicate exactly how the herbicide affected this ratio but the presence of some oxygen, during the day at least, suggests that some photosynthetic oxygen production is occurring in the beakers.

The mode of action of diquat is comprehensively reviewed by Summers (1980) and a summary is presented in Appendix 3 . Unlike herbicides such as terbutryne, diquat is not a specific inhibitor of photosynthesis but destroys the integrity of cell membranes, disrupting all metabolic processes. Thus, the herbicide is likely to have reduced the respiration of the Ranunculus as well as the photosynthesis. The ratio of these two processes will depend upon the effects of other organisms present, such as photosynthetic phytoplankton, and oxygen consuming decomposers, and upon the extent of the herbicide's influence on the Ranunculus. In these conditions, with the herbicide surrounding the plant, most of the stem is likely to have been affected, if the uptake of diquat can be assumed to occur all over the surface. If uptake is restricted to certain parts of the stem and if tissue penetration and translocation are poor, then the proportion of healthy to damaged tissue will influence the degree

of disruption to the diurnal oxygen cycles. The extent of visible decay on a stem may not reflect the actual extent of the herbicide's activity because microbial infestation of damaged tissue appears to start at particular points, such as breaks in the epidermis and leaf nodes.

Herbicide exposure period

The exposure times for diquat chosen for Experiment 1 were intended to include the periods over which diquat might remain in contact with plants, in flowing and static water. Diquat residues collected after a diquat-alginate trial in Crummock Beck, Cumbria, showed that a pulse of about 1mg l^{-1} diquat lasted in the treated area for twenty minutes (Barrett unpublished data). The period of exposure to diquat at a concentration of 1mg l^{-1} , will be influenced, in flowing water by various factors, some of which will be discussed in detail in other sections. These factors include: concentration of herbicide applied; water velocity; length of river treated, Section 7.7.2; removal of diquat from solution by adsorption Section 4.6.2.

In static water factors such as, water drift, turbulence, rate of dilution, adsorption of the herbicide, will be important in determining the length of time over which 1mg l^{-1} of diquat is available in the water. The concentration of diquat in the cultures was calculated to be 1mg l^{-1} at the time of application but it is likely that the concentration will have decreased during the exposure period. Even, in these sediment-free cultures, if adsorption of diquat onto the beaker walls was not great, there will have been some uptake of diquat by the plants in which the herbicidal effects were manifest.

There are many examples of studies of diquat and paraquat residues, remaining in static waters after herbicide treatment, (e.g. Coats et al. 1966, Yeo 1967, Frank and Comes 1967, Calderbank 1970). Diquat is thought to dissipate faster than paraquat (Yeo 1967). Depending upon the environmental conditions (particularly turbidity), diquat may remain detectable in lake water for several days (4-27) but the initial decline in concentrations from 1mg l^{-1} is rapid (e.g. 1.0-0.1mg l^{-1} in less than four days, Yeo 1967).

The twenty minute exposure period was chosen for Experiment 2 because of the interest in simulating the effects of a diquat application to flowing water.

Temperature

The range of temperatures chosen for Experiment 2 was based upon records of monthly mean temperatures from rivers in Scotland and southern England, for May-June, when the herbicide is most likely to be used. The records were provided by Water Authorities and River Purification Boards.

The interaction of the herbicide and temperature

Temperature may influence the outcome of a herbicide treatment in two ways:

1) Influencing the rate of plant kill
2) Influencing the final proportion of plants killed

The efficacy of a herbicide is usually rated in terms of the eventual proportion of vegetation removed. The rate at which this control is achieved is also important,in terms of the length of time before the risk caused by the plants (e.g. to flooding) is reduced,and the period over which other components of the ecosystem have time to adapt to the loss of vegetation.

There are two ways in which temperature can influence the effects of a herbicide:

a) Direct influence on the rate of uptake and biochemical activity of the herbicide, at the time of contact with the plant
b) Indirect influence on the rate of the decay processes, given the same degree of herbicidal activity

The inability of paraquat to cause chlorosis of leaf discs, at 1°C, was interpreted by Barnes and Lynd (1967) to indicate that the herbicidal activity was dependent upon enzymatic reactions, not just non-biological reduction. Increases in temperature were shown to increase the rate and magnitude of disc chlorosis. Cell damage, caused by paraquat, measured by membrane leakage, was found to be temperature dependent, being reduced at lower temperatures (Merkle et al. 1965). These results indicate that temperature may influence the biochemical activity of the bipyridinium herbicides, probably by its effect on the rate of metabolism of the treated plant and on the biochemical reactions involved in the herbicide's mode of action.

Mackenzie et al. (1971) showed that if Egeria densa was exposed to 0.25mg l^{-1} diquat for periods of less than 24 hours, an increase in

temperature improved the percentage of plant kill. For exposure periods of longer than 24 hours, 100% plant kill was achieved at all temperatures but the rate of kill was proportional to the temperature.

The interaction of exposure periods of less than 24 hours and temperature suggests that the temperature was influencing the rate of uptake of the herbicide, which could only occur during the period of exposure to the herbicide. For example, if a certain, threshold, amount of diquat (perhaps proportional to the plant biomass) had to be taken into the plant, to achieve 100% kill, whether this threshold was reached would depend upon the rate of uptake of the diquat and the length of time over which this uptake could occur. Thus, the threshold would be reached by rapid uptake over a short period, or over a longer period if the rate of uptake was slow.

Possible mechanisms of uptake of diquat will be discussed later in this section (3.4.4) but a rise in temperature is likely to increase the rate of uptake whether it is a process controlled by the plant's metabolism or a physical process dependent upon rates of diffusion.

The influence of temperature on the rates of kill (measured as a percentage of the original plant biomass), observed when exposure periods of over 24 hours were used, may have been both a direct effect on the rate of the herbicide's biochemical activity and an indirect effect on the rate of activity of the decay-organisms.

Experiment 2 was not continued for long enough to see whether the temperature only influenced the rate of damage or whether the percentage of damaged tissue at 9°C would have stopped short of 100%. The influence of temperature on the rate of decay of the plants which were not treated with herbicide, suggests that the decaying processes were strongly influenced by temperature.

If required, the influence of temperature on the uptake of diquat could be assessed by experiments using ^{14}C-labelled diquat, or by exposing plants to the herbicide, for short periods, at different temperatures and then maintaining all the plants, whilst decaying, at one, medium temperature.

Calcium concentration

The calcium concentrations used in Experiment 1 were rather extreme, and no natural waters (in which plants are likely to cause problems) would be totally devoid of calcium. The calcium concentrations in Experiment 2 were more realistic, being based upon the range of values occurring in Water Authority data.

In both experiments increases in the calcium concentration could be seen to antagonise the effects of the herbicide.

The interaction of the herbicide exposure period and the calcium concentration

Two ways in which calcium may influence the behaviour of the alginate formulation of diquat, have been mentioned in Section 2.2.3. The antagonism of calcium to the action of the dihalide salts of diquat (dibromide) and paraquat (dichloride), in solution, was observed by Parker (1966) and Barrett (unpublished data). No explanations for the mechanism of this antagonism were proposed.

The results of the experiments described here, show that the antagonistic effects of calcium, on the activity of diquat, can be reduced or overcome, if the period of exposure of the plants to the herbicide, is long enough.

A mechanism to explain these observations, in relation to diquat ions once in solution, will be discussed in the rest of this section (3.4.3 - 3.4.8).

3.4.3 The antagonistic effects of calcium on the activity of diquat

The points at which calcium may influence diquat, during the passage of the herbicide from solution to its point of action, are summarised below:

(1) Transport (diffusion, water currents) to plant surface

(2) Adsorption onto plant surface

(3) Uptake into plant tissue

(4) Translocation within the plant

(5) Penetration of cells and organelles (chloroplasts, mitochondria) to site of action

(6) Physiological mode of action

(1) Being a cation like diquat, the calcium ions in solution will not react directly with the diquat in solution. Diquat may be adsorbed and inactivated by precipitates of calcium carbonate in solution (calcite) or on the plant surfaces (marl) (2). This adsorption of diquat may be important in some calcareous waters and will be considered further in Section 4.6.2. No such precipitates were visible in the solution or on the plant surfaces in these experiments.

(6) The physiological mode of action of diquat (Appendix 3) is unlikely to be affected by the calcium concentrations in the water. This is because the sites of action (in the chloroplast in the light and in the mitochondria in the dark) are buffered from changes in external ion concentrations by the cell and organelle membranes. There are no obvious stages in biochemical action of diquat which are likely to be blocked by calcium ions.

This leaves the adsorption and uptake of diquat by plant tissues and cells (3) and by the organelles (5), or the translocation of the herbicide within the plant (4), as the most likely stages at which calcium ions can interfere with the activity of diquat.

3.4.4 The translocation and uptake of diquat by plants

The rapid uptake and translocation of diquat was shown in the original work, which first described diquat's herbicidal properties (Brian et al. 1958).

Translocation of diquat. The mechanism of translocation of diquat was determined from experiments by Baldwin (1963) and Brian (1966).

When a single leaf of a tomato plant was treated with diquat in the light, there was only localised damage. If a leaf was treated on a plant kept in the dark, when subsequently exposed to light, the whole plant was destroyed. This dispersal of the herbicide throughout the whole plant was restricted if the plant was immersed in water, but was not affected if the leaf petiole was killed by steaming. Autoradiographic examination, using ^{14}C -labelled diquat, showed that no movement of the diquat occurred in the dark but on subsequent exposure to light, the herbicide was distributed throughout the plant within five hours (Baldwin 1963). Brian (1966) showed that the downward movement of diquat and paraquat applied to the leaves of terrestrial plants, was increased if the environmental humidity was increased or if the soil was dry.

This evidence indicates that diquat is translocated in the xylem by the transpirational flow of water, the direction and velocity of which is determined by the external humidity. When diquat was applied in the light, the damage it caused to the leaf tissue prevented the herbicide from reaching the xylem, resulting in no movement around the plant. Application in the dark allowed the diquat to reach the xylem, but movement would have only occurred in the light, when the stomata would have opened and the transpirational flow increased.

There was little translocation of diquat in tomato plants submerged in water because of the lack of transpiration under such conditions. The vascular system, and xylem in particular, is much reduced in submerged aquatic plants. The transpirational flow seen in terrestrial plants is not possible underwater, although it is possible that some transient flow of water and dissolved salts may occur (Sculthorpe 1967).

Funderburk and Lawrence (1963a) using an autoradiographic method showed that there was little translocation of diquat or paraquat from shoots to roots, or vice versa, in submerged aquatic plants. It is interesting to note that phosphorus was seen to move in both directions.

Davies and Seaman (1968) observed some translocation of ^{14}C-labelled diquat in Elodea canadensis. The extent of this movement, which was the same in both light and dark, was very limited, and was concluded to be the result of diffusion within the Elodea.

These findings indicate why diquat is regarded as a contact herbicide if applied to aquatic vegetation. Calcium will not have had its antagonistic effect on the activity of diquat at the stage of translocation (4).

Adsorption and uptake of diquat. Diquat and paraquat were strongly adsorbed onto terrestrial plant tissue (Brian 1967). This process appeared to be reversible, seen by the exchange of labelled and unlabelled paraquat. The rapid initial adsorption was thought to be an ion-ion interaction, between the two positive charges on the nitrogen atoms of the bipyridinium herbicides, and the negative centres on the plant surface. This process was very rapid, such that within thirty seconds, 33% of the diquat applied to Beta vulgaris could not be washed off.

This primary stage of adsorption, possibly into the Donnan Free

space, was followed by a slower uptake, over two hours, into less accessible Donnan Free space. The third stage was a slow accumulation of the herbicide in the tissues (Brian 1967).

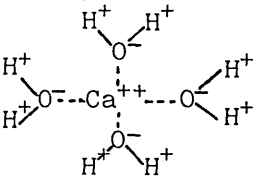
Similar studies were carried out on submerged Elodea canadensis, using ^{14}C-labelled diquat, by Davies and Seaman (1968). An initial increase in plant radioactivity, seen within fifteen seconds and continuing for 10-20 minutes, was attributed to adsorption onto the plant surfaces. The slower, long-term uptake which continued for 4.5 hours, was thought to be the result of metabolic accumulation. The uptake of diquat by Elodea had been partially inhibited by the respiratory inhibitors sodium azide and potassium cyanide (Seaman and Thomas 1966).

No specific mechanism for the uptake of diquat into aquatic plants has been proposed or tested. The following hypothesis as to how calcium ions may compete with diquat for uptake in aquatic plants, must, therefore, be highly speculative.

3.4.5 The competitive uptake of calcium and diquat by aquatic plants

The antagonism of calcium ions to the action of bipyridinium herbicides can be reduced by increasing the concentration of the herbicide (Parker 1966) or by prolonging the period of exposure. These observations suggest that this antagonism may involve a reversible competition between diquat and calcium ions. Reference to enzymatic inhibition may illustrate the difference between competitive and non-competitive antagonism, Fig. 22.

It has been suggested by staff at I.C.I. that the hydrated calcium ion is a similar size to the diquat ion (Barrett pers. comm.).



Hydrated calcium ion.

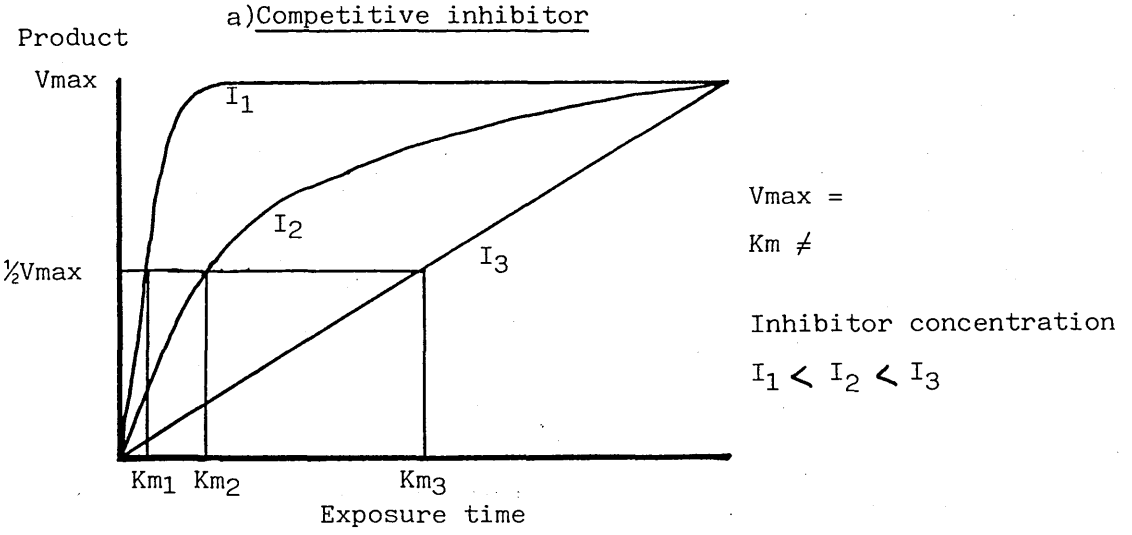
If this is correct, then competition between the ions at the stage of uptake into plant cells, through semi-permeable membranes, would seem likely, because ion size is important in this process.

In order to discuss these ideas further it is necessary to consider the mechanisms which have been proposed for the uptake of calcium ions into aquatic plants.

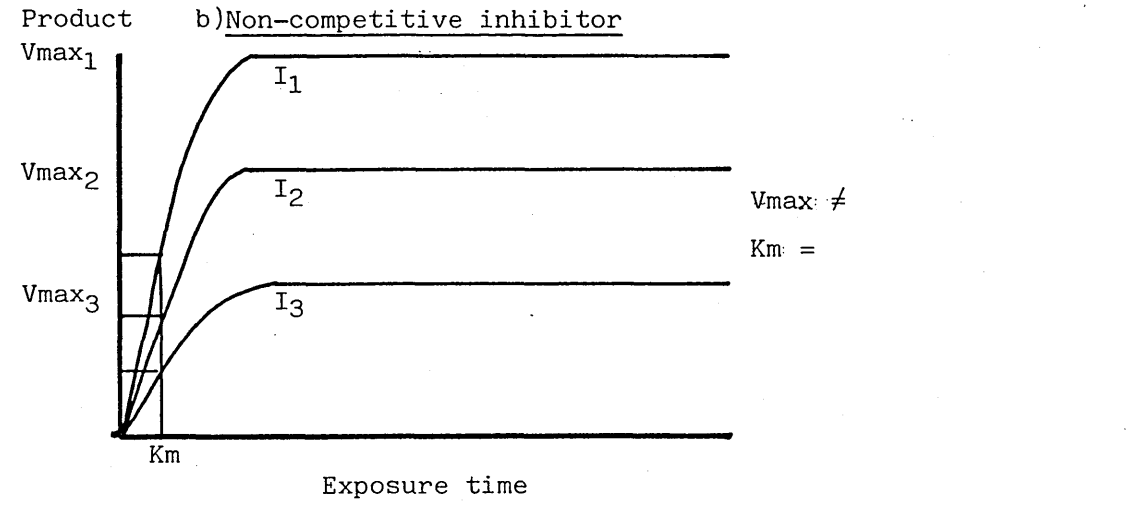
Fig. 22

Diagrams to illustrate competitive and non-competitive antagonism

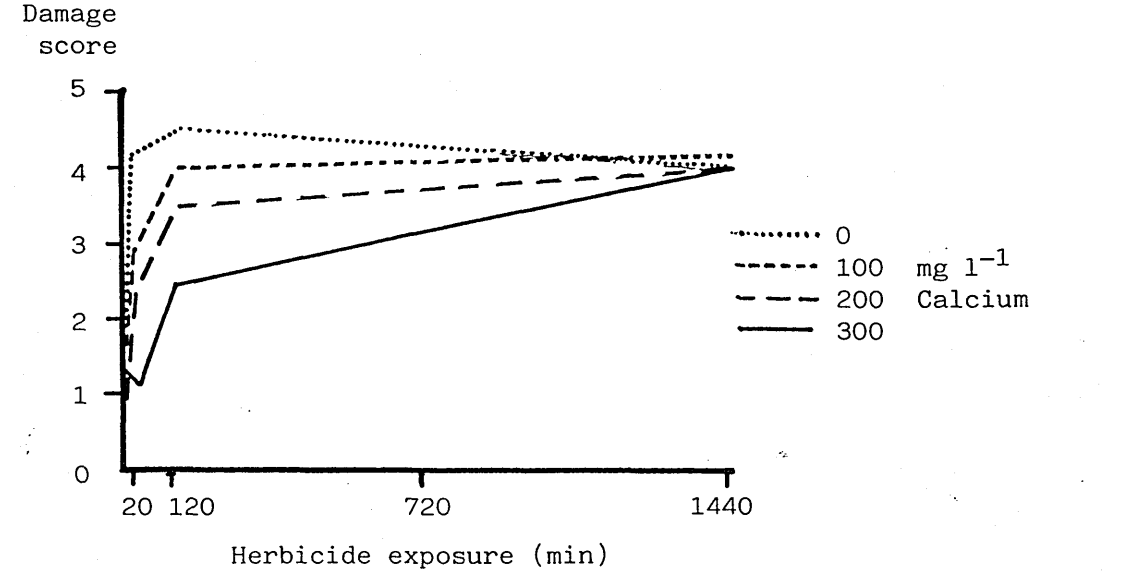
a) Competitive inhibitor



b) Non-competitive inhibitor



c) Damage score data from Experiment 1



A review of the early work on the uptake of cations by submerged macrophytes, such as the work of Arens, Steemann Nielsen and Lowenhaupt, is presented by Sculthorpe (1967). These authors proposed that the uptake of cations is linked to the photosynthetic utilisation of bicarbonate ions, as has been observed in many vascular macrophytes. Lowenhaupt (1956) proposed a mechanism for linking the two processes, which is summarised in Fig. 23.

This mechanism involved energy-rich coupling agents, produced in the chloroplasts, which carried cations and bicarbonate ions across the plasmalemma of the epidermal cells. The bicarbonate ions diffused to the chloroplasts whilst the cations diffused across the leaf. At the other side of the leaf the cations were pumped out by a mechanism similar to entry, with the hydroxyl ions released from the chloroplasts. Arens' observation that no cation transfer occurred in the dark was explained in that the release of cations from the plasmalemma was a light and oxygen dependent process.

For simplicity, the passage of the monovalent potassium cations has been illustrated in this discussion, but Lowenhaupt's work also involved the study of calcium ion transfer and the mechanisms could apply to both cations.

Lowenhaupt's mechanism assumed that both the influx and efflux of cations was maintained by energy consuming pumps. More recently a mechanism has been proposed which also requires light dependent ion pumps at the lower and upper leaf surfaces, but only for the active movement of hydrogen and hydroxyl ions respectively. The observation that free carbon dioxide increases near the lower leaf surface during bicarbonate utilisation, led to the proposal that a proton pump moves hydrogen ions out of the cell, for the extra-cellular conversion of bicarbonate ions to carbon dioxide and water. The carbon dioxide diffuses into the cell to the chloroplast (Prins et al. 1980).

The passage of cations across the leaf is a passive process driven either through the cell walls by the trans-leaf potential difference, or through the symplast by a proton motive force. The essential point is that the cation is transferred across the leaf to balance the net transport of negative ions (Prins et al. 1980, 1982). This mechanism is illustrated in Fig. 24.

These mechanisms depend upon the spatial separation of the sites of the hydrogen and hydroxyl ion extrusion. This separation is evident in the so-called polar-leaved plants, such as Elodea and

Fig. 23

Mechanism for the uptake of cations and bicarbonate proposed by Lowenhaupt (1956)

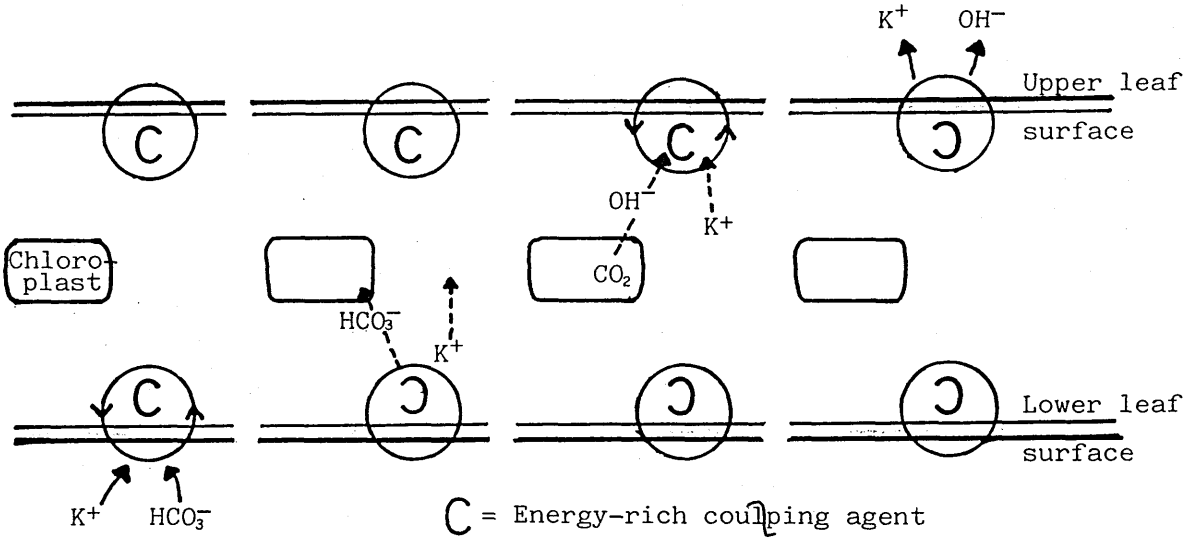
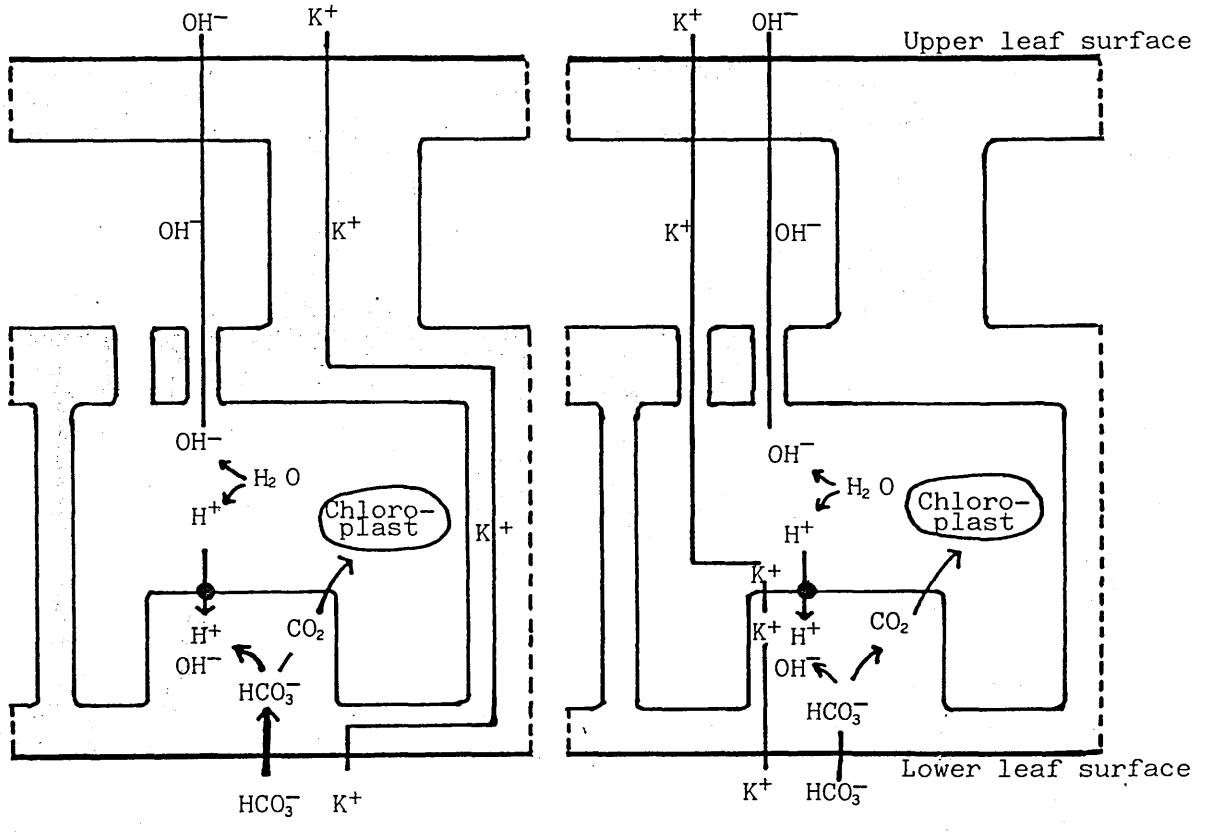


Fig. 24

Mechanism for the uptake of cations and bicarbonate proposed by Prins et al. (1980)



Potamogeton species. Specific sites of cation uptake were described by Arens, and Pate and Gunning (1972), and proton pumps may be placed in invaginations of the plasmalemma of such transfer cells.

If diquat and hydrated calcium ions are of similar size, the diquat ions may be able to substitute for calcium ions in these uptake mechanisms. The diquat ions may compete with the calcium ions for uptake, not necessarily at a physical point of entry, but for passage with 'free' bicarbonate ions which need a cationic charge balance.

Whether other cations, such as potassium and magnesium,would also compete with calcium and diquat for uptake, would have to be investigated. Parker (1966) noted that potassium nitrate and magnesium sulphate were the only salts, other than calcium compounds occurring in tap water, which might antagonise paraquat. Parker had also suggested that calcium antagonised the activity of diquat by interfering with uptake, not with the herbicide's effect on photosynthesis.

3.4.6 Explaining diquat accumulation and other observations, using the hypothesis of competitive uptake with calcium

These cation uptake mechanisms of Lowenhaupt and Prins et al., explain the transport, not the accumulation, of cations. A removal of cations at the upper leaf surface, is assumed to equal the uptake at the lower leaf surface. Small amounts of essential cations, such as calcium, are used or bound up in cells (particularly actively growing ones), for roles in maintaining membrane integrity, activating amylases and the formation of mitotic spindles. When this occurs another cation would have to be released at the upper leaf surface, to maintain the electrical potential balance across the leaf.

Diquat may be cycled through the leaf, which may explain the reversibility of uptake of labelled and unlabelled diquat, seen to exchange by Brian (1967). Since diquat's mode of action is catalytic, a sink for the herbicide will not be created by its use as a substrate. The diquat might exert its catalytic destruction whilst passing through the leaf. Since the site of action of diquat is in the chloroplasts (light) or mitochondria (dark), the diquat ions would have to cross the leaf via these organelles.

The bipyridinium herbicides accumulate in plant tissues, where the residues may persist for several weeks (e.g. Frank and Comes 1967, Way et al. 1971, Brooker and Edwards 1973a, Birmingham and Colman 1983). If diquat accumulates in plant tissues, more of it must be taken up than is effluxed. There are several ways in which this could be accommodated in the cation transport mechanism:

1) The uptake of diquat ions is balanced by the efflux of another cation in exchange

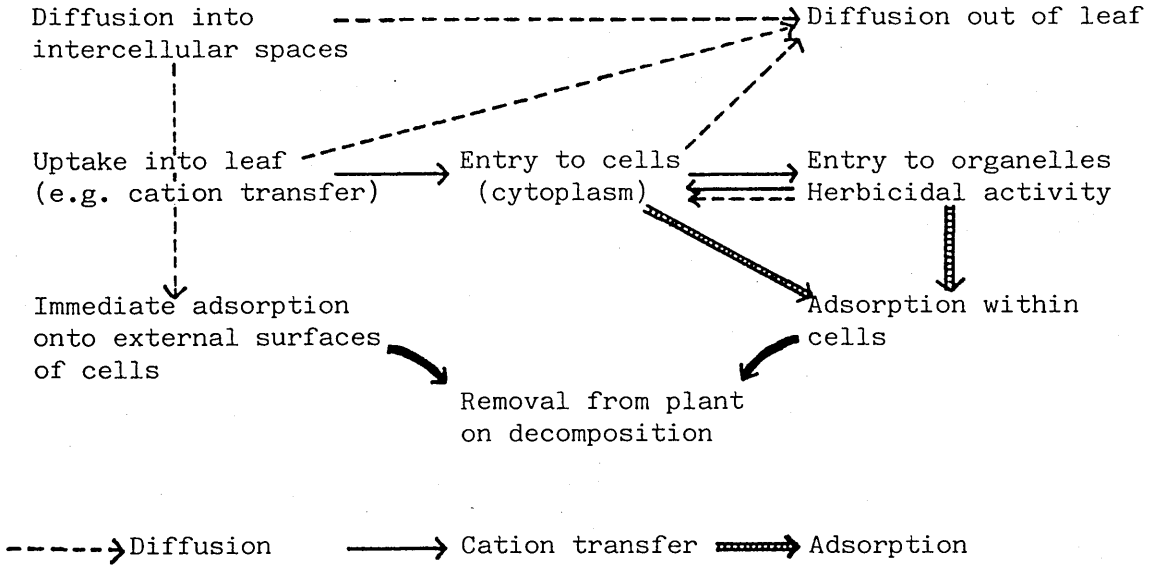
2) There is no cation efflux to balance the uptake of diquat, resulting in the eventual breakdown of the across-leaf potential difference. This may lead to the collapse of the transport system. This disruption must take several hours because uptake has been observed in Elodea over 4.5 hours (Davies and Seaman 1968).

3) The efflux pump is destroyed by the herbicide so that neither cations nor hydroxyl ions are removed. The influx pump is not affected until later resulting in an accumulation of diquat and other cations (and hydrogen ions).

Only the third suggestion does not assume that the diquat ions are retained within the cells in preference to other cations or against an electro-potential gradient. However, the influx pump would eventually stop because of an osmotic imbalance caused by the accumulating cations. To account for the long persistance times observed for diquat in plant tissues, the herbicide would have to be retained in the cells against diffusion, favoured by a concentration gradient out to the surrounding water. The concentration of diquat in water may become undetectable within four days but remains high in plant material for several weeks (e.g. Frank and Comes 1967).

The diquat residues which persist for long periods in plant tissues have mostly been adsorbed and may only be removed by treatment with 5 molar ammonium chloride (Birmingham and Colman 1983). This strongly adsorbed diquat is not herbicidally active. Only the diquat which enters the plant cells and organelles will be active and although some of this may diffuse into the Donnan free space as soon as the herbicide is applied, some may have been taken up by cation transfer mechanisms. Diquat diffusing into intercellular spaces will diffuse out again once the external concentration falls unless it has subsequently been adsorbed or transported into the cells.

The diquat which remains in plant tissues, after the external concentration has decreased, may be only the diquat ions which were immediately adsorbed and so were not involved in the herbicidal activity. More likely, the persistant residues will include ions which were taken into the cells, exerted their herbicidal properties and were subsequently adsorbed. It is not evident exactly where in, or on, the plant material the adsorbed diquat ions are held nor how rapidly adsorption occurs. Thus, it is not possible to determine what proportion of diquat ions contribute to the herbicidal activity or the relative importance of the following routes in diquat accumulation.



Apart from having to accommodate the observations that diquat accumulates in plant tissues, any proposed uptake mechanism must also take various other findings into account.

The presence of calcium may be antagonistic to the efficacy of a cationic herbicide, such as diquat, but an interaction in which calcium stimulated the uptake and efficacy of the anionic herbicide 2,4-D amine, was observed with Myriophyllum spicatum (Stanley 1975). The uptake of phosphate by Myriophyllum was also enhanced by the presence of calcium. The transfer of these anions may be unrelated to the cation transport, or the anions could be competing with the bicarbonate ions for uptake.

An increase in calcium concentration may favour the formation of marl at the upper leaf surfaces, by pushing the following equation

towards calcium carbonate production:

$$Ca^{++} + HCO_3^- + OH^- \rightleftharpoons \underset{\text{marl}}{CaCO_3} + H_2O$$

(Wetzel 1960)

An increase in marl formation would reduce the concentration of bicarbonate ions available for uptake and this might improve the competitive uptake of other anions instead. A knowledge of the mechanisms controlling the activity of the bicarbonate-cation influx and efflux pumps, would be necessary to assess the feasibility of such proposals.

Calcium has been observed to be antagonistic to the uptake of another cation, zinc (Pickering and Pvia 1969). The three stages of zinc uptake observed were very similar to those described for the uptake of diquat (Brian 1967), and the antagonism of calcium was attributed to competition for uptake.

However, the mechanism for the uptake of zinc may not be the same as for diquat because the observations of zinc uptake were made on the aquatic moss, Fontinalis antipyretica. Steemann Nielsen (1946) demonstrated that Fontinalis could only use carbon dioxide, and not bicarbonate ions, as a photosynthetic carbon source. Thus, either a different mechanism of competitive uptake for calcium and zinc must occur in Fontinalis, or the cationic uptake mechanism suggested for diquat is not exclusively related to bicarbonate utilisation.

It is not just the uptake, or availability, of diquat to plants which appears to be influenced by the presence of calcium ions. The concentrations of diquat that cause acute toxicity to fish are markedly higher in calcareous waters (Simsiman et al. 1976). For example, ten times more diquat was needed to cause the same level of toxicity to Fathead Minnows, in water with 299mg l^{-1} calcium, as was needed in water with 22mg l^{-1} calcium (Surber and Pickering 1962).

These findings might suggest that calcium is likely to affect the activity of diquat for a variety of uptake mechanisms. A direct competition for uptake between similarly sized ions would seem likely.

A final observation concerning the uptake of diquat by macrophytes, is the fact that copper appears to have a synergistic effect on the activity of diquat (Sutton et al. 1970). Advantage of this fact is often taken by water managers in the U.S.A., who use mixtures

of diquat and copper sulphate (e.g. Haller et al. 1983).

Using ^{14}C-labelled diquat, the influence of copper was seen to be to specifically increase the uptake of diquat ions (Sutton et al. 1972). Copper was also found to increase the movement of herbicides, both anionic and cationic, through the epidermal tissue of isolated Potamogeton nodosus leaves (Pringle and Anderson 1980).

As a cation, copper might have been expected to compete for uptake with diquat. The synergism of copper to the uptake of diquat was attributed to an increase in cell membrane permeability (Sutton et al. 1972). This may be a sufficient explanation, rather than trying to include the presence of copper in the cation transfer mechanism. This is because the copper, in its action as a heavy metal (inactivating enzymes and precipitating proteins), would probably destroy any active cation uptake mechanisms, fairly rapidly.

3.4.7 Some experiments which might help to explain the uptake of diquat and the antagonism of calcium

Evidence to confirm, or refute, the proposed mechanism for the uptake of diquat and calcium antagonism, might be obtained by the following experiments:

1) Using ^{14}C-labelled diquat, measure the rate of uptake of the herbicide, and its herbicidal effect, in a range of calcium concentrations. The concentrations of diquat and calcium should be quantified not just estimated from dose rate. If a suitable calcium isotope were available, the effect of diquat concentrations on the rate of uptake of calcium could be compared.

2) The rate of uptake and the activity of diquat could be compared when plants were exposed to the herbicide in the light or in the dark. Subsequent maintenance of the plants would be in the light.

3) The uptake and activity of diquat could be estimated in other conditions in which bicarbonate utilisation is low, e.g. low pH, with plants such as Fontinalis antipyretica. Comparisons could be made with results from conditions favourable to bicarbonate use.

4) The effects of temperature on the uptake of diquat could be

examined. Not only would an increase in temperature accelerate any metabolic processes involved in an active uptake mechanism, but the rate of photosynthesis would increase rapidly stripping the water of free carbon dioxide, and so increasing the need for bicarbonate utilisation and uptake.

3.4.8 The limitations of small-scale static experiments and some alternative suggestions

The laboratory experiments described in this chapter, have illustrated certain important influences that environmental factors may have on the efficacy of diquat. The conditions of these experiments were extremely artificial and the interaction of other variables which may be important in the field, would have been removed or controlled.

The two major limitations of these experiments, in terms of investigating the efficacy of diquat-alginate in watercourses were:

1) The use of static cultures

2) The need to dilute the diquat-alginate down to a solution, because of the small-scale, rather than applying strings of the Midstream formulation which would gel in the water.

In questioning the validity of extrapolating conclusions drawn from static laboratory culture to flowing systems, one needs to consider which processes would be affected by water movement. For example, it is unlikely that the effects that different temperatures have on diquat's activity, would vary in static or flowing culture. However, if the interactions with herbicide formulation or exposure period (both of which are affected by water movement) are to be considered, then the extrapolation of the influence of temperature in static cultures,to flowing systems, would not be as straightforward.

Even in the lentic environment, diffusion and water dispersal,by currents and wind action, are likely to reduce the contact time of the herbicide with the plants, compared to that in a well-mixed beaker. The situation is even less realistic if conclusions are to be applied to flowing water.

These experiments had the additional problem of trying to

culture in static conditions, plants taken from flowing water. If these plants are adapted to flowing water, their health may deteriorate in static culture and will limit the duration of the experiments.

The inability to use diquat-alginate in the viscous formulation was not as important in static culture as it would have been in a small-scale flowing system. It was worth using the diluted alginate formulation, rather than the Reglone solution, because of the suggestions by Barrett (1981b), that the improved performance of diquat in alginate, is not just due to the physical binding of the herbicide onto the plants.

Laboratory scale flowing systems are bound to be more complex than static ones, because even at the simplest level, of piping water in and out of beakers, a constant supply of suitable water or culture medium is needed. This is a particular problem if manipulation of specific ions, such as calcium, is intended.

Small-scale flowing systems have been developed for physiological studies (e.g. Westlake 1967). Guttering pipes were used successfully for experiments in which the removal of diquat from flowing water was measured, in the presence and absence of plants (Barrett and Newman unpublished data). A limited supply of distilled water was not a problem for these experiments, lasting only a few minutes or hours, but observations over several days would not have been possible. The pattern of flow, examined using injections of dye, in the guttering tended to be highly laminar across the water surface. Little mixing of water below the surface occurred, especially in the presence of submerged plants.

A flume system was developed by Dawson (pers. comm.) which was designed to ensure a well mixed flow pattern. With his permission a copy of this system, on a scale large enough to allow the use of fine strings of diquat-alginate (approx. 3m long), has been built at Glasgow University. Several hundred grammes, fresh weight, of Ranunculus sp. have been cultured in this channel for several weeks. Experimental work has not yet been possible in this system but it has great potential as a medium for experiments using diquat-alginate.

3.5 Conclusions of the Laboratory Experiments

1) Damage scores and dissolved oxygen concentrations were the most suitable parameters for assessing the activity of diquat-alginate on Ranunculus stems, in static culture.

2) The rate of activity of diquat was increased by:
   increased period of exposure to the herbicide
   increased water temperature
   decreased calcium concentration of the water

3) The antagonism of calcium to the activity of diquat appeared to be a competitive process, reduced by increasing the period of exposure to the herbicide. This competition may occur at the stage of uptake into the plants.

4) The initial rapid uptake of diquat may be by passive diffusion. An hypothesis may be proposed that the active uptake of diquat into leaves is the same as for other cations, such as calcium. Calcium uptake is thought to be related to the photosynthetic utilisation of bicarbonate as a carbon source for some submerged macrophytes.

5) Experiments with ^{14}C-labelled diquat could be used to test these hypotheses.

6) Small-scale, static-culture experiments did not allow the expression of the physical characteristics of diquat-alginate. Such conditions were highly artificial for the growth of species adapted to flowing water. Small-scale, through-flow flumes can be constructed which would provide a more realistic medium for laboratory experiments concerned with herbicide use in flowing water.

CHAPTER FOUR

FIELD TRIALS OF WEED CUTTING AND THE USE OF DIQUAT-ALGINATE IN RIVERS

I. COMPARISON OF WEED CONTROL METHODS WITHIN EACH RIVER

4.1 Introduction

It has been proposed that all experiments can be classified as mensurative or manipulative (Hurlbert 1984). Mensurative experiments involve making measurements at one or more points in time or space, so that time or space are the only experimental variables. Habitat surveys and population sampling are examples of this type of experiment, and are usually conducted outside the laboratory.

Manipulative experiments usually involve the imposition, by the experimenter, of some external factor(s). Two or more treatments are applied to different experimental units with the intention of comparing the effects of such manipulations. The laboratory environment tends to be favoured for this type of experiment, so that factors other than those being manipulated, can be kept constant or regulated. This approach is acceptable for the study of manipulations which are encountered in laboratory, or laboratory-like, conditions, but there is almost bound to be an artificial oversimplification, if treatments which are ultimately to be applied in field conditions, are so examined.

The conditions encountered in the field are the opposite extreme to those in the laboratory, with a lack of control over most, if not all, environmental variables. The experimenter's manipulative powers are more limited in the field, compared to the laboratory. The environmental conditions, for manipulative experiments in aquatic habitats, especially flowing water, are particularly difficult to control, compared with, for example, a well defined plot of soil in a uniform field.

Despite this lack of control, or perhaps because of it, the importance of field trials, should not be underestimated. The risks of error in trying the extrapolate to the field, results from laboratory experiments may be very high (Barrett 1981a).

Field work in the aquatic environment poses various problems which are not encountered in terrestrial habitats. For example, the practical difficulty of observing and quantitatively assessing organisms underwater. Some of these problems, with particular reference to experiments using aquatic herbicides, have been addressed by Robson and Barrett (1972). These authors, rather discouragingly, begin one paragraph with the line,

"Flowing water should be avoided whenever possible.....".

To be fair, this was written before the development of diquat-alginate as a flowing water herbicide, but when trying to design experiments in this environment, it may often be tempting to agree!

Most of the examples of field trials of aquatic weed management techniques, in the literature, either only make comparisons between treated and untreated sites, or between different levels of the same treatments. Comparisons between different types of treatment are often only made after publication, or at best, over different seasons. To provide reliable comparisons, which may be statistically valid, methods of weed control, and their effects, should be directly compared and not studied in isolation (Barrett 1981a).

Direct comparisons of emergent weed control methods, by manual clearance or using herbicides, were made in drainage channels by Brooker (1976a, 1976b). Mechanical cutting and herbicide use for controlling submerged weeds in canals, were directly compared in field trials by Eaton et al. (1981), and Murphy and Eaton (1981).

The field work described in this,and the two following chapters, was designed to provide a direct comparison of manual or mechanical weed cutting methods with the use of diquat-alginate. Comparisons of the efficacies and the ecological effects, of the two management techniques were made in 1984 in one canal and three river sites.

The results of the river trials, comparing weed control methods within each river, are presented in this chapter. Comparisons of the trials, between the river sites, are considered in Chapter Five. The trial in the canal is discussed in Chapter Six.

4.2 Experimental design for aquatic field trials

4.2.1 A review of experimental designs used in aquatic field trials

The subject of experimental designs for use in field work is considered, often at great length, by most statistical text books, particularly those aimed at ecological and agricultural research (e.g. Ridgman 1975, Little and Hills 1978, Mead and Curnow 1983). Such texts are almost exclusively concerned with terrestrial experiments. The particular problems encountered in aquatic, and more specifically, in flowing water systems are rarely discussed.

The critical features of experimental design were considered, by Hurlbert (1984), to be:

Controls, Replication, Interspersion

Controls

Untreated control, or reference, experimental units are extremely important when biological systems, especially ones as complex and changeable as aquatic habitats, are being studied and manipulated. However, it is often difficult to find suitable experimental units, for replication. This will depend upon the size and nature of the experimental units being studied. In the Hubbard Brook Programme, in which whole river catchments were studied, it was possible to find similar catchments for comparison (Likens 1985). More commonly, if a whole ecosystem, such as a lake, is being treated, it is difficult to find two sites which do not significantly differ from each other prior to treatment.

One way to overcome this problem, of finding more than one, similar experimental unit, is to use sequential, rather than concurrent controls. A pre-treatment survey of the site is used for comparison with the post-treatment observations (e.g. Brooker and Edwards 1973a). The duration of such pre-treatment studies, and the season in which they are carried out, will depend upon the conditions anticipated for the post-treatment study. Long-term monitoring of sites would be necessary if the temporal changes or annual variations were likely to be greater than the effects of the treatment itself, (e.g. Westlake and Dawson 1982).

It is usually easier to assign control units if the experimental units are subdivisions of an aquatic system, such as a small plot in a

lake (e.g. Barrett and Logan 1982), or a limited length of river (e.g. Morrison and Courtney 1981). One problem, particularly important in work with herbicides, is that of isolating the plots, so that, for example, the herbicide does not spread from the treated to control plots. In static water, barriers can be used to isolate plots but these are usually expensive, and artificially limit the movement of organisms and chemicals, in addition to the herbicide. Usually plots in static water are isolated by leaving gaps between them. These gaps have to be sufficiently large that diffusion and water currents are unlikely to carry the herbicide between treated and untreated plots. The risk of contamination of control plots is greater in flowing water, unless the controls are placed upstream of the treated plots (e.g. Barrett and Murphy 1982).

Some confusion in terminology could arise here, because of the use of the word 'control' in relation to the management of weeds. Whenever possible reference will be made to 'untreated' experimental units.

Replication

Replication in experiments is desirable so that differences between, for example, a treated and an untreated plot, can be assigned to the effect of the treatment with some confidence, and may not be just due to random variation between the plots. If certain statistical analyses are to be used to quantify the differences between the treatments, then replication is essential. Thus, an estimate of the within treatment variation, or error, can be made, upon which the significance of any comparisons between treatments, can be judged.

If the provision of control units presented a problem, then finding sufficient experimental units for replication is even more difficult, and few trials in whole ecosystems, such as lakes, are replicated. Sequential replication is sometimes attempted, but the same problems of temporal variability as found using sequential controls, would be encountered (e.g. Way et al. 1971).

Replication is usually possible with smaller experimental units, but the problem of the downstream contamination of replicates, with chemicals, arises in flowing water so that treatment replication is often sacrificed in rivers (e.g. Morrison and Courtney 1981).

If the replication of treatments is possible, then the number of replicates per treatment has to be decided. It is possible to

estimate the optimum number of replicates for an experiment, if an estimate of the expected variability is available (Mead and Curnow 1983, page 285). These estimates of the optimum number of replicates usually have to be ignored because of resource limitation, (e.g. time, area, manpower). The few replicates possible as a result of these limitations, are frequently unsatisfactory, especially if a range of habitats or populations are to be sampled. Some factors, such as water chemistry, may show little variation between sections of a river, but macroinvertebrate populations can vary considerably. For example, it was estimated in one small, fairly uniform riffle in a creek, that for 95% confidence in sampling one population, 194 Surber samples (1 ft^2) were needed for dry weight assessment. Estimates of the total invertebrate number would need 73 samples (Needham and Usinger 1956).

Interspersion

In experiments with low levels of replication, there has been some debate as to whether a complete randomisation, a random block, or a systematic distribution of replicates is most suitable (Bourdeau 1953, Hurlbert 1984). In static water, in which the separation of treatments is possible, a randomised block design is likely to be most appropriate for statistical analysis (e.g. Barrett and Logan 1982).

In flowing water, unless the channel is wide enough for parallel replicates, the linear dispersal of the replicates will be largely determined by the degree of downstream interference expected between treatments. This interference may include the treatment itself (e.g. herbicide) or any mobile results of the treatment (e.g. released nutrients, emigrating fauna).

Commonly the replicates of a particular treatment will be placed together, with the controls upstream (e.g. Brooker 1976a). This segregation of treatments rather defeats the object of having replicates. There is the risk that locational differences upstream and downstream, prior to sampling, or arising during the experiment (nondemonic intrusion), may be interpreted as differences due to the treatments (Hurlbert 1984). Examples of experimental designs with the treatment replicates divided between blocks, in rivers are rare (e.g. Logan 1984).

As if the experimenter working in the aquatic environment does not have enough to worry about, there is the risk with unattended long-term experiments that someone will visit the experimental site and provide their own manipulation. These events, and the demonic intrusion described by Hurlbert (1984) have to be stoically accepted as part of Murphy's Law and its corollaries (Murphy pers. comm.).

4.2.2 The experimental designs used in the river trial

The limitations on resources of time, manpower and the lengths of river available, were such that only two replicates of each treatment were used in most of the field trials. Definitions of some terms which will be used in describing the field trials are as follows:

Site	Total length of a river studied
Treatment	Management regime (i.e. herbicide, cutting, untreated)
Section	Treatment replicate, one of the 100m or 200m lengths of river subject to a specific treatment (e.g. upstream herbicide replicate)

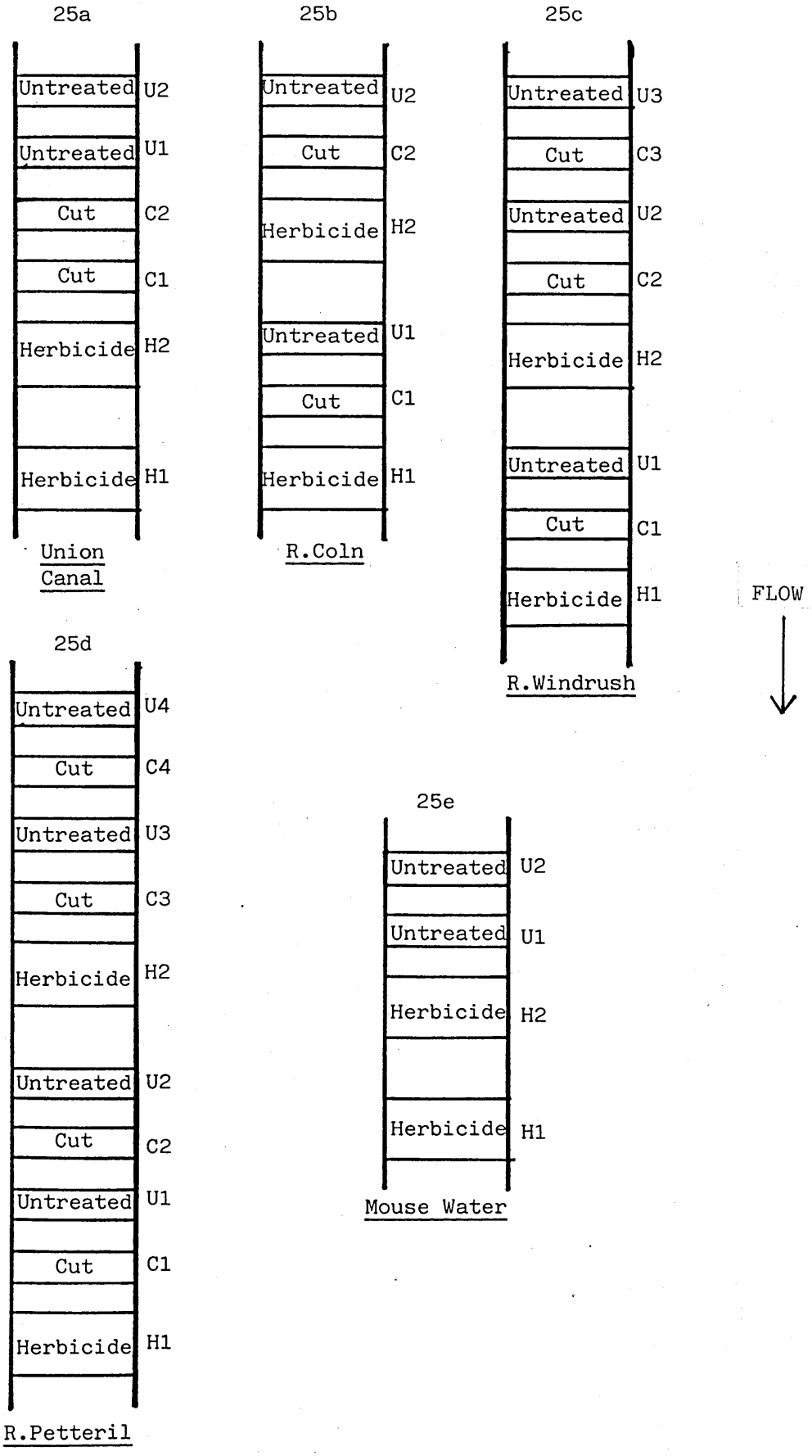
Three treatments, herbicide, cut and untreated, were compared in three of the rivers, R.Petteril, R.Windrush and R.Coln. A cutting treatment was not applied in the Mouse Water. Untreated and cut sections were 100m long, whilst lengths of 200m were treated with herbicide. Only the middle 100m of the herbicide treated sections were sampled, so that any edge effects would be avoided.

In order to avoid the risk that herbicide residues passing downstream might influence other treatments, a segregated experimental design (Fig. 25a) was initially considered. With such segregation it could not be assumed that differences which might occur between,(for example, the untreated and herbicide) sections only arose as a result of the treatments. Some unknown, or unquantified, change in the character of the river, as it passes downstream over the whole site, might be responsible for such differences.

The gross characteristics of many rivers change from narrow, steep, upland streams to wide, meandering lowland rivers. Many attempts have been made to zone and classify river communities in relation to these longitudinal changes (Hynes 1970). On a smaller scale, biological changes, such as the 2-3 month delay in the date of

Fig. 25

Experimental designs used in the field trials



flowering of Ranunculus penicillatus var. calcareus, have been observed over 10km of the River Piddle (Dawson 1980).

Such consistent upstream to downsteam changes may not be important in trials in which sites are only 1-2km long. Unless the river channels are very uniform over these distances, there may be chance differences between the upstream and the downstream halves of the site (e.g. distribution of riffles and pools).

In an attempt to allow for such environmental heterogeneity, a basic two-block experimental design (Fig. 25b) was proposed. With this design, differences between the upstream and downstream halves of the site could be quantified in the analysis of variance.

Using the two-block design, the risks of herbicidal effects passing downstream from the upstream herbicide section (H2) must be minimised. The maximum previously recorded distance, of visible downstream effects from an application of diquat-alginate, was 200m. These effects were observed in the swiftly flowing (mean flow 0.5m s^{-1}) and shallow (mean depth 0.25m) Cumwhitton Beck in Cumbria, and in the very soft water (less than 6mg l^{-1} calcium) and fast-flowing River Spey, Morayshire (Barrett and Murphy 1982, Barrett pers. comm.). These results were exceptional, usually downstream herbicide effects are only seen up to 20-30m below the treated area. This distance will depend upon water velocity, depth, presence of plants, and other features of the river which will trap the gelled tadpoles of diquat-alginate and which will adsorb diquat residues.

The risks of herbicide drift affecting downstream sections, can be reduced by the provision of a large, unsurveyed gap below the upstream herbicide section. Robson and Barrett (1972) recommended buffer zones of no less than 100m but in view of the experiences with diquat-alginate in Cumwhitton Beck and the River Spey, a gap between the blocks of at least 250m was provided. If possible these zones included deeper reaches with slow flow so that the tadpoles and diquat residues were more likely to be detained and adsorbed by plants and sediments, on their passage downstream.

This experimental design assumes that the brief pulse of soluble unbound diquat, which will be carried downstream immediately after the application of the herbicide, will have no significant herbicidal effects in the downstream block. This assumption is reasonable in view of the evidence that liquid applications of diquat are ineffective in flowing water (Barrett 1978b, 1981b, 1981c).

Due to the highly variable nature of the river, caused by many meanders, and the limited length of site available, the simple two block design (Fig. 25b) was used in the R.Coln.

A sufficient length of river was available in the R.Windrush to allow the slightly more complex design in Fig 25c. In this design, if any significant downstream effects were observed, from H2 into U1 and C1, these sections could be ignored. Instead the two upstream cut (C2,C3) and untreated (U2,U3) sections would be compared with the herbicide sections.

The R.Petteril is a shallow, fairly swift-flowing stream, which means that it is likely to be prone to the downstream drift of diquat-alginate. For this reason the most sophisticated experimental design (Fig. 25d) was used. If drift from the upstream herbicide section affected the sections in the downstream block, the upstream sections (C3,C4,U3,U4) could be compared with the herbicide sections(H1,H2). The effects of the downstream herbicide drift could be quantified by analysing the results from the affected section U1,U2,C1,C2. This design could be accommodated in the R.Petteril because a long site was available, the channel was fairly uniform and the co-operation of the North West Water Authority was particularly valuable. As a result, this site was the most intensively studied.

The design used in the R.Windrush (Fig. 25c) was originally intended for the Mouse Water but as a result of engineering works at the downstream end of the site, the length of the river to be surveyed was reduced and the cutting treatment abandoned. Downstream herbicide effects were anticipated, so a segregated design (Fig. 25e) was used, avoiding the risks of herbicide drift affecting either of the untreated sections.

The arrangement of treatments within the two blocks was not random because of the necessity of placing the herbicide sections downstream of the others. Cut and Untreated sections were systematically distributed along the site.

The lack of independence between sections, caused by the passage of water between them and the lack of randomisation within the blocks were important characteristics of the experimental design. Caution has to be observed when applying and interpreting statistical analyses to data from such complex and variable systems.

4.3 The River Sites

4.3.1 The criteria for site selection

The rivers and sites used in these trials were chosen using four criteria.

1) The presence of a submerged macrophyte, known to be susceptible to diquat-alginate, was chosen as a common link between all of the sites. Rivers in which species of the Ranunculus subgenus Batrachium were known to occur, were identified by reference to Haslam (1982a, 1982b).

2) In view of the importance that the calcium concentration of water has on the action of diquat-alginate, a range of sites throughout the country was chosen. The aim was to include some highly calcareous rivers of southern England and some less calcareous rivers of nothern England and Scotland.

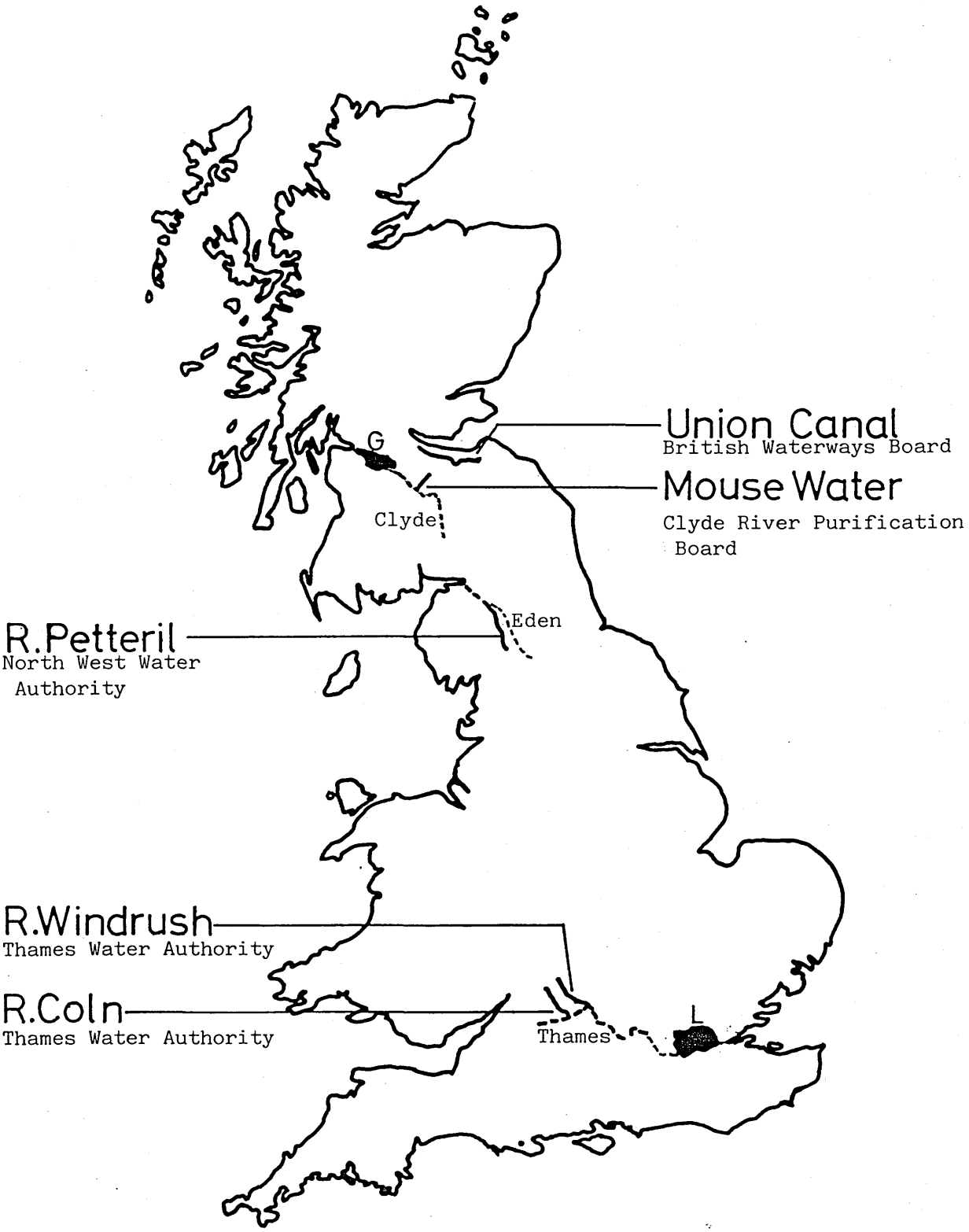
3) The specific rivers were selected with the help of biologists from the Water Authorities (W.A.) and River Purification Boards (R.P.B.) in the appropriate areas. These people could provide information on accessibility, the plant growth to be expected, and the details of any past management. When available the W.A./R.P.B. staff provided information on water discharges and chemistry from 1983 and preceding years.

4) Brief visits were made to the rivers, finally chosen, in October and November 1983. Uniform lengths of river which were suitable for sampling by wading and had vehicular accessibility were selected. Visits at this time of year had the disadvantage that many of the submerged plants had died back, so that it was difficult to anticipate the extent and composition of the macrophytes in the 1984 season.

The four rivers selected for the trials are shown in Fig. 26. In addition to obtaining the permission of the W.A.s and the R.P.B. to use the herbicide, riparian owners and other interested organisations, such as fishing clubs, were informed of the intended trials.

Fig. 26

Map of the sites for the field trials



4.3.2. Site descriptions

The mean physical and chemical characteristics of each river, just prior to treatment, are summarised in Table 4 and Table 5 respectively. These values were derived from measurements made in each section of each river by the methods described in Section 4.4.2.

River Petteril National Grid Reference NY498349 - NY 492367

The R.Petteril, which has moderately soft-water, rises in hills in the north-east of the English Lake District, and for most of its length runs parallel to the R.Eden. The R.Petteril flows through mixed arable and pasture farmland. Although it is too small to have required management, there have been occasional problems with silage pollution. Access to the site was at Plumpton Hall, and the river is fished by the Penrith Angling Association.

River Windrush N.G.R. SP393058 - SP384067

The R.Windrush rises in the Cotswold Hills, and the upper two-thirds flow over oolite rocks. At Witney, Oxfordshire, the river divides into two parallel arms which rejoin just upstream of the confluence with the R.Thames. The studied section was on the eastern arm, 5km upstream of the junction with the Thames and at this stage the river flows over clay.

The surrounding land is used for mixed grazing and arable farming and there are a number of flooded gravel pits, with one active quarry adjacent to part of the studied section. The vegetation further upstream is indicative of serious pollution, with a low species diversity and a predominance of Potamogeton pectinatus, (Haslam 1976).

The river has been managed in the past by Thames Water Authority but this cutting and removal of vegetation was considered uneconomic and was stopped in the 1970's. The site is fished by the Cotswold Fly Fishers.

River Coln N.G.R. SP146030 - SP147037

The R.Coln also rises on oolite rock in the Cotswolds. It mostly flows over clay but has a considerable input of limestone water from other tributaries. The site chosen was 8km upstream of the confluence with the Thames, just north of Fairford, on the Ernest Cook Trust Estate. The river here is managed as a fly fishery and there

are considerable financial interests in maintaining it in a suitable condition for anglers. The Estate Water Bailiff is responsible for the regular macrophyte management, which involves the manual removal of specific beds of weed, which reach the water surface and hinder casting.

Mouse Water N.G.R. NS941491 - NS936496

The Mouse Water is a tributary of the R.Clyde, rising on limestone in the west of the Pentland Hills. The limestone origin caused the water to be harder than had been anticipated. Just downstream of the studied site, the river runs through Ryeflat Moss, from which in the past peat has been extracted. The site chosen was just upstream of the confluence with the Dippool Water, and is in grazing farmland. The river is organically enriched and there have been problems with silage spills.

Major engineering works, to straighten the Mouse Water where it regularly floods, downstream of the Dippool confluence, were started in May 1984. These works influenced the choice and size of the site, but did not subsequently affect the study.

Three of the rivers were included in the river classification by Holmes (1983). This classification was based upon bank, emergent and submerged vegetation. A summary of the classes into which the three sites (Petteril, Windrush, Coln) fell, is presented in Table 6.

Table 4

A summary of the physical characteristics of the rivers just prior to treatment

	Depth (cm)	Width (m)	Water Velocity Surface ($m\ s^{-1}$)	Water Velocity 0.6 x depth ($m\ s^{-1}$)
Petteril				
Mean	15.5	5.36	0.33	0.23
Number of readings	30	10	26	15
Standard error	1.2	0.36	0.03	0.02
Minimum	5.0	4.0	0.09	0.07
Maximum	33.0	7.6	0.61	0.43
Windrush				
Mean	48.2	9.7	0.40	0.29
Number of readings	32	8	32	30
Standard error	2.7	0.33	0.02	0.02
Minimum	18.0	8.5	0.21	0.16
Maximum	70.0	10.9	0.76	0.59
Coln				
Mean	39.9	9.42	0.38	0.30
Number of readings	30	6	30	29
Standard error	2.4	0.53	0.02	0.02
Minimum	12.0	7.7	0.21	0.11
Maximum	64.0	11.0	0.83	0.63
Mouse Water				
Mean	35.6	5.3	0.13	0.11
Number of readings	12	4	11	8
Standard error	2.6	0.66	0.03	0.01
Minimum	19.0	4.0	0.07	0.07
Maximum	47.0	7.0	0.39	0.14

Table 5

A summary of the chemical characteristics of the rivers just prior to treatment

	R.Petteril n=10	R.Windrush n=8	R.Coln n=6	Mouse Water n=4
Date	31.5.84	25.5.84	19.6.84	28.6.84
Start time	12:40	09:10	09:00	11:00
Water temperature (°C)	17.2 (0.08)	14.3 (0.16)	18.3 (0.2)	13.6 (0.2)
Dissolved oxygen (% saturation)	163.4 (2.93)	161.3 (7.3)	118.6 (1.0)	130.5 (16.8)
pH	7.96 (0.03)	8.34 (0.1)	6.72 (0.14)	7.79 (0.13)
Conductivity (μ mohs cm^{-1})	435.6 (2.56)	488.7 (7.4)	478.3 (4.8)	616.2 (2.4)
Total water hardness (mg l^{-1} $CaCO_3$)	*164.7 (2.4)	240.3 (8.9)	*296.0 (1.6)	349.5 (1.7)
Calcium ion concentration (mg l^{-1} Ca^{++})	*62.0 (2.0)	106.7 (3.3)	*118.3 (1.7)	81.0 (2.3)
Nitrate ion conc. (mg l^{-1} NO_3^-)	*10.2 (0.17)	35.7 (0.7)	*24.0 (0.2)	11.4 (0.1)
Reactive phosphorus (mg l^{-1} P)	*0.20 (0.01)	0.12 (0.004)	*0.07 (0.001)	0.03 (0.006)

* = mean of 3 samples of readings from top, middle and bottom of the total length of the site.

( ) = standard error

Table 6

A summary of river classification by Holmes (1983) as related to the experimental sites

R.Windrush site

Class A1vi Fast flowing calcareous small rivers on mixed substrate

Fast flowing, small rivers from chalk or oolite catchment. Calcareous water with a stable flow regime, flowing over substrates of sand, gravel and occasionally silt and clay. Shallow banks with a high water table, usually surrounded by intensively farmed land. Rarely greater than 15m wide. Enrichment of the water favouring submerged species which are free-floating or have shallow roots.

Diversity of speices including fine-leaved Ranunculus fluitans R.calcareus, Myriophyllum spicatum, Zannichellia palustris and broad-leaved Potamogeton perfoliatus, Callitriche spp. Chalk stream flora with species reflecting enrichment e.g. P.pectinatus.

R.Coln site

Class A3ii Small, silted, enriched chalk rivers

Stable flow from chalk or oolite aquifers, but narrower, with coarser substrates and less stable flow than the classic chalk rivers in Class A3i. Clear base-rich water. Water table high on banks. Above average species richness, although less than classic chalk rivers.

R.Petteril site

Class B3iii Small streams in hill districts traversing alkaline rocks

Streams rising on moorland over 200m high, at lower altitudes flowing over limestone, sandstones,grits and mudstone. Starting as oligotrophic, slightly acidic streams, becoming more basic and nutirent rich. Moderate to rapid water flow over rock, pebbles, fine silt or clay. Banks lower than 1m because of stable flow from underlying limestone.

Aquatic plants rare, e.g. Ranunculus fluitans, R.calcareus Potamogeton crispus, Callitriche stagnalis, and taxa prefering faster flow on cobbles, e.g. Bryophytes.

4.4 Methods Used in the River Trials

4.4.1 The weed control treatments

Cutting and removal

Cutting the vegetation in the R.Petteril was a brief operation in which only the Ranunculus beds were removed. A man with two sickles cut the macrophytes' stems at the base, and flicked the plants onto the bank. This was only carried out in the two upstream cut sections.

The R.Windrush was cut by a team of men, with scythe blades on chains, which were dragged upstream, from the banks, in the three 100m cut sections. The cut material was removed with rakes and was dumped on the banks.

Weed beds were removed from the R.Coln as required by the Estate, during late June-early July. Despite requests that cutting should be limited as far as possible in the untreated section, especially avoiding the permanent transects, and that as much vegetation as possible should be removed from the cut sections, there were places where the opposite occurred. Although a few beds were cut in the herbicide sections, none of these were in the transects, or affected the sampling.

Herbicide application

The herbicide treatments were applied as early in the summer as possible when the vegetation would be actively growing, but before most of the weed beds had reached the water surface.

The commercial formulation of diquat-alginate, Midstream, was applied from a Jetpack sprayer with a 2mm nozzle in accordance with the manufacturer's instructions, and following normal safety procedures (MAFF 1985).

The dose-rates were calculated per area of water surface treated, so that 100 l ha^{-1} of Midstream was applied to give a nominal concentration of 1mg l^{-1} of diquat. This is the recommended dose-rate for water over 30cm deep.

The application of Midstream was calibrated by recording the time taken to pump one litre of herbicide from the sprayer, at a given delivery pressure. At 15 p.s.i. pressure this took 43 seconds. Each

200m section to be sprayed, was divided into 50m long stretches and the area to be treated, and hence the volume of Midstream to be applied, was calculated for each stretch. The time to pump the required volume was estimated. Each stretch was treated by walking the 50m length in the specified time, whilst pumping out the Midstream at a constant pressure.

In the R.Windrush only 100m long sections of river were treated with herbicide.

4.4.2 The ecological monitoring of the river sites

Pre-treatment surveys were carried out, at least once, at all sites, followed by one survey shortly after treatment, and at least one later in the season. The timing of the treatments and sampling surveys, are illustrated in Fig. 27.

Observations

On each sampling survey notes were made about the conditions of the rivers and the vegetation. Photographs were taken of each section, permanent transect, and interesting macrophytes.

Plant biomass

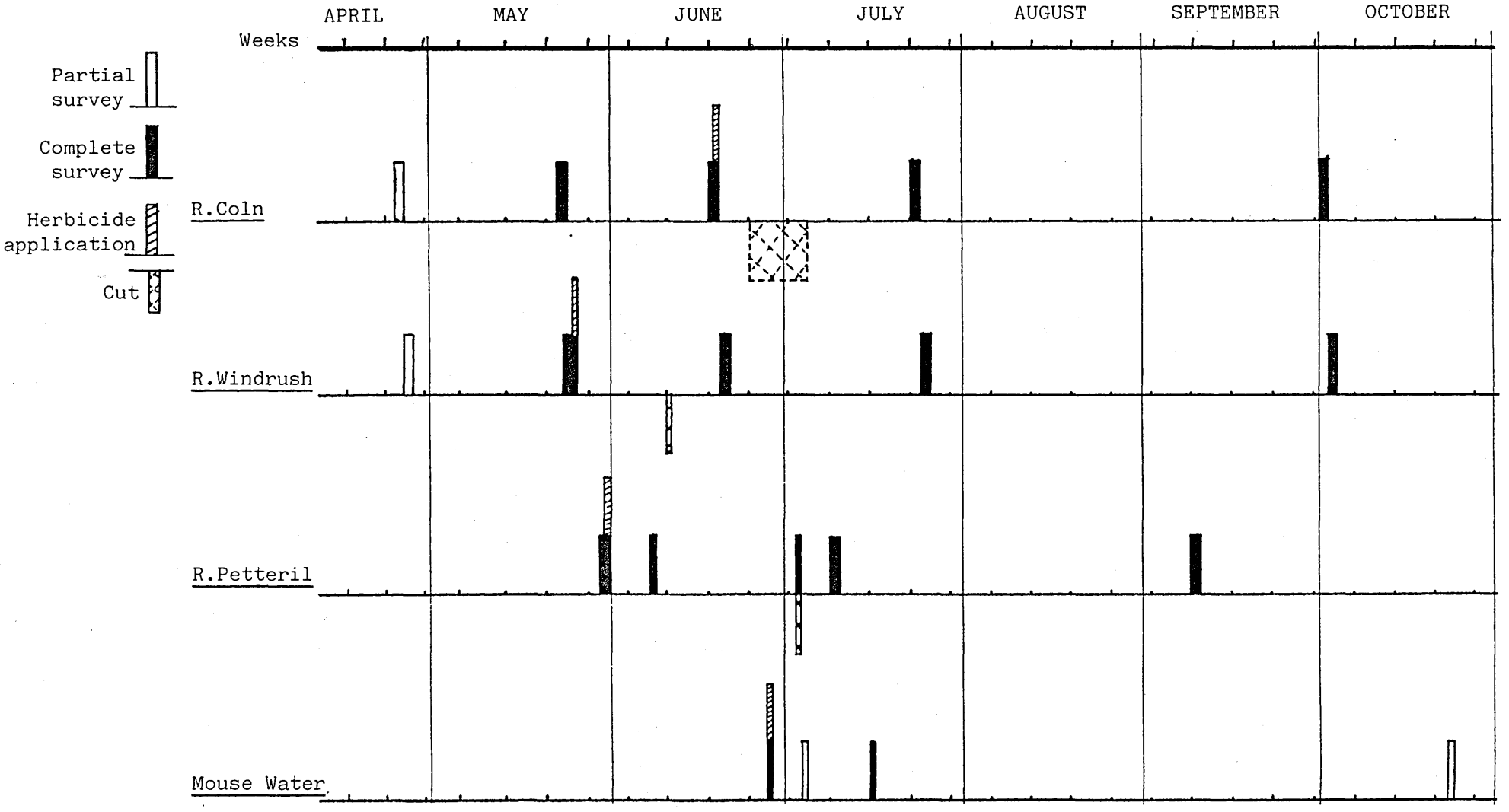
Samples of vegetation were taken from weed beds, or from areas recorded to have had weed beds prior to treatment, using a Lambourn sampler (Hiley et al 1981). The sampling area was 0.05m^2 and material was removed to a depth of 6cm. The vegetation was cut out using shears and the substrate was disturbed using a trowel.

It had originally been intended that five Lambourm samples would be taken per section, five being the minimum number used by Wright et al. (1982). The severe limitations on time forced this number to be reduced to two, a factor which was found to impose considerable constraints on the subsequent statistical analyses.

The samples collected in the field were returned to the laboratory in plastic bags, where the vegetation was sorted into species. The plants were spun for 30 seconds in a 3000 r.p.m. spin-dryer, and were then dried at 60°C to a constant weight. Plant species were identified in the field using Haslam et al. (1975), or back in the laboratory using Holmes (1979) or Clapham et al. (1962).

Fig. 27

Timetable of fieldwork



Vegetation mapping

Permanent transects, not destructively sampled, were marked with wooden posts in each section. Five metre long transects were used in all rivers except the R.Windrush where 3m transects were used, to compensate for the greater width. The transects were sited so as to include Ranunculus beds typical of the river flora, and where the left bank, from which the mapping was always started, was straight.

The vegetation was mapped using the Rectangles Method of Wright et al. (1981), in which the dominant species, or substrate,in a 0.5m x 1m quadrat, is recorded. The method was modified so that for each $0.5m^2$ rectangle with an equal cover of two species or substrates, this was recorded as a $0.25m^2$ rectangle for each. This modification provided a slightly more sensitive recording of the area covered, which was important in rivers with plants which tended to occur in long and narrow beds. At the time of mapping a tape-measure was suspended across the river, to indicate the 0.5m divisions, and a metre rule was used to show the length of each rectangle. Rectangles with two equal species or substrates, were sub-divided lengthways.

The resulting data were used to assess the percentage frequency of each species, or substrate, by dividing the number of $0.25m^2$ rectangles in which a species occurred, by the total number of $0.25m^2$ rectangles in the transect. Simplified maps, with some of the species grouped together, were produced on a Commodore personal computer.

Benthic and macrophyte-associated fauna

The macroinvertebrates associated with the vegetation, and those disturbed from the top 6cm of benthos within the Lambourn sampler, were sorted from the vegetation by repeated washings, in the laboratory. Throughout this procedure nets were used with an equal mesh size to that on the Lambourn sampler. The mesh had 355μm aperture and this size was likely to retain a large proportion of the life-cycles of the expected invertebrates(Zelt and Clifford 1972).

The samples, from which most of the vegetation had been removed, but which still contained considerable amounts of sediment, were preserved in 70% alcohol.

Macroinvertebrates were preserved from all the macrophyte samples but this provided far too many samples to sort and count. Only a few samples were selected for counting, depending upon the macrophtye

results. Some of the macroinvertebrate samples were too large to sort completely, so they were all sub-sampled to ⅛ of their original size. This was achieved using a sub-sampler which split the material into two halves. The half of the sample to be retained was chosen each time by tossing a coin to prevent bias. Volumes of water divided by the sub-sampler were found not to vary from their expected halves.

Easily identifiable taxa of macroinvertebrates from the sub-samples, were counted using a stereo-microscope, and specimens were selected at random for identification to species.

Water chemistry

Water chemistry measurements and samples were taken from the downstream end of each section, at the same time of day for each survey. To minimise the risks of interference, the sections were sampled by working progressively upstream.

The following five parameters were measured in the field:

1) Air temperature
2) Water temperature } Mercury and glass thermometer
3) Dissolved oxygen concentration
4) pH } Digital meters, listed in Section 3.2.2
5) Conductivity — Simac analogue meter

Water samples were collected and kept in five litre carboys, in the dark at 4°C, for laboratory analysis within 1-4 days.

a) Calcium concentrations.

Measured using a calcium ion-selective electrode, with the direct potentiometry method specified by the manufacturers Russell pH Ltd. Calcium chloride calibration curves were used.

b) Total water hardness.

Assessed by a titration method with E.D.T.A*., using B.D.H. total water hardness indicator tablets (British Drug Houses Chemicals Ltd 1973).

c) Nitrate ion concentrations.

Measured using a nitrate ion-selective electrode, from Russell pH Ltd. Sodium nitrate standards were used for calibration.

*E.D.T.A.= Ethylene diamine tetra-acetic acid.

d) Reactive phosphorus.

One litre samples were collected in acid-washed borosilicate bottles and were analysed by the standard spectrophotometric method (H.M.S.O. 1981).

Depth profiles and flow transects

Water depth and flow velocity were measured at regular intervals across each section, on the downstream edge of the permanent transects.

Flow was measured with an Ott propeller flow meter, just below the water surface and at 0.6 x water depth. The latter measurement provides an estimation of the mean flow velocity (Grover and Harrington 1943, Linford 1961).

Photosynthetically active radiation (P.A.R.) measurements

In June 1984 a light meter suitable for use underwater, became available. By taking readings from just below the water surface (I_o), and from a known depth (I_d at depth d), the P.A.R. extinction coefficient (ϵ) could be calculated.

$$\epsilon = \frac{\log_e \left[\frac{I_o}{I_d}\right]}{d}$$

Diquat residues

Immediately after the herbicide application and at regular intervals thereafter, one litre water samples were collected from the downstream end, and in some cases from a point further downstream, of the treated sections. The water was collected from the bank using a bucket on a pole, so that sediments were not disturbed. Samples were also taken from upstream of the treated sections, to check that there was not a coincidental addition of diquat from another source.

4.4.3 Methods for the quantitative analysis of diquat ions

As a result of the complex chemical nature of the diquat (2,2' bipyridine) ion, there are several analytical methods which can be used to identify and quantify diquat residues. The four principal methods are summarised in Table 7. Comprehensive reviews of the methods are provided by Simsiman et al. (1976) and Summers (1980).

The approach selected will depend upon the source of the diquat residues, and the sensitivity and accuracy of measurements required. The analysis of soil, plant, or animal tissues, or the assessment of very low concentrations of diquat, would additionally require methods of extraction and concentration. These processes usually involve chromatographic separation, using cation-exchange resins, such as Permutit Zeo-Karb 225 (Calderbank and Yuen 1966). With sensitive instruments these methods can result in the detection of 0.001mg l^{-1} diquat (I.C.I 1972).

In the present study only concentrations above 0.02mg l^{-1} were considered to be important (the concentration considered safe for irrigation MAFF 1985). The time-consuming step of ion-exchange concentration was unnecessary. The simplest analytical method of directly measuring the U.V. absorption of the diquat ion, at 310nm was chosen (Yuen et al. 1967).

The maximum absorption of the reduced cation radical, at 378nm, is stronger than that of the unreduced diquat ion, at 310nm (Summers 1980). The diquat ion can be reduced by sodium dithionite and sodium metabisulphite (Calderbank and Yuen 1966). This analysis was tested on a few water samples using the method of I.C.I. (1972), but the results obtained were not reproducible, so the method was not pursued. This particular analysis is usually performed on clean samples, concentrated on an ion-exchange resin, so it is likely that there was some interference from another constituent of the river water.

Diquat analysis by direct U.V. spectrophotometry

The U.V. absorption of the river samples was measured using a Philips Pye Unicam SP8-500 UV/VIS spectrophotometer. Calibration standards were prepared from diquat crystals provided by I.C.I. and covered a range of 0.01-0.5mg l^{-1} of diquat. The limit of sensitivity of the method was considered to be 0.05mg l^{-1}. Glass cells with a 1cm pathlength were used.

Table 7

Methods for the quantitative analysis of diquat residues

Method	Advantages	Limits of detection
Spectral absorption		
a) Direct absorption of U.V. radiation at 310nm wavelength, by diquat ions in solution.	Relatively quick and easy method, when there is little interference from other cations.	With cation-exchange concentration Potato tubers 0.01ppm water 0.003ppm
Calderbank et al. (1961), Yuen et al. (1967), Carlstrom (1968).		
b) Absorption of radiation at 378nm by the green radical cation of diquat, reduced by sodium dithionite or a sodium dithionite/sodium metabisulphite mixture.	Stronger absorption maximum than at 310nm and less interference from cationic constituents of the source tissue.	As above.
Calderbank et al. (1961), Calderbank and Yuen (1966).		
Polarography		
Polarographic reduction of diquat produces two distinct one-electron reduction waves. Half-wave potential of the first wave is at -0.36 V and of the second wave at -0.75 V.	This method is not commonly used for analysis, but with cation-exchange concentration, recovery of diquat from food crops of 84-97% has been obtained.	0.5ppm With concentration the limit with respect to the raw sample is 0.01-0.1ppm.
Engelhardt and McKinley (1966).		
Chromatography		
a) Paper and thin-layer. Several methods used to determine the purity of samples, to identify diquat and its breakdown products and to separate diquat from plant or animal tissues.	Simple, standard laboratory procedures. Can be used with radio-isotope labelled diquat.	Depends upon exact method.
Reviewed by Calderbank (1968), Summers (1980).		

(Continued)

Table 7 (continued)

Chromatography (cont.)		
b) High-pressure liquid chromatography has been investigated for determining paraquat in urine in poisoning cases. Can separate from diquat, which could be analysed by this method. Pryde (1975), Pryde and Darby (1975).	Rapid, good precision and readily adapted for quantitative analyses. No cation-exchange concentration needed.	Paraquat has been detected to 0.001ppm in urine.
c) Gas chromatography. This method can only be used if the diquat is first converted into a volatile derivative e.g. by hydrogenation.	Not practical.	
Bioassay		
Plant material, commonly Lemna minor is bleached by diquat. The time for chlorosis in a sample, is compared with the time for chlorosis in standard solutions. The effects of environmental factors, such as water hardness and light, on the herbicide's activity, have also been assessed by this method. Funderburk and Lawrence (1963b), Blackburn and Weldon (1965), Parker (1965).	Although a relatively slow method, 24 hours for 1ppm but several days for lower concentrations, this method is very sensitive and measures the amount of diquat actually available to plants.	0.0005ppm in solution.
Other bioassay methods include measuring the % of water melon cotyledons which sink in 24 hours in solutions of diquat, in the light. DaSilva et al. (1976)		Tested to (approx) 0.2ppm

By scanning the absorption range from 200-350nm for the standard solutions, and some of the river samples, 309nm was found to be the wavelength at which maximum absorption was detected.

There was found to be considerable background interference in the river samples when compared with the standards, which had been prepared with distilled water. This was corrected by using an untreated sample, from the same river as the residue sample, as the blank in the reference cell. A distilled water blank was used with the calibration standards. Samples with more than 0.5mg l^{-1} diquat, and their reference blanks, were diluted as necessary, with distilled water.

A new calibration curve was produced each time that a set of samples was analysed, and a standard solution was checked between every ten readings to ensure that there was no drift.

For maximum stability and reproducibility, Yuen et al. (1967) recommended buffering samples with sodium acetate and acetic acid, to pH 4.05. The pH of the untreated river samples was measured, after storage, and they were all within a range of 7-8 pH. The buffering capacities of the samples would have been different and the concentration calculations would have been complicated, by adding variable quantities of acetic acid, to obtain a pH of 4.05.

To test the stability of the diquat in river water, solutions of 0.4mg l^{-1} diquat were prepared by adding 8ml of a 25mg l^{-1} diquat standard to 492ml of each river water. These solutions were well mixed and after five hours they were analysed, in comparison with two standard 0.4mg l^{-1} solutions made with distilled water.

The U.V. absorption of the 0.4mg l^{-1} solutions prepared with river water, did not differ significantly from the solutions prepared with distilled water. From these results, it was considered unlikely that the characteristics of the river water, such as pH, would affect the diquat analyses.

4.4.4 The storage of diquat residues

It was not possible to analyse the diquat residue samples immediately after collection, so they were frozen, in partially filled one litre polypropylene bottles. To assess the effects that storage might have on diquat, three standard diquat solutions containing 0.4mg l^{-1} diquat were also frozen. Three standard solutions were stored in the dark at 4°C.

The standards were thawed and analysed after 28 days. There were no significant differences in the measured diquat concentrations of the solutions, before and after freezing or cold storage. The river samples were frozen to prevent the growth of any cold-tolerant algae or bacteria. When not in immediate use, the thawed samples and standard solutions were kept in the dark at 4°C.

Preventing the exposure of diquat to light is important because photochemical degradation occurs. Diquat is rapidly degraded in U.V. light (240-260nm range) to volatile products (Funderburk et al. 1966). One hundred percent loss of diquat from aqueous solution, after exposure to U.V. light for 24 hours has been reported (Slade and Smith 1967). The photochemical degradation of diquat is slower in sunlight, (70% loss in 21 days, 90% loss in 35 days) because sunlight has little radiation of wavelengths less than 300nm. Degradation rates similar to those in sunlight were obtained when U.V. light was passed through borosilicate glass, which filters out radiation of wavelengths less than 300nm (Smith and Grove 1969).

No decomposition of diquat occurs in the dark (Funderburk et al. 1966), so the samples were kept under covers in the laboratory during analysis.

4.4.5 Statistical analyses of the field trial data

Whenever appropriate, analyses of variance have been carried out to compare treatments at different sampling times, within each river. As with the laboratory analyses, time was not treated as a simple factor, but a split-plot analysis was used (Section 3.2.3). Tests of least significant difference, and transformations of the data were applied if necessary (Section 3.2.3).

If multiple samples had been taken per section (e.g. macrophyte biomass, macroinvertebrates) these could not be treated as replicates because they came from the same treatment replicate. To have analysed these sub-samples as replicate samples of a treatment, would have been to use pseudoreplication (Hurlbert 1984). Multiple samples per experimental unit do not increase the number of degrees of freedom available for testing a treatment effect (Hurlbert 1984).

When sub-samples have been collected from a section, their mean has been used in the ANOVA. The variability of these means, indicated by their standard errors, have been listed. All ANOVA's were carried out using the GENSTAT package on the Edinburgh University computer.

4.5 Results of the River Trials

The results of the river trials have been presented in the following order:

Macrophytes - Observations, biomass,cover
Diquat residues
Depth profiles and flow transects
Light transmission
Water chemistry
Macroinvertebrate communities

The data from each river are presented within these headings in the order: R.Petteril, R,Windrush, R.Coln, Mouse Water.

Some of the data from the R.Petteril have been published (Fox et al. 1986) but the complete results are presented here.

4.5.1 Macrophytes

R.Petteril

Observations

The stony substrate of the R.Petteril caused some difficulties in trying to embed the Lambourn sampler. There was a substantial covering of the filamentous alga Cladophora glomerata in June and July. The other dominant macrophyte was Ranunculus penicillatus var. vertumnus. The Ranunculus was localised, particularly at the upstream and downstream ends of the site, to large, shallow beds of flowering plants. A few small beds of Ranunculus fluitans were found throughout the site.

One week after the herbicide treatment, it could be seen that there had been a dramatic effect on the treated Ranunculus. Chlorotic, limp and leafless remains of Ranunculus beds were found in both of the herbicide sections. These symptoms, or losses, of Ranunculus were apparent for several hundred metres downstream of the site and local inhabitants commented on the 'overnight' disappearance of the flowering plants. The effects of the upstream treatment had been to remove and damage the Ranunculus from all four sections between the two herbicide replicates. For example, a large weed bed on a shallow riffle in the downstream cut section (C1), about 800m downstream of the herbicide application (H2), was removed. The effects of the

diquat-alginate treatment on Ranunculus in the downstream herbicide section, can be seen in Plates 1a and 1b.

The Ranunculus in all of the sections upstream of the herbicide application, was healthy and in flower. The Cladophora in the herbicide treated sections was not noticeably reduced or affected; nor was the other non-vascular macrophyte present, the moss Rhynchostegium riparioides.

By July, forty days after the herbicide application, all the Ranunculus had disappeared from the herbicide and intervening sections, leaving patches of bare substrate, with very little root stock remaining. Cladophora was still present, but was reduced in comparison with the pre-treatment cover.

Ranunculus in the cut sections was reduced to cut stems, stumps and root stock. The cutting procedure, seven days earlier, had removed all individual weed beds, but the remains were greater than those left by the herbicide. The Ranunculus in the untreated sections was no longer flowering, but the beds had become much larger and broke the water surface.

By September, no regrowth of Ranunculus had occurred in the herbicide sections. Small sprigs of Ranunculus were found amongst shallow stones in one of the sections between the two herbicide replicates. Some Zannichellia palustris had started to grow in the downstream herbicide section, around where the Ranunculus beds had been. This species had been noticed previously, in sections upstream of the herbicide applications, and by September had formed quite obvious beds near the river margins in these places.

The Cladophora cover was reduced in the whole site and no longer dominated the river. The Ranunculus in the cut sections had shown little regrowth since July, but the root stock was still visible. The Ranunculus in the untreated sections appeared to be healthy, but the cover had been reduced from its summer level, as loose material was washed away.

Biomass

A table with the complete biomass data, for each section, is presented in Appendix 4a.

As a result of the downstream effects of the herbicide treatment, the sections between the herbicide replicates were not used for comparison with the cut and untreated sections, but were analysed as

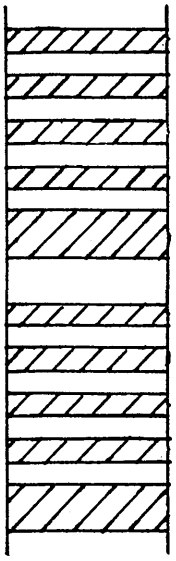
Plate 1

Ranunculus bed in the R.Petteril from which a Lambourn sample has been removed ($0.05m^2$)

Plate 1a. Prior to herbicide application 31.5.84

Plate 1b. Eight days after herbicide application

separate treatments, of herbicide drift.



P1 + P6 = Herbicide

P2 + P3 = Drift d/s - Untreated with herbicide influence, downstream pair

P4 + P5 = Drift u/s - Untreated with herbicide influence, upstream pair

P7 + P9 = Cut

P8 + P10= Untreated

u/s = upstream
d/s = downstream

The biomass data were analysed with (total), and without (macrophytes only), the filamentous algae. A $\log_e$ (X x 100 +1) transformation was used on the biomass data for the ANOVA, but, as described in Section 3.2.3, the untransformed data have been plotted.

The mean biomasses of macrophytes only, for all treatments, are shown in Fig. 28a. A partial survey of herbicide and untreated sections, was carried out eight days after the herbicide application (d.a.h.). The results of this survey were analysed with the herbicide and untreated data only (Fig. 28b, macrophytes only).

In the analysis of the macrophytes only, from all treatments, the time factor and the time * treatment interaction were significant, ($F_{2,10}$ = 39.17 and $F_{8,10}$ = 8.59 respectively). There were no significant differences between the treatments prior to the application of the herbicide.

By 10.7.84, 40 days after the herbicide application and 7 days after the cut (d.a.c.), the biomasses in all the treatments were significantly different from the untreated sections. All treatments, except the drift u/s, significantly differed from their pre-treatment biomasses.

By 11.9.84 the biomasses in the herbicide and untreated sections were not significantly different, but each had significantly changed from the July sample. The biomasses in the cut and drift u/s sections were significantly reduced from their May and July values, and were significantly less than in the herbicide sections.

Fig. 28

Biomass of macrophytes only (-algae) in R.Petteril

Fig. 28a

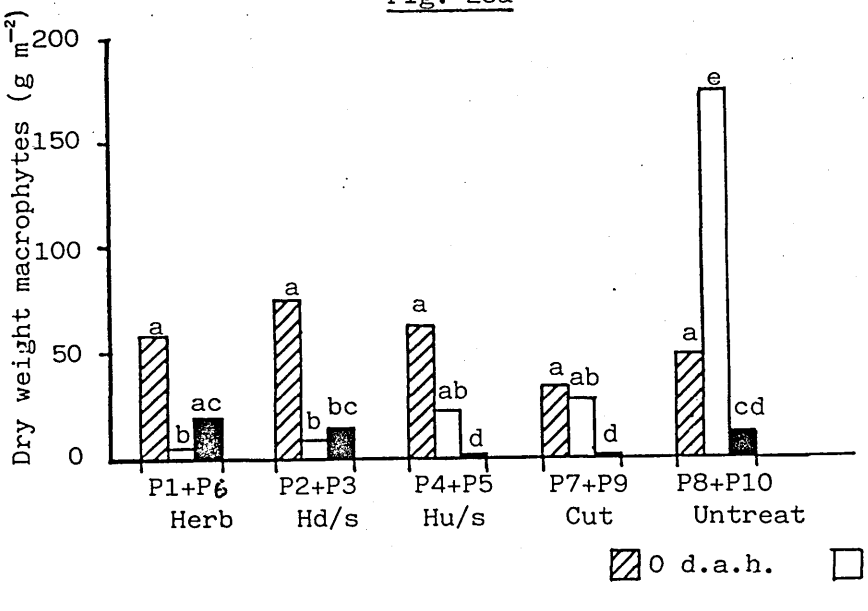
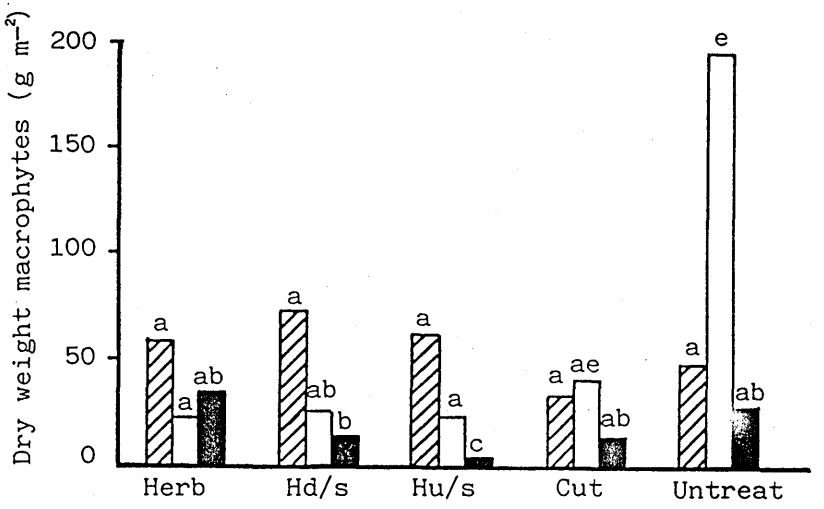
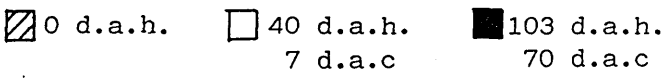


Fig. 29

Biomass of total macrophytes in R.Petteril

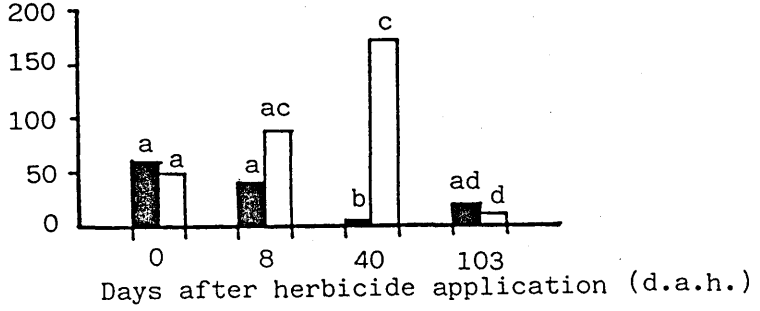
Fig. 29a





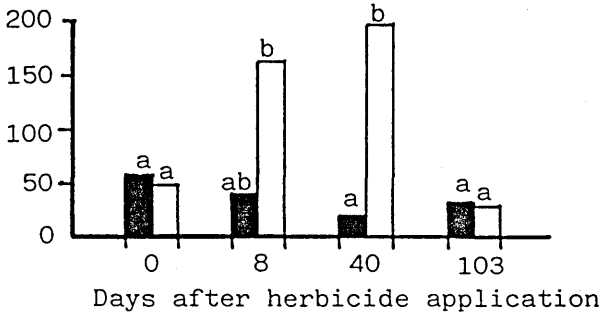
Dry wt macrophytes ($g\ m^{-2}$)

Fig. 28b



Dry wt macrophytes ($g\ m^{-2}$)

Fig. 29b



Analysis of the herbicide and untreated data, from the four sampling times (Fig. 28b), showed that the time factor ($F_{3,6}$= 8.41) and the time * treatment interaction ($F_{3,6}$ = 16.48) were significant. Although plant biomasses from the 8.6.84 (8 d.a.h.) were not significantly different from the pre-treatment values, they do show intermediate values between the pre-treatment and July samples. This suggests that some loss of biomass had occurred in the herbicide sections, during the 8 days after treatment, whilst the untreated plants had continued to grow.

When the biomass data including the filamentous algae were analysed (Fig. 29a and 29b), the time factor ($F_{2,10}$= 9.97) and the time * treatment interaction ($F_{8,10}$ = 3.14) were significant. The biomasses in the herbicide and cut treatments did not significantly alter throughout the summer, but the biomass in the untreated sections had significantly increased by July.

Only the time * treatment interaction ($F_{3,6}$=4.88) was significant when the herbicide and untreated total biomass data were analysed (Fig.29b). The untreated biomass had significantly increased from its pre-treatment value, by 8 days after the herbicide application.

The post-treatment biomasses were calculated as percentages of the pre-treatment values, and these %-biomass change values were analysed in the same ways as the biomass data. The %-biomass change values for all treatments, without algae, are presented in Fig.30a. All factors and interactions were significant in this analysis, (Treatment $F_{4,4}$= 30.78, time $F_{1,5}$= 14.58, time * treatment $F_{4,5}$= 8.15, blocks $F_{1,4}$= 9.09). By 10.7.84 the biomass in the herbicide sections was only 9% of the pre-treatment value. This was significantly different from the cut sections, which were 98% of their pre-treatment values, and from the untreated sections, where the biomass had increased to 370% of the May biomass.

Only the time ($F_{2,4}$= 7.79) and the time * treatment ($F_{2,4}$=16.89) factors were significant when the herbicide and untreated data were analysed (Fig. 30b). The %- biomass change by 8 days after herbicide application, was not significantly different for the two treatments.

Analysis of the % change in total biomass (Fig 31), did not show a significant time * treatment interaction ($F_{4,5}$= 1.75). Time was the only significant factor ($F_{1,5}$= 8.13) showing a reduction by September.

Fig. 30

Percentage change in biomass of macrophytes only (-algae)

Fig. 30a

Fig. 30b

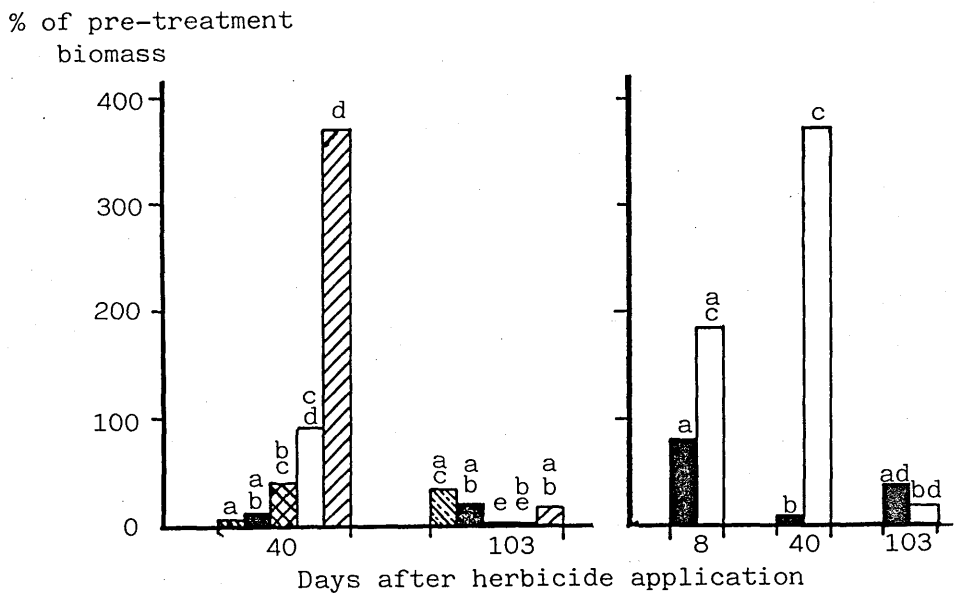
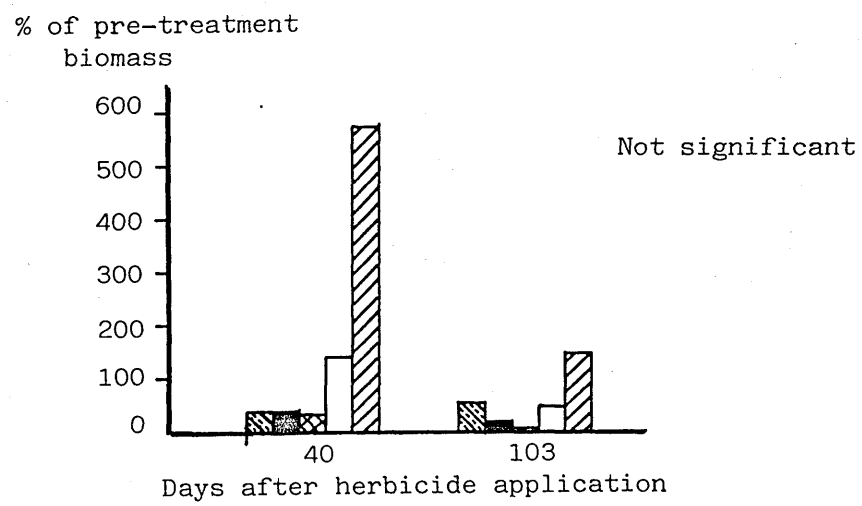


Fig. 31

Percentage change in total biomass



Figs 30a + 31

- Herbicide
- Drift d/s
- Drift u/s
- Cut
- Untreated

Fig. 30b

- Herbicide
- Untreated

Cover

Some examples of the simplified vegetation maps are shown in Fig. 32. The complete set of maps, for all sections and sampling dates, is presented in Appendix 5a.

The ANOVA of %-frequency was carried out on the data for submerged plants only, with or without the algae. These data did not need to be transformed for the ANOVA.

When the %-frequency of macrophytes only (Fig. 33a), was analysed, the time * treatment interaction ($F_{8,10}$= 5.78) was significant. There was a significant decrease in the %-frequency of macrophytes only, with time ($F_{2,10}$= 32.21) and there was a significantly greater %-frequency in the upstream block ($F_{1,4}$= 15.74).

Prior to the herbicide application, there were no significant differences, in the %-frequency of macrophytes only, between the treatments. By 40 days after the herbicide application, the %-frequency of vegetation in all the sections affected by the herbicide, was significantly less than the pre-treatment values. The cut and untreated sections did not show a significant decrease in %-frequency of macrophytes, until September.

Analysis of %-frequency of the total submerged macrophytes (Fig 33b), showed that time was the only significant factor, with only a significant reduction in September ($F_{2,10}$= 15.63).

As with the biomass data, the percentage changes from the pre-treatment values, of the %-frequency of macrophytes, were calculated. Excluding the algae (Fig. 34a), the treatment ($F_{4,4}$= 7.22), time ($F_{1,5}$ = 20.66) and time * treatment ($F_{4,5}$= 31.03) factors were significant. If algae were included in the analysis, only the time factor ($F_{1,5}$= 10.39) was significant (Fig. 34b).

Fig. 32

Examples of vegetation maps from the R.Petteril

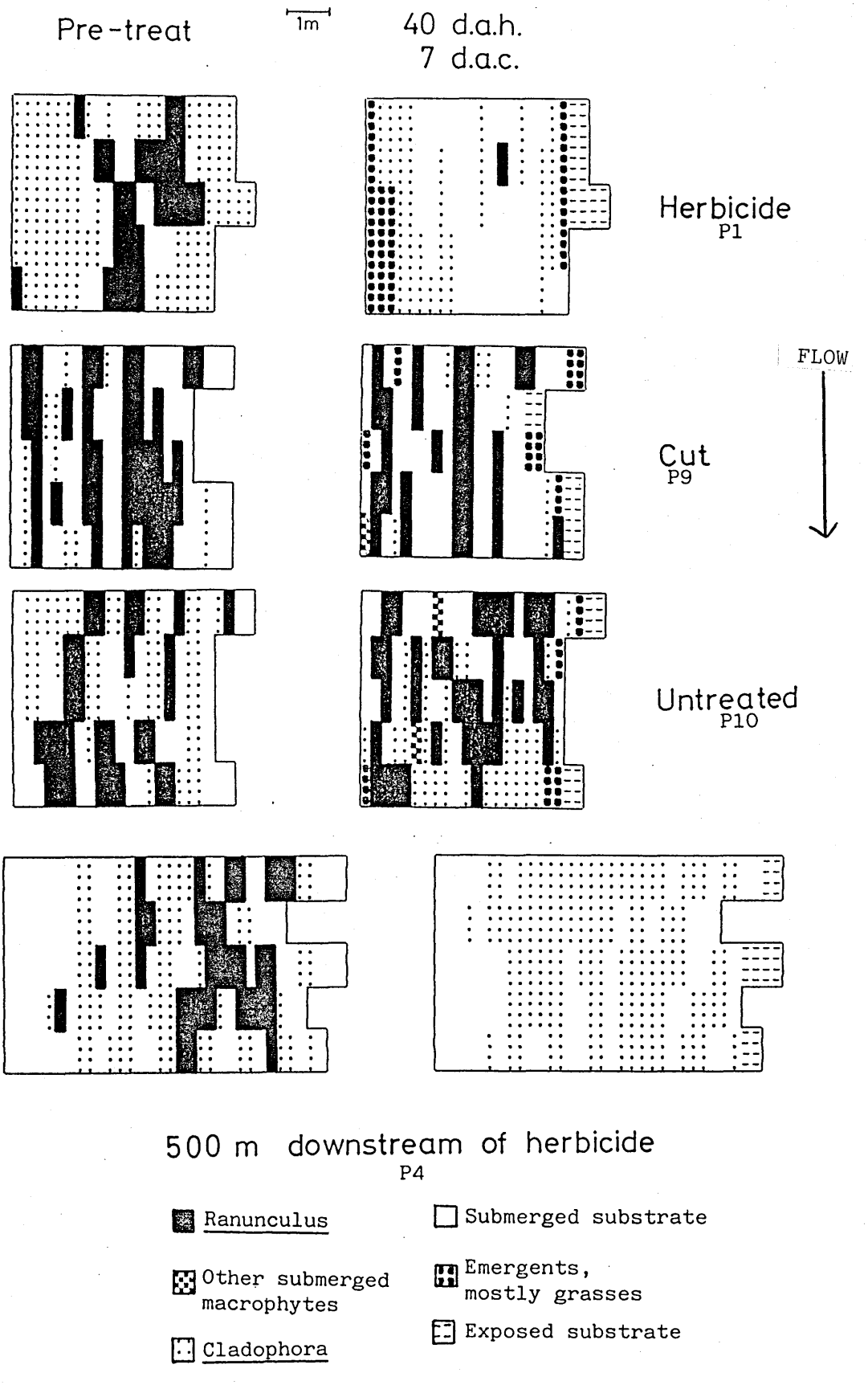
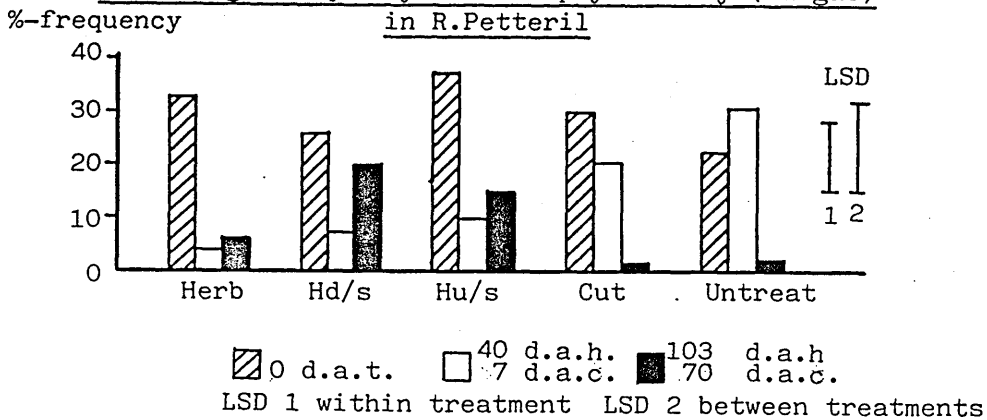


Fig. 33a

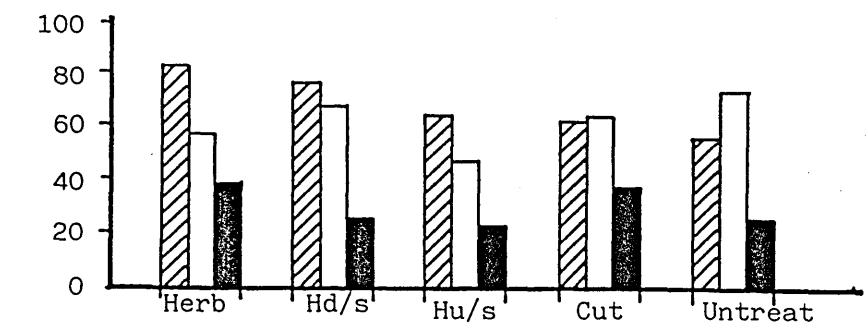
Percentage frequency of macrophytes only (-algae) in R.Petteril



LSD 1 within treatment LSD 2 between treatments

Fig. 33b

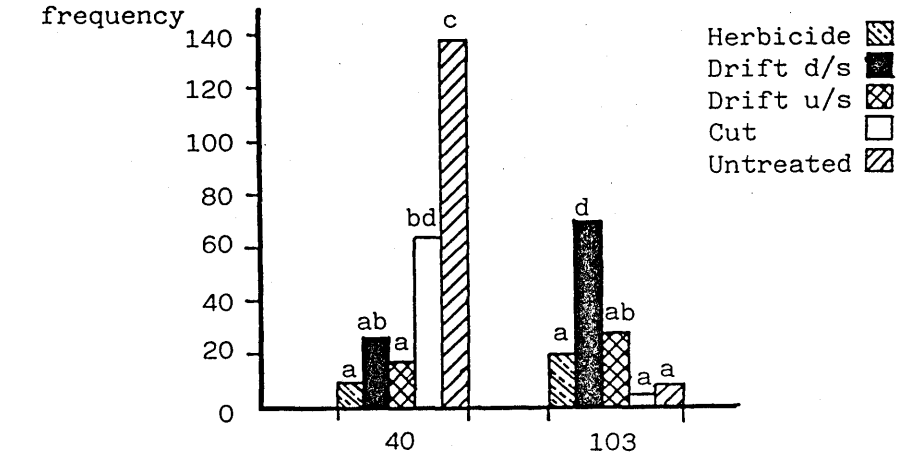
Percentage frequency of total macrophytes



Not significant

Fig. 34a

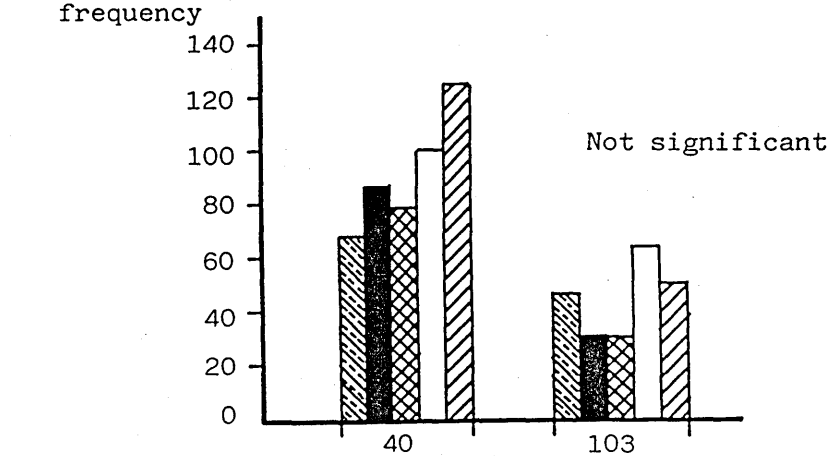
Percentage change in frequency macrophytes only



Days after herbicide application

Fig. 34b

Percentage change in frequency total macrophytes



Days after herbicide application

R.Windrush

Observations

The sections in the downstream block were deeper and slower flowing than those in the upstream block. The water was frequently very turbid so observations were difficult if the submerged vegetation was not visible from the bank.

Ranunculus penicillatus var. calcareus occurred in all of the sections, often associated with Myriophyllum spicatum and two or three species of Potamogeton. Potamogetom pectinatus was the predominant species of the genus.

In mid June, 26 days after the herbicide application and 10 days after the cut, there was little indication that the herbicide had had any effect. Only in the downstream herbicide section were some Ranunculus stems,without leaves,seen at the water surface. The volume of plants in the water column, which could be felt, but not seen, was not substantially reduced. In the upstream herbicide section, the Ranunculus had not only grown but was still flowering and showed no phytotoxic symptoms at all. The previous dominance of Ranunculus was being reduced naturally by the rapid spread of Potamogeton pectinatus.

In all the cut sections, the weeds had been noticeably reduced, to stem stumps, in the centres of the channels, whilst the edges had been much less efficiently cleared. The overall effect was a considerable reduction in the obstruction to water flow. Plate 2.

Ranunculus was growing well and was still flowering, at the water surface, in all the untreated sections. Potamogeton pectinatus was starting to become dominant in the uppermost section.

By the end of July (61 d.a.h. and 45 d.a.c.), the Ranunculus in the downstream herbicide section was still present, but had been reduced to mainly old stems in the lower part of the water column. Potamogeton species were spreading over the water surface, where the Ranunculus had been. The Ranunculus in the upstream herbicide section was still healthy, although no longer flowering, but was being swamped by Potamogeton pectinatus.

Some regrowth of Ranunculus was evident in the cut sections. This was most obvious in the top cut section where the Ranunculus, on a shallow ridge, was growing rapidly, and was of uniform length.

Cladophora glomerata and other algae (e.g. Enteromorpha sp.) formed thick blankets over many of the upstream sections. In the

Plate 2

R.Windrush: upstream end of cut section W5

(Note abrupt disappearance of flowering Ranunculus.)

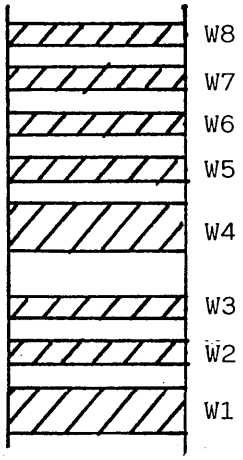
untreated sections the water flow was restricted, by the macrophytes and algae, to narrow channels between the weed beds.

By October there was little overall change from July, although some of the algae had been lost. Neither of the herbicide sections showed further damage, or regrowth, of Ranunculus. The cover of Potamogeton pectinatus in the upstream herbicide section was at its greatest in October. The density of Ranunculus in the upstream cut section appeared to be back to its pre-cut density.

Biomass

The complete biomass data, for each plant species and section, are presented in Appendix 4b.

Since the herbicide was not seen to have any effect in the upstream replicate, the downstream cut and untreated sections (C1, U1) were used in the analyses to compare all three treatments.



W1 + W3 = Herbicide
W2 + W5 = Cut
W3 + W6 = Untreated
} Comparison of all three treatments

W2 + W5 + W7 = Cut
W3 + W6 + W8 = Untreated
} Comparison of cut and untreated sections only

For the ANOVA the biomass data were transformed by $\log_e$ (X x 100 +1). The analyses with all three treatments did not show significant time * treatment interactions whether or not the algae were included in the biomass (Fig. 35).
Time was the only significant factor ($F_{3,9}$=15.34 without algae) in either analysis with a significant reduction in biomass between the May and June samples and between July and October.

e.g. macrophytes only	May	June	July	October
biomass (g m^{-2})	289	173	172	59

If the herbicide treatment was excluded from the analysis and the three cut and untreated sections were compared, the analysis of macrophytes only, did not show a significant time * treatment interaction. Only the time factor was significant.

Fig. 35

Biomass of macrophytes in R.Windrush

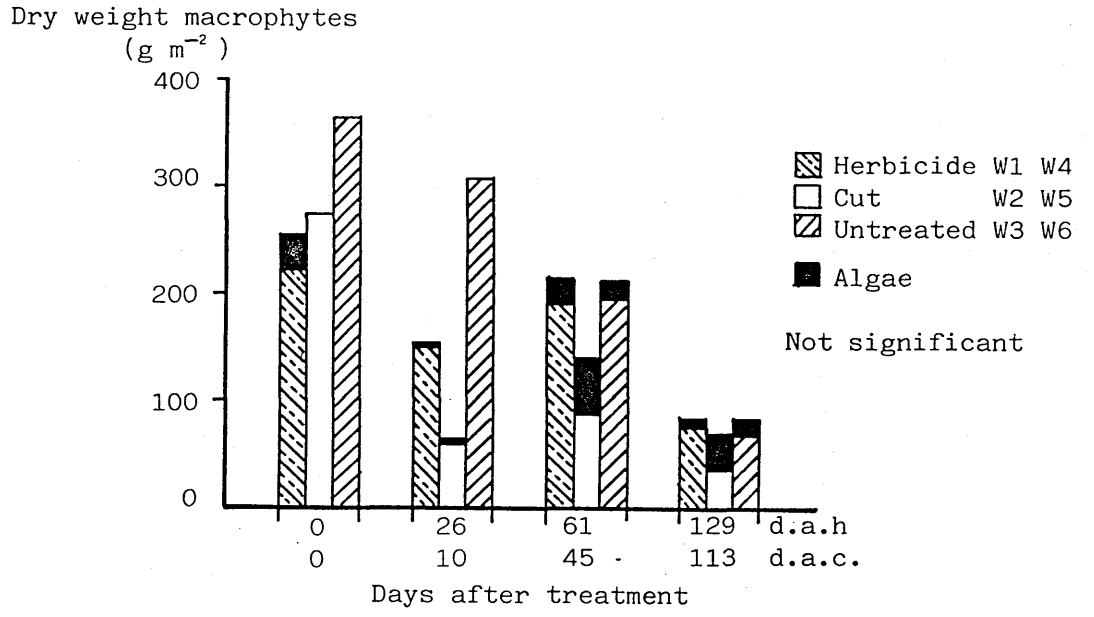


Fig. 36

Biomass of macrophtyes in R.Windrush

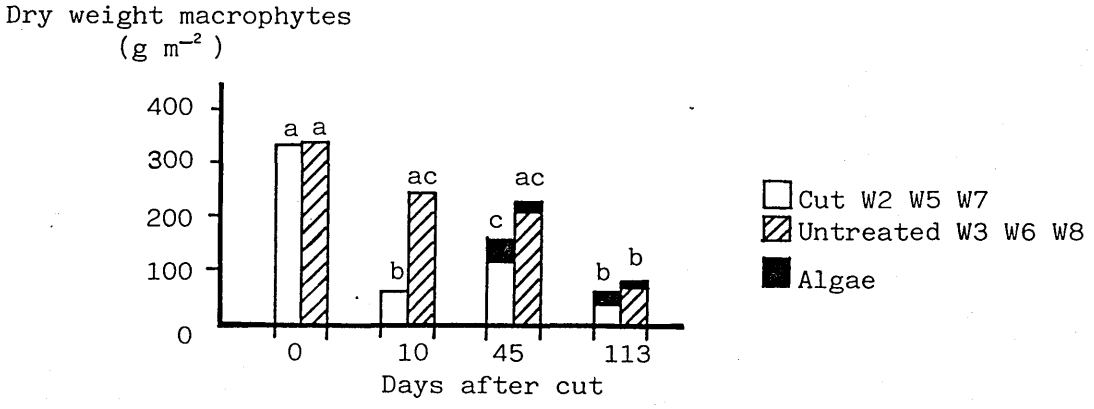
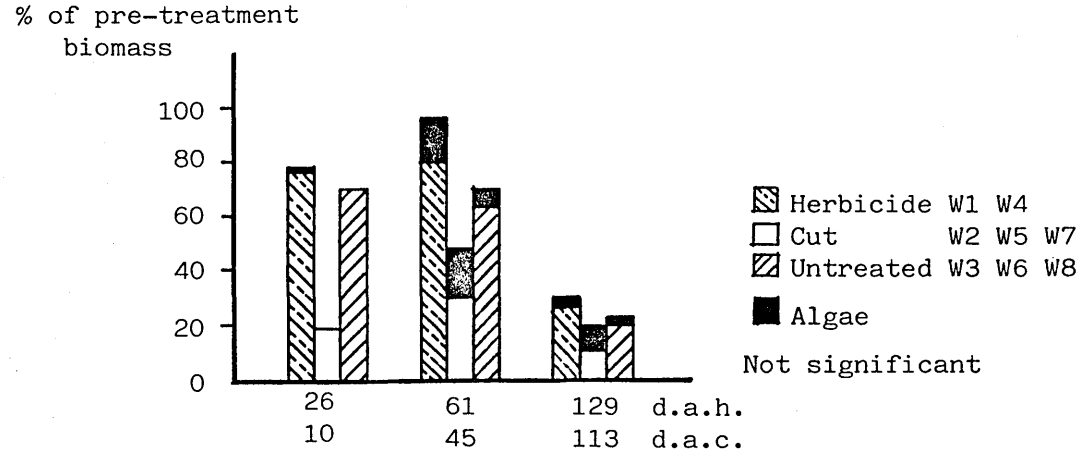


Fig. 37

Percentage change in biomass



However, when the comparison of the cut and untreated sections was repeated, including the algae (Fig. 36), the time * treatment interaction was significant ($F_{3,12}$= 4.51), as well as the time factor ($F_{3,12}$= 26.85). The biomass in the cut sections, 10 days after the cut, was significantly less than in the untreated sections, and less than the pre-treatment values. By 45 days after the cut, the biomass in the cut sections had significantly increased from the June values. The biomass in the untreated sections decreased throughout the summer, but the reduction was only significant between July and October.

The fact that the total biomass data were significant for the time * treatment interaction, but not the data excluding the algae, was because of the reduced variability in the July samples, specifically the biomass from the downstream cut section.

The percentage biomass changes were calculated. Only the time factor was significant when these data were analysed, for all treatments, with or without algae (Fig. 37). The analysis of cut and untreated sections only, also resulted in time being the only significant factor.

Cover

Examples of the simplified vegetation maps, from some of the sections in the R.Windrush, are presented in Fig. 38. The complete set of maps is given in Appendix 5b.

The data were not transformed for the ANOVA. In comparisons of the three treatments for Ranunculus, macrophytes only and for total vegetation %-frequency (Fig. 39a), only the time factor was significant.

Comparison of the %-frequency of submerged vegetation in the cut and untreated sections (Fig. 39b), did not result in a significant time * treatment interaction. Time was significant (Fig. 39c) for Ranunculus, macrophytes only and total vegetation. The treatment and blocks factors were significant for the submerged macrophyte data, with or without algae, with always a greater %-frequency of vegetation in the untreated sections.

When percentage changes in frequency of vegetation were analysed for all treatments, or just for the cut and untreated sections, none of the factors nor the interaction were significant (Fig. 40a, 40b).

Fig. 38

Examples of vegetation maps from the R.Windrush

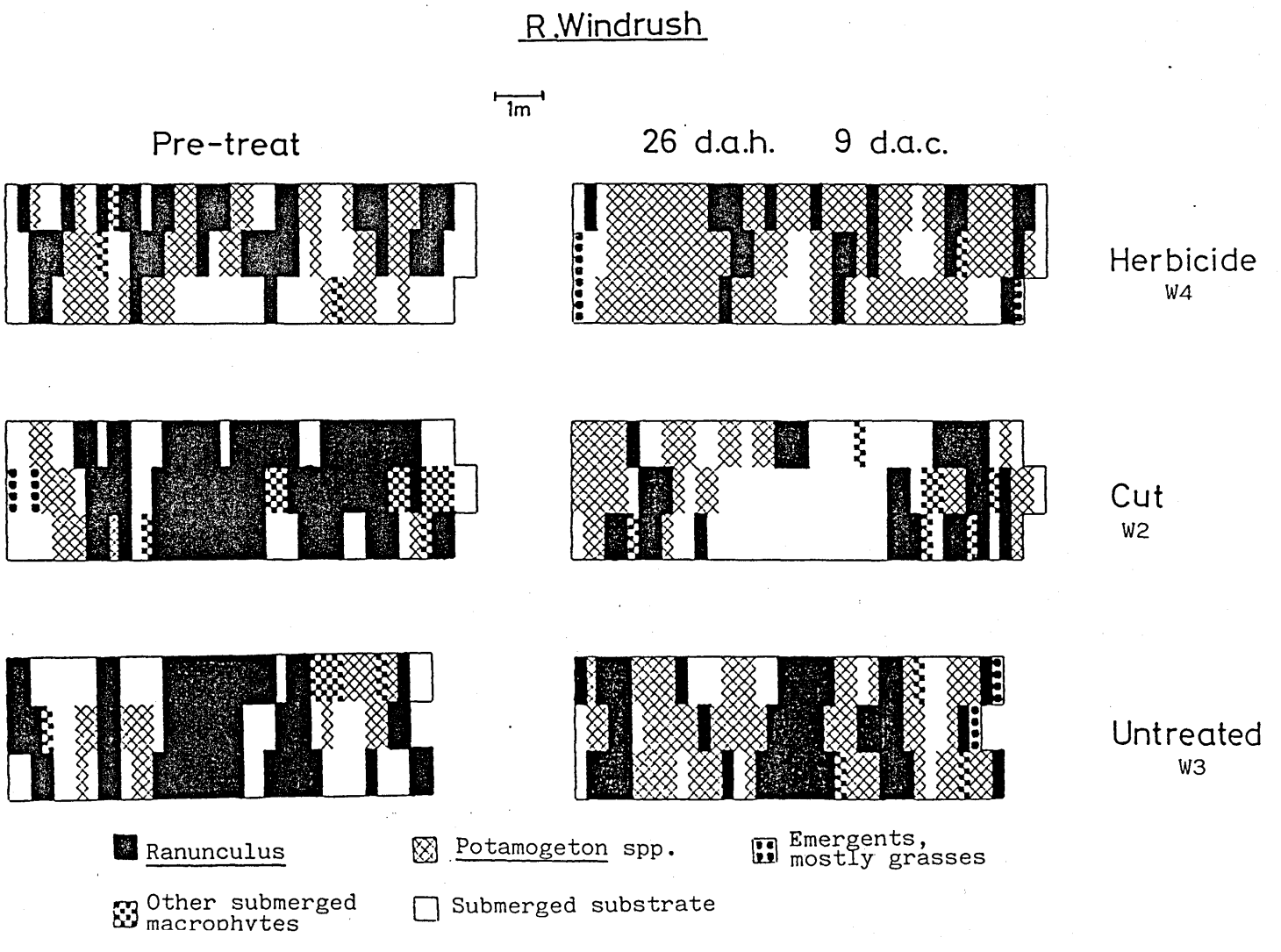
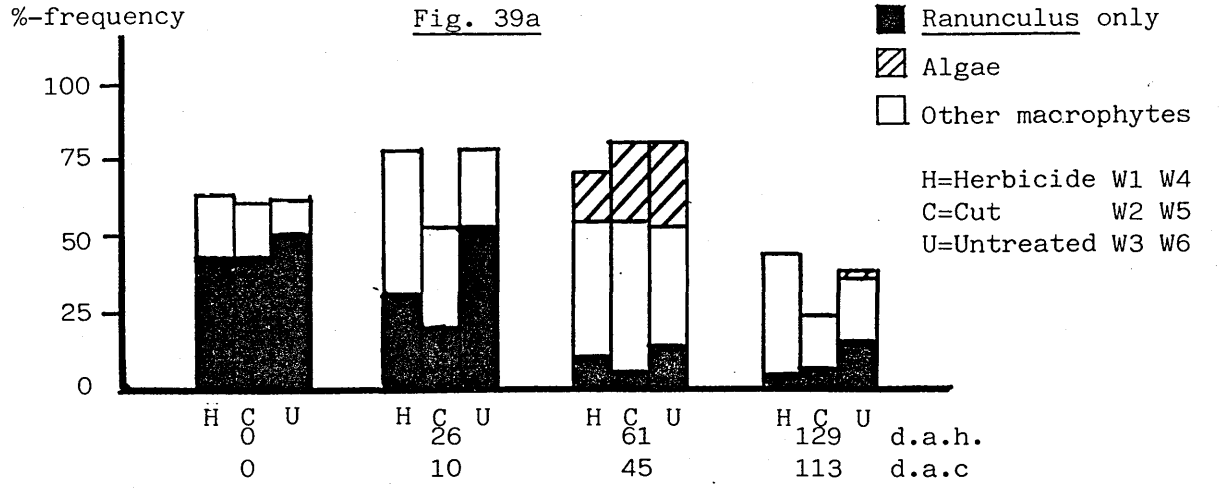


Fig. 39

Percentage frequency of macrophytes in R.Windrush



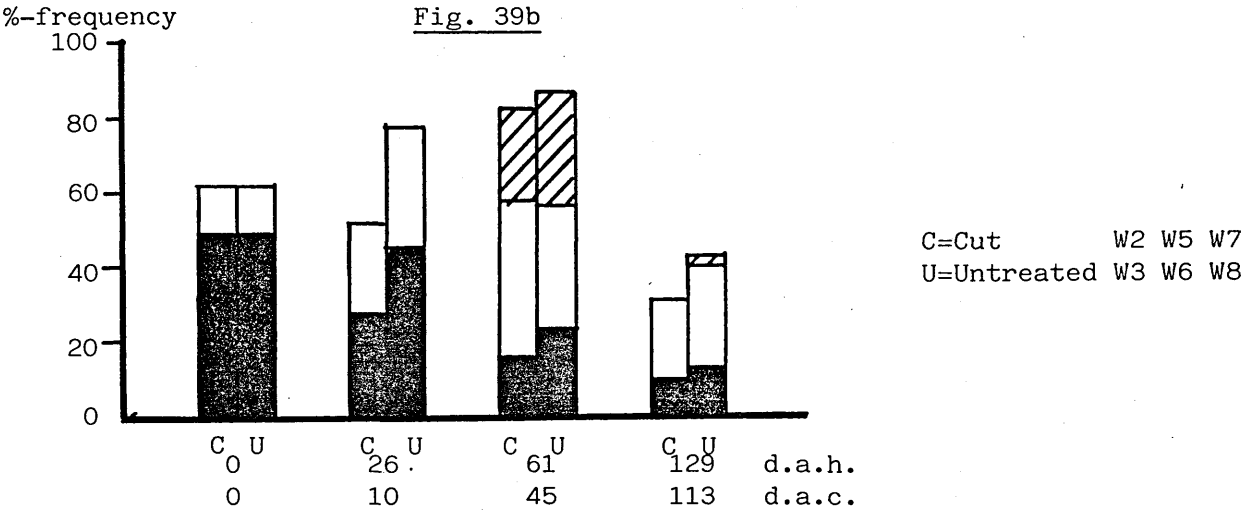


Fig. 39c

Data from all cut and untreated sections

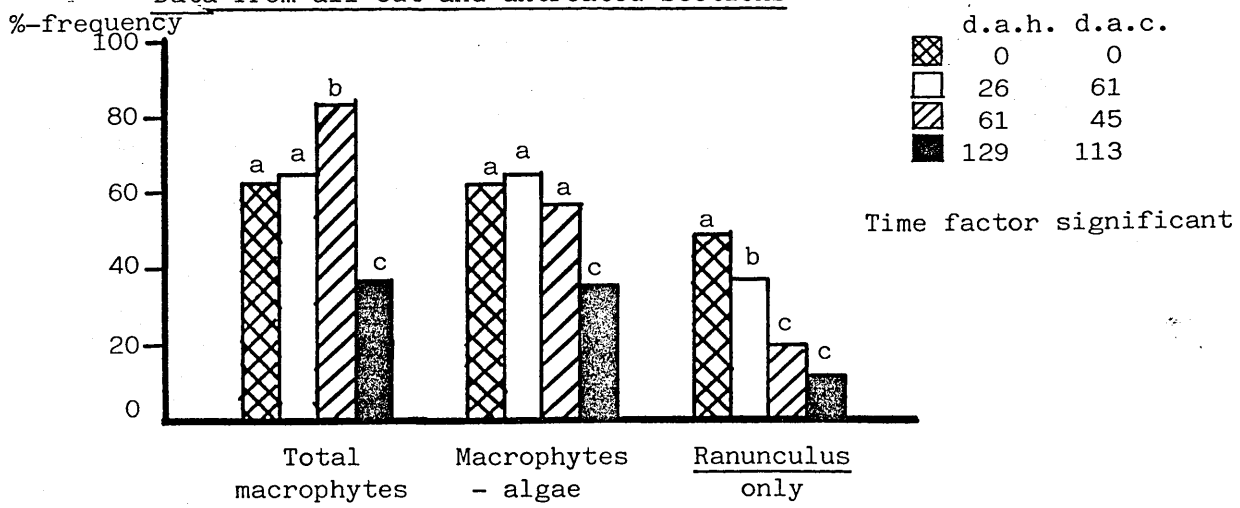


Fig. 40a

Percentage change in frequency: total macrophytes in R.Windrush

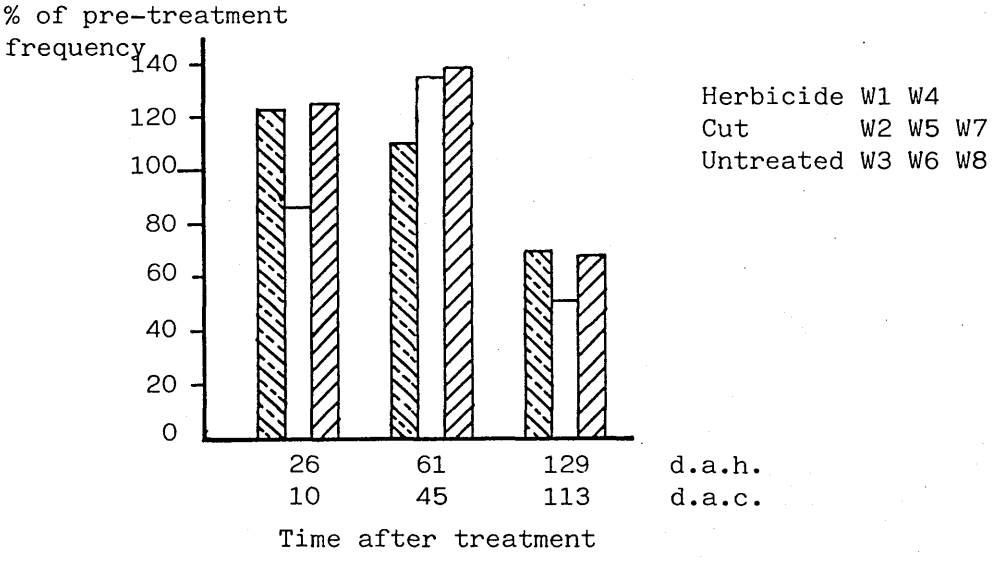
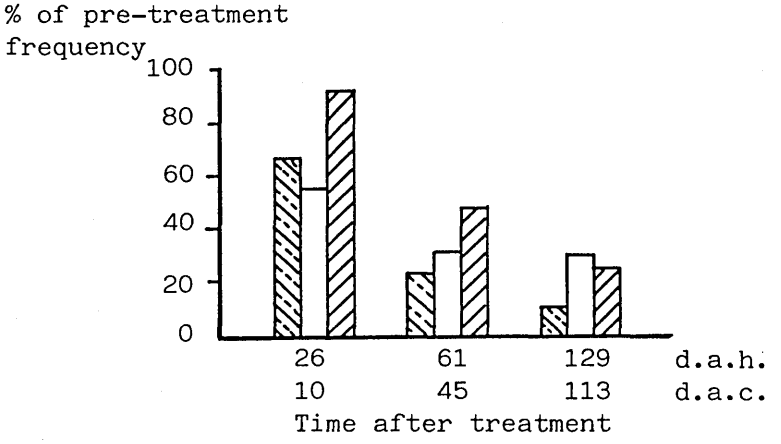


Fig. 40b

Percentage change in frequency: Ranunculus only



R.Coln

Observations

Ranunculus penicillatus var. calcareus was the dominant species, forming large, flowering beds which reached the water surface in all sections by mid June.

By the end of July, 36 days after the herbicide application, there were obvious phytotoxic symptoms in the herbicide sections. Ranunculus in the downstream herbicide section had been stripped of its leaves so that only layers of the tough, old stems remained. This residual material was mostly marl and sediment encrusted (Plate 3a,3b).

Schoenoplectus lacustris (Scirpus lacustris) growing amongst the Ranunculus was apparently unharmed by the herbicide, although some leaves had lost their tips. Similar damage was seen in the upstream herbicide section, but the remaining stems rose higher in the water column. This section had a layer of filamentous algae, a species of Cladophora, the only section to do so.

Parts of the downstream cut section had been cut, but the effect was to remove surface weeds rather than whole beds. There were some symptoms, such as leaf loss, in the cut and untreated downstream sections, suggesting that the Ranunculus had been damaged by herbicide from the upstream application.

The only damage in the upstream cut and untreated sections was where the ends of some of the Ranunculus beds had been cut. Although this was not limited to the cut sections, the Ranunculus in the untreated section was bright green and fully leaved, contrasting with the brown, leafless stems of the herbicide damaged plants.

By October, 106 days after the herbicide application, the old stems of Ranunculus from the herbicide sections had mostly disappeared but a few short, single stemmed shoots of bright green Ranunculus had grown from the river bed.

There was evidently some regrowth from cut stems in the downstream cut section. These shoots were shallow in the water column but this regrowth was much denser than in the herbicide sections.

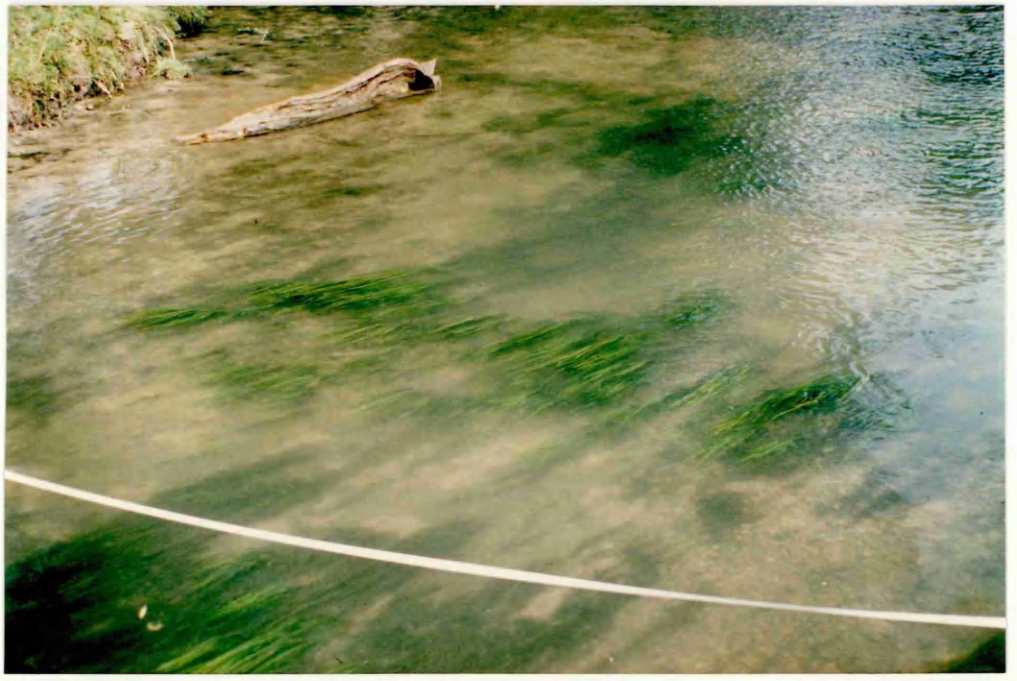
Ranunculus was still healthy in the upstream cut and untreated sections, but there had been some losses because the weed beds no longer broke the water surface and were smaller than in July.

Plate 3

Submerged macrophytes in the downstream herbicide transect in the R.Coln, before and after treatment

Plate 3a. Flowering Ranunculus beds prior to herbicide application on 20.6.84

Plate 3b. Schoenoplectus and remains of Ranunculus stems thirty six days after herbicide application



Biomass

The complete biomass data, for each species and section, are presented in Appendix 4c. The data did not need to be transformed for the ANOVA. It should be noted that both the May and June data were collected prior to the herbicide application.

When the biomass data (with or without algae) were analysed for all treatments and sampling times (Fig. 41a), the only significant factor was time, with a significant reduction in biomass in October (Fig. 41b).

Even if only the herbicide and untreated sections were analysed, and only from the June and July sampling times, the time * treatment interaction was still not significant. This lack of significance was rather surprising because there was a decrease in biomass after herbicide treatment, whilst the biomass in the untreated sections increased over the same period. The lack of significance is the result of the great variability between the replicates, especially the July samples.

		Herbicide	Cut	Untreated
Biomass ($g\ m^{-2}$)	d/s	112	79	130
	u/s	29	172	220

This variability between replicates was apparent when the percentage change in biomass was calculated, whether the May, or the June samples were used for comparison (Fig. 42a, 42b). There were no significant factors or interactions from the analysis of these data.

Cover

Some of the simplified vegetation maps are given in Fig. 43, the complete set are presented in Appendix 5c.

There were no significant factors or interactions in any of the analyses of %-frequency of Ranunculus or total submerged macrophytes (Fig. 44a). The analyses of the percentage change in %-frequency of vegetation (Fig. 44b) did not show any significant factors either.

Fig. 41

Biomass of macrophytes in R.Coln

Fig. 41a

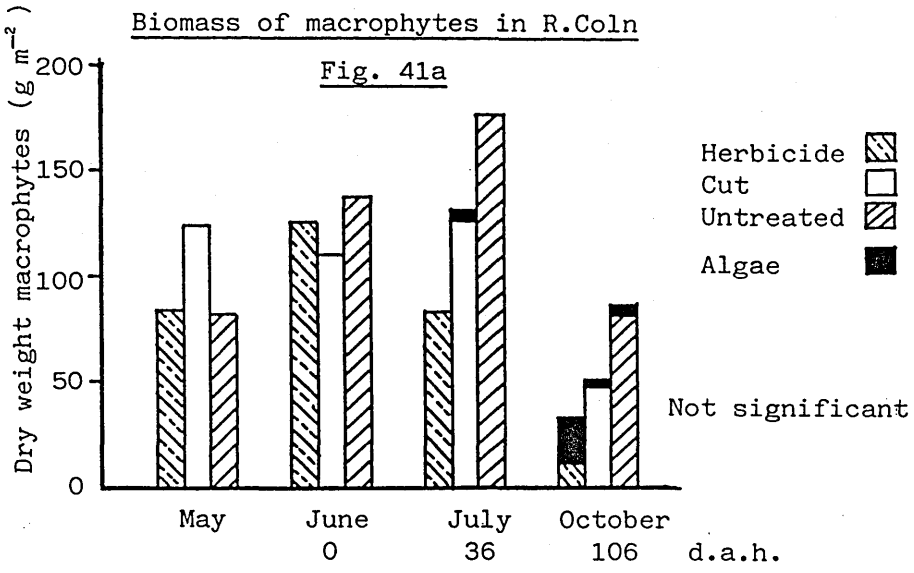


Fig. 41b

Time factor significant

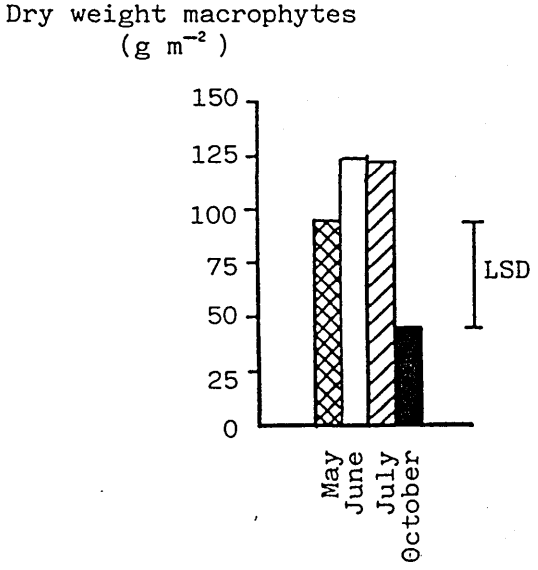


Fig. 42

Percentage change in biomass of macrophytes only

Fig. 42a

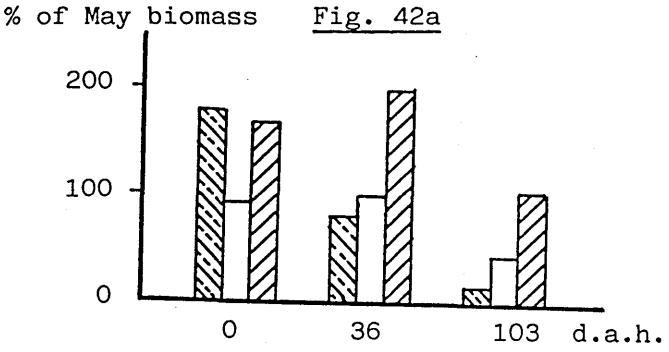


Fig. 42b

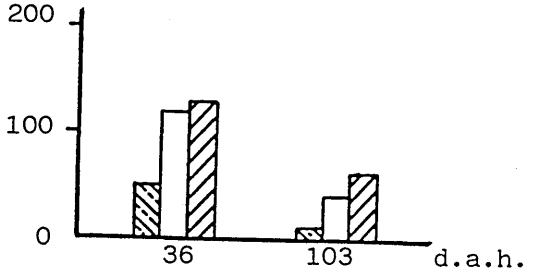


Fig. 43

Examples of vegetation maps from the R.Coln

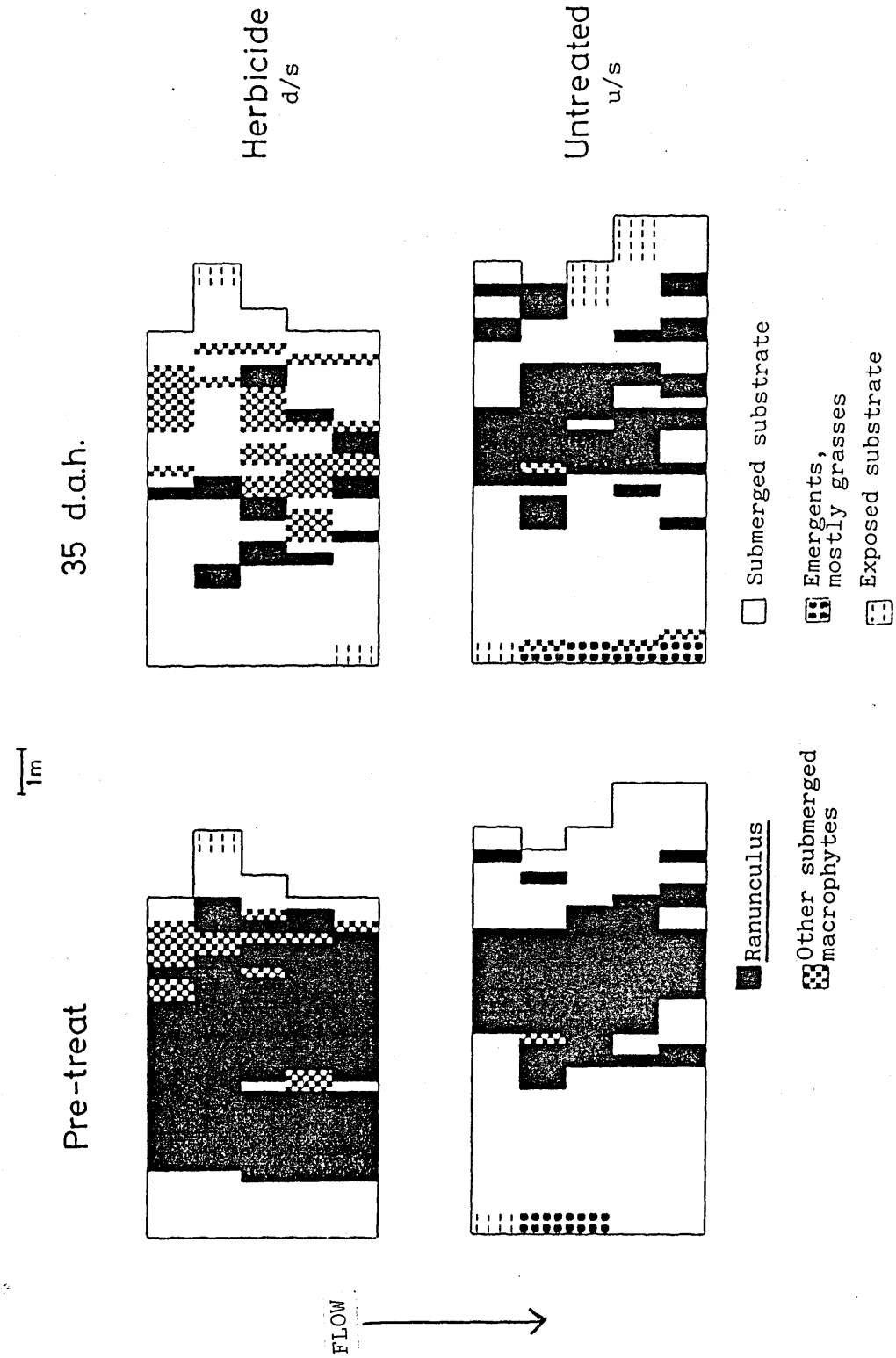


Fig. 44a

Percentage frequency of macrophytes in R.Coln

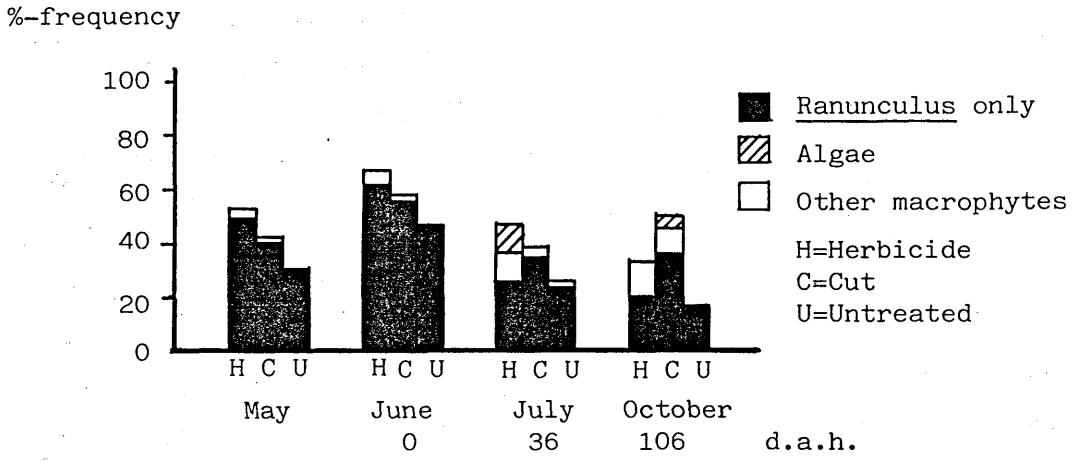
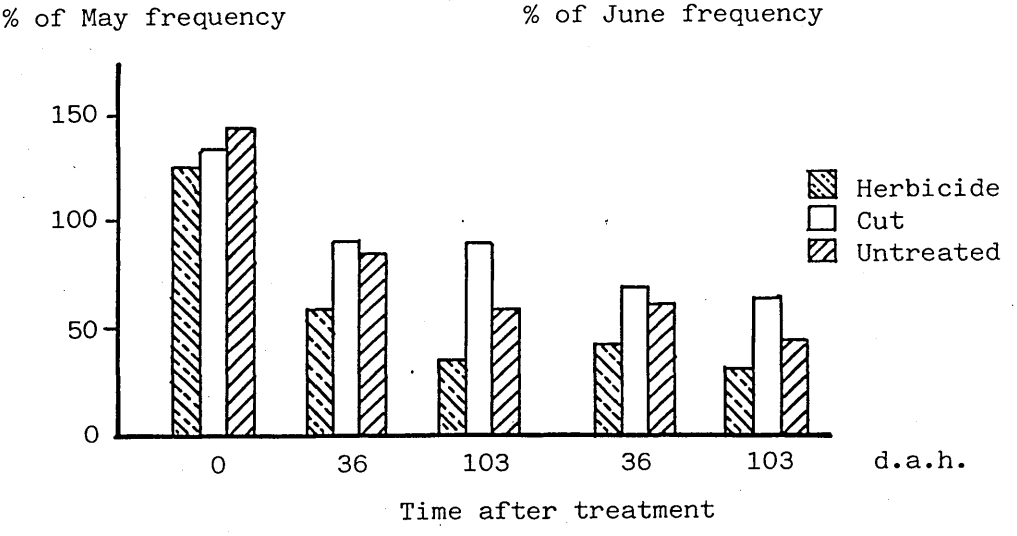


Fig. 44b

Percentage change in frequency:Ranunculus only



Mouse Water

Observations

The site was composed of two distinct macrophyte communities. The downstream half, which was shallower and of more rapid flow, was dominated by Cladophora sp. There were small beds of flowering Ranunculus aquatilis in the shallows, which were interspersed with deep areas with Potamogeton natans and Sparganium emersum. The upstream half of the site was deeper and slower flowing, and was dominated by P.natans and S.emersum. Only one small bed of Ranunculus could be found in each untreated section.

Six days after the herbicide treatment, there were obvious signs, in both herbicide sections, that the Ranunculus was extremely unhealthy, with chlorosis and a loss of turgidity. Healthy Ranunculus was not found until reaching a point 400-500m downstream of the treated sections. Other symptoms of phytotoxicity, such as chlorosis of S.emersum, could be seen 200-300m downstream.

The S.emersum in the treated sections was chlorotic underwater, but where it had been hit by the diquat-alginate above the water surface, the plants were brown and burnt. Similar symptoms were seen on directly hit P.natans leaves, and on emergent vegetation which had been sprayed.

By 18 days after the herbicide application, little Ranunculus remained in the herbicide sections. Some submerged P.natans plants, found in shallow, swifter-flowing water, had apparently lost their leaves. The S.emersum and the floating-leaved P.natans plants, showed no further damage.

The vegetation in the untreated sections continued to grow. In the middle of the site the P.natans leaves almost completely covered the water surface,and the dense growth of stems made wading difficult. The upstream end of the site became choked with S.emersum and S.erectum.

The October visit to the Mouse Water was postponed by storms. By the end of the month the water level had risen by 0.5m from the previous visits,and was too fast for wading. There were signs that the river had flooded to at least 1m above the summer levels. The water was peaty and turbid so it was not possible to see the submerged plants. Many of the floating leaves of the P.natans had been ripped away, leaving leafless stems. There was no sign of any Ranunculus although any regrowth might have been swept away.

Biomass

The complete biomass data are given in Appendix 4d. With the limited number of treatments and sampling times, it was unlikely that the time * treatment interaction would be significant, especially since there were large reductions in biomass in both treatments (Fig. 45a). The time factor was significant, whether or not the algae were included in the biomass analysis (Fig. 45b). There was no significant difference between the two treatments, when the %-biomass changes were analysed (Fig. 45c).

Cover

The simplified vegetation maps are presented in Fig. 46.

The %-frequency of submerged macrophytes (with or without algae) remained constant over the two sampling times (Fig. 47a). Only the Ranunculus data showed any difference in the percentage change in %-frequency, (Fig. 47b) but this difference was not significant.

Fig. 45

Biomass of macrophytes in Mouse Water

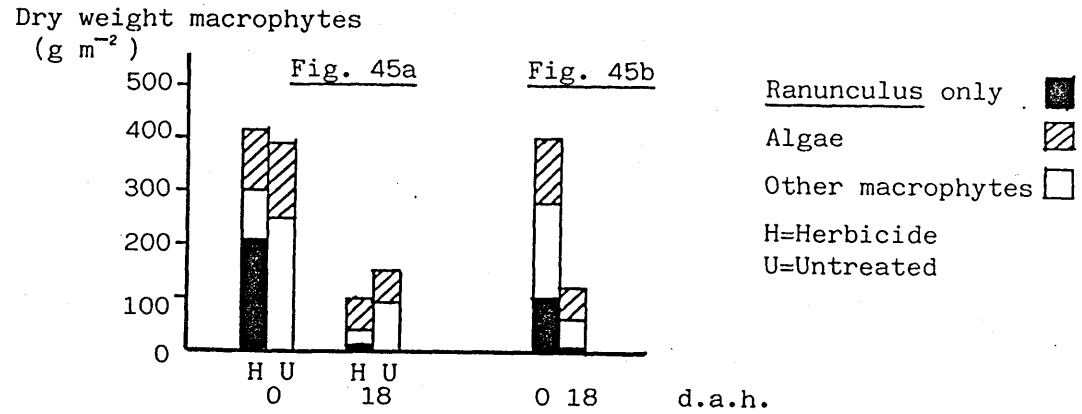


Fig. 45c Percentage change in biomass

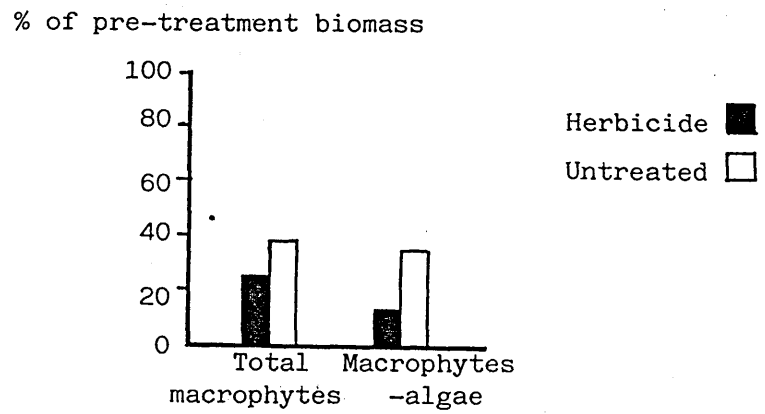
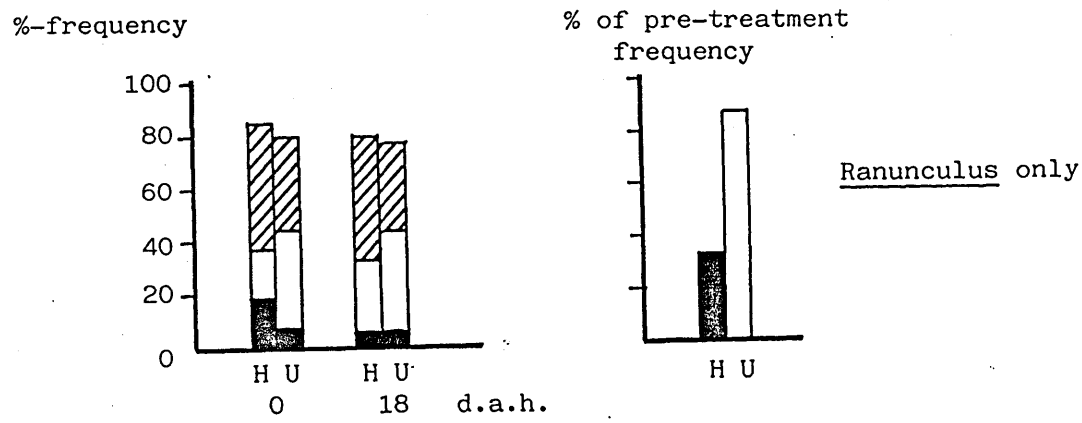


Fig. 47a

Percentage frequency of macrophytes in Mouse Water

Fig. 47b

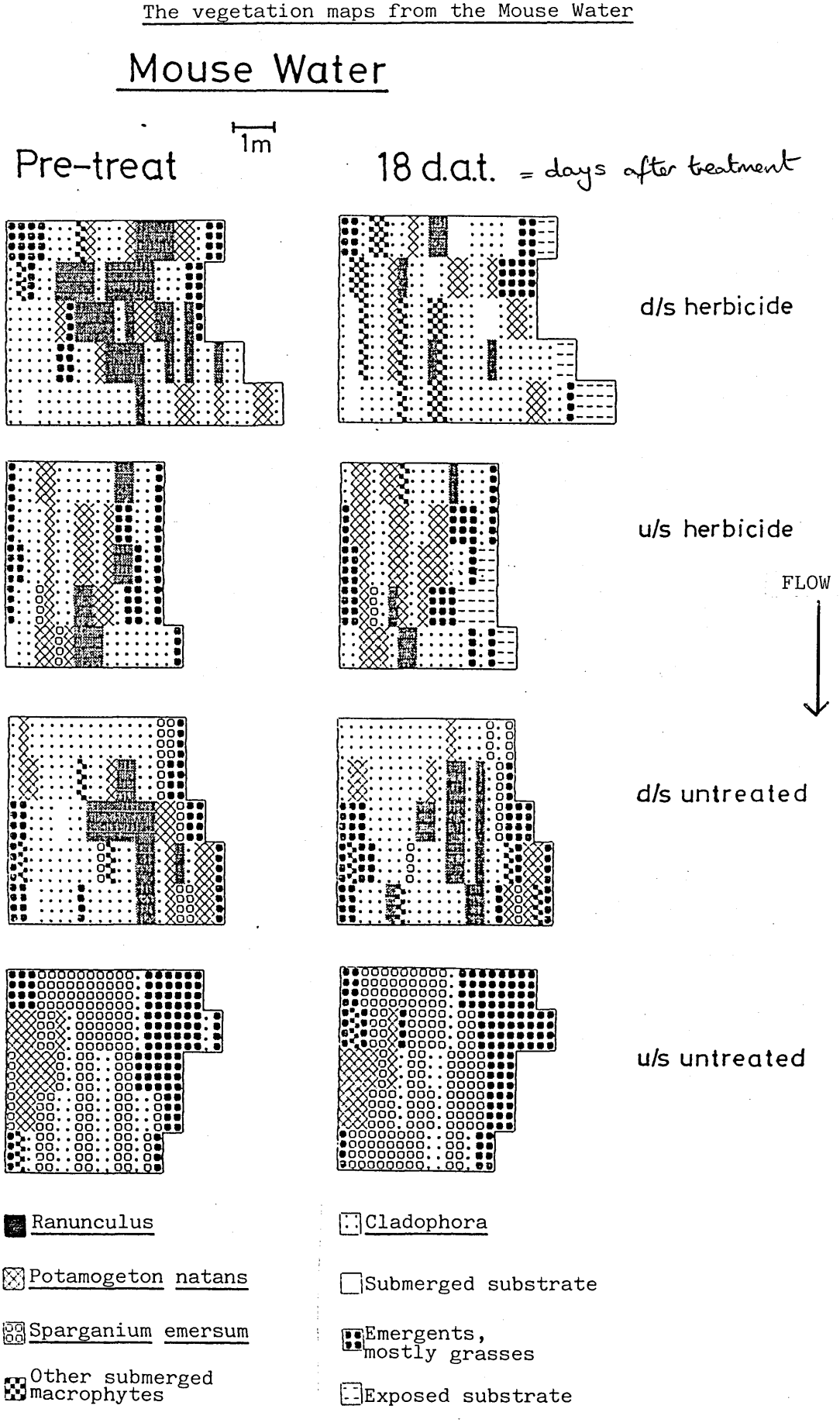
Percentage change in frequency



Key as Fig. 45a

Fig. 46

The vegetation maps from the Mouse Water



4.5.2 Diquat residues

The diquat residues collected after application, from each herbicide section, have been plotted in Fig. 48a-d. Each point was determined from the mean, of the analysis of three sub-samples, taken from a single river sample. The standard errors of these means were small. These errors would indicate the variability of the diquat analysis, not of the distribution of residues in the water. Diquat residues were not detected in any of the samples taken from upstream of the herbicide sections.

The areas under the curves of residue concentration against time after application, have been calculated, both for the full time of sampling and for a minimum time of 40 minutes. These areas are the herbicide concentration x time products of each section and are referred to as the 'availance' values.

Availance is defined by:

$$\int_0^\infty C\ dt$$

C = diquat concentration
t = time

the product of availability and time for an infinite time. This differs from exposure, which is defined if the time of contact with the herbicide is finitely limited (Hartley and Graham-Bryce 1980).

R.Petteril

Diquat residues could be detected in the downstream herbicide section, within one minute of the start of the herbicide application. These residues reached a very high peak within 5 minutes. The concentrations fell rapidly over the following 15 minutes and then declined more slowly.

Residues were detected in the upstream section after only one minute, but declined after 5 minutes. The diquat concentration subsequently increased again, after 10 minutes but the sampling programme did not last long enough to show the maximum concentration or duration of this peak. The availance values for the two sections were quite different up to 40 minutes,but may have been more similar if the sampling had continued for longer, especially in the upstream section.

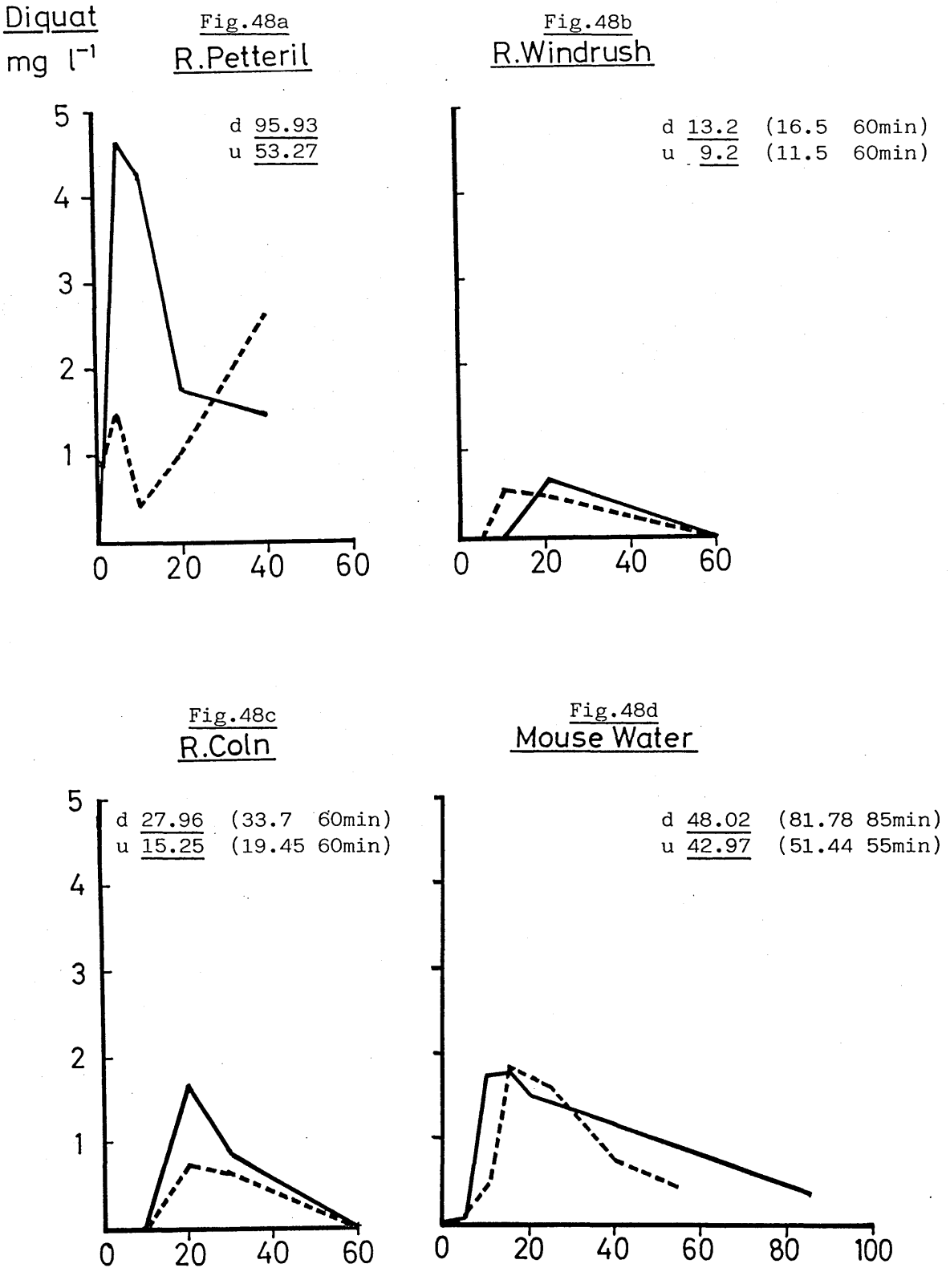
Of the water samples taken from the upstream edge of section P5 (about 250m downstream of the upstream herbicide section), a trace of diquat was detected after 20 minutes and after 40 minutes this rose to 0.05mg l^{-1}. It is possible that higher concentrations might have

Fig.48

Diquat residues from the river trials

Availance values (mg l^{-1}.min) over 40 minutes (total time)

d=downstream section
u=upstream section



been collected later, if a pulse of diquat was passing downstream.

R.Windrush

Diquat residues were only detected from the downstream herbicide section 20 minutes after the start of the application. This single residue value, makes the availance estimation highly dubious because the herbicide concentration might have fallen to zero at any time before the next sampling time.

Residues were detectable 10 and 20 minutes after treatment in the upstream section. The availance value for this section is slightly more credible than that for the downstream section, but the two values are, in fact, in fairly good agreement.

R.Coln

Diquat residues were not detectable in either herbicide section until 20 minutes after the start of the application of Midstream. A high peak of diquat (greater than the intended dose of 1 mg l^{-1}), was seen in the downstream section, but the concentration decreased after a further 10 minutes.

The residue concentrations detected in the upstream section were lower than those in the downstream replicate, but the timing of the peak was the same.

Samples taken from the upstream edge of section C3 (250m downstream of the top herbicide section), did not contain any detectable diquat until 60 minutes after treatment. At this time 0.2mg l^{-1} was detected, which was nearly 30% of the maximum value found in the upstream herbicide section.

Mouse Water

The residue data from the Mouse Water were the most satisfactory of all the rivers. Traces of diquat were detectable in both herbicide sections, by 5 minutes after the start of the herbicide applications. The residue concentrations in both sections,rapidly increased to peaks of nearly 2.0mg l^{-1} (the maximum permitted dose, MAFF 1985), by 15 minutes after treatment. The concentration fell slowly over the following 70 minutes, in the downstream section, by which time the concentration was still quite high, 0.32mg l^{-1} . The decline in residues was slightly faster in the upstream section, but after 40 minutes, the availance values from the two sections were similar.

4.5.3 Depth profiles and flow transects

Depth profiles at each section transect, from each sampling date are shown in Fig. 49a-d. The depths of the macrophytes were also recorded, and the approximate area of the channel cross-section filled with vegetation, has been illustrated. Histograms of the mean depths and flow velocities, for each section and sampling time, are presented in Appendix 6. A summary of these data, as mean depths and flow velocities per river, for each sampling time, is given in Table 8. Standard errors have been indicated when applicable, but no futher statistical analyses were carried out.

R.Petteril

The coarse, stony substrate in the shallow R.Petteril, caused quite substantial variations in depth measurements within small areas. Water depth decreased slightly in all sections throughout the summer. The cross-sectional area of vegetation increased in many sections between May and July, but was reduced to zero in all sections by September. The vegetation in the herbicide (P6) and the cut (P7) sections, in July was filamentous algae.

There was little pattern to the flow velocity measurements, but they were difficult to take in such a shallow, weedy stream.

R.Windrush

The mean water depth declined in all sections throughout the summer, but the reduction was not particularly marked after the weed cut. The cross-sectional area of vegetation increased from May to July in all sections, and then showed a marked reduction in October. The effect of the cut on the cross-sectional distribution, especially in the centre of the channel, could be seen in section W2 and W7, but not in W5. The marked change in distribution in the downstream herbicide section W1, between June and July was interesting, but difficult to explain.

Flow velocities tended to decrease over the summer in all sections. There was not a noticeably greater reduction in velocity after cutting, compared with the untreated sections. Flow measurements were often impossible to take because of the obstruction caused by the large volumes of vegetation.

Depth profiles of the rivers at the transects

Fig 49a R.Petteril

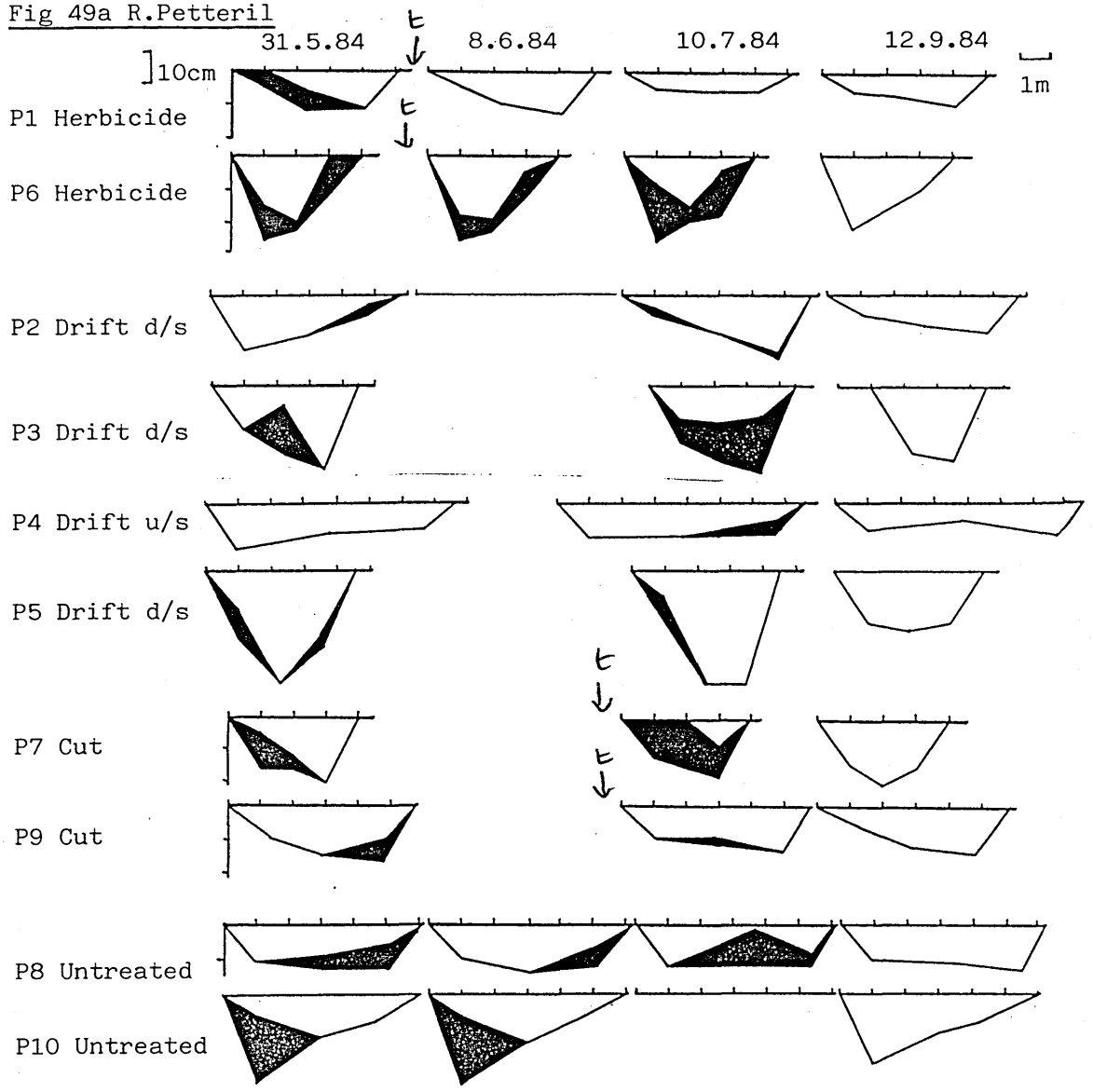


Fig. 49b Mouse Water

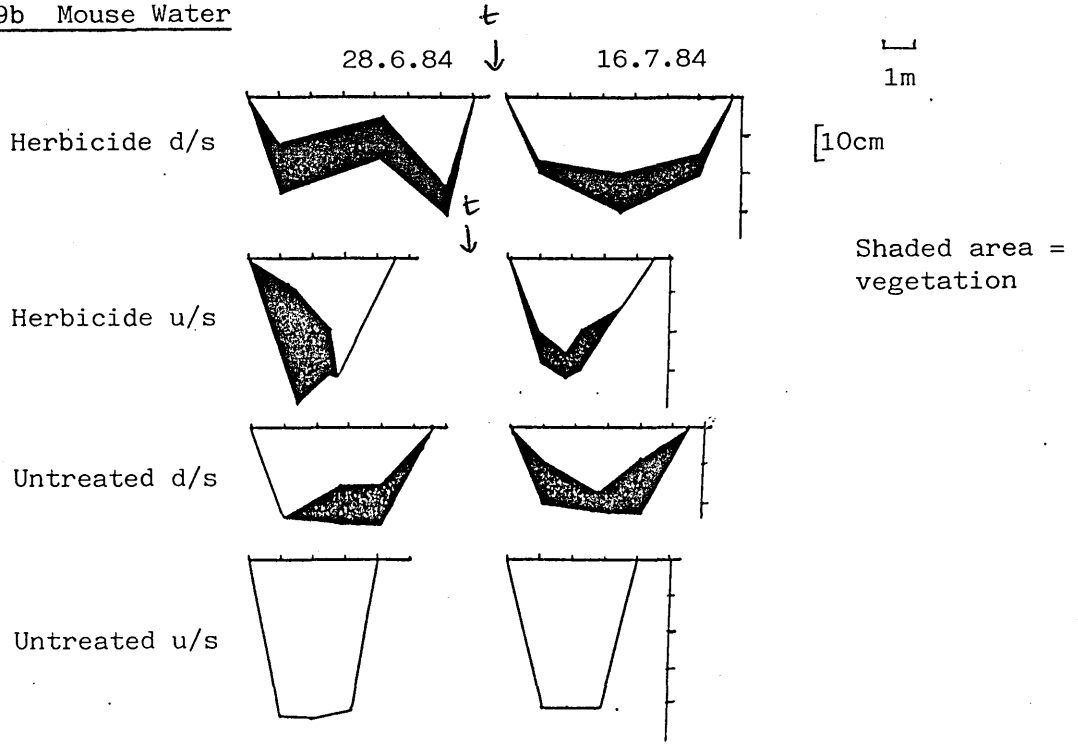


Fig. 49c R.Windrush

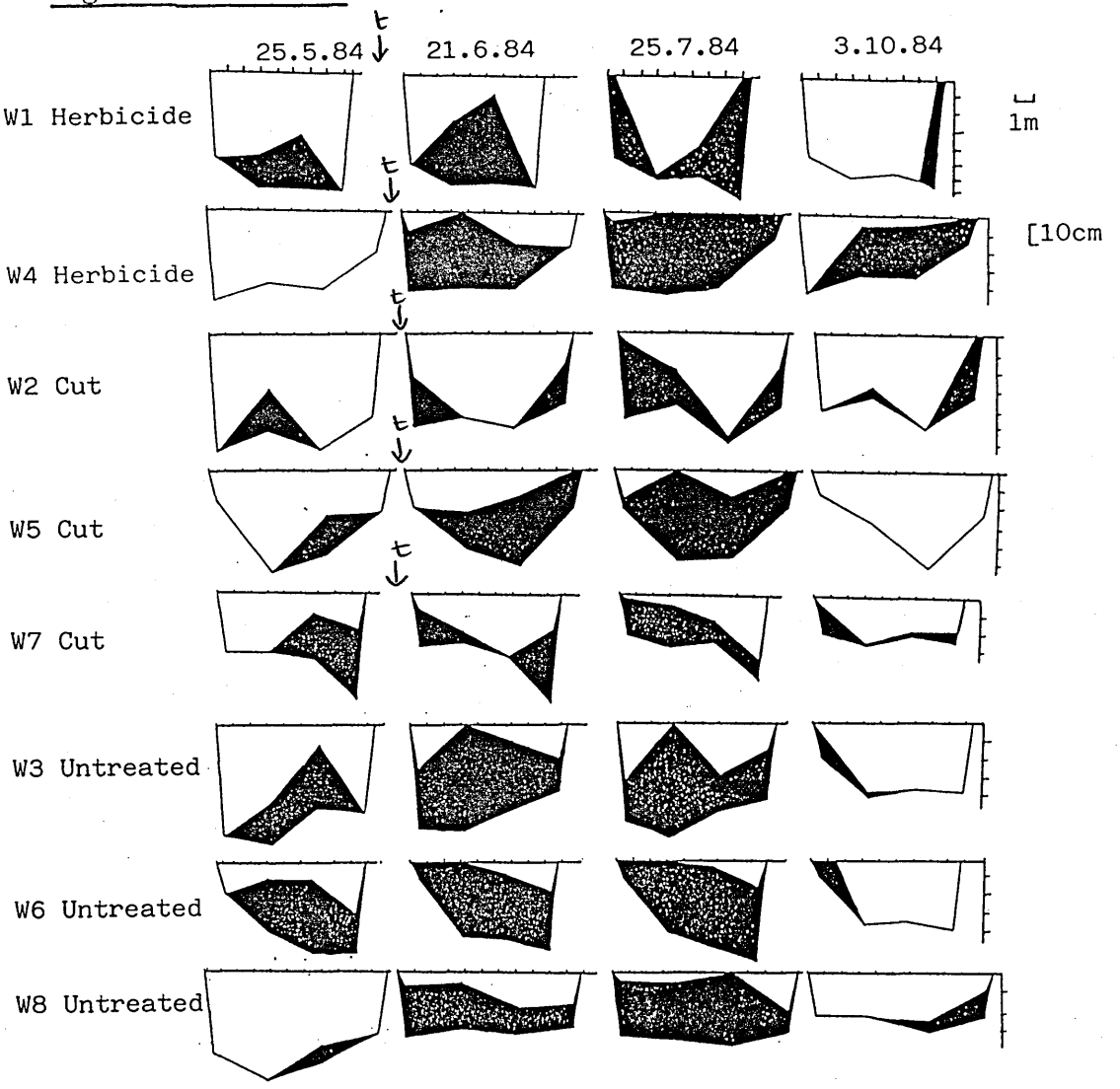


Fig. 49d R.Coln

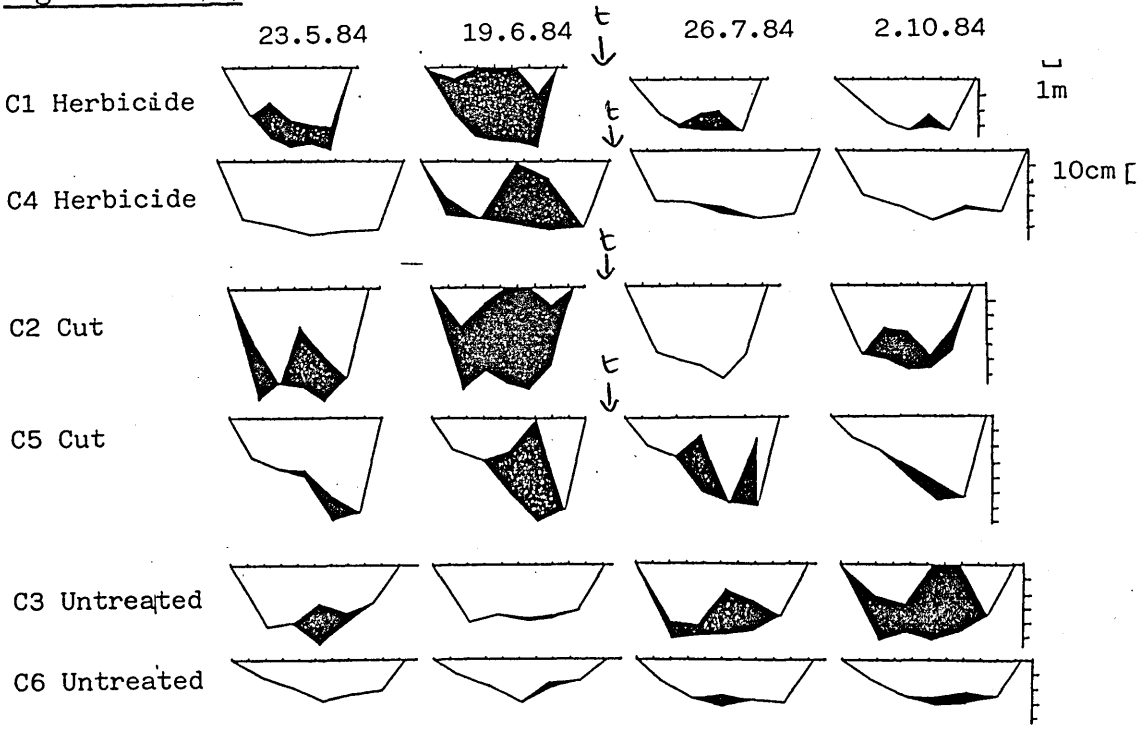


Table 8

Mean depths and water velocities in the river sites

	Depth	Water Velocity	
		Surface	0.6 x depth
	(cm)	(m s^{-1})	(m s^{-1})
R.Petteril			
May	15.5 (1.23)	0.33 (0.03)	0.23 (0.02)
June	14.3 (2.03)	0.03 (0.04)	0.21 (0.03)
July	15.3 (1.53)	0.26 (0.02)	0.18 (0.03)
September	12.1 (0.99)	0.32 (0.03)	0.21 (0.02)
R.Windrush			
May	49.6 (2.98)	0.40 (0.02)	0.29 (0.02)
June	44.3 (2.55)	0.29 (0.02)	0.20 (0.02)
July	44.2 (2.64)	0.24 (0.03)	0.19 (0.03)
October	35.4 (2.39)	0.26 (0.02)	0.19 (0.02)
R.Coln			
May	39.9 (2.44)	0.38 (0.02)	0.30 (0.02)
June	38.1 (2.33)	0.35 (0.02)	0.31 (0.02)
July	31.9 (1.98)	0.26 (0.02)	0.20 (0.02)
October	30.2 (2.07)	0.25 (0.02)	0.21 (0.01)
Mouse Water			
June	35.6 (2.57)	0.13 (0.03)	0.11 (0.01)
July	31.3 (2.62)	0.12 (0.02)	0.1 (0.01)

R.Coln

Water depth decreased in all sections throughout the summer, with noticeably larger reductions after the herbicide treatment, and the cut, in the downstream sections, C1 and C2. The cross-sectional area of vegetation had also markedly declined by July in the herbicide and cut sections. However, there was a similar reduction in the untreated sections at this time, so it is not possible to attribute these changes simply to the herbicide or cut treatments.

Flow velocities decreased in all sections throughout the summer.

Mouse Water

There was a slight reduction in water depth between the sampling times, in all sections, but no distinct changes in the cross-sectional areas of the vegetation. Flow was only noticeably reduced in the upstream herbicide section. The surface flow velocity was less than that at 0.6 x depth, in the upstream untreated section. This would have been because this section lacked submerged vegetation but was dominated by floating-leaved species.

4.5.4 Water Chemistry

R.Petteril

The mean values for all the chemical parameters measured at each sampling time are presented in Table 9a. There was very little, or no, variation between the sections for any parameters except dissolved oxygen (Fig. 50a). For all parameters,other than dissolved oxygen, the differences between sampling times were greater than any variation between sections within a sampling time. Nitrates and reactive phosphorus both had their minimum concentrations in July, trends that were also seen in the data from the North West Water Authority.

The dissolved oxygen readings do not show particular patterns that could be related to the management of specific sections. It is likely that the diurnal changes during the period over which the readings were taken, and localised variations on plant density or organic inputs, would have had a greater influence on dissolved oxygen values. However, it is interesting that the greatest variation in dissolved oxygen readings occur in July, when the maximum effects of the treatments were observed. When dissolved oxygen concentrations

were plotted per section, with plant biomass or % frequency of vegetation, there appears to be more of an inverse, than a direct, relationship between the macrophytes and dissolved oxygen (Fig. 50b).

The most important fact, is that the water was always super-saturated with dissolved oxygen. It would appear unlikely, from these data, that oxygen depletion (resulting from plant decay) sufficient to cause distress to the stream fauna, would have occurred. These data were collected in the afternoon, so it is not possible to predict the conditions at night.

R.Windrush

The mean water chemistry data for the R.Windrush are presented in Table 9b. The only variations observed between sections, had an upstream to downstream gradient. These patterns, principally seen for dissolved oxygen and pH, were related to the diurnal changes occurring during the morning sampling period,because readings were started at about 9am and were taken moving progressively upstream.

There was little variation in most parameters, including nitrates and reactive phosphorus, throughout the season. There was good agreement between these data and those of Thames Water Authority.

R.Coln

The means for all parameters, per sampling time are listed in Table 9c. There was little variation between sections for any parameter, and these data corresponded to those from Thames Water Authority.

Mouse Water

The mean chemical data for the Mouse Water are presented in Table 9d.

Dissolved oxygen was the only parameter which exhibited obvious differences between the sections (Fig. 51). The same pattern of dissolved oxygen concentrations is seen before, and after, the herbicide treatment, and the lower concentrations occur in the untreated sections.

Table 9a,9b

Mean water chemistry data from the field trials

River	9a R.Petteril				9b R.Windrush			
Date	31.5.84	8.6.84	10.7.84	12.9.84	25.5.84	21.6.84	25.7.84	3.10.84
Start time	12:40	10:30	15:22	14:05	09:10	09:00	09:30	09:45
Water temperature (°C)	17.2 (0.08)	17.2 (0.2)	20.2 (0.07)	13.5 (0.03)	14.3 (0.2)	19.6 (0.2)	17.2 (0.1)	10.2 (0.3)
Dissolved oxygen (% saturation)	163.4 (2.93)	160.5 (6.3)	203.9 (7.2)	121.2 (1.7)	161.3 (7.3)	121.2 (3.6)	109.6 (1.4)	123.3 (5.5)
pH	7.96 (0.03)	8.24 (0.06)	8.92 (0.02)	8.33 (0.3)	8.34 (0.1)	7.91 (0.04)	8.28 (0.07)	8.08 (0.07)
Conductivity (u mohs cm^{-1})	435.6 (2.56)	427 (3.4)	440.3 (4.2)	-	488.7 (7.4)	521.4 (1.4)	505.0 (1.9)	473.6 (9.7)
Total water hardness (mg l^{-1} $CaCO_3$)	164.7 (2.4)	-	229.1 (1.8)	222.3 (1.4)	240.3 (8.0)	280.7 (3.2)	246.1 (0.6)	270.5 (0.82)
Calcium ion conc. (mg l^{-1} Ca^{++})	62.0 (2.0)	65.3 (1.3)	69.5 (0.7)	59.1 (0.7)	106.7 (3.3)	124.9 (2.4)	100.3 (0.4)	98.1 (0.5)
Nitrate ion conc. (mg l^{-1} NO_3^-)	10.2 (0.17)	11.0 (0.2)	2.65 (0.25)	8.02 (0.07)	35.7 (0.7)	30.3 (0.21)	26.8 (0.3)	32.3 (0.3)
Reactive phosphorus (mg l^{-1} P)	0.20 (0.01)	0.25 (0.01)	0.09 (0.006)	0.14 (0.004)	0.12 (0.004)	0.10 (0.003)	0.07 (0.005)	0.10 (0.001)

( ) = standard error

Table 9 (continued) 9c,9d

Mean water chemistry data from the river trials

River	9c R.Coln				9d Mouse Water	
Date	23.5.84	19.6.84	26.7.84	2.10.84	28.6.84	16.7.84
Start time	14:05	09:00	09:10	09:30	11:00	11:30
Water temperature (°C)	14.7 (0.2)	18.3 (0.2)	14.6 (0.1)	10.5 (0.2)	13.6 (0.2)	14.3 (0.3)
Dissolved oxygen (% saturation)	142.0 (2.5)	118.6 (1.0)	118.8 (2.2)	111.8 (1.3)	130.5 (16.8)	95.2 (16.2)
pH	7.81 (0.16)	6.72 (0.14)	7.95 (0.11)	8.04 (0.04)	7.79 (0.13)	7.61 (0.07)
Conductivity (u mohs cm^{-1})	451.7 (13.5)	478.3 (4.8)	444.2 (3.3)	456.7 (4.9)	616.2 (2.4)	630.7 (3.5)
Total water hardness (mg l^{-1} $CaCO_3$)	-	296.0 (1.6)	251.7 (0.2)	260.3 (0.3)	349.5 (1.7)	349.5 (2.1)
Calcium ion conc. (mg l^{-1} Ca^{++})	98.3 (1.7)	118.3 (1.7)	100.7 (0.4)	91.8 (1.7)	81.0 (2.3)	85.5 (0.9)
Nitrate ion conc. (mg l^{-1} NO_3^-)	25.2 (1.4)	24.0 (0.2)	22.9 (0.15)	25.5 (0.3)	11.4 (0.1)	10.4 (0.15)
Reactive phosphorus (mg l^{-1} P)	0.12 (0.004)	0.07 (0.001)	0.04 (0.005)	0.05 (0.004)	0.03 (0.006)	0.03 (0.006)

Fig.50a

Percentage saturation of dissolved oxygen in the R.Petteril

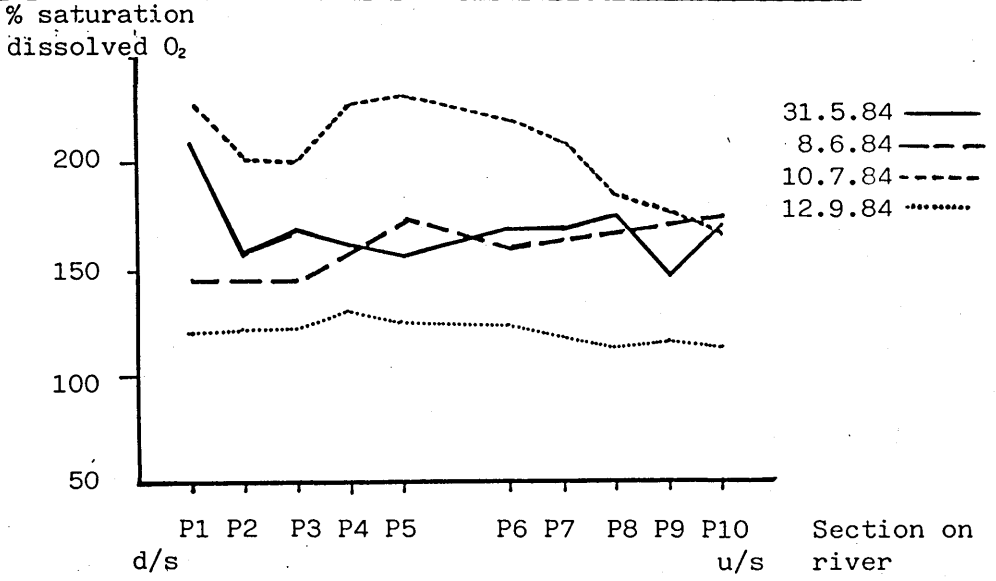


Fig. 50b

Dissolved oxygen on 10.7.84 compared with plant biomass

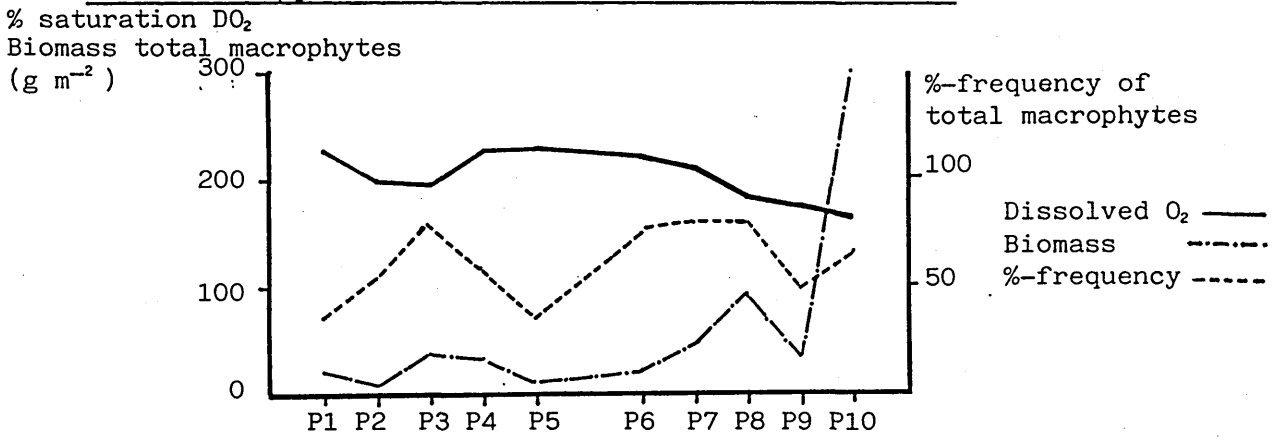
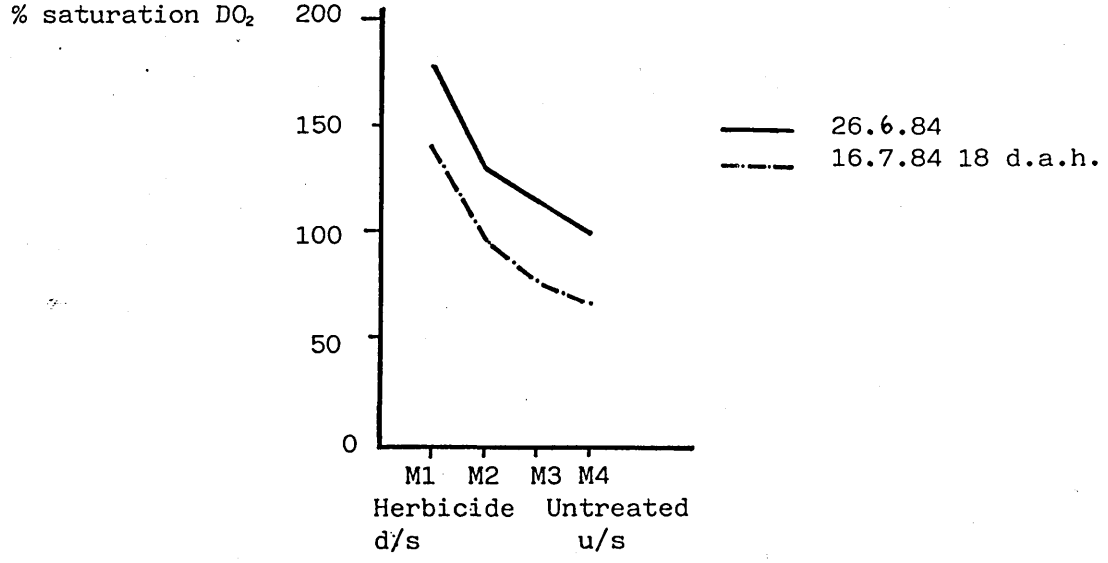


Fig. 51

Dissolved oxygen in the Mouse Water



4.5.5 Light (P.A.R.) transmission

The mean light extinction coefficients per river and sampling time, are presented in Table 10. Data were not available early in the season, because of the lack of suitable equipment. Thus, the only pre-treatment measurements were taken in the R.Coln.

At the beginning of the summer, suspended material was filtered out of the water samples, and was extracted in acetone, to determine the chlorophyll content of the waters. The procedure was stopped when it became evident that the chlorophyll concentrations were very low and would be of little value. In retrospect, it appears that a method of measuring turbidity, or concentrations of suspended solids, would have been more useful, especially for the pre-treatment samples.

Some data for suspended solids have been obtained from the Water Authorities, and these are shown in Fig. 52a-c.

R.Petteril

Light measurements were difficult to take in the shallow and turbulent riffles of the R.Petteril. There were large variations between readings, especially in September. The stream water appeared to be reasonably clear, and the high extinction coefficients were likely to have been caused by the scattering and reflection of light entering the turbulent surface waters. The concentrations of suspended solids were very low at the beginning of the summer, when the herbicide was applied, and increased later, when the light readings were taken.

R.Windrush

The water in the R. Windrush was very turbid, especially early in the season, when it was impossible to see the channel bottom in many sections. Provided the light readings were taken in unvegetated areas, the variation between readings was not as great as in the R.Petteril.

The data for suspended solids came from points 11.5km upstream from the site, and 5.5km downstream. Both of these sampling points were on undivided parts of the river, whereas the study site was on the eastern arm of the divided river. It is difficult to determine which of the two sets of data, best represent the conditions at the study site. The high values at the upstream sampling point indicate that concentrations of suspended solids may be high in this river during the summer.

R.Coln

The extinction coefficients in the R.Coln were reasonably consistent, and the water was noticeably turbid throughout the summer. As with the R.Windrush, data were available for suspended solids, from two sampling points. One was 7km upstream and the other 11km downstream of the experimental site. There were considerable differences between the values from these sampling points, with the higher concentrations of suspended solids at the downstream point.

Mouse Water

The mean extinction coefficient for the Mouse Water had a large standard error. This was the result of differences between the upstream and downstream halves of the site.

	upstream	downstream
Extinction coefficients	1.54 1.57	2.11 2.22

This difference was likely to be due to the more turbulent nature of the downstream half of the site, causing a greater scatter of light entering the water, and increasing the suspension of particulate matter.

Table 10

Mean light extinction coefficients from the river trials

	R.Petteril	R.Windrush	R.Coln	Mouse Water
June	-	1.698 (0.089)	1.289 (0.076)	-
July	1.821 (0.26)	1.042 (0.113)	1.190 (0.081)	1.859 (0.50)
Sept/Oct	1.259 (1.06)	1.016 (0.086)	1.320 (0.172)	

( ) = standard error

Fig. 52

Concentrations of suspended solids in the rivers throughout 1984 from data provided by the Water Authorities

Fig. 52a R.Petteril

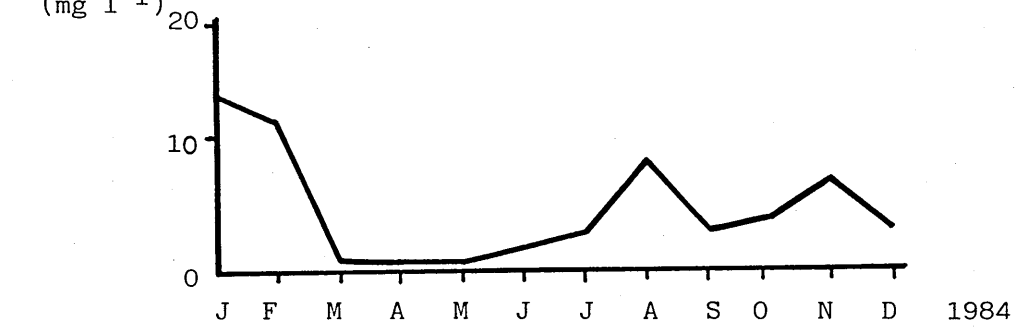


Fig. 52b R.Windrush

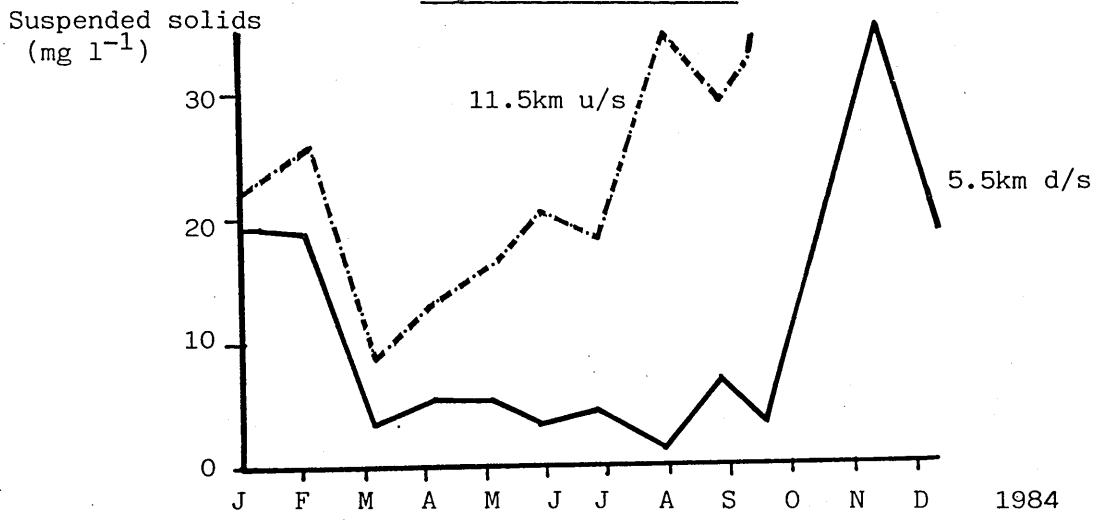
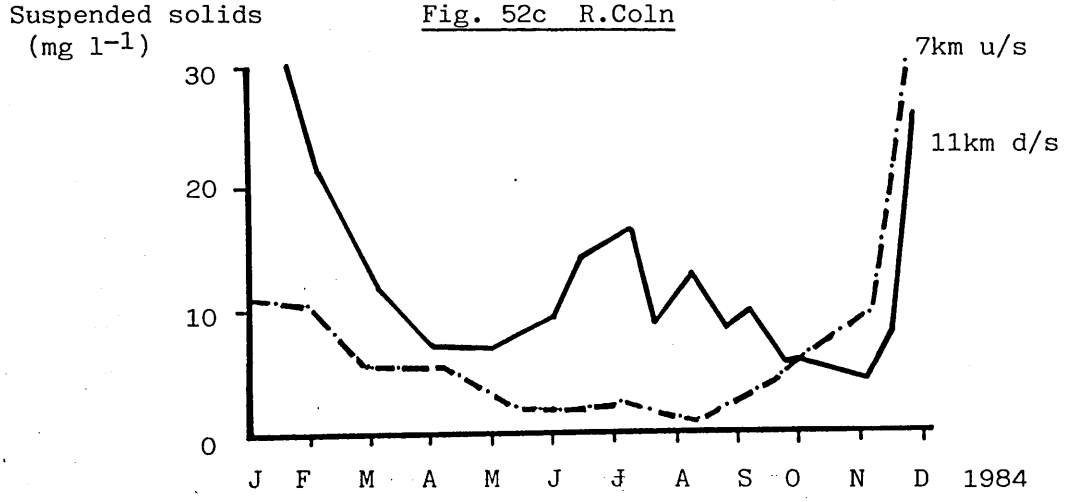


Fig. 52c R.Coln



4.5.6 Macroinvertebrate communities

As had been anticipated, the macroinvertebrate data showed great variability. The two sub-samples per section were quite inadequate to provide statistically significant, and convincing, quantitative descriptions of the macroinvertebrate communities.

However, these data provided a qualitative basis for considering whether the treatments caused any gross changes in the diversity of the macroinvertebrate fauna.

The data have been presented in four ways.

1) Histograms showing the mean numbers of individuals per sampling unit (⅛ of a sample from 0.05m^2 river bed), for each treatment and sampling time. These histograms were only produced for the major taxa (Fig.s 53-55).

Simple ANOVAs (without the blocks factor) were carried out on these data on the Glasgow University main-frame computer using the MINITAB statistical package (Ryan et al. 1976). Only analyses which showed variance ratios which were significant at the 5% level have been indicated.

The positive halves of the two-sided confidence intervals, calculated at the 95% level, for each mean have been indicated on the histograms. These confidence intervals were calculated using MINITAB.

Percentage changes in numbers per treatment, between the pre- and post-sampling times, have been calculated, but have only been illustrated when differences between treatments, or sampling times were significant.

2) Species lists for each site are presented in Appendix 7 . These lists were compiled from samples, selected at random, from each site, and so include species from any section or sampling time. These lists should provide a more detailed view of the composition of the communities associated with each site. A comprehensive list of the species that may be found in the R.Coln, at Fairford, is given by MacKey et al. (1982).

3) Species diversity indices were calculated from the pooled data from each treatment and sampling time. These indices are listed in Table 11.

The Shannon-Weaver species diversity indicies were estimated using a computer programme developed by the U.S. Department of Agriculture (1984). The use of this index,based upon the information theory function of Shannon (1948), has been discussed by Hellawell (1978).

4) Correlations were tested (using MINITAB) between the numbers of invertebrates in the major taxa, and the biomass of vegetation, for each site. These data (Table 12) could be calculated because the macroinvertebrate samples were collected with the plant samples, although it should be noted that the macroinvertebrate samples, and not the vegetation, had been sub-sampled by one-eighth.

The following comments describe the apparent trends in the data. These trends are not statistically significant unless specified, by the use of the term significant.

R.Petteril

The macroinvertebrate data for the major taxa, in the R.Petteril are illustrated in Fig. 53.

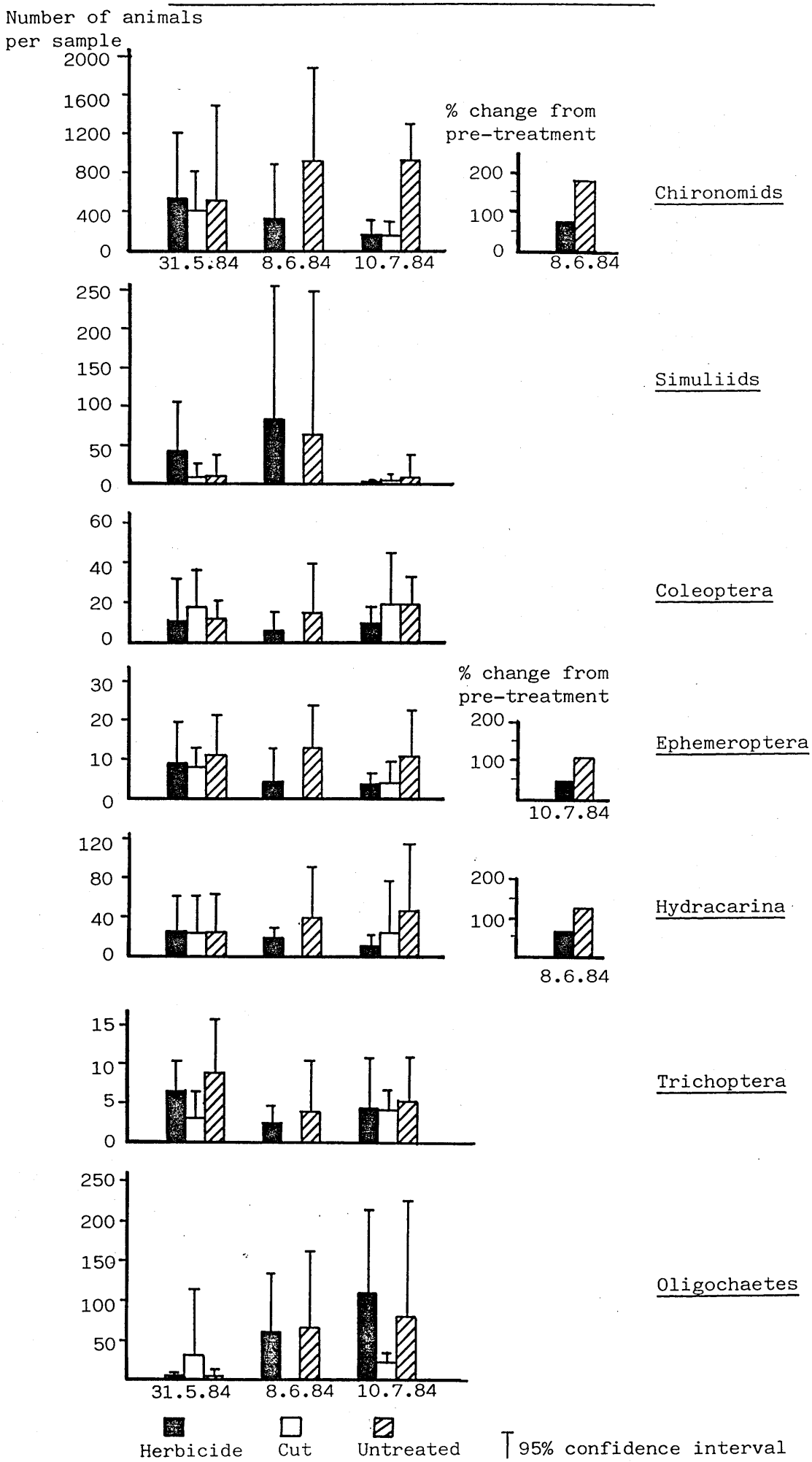
The number of chironomids in the herbicide and cut samples declined after treatment, whereas there was an increase, or a maintenance of numbers in the untreated samples, during the summer. The herbicide and untreated samples showed significant differences in the percentage change between the sampling times,immediately before and after the herbicide application.

The maximum number of simuliids occurred at the second sampling time, regardless of the treatment. The Coleoptera and Trichoptera numbers showed no clear pattern with time, and there were no differences between the treatments.

The Ephemeroptera and Hydracarina showed increasing, or stable, numbers throughout the summer, in the untreated sections, but numbers in the herbicide sections (and for Ephemeroptera in the cut sections) declined. The percentage changes for the herbicide and untreated sections were significantly different between the pre-treatment and the July, or June, post-treatment samples, for Ephemeroptera and Hydracarina respectively.

Oligochaete numbers increased fairly dramatically throughout the season in the herbicide and untreated sections. Numbers in the cut sections showed little change.

Macroinvertebrate data from the R.Petteril



The simuliids, Ephemeroptera and Hydracarina showed significant correlations with the plant biomass (± algae). Total numbers of invertebrates and numbers of chironomids were only correlated to plant biomass if the algae were included. Coleoptera and Trichoptera were only correlated with the plant biomass without algae.

The species diversity indices varied between sections prior to treatment, being lowest in the untreated sections. The indices increased through the summer in all samples, and the variation between samples, within a treatment, became smaller.

R.Windrush

Chironomid and gastropod numbers declined, with time, in both the cut and untreated sections. Numbers of Ephemeroptera fell after the cutting treatment, whilst numbers in the untreated sections did not change. The percentage change in numbers of Ephemeroptera, between the two sampling times, was significantly different for the two treatments. Data for the R.Windrush are presented in Fig. 54.

The other major taxa, Coleoptera, Trichoptera, Hydracarina and Malacostraca, all showed opposing trends for the two treatments, with declines in the cut sections and increases in the untreated sections.

Only the numbers of Trichoptera showed a significant correlation with the plant biomass. The species diversity indices remained the same, over the sampling times, in the cut sections. The indices were initially lower in the untreated sections, but by June had increased to equal the values in the cut sections.

R.Coln

Macroinvertebrate data for the R.Coln are illustrated in Fig. 55. All of the taxa illustrated, with the exception of the oligochaetes, showed increases in numbers in both treatments, between the June and July sampling times. Total numbers of Trichoptera significantly increased between the two sampling times. The numbers of Coleoptera differed significantly between the treatments.

Only the Hydracarina showed a significant interaction between time and treatment, with a significantly greater increase in numbers between June and July in the untreated sections, compared to the herbicide treated ones. The oligochaete numbers decreased in the herbicide sections, but over the same time increased, from nothing in the untreated sections.

Total numbers of macroinvertebrates, Coleoptera, and Hydracarina were significantly correlated with plant biomass. Diversity indices increased with time in both treatments.

Fig. 54

Macroinvertebrate data from the R.Windrush

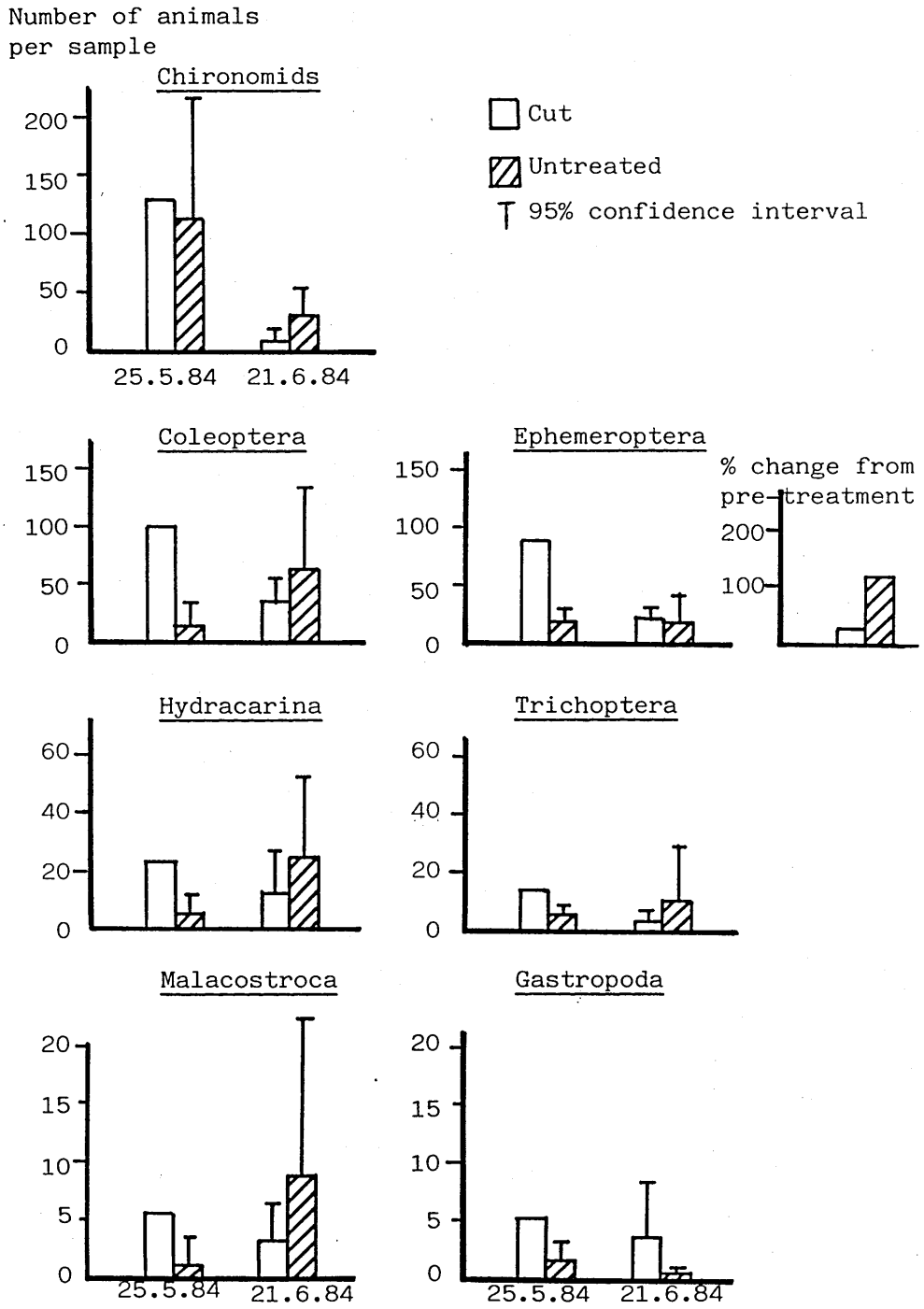


Fig. 55

Macroinvertebrate data from the R.Coln

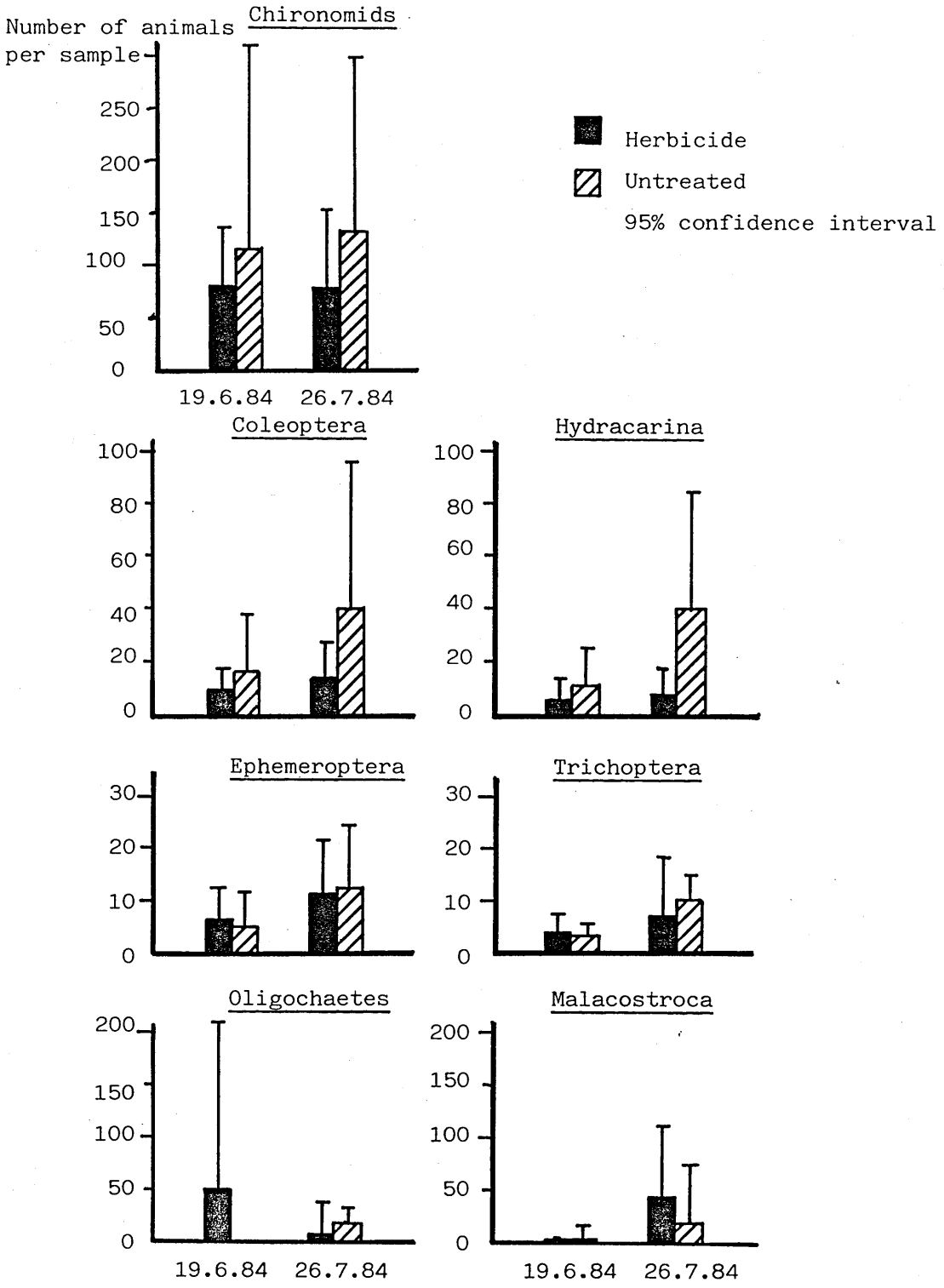


Table 11

Shannon-Weaver diversity indices calculated for the macroinvertebrate data from the river trials

		May	June	July
R.Petteril				
	Herbicide	0.7851	1.1649	1.3346
	Cut	0.9771	-	1.3763
	Untreated	0.5328	0.8022	0.7708
R.Windrush				
	Cut	1.8167	1.7973	
	Untreated	1.1206	1.8299	
R.Coln				
	Herbicide		1.5799	1.8122
	Untreated		1.5756	1.8815

Table 12

Macroinvertebrate taxa showing significant correlations between animal numbers and plant biomass

Correlation coefficients with:	Total plant biomass	Biomass of plants excluding algae
R.Petteril (d.f.=30)		
All taxa	0.466	
Chironomidae	0.387	
Simuliidae	0.395	0.463
Ephemeroptera	0.589	0.558
Coleoptera		0.403
Trichoptera		0.449
Hydracarina	0.626	0.737
R.Windrush (d.f.=11)		
Trichoptera	0.754	
R.Coln (d.f.=14)		
All taxa	0.547	0.535
Chironomidae	0.519	0.509
Coleoptera	0.585	0.574
Hydracarina	0.583	0.570

d.f. = degrees of freedom

4.6 Discussion of the River Trials

This discussion will be limited to comparisons between treatments and sampling times, within each site. A discussion of the comparisons which can be made between sites will be presented at the end of Chapter Five.

4.6.1 R.Petteril

a) Macrophytes

The observations made at the site on the R.Petteril throughout the summer of 1984, agreed well with the description of the site provided in the river classification of Holmes (1983).

The only major difference in the submerged flora was the presence of Zannichellia palustris, which was not included in the species list of the class B3iii. Zannichellia was not observed at the beginning of the summer, but developed later in the downstream herbicide section and in areas of the river upstream of the site. On visits to the site in 1985 and 1986, it was noticed that the distribution and size of beds, of Zannichellia had increased. The beds of Zannichellia were commonly established at the upstream ends of Ranunculus beds, and subsequently grew over much of them. This is an interesting interaction which might be worth further investigation.

The appearance of Zannichellia might be associated with an enrichment of the stream, which might also explain the dominance of the filamentous algae. Agricultural enrichment from fertilizers, might explain the high phosphorus concentrations in June and July. Cattle had access to the river along much of the site, causing considerable bank erosion and enriching the water with dung.

Untreated sections

The Ranunculus in the untreated sections (P8 + P10), flowered in May and June, but continued to increase in biomass and cover into the mid-summer. By September a considerable loss of biomass had occurred. The autumn wash-out in such a shallow stream, would be severe, with the increase in rain run-off from the hilly catchment.

Cutting treatment

Although there was little reduction in biomass before and after cutting, the weed growth was checked and did not show the great increases evident in the untreated sections. Had the cut been made

earlier in the season it is likely that a substantial regrowth would have occurred, presenting more of a problem later in the season (Ham et al. 1982). The specific cutting of fairly mature Ranunculus plants, which would have to compete with the well-established cover of Cladophora, was probably responsible for the limited regrowth.

Herbicide treatment

The herbicide effectively cleared a central channel in the stream, killing the Ranunculus plants back so completely, that the root stock did not regrow later in the season.

The effects of the herbicide were apparent much further downstream than the tadpoles of diquat-alginate are likely to have been carried. Most of the downstream effects must have been caused by the diquat released into solution. Although the residue data do not indicate how much longer than 40 minutes after application, the diquat would have continued to have been released from the treated sections, it is likely that the extremely high (albeit short-lasting) concentrations of herbicide, would have contributed to the downstream effects.

It is likely that a half-dose, of 0.5 litres Midstream per $100m^2$ of water surface, (recommended for water less than 30cm deep) would have been sufficient in the R.Petteril. Such a reduction in dosage would probably not reduce the effects on the plants to which the diquat-alginate was directly applied, but would reduce the severity of the downstream effects.

The availance (calculated from available data) in the downstream section was double that in the upstream section. It is not clear why the dispersal of diquat residues was different in the two sections: they did not significantly differ in water quality, or quantity of vegetation, and the flow rates were comparable. The upstream section was, however, narrower and deeper. A more comprehensive survey of water velocities and flow pattern might help to explain the variability of these results.

Although Cladophora is classed as being moderately susceptible to diquat-alginate, the herbicide had no detectable effect on the algae. This may have been because cleared areas could be rapidly recolonised by propagules carried downstream from upstream sources. More damage to the algae might have been expected, with such high concentrations of diquat. If there was little penetration by the

diquat, of the thick algal beds, then much of the herbicide would have been lost in the flowing water,to sediments and other sinks (Simsiman et al. 1976). In swift-flowing waters a balance must be sought between the concentration of the herbicide and the brief time that it spends in contact with the plants once released from the alginate. This availance will be influenced by any factors which enhance or reduce the activity of the herbicide.

In the moderately soft-water R.Petteril, with little suspended material and clean plant surfaces, the diquat may have had a relatively long persistance prior to adsorption onto plant or sediment surfaces. The rapid flow, combined with the apparently low uptake by the algae, may explain the rapidity and severity of the herbicide's action on susceptible species, and how the diquat remained active well downstream of the point of application.

b) Water chemistry

No changes in water chemistry were observed which could have been attributed to the weed management treatments. It is possible that short-term effects, such as a reduction in dissolved oxygen concentrations downstream of the plants decaying after the herbicide-kill, might have occurred between the survey dates. The rapid inflow of fresh water from upstream and the presence of such large quantities of healthy algae, would have diluted and minimised any changes.

c) Macroinvertebrate communities

Cutting treatment

The numbers, or changes in numbers, of the macroinvertebrates in the cut sections were never significantly different from those in the herbicide or untreated sections. The reductions in numbers of chironomids and Ephemeroptera in the cut sections, between May and July, were very similar to the changes which occurred in the herbicide treated sections. However, there was not a significant reduction in plant biomass in the cut sections over this period, indicating that a reduction in the densities of these animals on the vegetation had occurred.

There are two possible explanations for these observations:

1) The numbers of macroinvertebrates were closely correlated to the biomass of plants. The herbicide and untreated sections, in July,

had the extremes of plant biomass and, it would be expected, the extreme numbers of invertebrates. The plant biomass in the cut sections was intermediate between the other treatments and had not changed from the pre-treatment value. Thus, the numbers of macro-invertebrates would be expected not to change between May and July, and in July to be intermediate between the numbers found in the herbicide and untreated sections. This was clearly illustrated by the numbers of Hydracarina.

Based on these assumptions, the numbers of chironomids and Ephemeroptera in the post-cut samples, were lower than expected. This may have been the direct result of the physical disturbance caused by the cutting procedure, seven days earlier, from which these animals had not yet recovered. Also the vegetation remaining after the cut, mostly stems and roots, may not provide as hospitable an environment for the invertebrates, as the same biomass of whole plants provided, prior to the cut. This would be of particular importance to species which were dependent upon the Ranunculus leaves to provide shelter from predators and water currents.

2) Rather than the density of macroinvertebrates being lower than expected in the cut sections, in July, the density of animals on the herbicide treated plants may have been higher than expected. In the herbicide treated sections, the loss of plant biomass will have occurred over a longer period, than in the cut sections where plant removal was immediate. This might have allowed the macro-invertebrates time to migrate from the distal necrotic tissues to the bases of the plants, or the substrate surface, which was then sampled in July. Animals on the plants which were removed by cutting would have been immediately and completely lost from the system. There would have been no time for the animals on the removed tissue to have crowded onto the remaining stems.

The numbers of Ephemeroptera and chironomids were found to correlate with the total plant biomass so that these suggestions may explain the distribution of these animals, assuming that the trends in the data are real, although not statistically significant.

The low numbers of oligochaetes after the cutting treatment may have resulted from the physical disturbances caused by the cut itself, and by the sudden removal of the plants. Silt, which would have accumulated in the low water velocities, in the centres of the

weed beds, would have been rapidly removed on exposure to the water currents. Oligochaetes predominantly feeding on the detritus in these accumulations of silt, would also have been suddenly swept away.

Much of the silt in the herbicide treated weed beds would also have been lost but not as suddenly as in the cut sections. This would have allowed the worms time to migrate deeper into the benthos before the plants and silt were washed away. The July sample was taken 40 days after the herbicide treatment, allowing a longer recovery period, than the 7 days after the cut. The decaying plants in the herbicide sections, may also have provided a large food resource of detritus for oligochaetes.

Herbicide treatment

It was not possible from these data to distinguish whether any reductions in numbers of macroinvertebrates in the herbicide sections were due to the direct effects of diquat (e.g. toxicity), or were indirect effects resulting from the loss of macrophytes.

The three taxa which did show significant reductions in numbers, after the herbicide treatment, also showed significant correlations between the numbers of animals and the total plant biomass. Thus, it would seem likely that the reductions in numbers of chironomids, Ephemeroptera and Hydracarina in the herbicide sections occurred as a result of the loss of macrophyte habitat. After the treatment of a reservoir with paraquat, Brooker and Edwards (1974) observed significant decreases in the numbers of Chironomids and Trichoptera. They concluded that these population reductions were the result of the loss of the macrophyte habitat.

A lack of correlation between the numbers of an invertebrate taxa and the plant biomass does not necessarily indicate that the animals do not use the plants as a substrate. Seasonal population changes may mask the the correlation, or the population density on the plants may change. For example, a species with a particular preference for Ranunculus as a habitat (e.g. Brachycentrus subnubilus, Trichoptera) may increase in density on the plants remaining after weed control, if migration to other substrates was not favoured. Population changes,measured by numbers per unit of substrate area, might not be detected if changes in macroinvertebrate density occur.

The oligochaete worms were the only taxa to show an increase in numbers, between May and July, which was greater in the herbicide

treated sections, than in the untreated ones. This difference between the treatments was not significant but the oligochaetes, which were predominately detritus feeding genera (e.g. Tubifex), may have benefited from the increase in decaying plants in the herbicide sections. Similar observations were made after the treatment of a reservoir with a diquat and copper sulphate mixture (May et al. 1973).

Low species diversity indices were associated with high numbers of chironomids (e.g. in all the pre-treatment samples and the June and July samples from the untreated sections). This relationship was found because the numbers of taxa did not differ much between samples, and the chironomids were the most numerous taxa. The diversity of a sample is based upon the ratio of $\frac{\text{Number of individuals}}{\text{Number of taxa}}$.
The diversity indices were relatively high for the cut and herbicide treated samples because the chironomid numbers had been reduced by the removal of macrophytes.

4.6.2 R.Windrush

a) Macrophytes

The physical and flora characteristics of the site surveyed on the R.Windrush, matched the description of the river class A1vi (Holmes 1983) in which this part of the river occurred. The flora was species-rich with a diversity of fine-leaved species (e.g. Ranunculus penicillatus var. calcareus and Myriophyllum spicatum) and of broad-leaved species (e.g. Potamogeton lucens and P.perfoliatus). The predominance of P.pectinatus suggested that there was nutrient enrichment of the water, which was supported by the consistently high nitrate concentrations (with a range of 26-37mg l^{-1} nitrate).

Untreated sections

The Ranunculus flowered in late May to the end of June. The biomass and cover of Ranunculus declined after June. This reduction may have been due to the decline from maximum biomass that has been associated with the cessation of flowering (Dawson 1978). The progressive replacement of Ranunculus by P.pectinatus, which spread across the water surface, may have also contributed to its decline. The algal cover in July would have added to the shading of the Ranunculus. Algae caught in the stems of the macrophyte, may have

increased water resistance and would have resulted in greater stress and breakage of Ranunculus stems.

Cutting treatment

The cutting treatment in the R.Windrush was effective, with a significantly smaller biomass of vegetation in the cut sections, ten days after treatment, than in the untreated sections or in the pre-cut samples. The method of cutting ensured that the centre of the channel was efficiently cleared whilst leaving a fringe of submerged macrophytes along each edge. This pattern of clearance would allow an efficient discharge of water and would leave sufficient areas of submerged vegetation to provide a habitat for macroinvertebrates and fish.

By 45 days after cutting, the biomass of vegetation in the cut sections had significantly increased from the amount found 10 days after the cut. This increase in biomass was not due to the spread of submerged plants from uncut areas, but was seen to be the result of regrowth from cut stems. The macrophyte stems were not cut right back to the substrate but, depending upon the unevenness of the river bottom, stems of 20cm, or more, remained. In the top cut section (W7) most of the regrowth was of Ranunculus. This Ranunculus occurred on a shallow ridge across the river, so that the cut stems were well positioned to benefit from the increased exposure to light, after the removal of the overlying vegetation.

Potamogeton perfoliatus and P.pectinatus regrew most vigorously in the middle cut section (W5). In the downstream cut section (W2) a layer of Cladophora had taken advantage of the increased light penetration and covered most of the substrate. By 45 days after the cut, some of the P.perfoliatus and Myriophyllum beds, which had been less severely cut, were growing over the algal mat.

By October the vegetation in all sections had been reduced to a low biomass, and the wash-out had been most severe in the centre of the channel. This left the cover of vegetation in all sections looking like the post-cut sections.

The chain method of cutting is useful for clearing a central channel, but the cut material was removed with rakes, making the whole process highly labour intensive. It was the most suitable cutting method for small-scale trials, but high labour costs would make it an uneconomic proposition for rivers with great lengths to be managed.

Parts of the site on the R.Windrush would have been deep enough for a weed cutting boat, but there were shallow riffles (e.g. downstream end of section W7) over which a boat could not have passed. Under these circumstances, an effective herbicide, which could be used to selectively remove the vegetation from the centre of the channel, would be extremely useful.

Herbicide treatment

The macrophytes in the upstream herbicide treated section were seen to be just as healthy a month after treatment as they had been before the herbicide application. No symptoms of diquat's action (leaf loss or chlorosis) were identified in any species. Observations were more difficult in the turbid, deep water of the downstream section. Although a few Ranunculus stems, which had lost leaves, were seen at the water surface, the material collected from the rest of the water column appeared to be undamaged. The reductions in plant biomass after the herbicide application were not significant and the percentage of substrate covered by vegetation increased.

There was little evidence that the herbicide had had much effect in the R.Windrush. Diquat residues were only detected by 10 or 20 minutes after the start of the herbicide application. The maximum concentration of diquat detected was 0.66mg l^{-1} so that the objective concentration of 1.0mg l^{-1} may not have been achieved. More residue samples would have been needed to provide an accurate estimate of the availance of the diquat.

The lack of herbicidal activity in the R.Windrush may be attributed to several factors, which almost certainly had a combined antagonistic effect.

1) The calcium concentration of the river water, at the time of treatment, at 107mg l^{-1}, was fairly typical of a chalk stream (Westlake et al. 1972). In the laboratory experiments, the antagonism of calcium to the action of diquat was evident at a concentration of 100mg l^{-1} calcium. It is likely that the calcium ions in the R.Windrush water contributed to reducing the activity of the diquat-alginate.

2) Several of the macrophyte species found in the herbicide sections are recognised as being only moderately susceptible to diquat-alginate:

Susceptible	Moderately susceptible	Unknown
Ranunculus spp.	Potamogeton pectinatus	P.perfoliatus
Myriophyllum spicatum	P.lucens	Schoenoplectus lacustris
	Filamentous algae	Fontinalis antipyretica

3) At the time of the herbicide application, the water was extremely turbid, especially in the downstream herbicide section. No data were available for light transmission at this time, but in the downstream herbicide section, a metre rule could not be seen deeper than about 15cm from the water surface.

The origin of this turbidity, which decreased during the summer, was a matter of some dispute between the Thames Water Authority and some of the local anglers. The turbidity problem had, apparently, arisen only a few years prior to 1984, after the regular weed management in the R.Windrush was stopped.

It was suggested by the Water Authority that the turbidity was caused by a suspension of calcite (calcium carbonate). Greater quantities of calcite were being precipitated out than previously because the increased biomass of unmanaged plants was removing more carbon dioxide from the water during photosynthesis. The removal of carbon dioxide from the water raises the pH, which is one of the conditions that favours the precipitation of calcite (House et al. 1986a).

The capacity of the R.Windrush water to precipitate calcite, was examined in 1985. There was no evidence that the precipitation of calcite was particularly high; certainly no more than in the R.Frome which does not have a problem with calcite turbidity (House unpublished data).

The alternative suggestion was that the turbidity was caused by suspended clays. These clays had come from the river banks, on the outside of bends, that were being undercut. The problem had become worse in the summers since weed management had stopped because the water was no longer able to flow away down the cleared centre of the channel.

Some evidence to support this theory came from an X-ray analysis of residues filtered out from the water, at the Weed Research Organisation, Oxford, in September 1984. The results of this analysis showed high proportions of silicon and aluminium in the suspended solids. Silicon and aluminium are major components of many clays (W.R.O. unpublished data).

In fact the exact nature of the suspended materials is not particularly important because both calcite and clays will adsorb diquat.

The highly polar diquat cation is strongly adsorbed onto clays, many organic materials and soil colloids, which are predominantly negatively charged. A considerable amount of work has been carried out to study the differential adsorption capacities, for diquat, of different types of clays. This research has been focused on both the terrestrial (e.g. Weber et al. 1965) and the aquatic (e.g. Coats et al. 1966) uses of diquat. A review of this research is provided by Summers (1980).

The adsorption of diquat onto clays and other components of soil, is important because this property is responsible for the short persistence of the herbicide. In terrestrial environments this is desirable so that if diquat reaches the soil during a foliar application, it is immediately rendered biologically inactive and will not affect later seedling growth, which may be of a crop. In flowing water short persistence is very important in limiting the spread of the herbicide's effect. The adsorption of diquat onto organic and clay materials, and the fate of the herbicide in water is reviewed by Simsiman et al. (1976).

It has been suggested that clays could be used to remove diquat from potable water (Faust and Zarins 1969). This apparent advantage becomes a problem if the clays or sediments are suspended around, or cover, the target plants, reducing the concentrations of herbicide that are biologically active.

This problem of diquat adsorption has been discussed in relation to the suspended materials and aggregates of particulate matter on plant surfaces, found in many of the turbid inland waterways in Australia (Bowmer 1982a, 1982b). A method for estimating the additional amount of diquat that would be needed to saturate these adsorption sites is suggested, but increasing the dose rates of diquat would soon become uneconomic.

The high turbidity of the river water and the coating of sediments found on some plants, were likely to have contributed to reducing the efficacy of the diquat and the concentration of residues found in the water.

b) Water chemistry

No changes in the water chemistry, that could be related to the treatments, were identified. Any effects that a reduction in plant biomass might have (e.g. reduction in the photosynthetic production of dissolved oxygen) are unlikely to be detected over the short lengths of river which were cut. If a much greater length of river had been managed by cutting, any effects on the water chemistry would be reduced by the presence of the submerged vegetation remaining at the edges of the channel.

c) Macroinvertebrate communities

The variations within the treatments were so great that for most taxa no significant differences between the treatments or sampling times could be identified.

Some of the changes in numbers appear to be large, and in several cases; Coleoptera, Trichoptera, Hydracarina and Malacostraca, there was a decrease, between sampling times, in the numbers in the cut sections, whilst the numbers in the untreated sections increased. However, these differences were not significant, even if the percentage changes (greater than 100% in the untreated sections, less than 100% in the cut ones), were considered.

The only significant changes in numbers were detected for the Ephemeroptera and simuliids. The simuliid data were distorted by a single very high count of 236 in one sample, compared to less than 8 in all others.

Only the Trichoptera showed a significant correlation between animal numbers and plant biomass. It is possible that the reduction in numbers of these animals in the cut sections (seen when the numbers in the untreated sections increased) resulted from the loss of the macrophyte habitat. Reductions in numbers in the cut sections, of taxa not correlated to the plant biomass, may also have been due to the loss of habitat, or may have been a direct result of the disturbance caused by the cutting process, 10 days earlier.

The species diversity indices were similar for all but the June samples from the untreated sections. These were lower than the pre-cut samples because although they both had a high proportion of chironomids (which would tend to reduce the diversity) the pre-cut samples had a higher number of taxa.

4.6.3 R.Coln

a) Macrophytes

The characteristics of the experimental site chosen on the R.Coln, matches the river class A3ii description of a small, silted, enriched chalk stream (Holmes 1983), reasonably well.

Untreated sections

The dominant Ranunculus penicillatus var. calcareus flowered during May and June, and the biomass continued to increase, in the upstream section, until July. The biomass had decreased throughout the site by October.

Cutting treatment

The macrophytes in the cut and untreated sections were cut by the Estate Water Bailiff, whenever they were considered to impair fly-fishing. There was no evidence from observations of the site, and from the biomass and mapping data, that the distribution or amount of vegetation in the cut sections was different from that in the untreated areas.

Herbicide treatment

The lack of significance in the difference in macrophyte biomass and cover, between treatments and sampling times, in the R.Coln, was disappointing. This lack of significance was due to the large variations within treatments (i.e. between replicate sections).

The action of the herbicide was evident from the observations of the sections (Plate 3). In the downstream section, in particular, the large beds of Ranunculus which had reached the water surface in June, had been reduced to a layer of thick, old, silt and marl encrusted stems. These remains, lying close to the bottom, were not causing an obstruction to water flow or fishing. However, the presence of these stems did keep the cover scores high, in the vegetation mapping, so that there was little change in the % frequency of

Ranunculus after the herbicide treatment.

The remaining stems would also have been heavier than the flowering stems which had predominated prior to the herbicide application. The older stem at the base of the weed beds would be composed of stronger, denser tissues, with less aerenchyma, which could support the pulling stresses on the buoyant, flowering stems. The herbicide would have mostly removed the buoyant vegetation, leaving the heavier material, so that the reductions in biomass would not have been as great as expected, for the volume of tissue lost.

The R.Coln had a high calcium concentration at the time of the herbicide application, 118mg l^{-1}. The water was slightly turbid and the herbicide was applied late, at the end of June, to avoid the Mayfly hatch. All these factors would have been expected to reduce the efficacy of the herbicide.

If the herbicide had been applied earlier in the summer, before the Ranunculus had reached the water surface, it is likely that the reductions in plant biomass and cover would have been greater. It is possible that the diquat-alginate did not completely penetrate the weed beds, to reach the supporting stems, which were left behind.

The encrustations of silt and marl on the older stems may have adsorbed and inactivated any diquat which did reach the bottom of the weed beds, before it came into contact with plant tissue. Some of the older stems may have been buried in silt, which had accumulated within the weed beds. These stems would have only been exposed once the silt was washed away, following the removal of the younger Ranunculus stems.

The origin of the turbidity in the R.Coln was unknown. Some may have been due to calcite precipitation but there were suggestions, from the riparian owners, that a fish farm upstream might be releasing dirty water. There was no evidence to substantiate this claim, which would have been a matter for Thames Water Authority to investigate. The Water Authority data did show that the concentrations of suspended solids were greater downstream of Fairford, than upstream, so that it is possible that there may have been an input of material just upstream of the experimental site.

The maximum concentration of diquat residues sampled from the downstream section was 1.65mg l^{-1}, suggesting that a concentration of at least 1.0 mg l^{-1} might have been achieved throughout most of

the section, despite the turbidity.

The maximum diquat concentration in the upstream herbicide section, 0.71mg l^{-1} , was less than half of that in the downstream replicate. There was no apparent reason for this difference in maximum concentrations, and the consequent difference in availance. The upstream section had a lower pre-treatment biomass of plants, so that the uptake of diquat might have been expected to be less than in the downstream section. Diquat was detected over a longer period in the upstream section.

The detection of 0.21mg l^{-1} diquat, 60 minutes after the start of the herbicide application, in the downstream untreated section, was an interesting result. This observation supports the suggestion that the loss of macrophyte cover and lack of gain in biomass in the downstream untreated section, compared with the upstream one, may have been the result of diquat drift from the upstream herbicide section.

b) Water chemistry

The water chemistry did not appear to be affected by any of the weed control treatments.

c) Macroinvertebrate communities

The Hydracarina was the only taxon which showed a significant difference in numbers of animals between the two treatments, over the two sampling times. The Coleoptera and chironomids showed a similar pattern, with a larger increase in numbers in the untreated sections than in the herbicide treated ones. All three taxa showed a significant correlation between the numbers of animals and the plant biomass. The increase in numbers in the untreated sections may have resulted from the increase in biomass of plants, providing more of a suitable habitat for these taxa.

The numbers of Hydracarina, Coleoptera and chironomids in the herbicide treated sections did not change between the two sampling times, but the biomass of plants decreased. The density of animals on the remaining vegetation may have increased as a result of an immigration of animals from decaying tissues.

The oligochaete data was distorted by a single, very high, pre-treatment value of 200 worms, in one of the herbicide sections. None of the taxa showed reductions in the numbers of animals between sampling times in the herbicide sections. Species diversity increased in all treatments as numbers in taxa other than chironomids increased.

4.6.4 Mouse Water

Diquat-alginate appeared to be very effective on the Ranunculus occurring in both the herbicide treated sections, and for a considerable distance downstream. The Ranunculus showed typical symptoms of diquat toxicity within 6 days of treatment. Sparganium emersum and Potamogeton natans showed some symptoms of phytotoxicity, but loss of damaged tissues appeared to be small.

Neither of the quantitative assessments of the herbicide's activity showed significant differences between the herbicide and untreated sections. The macrophyte cover was similar, in both treatments, before and after the herbicide application. The plant biomass decreased with time in both treatments.

The reduction in plant biomass, but not cover, in the untreated sections may have occurred because the June biomass sample included a large proportion of submerged P.natans stems, which were not present in the July samples. Growth of these submerged stems may have been limited by shading from the floating leaves of P.natans or S.emersum. If the light penetrating the surface canopy was reduced sufficiently to limit photosynthesis of submerged leaves to less than the compensation level, then the biomass of these leaves might be reduced as they become moribund and are washed away.

The shading of the submerged vegetation would have been exacerbated by the brown colour and suspended silt loading of the water.

In some of the deeper, slower flowing areas of the river, where the cover of floating-leaved vegetation was often very high, the stream bottom was covered with a thick layer of silt. Organic enrichment of the Mouse Water had been reported by the Clyde River Purification Board in the past, and it was likely that these sediments had a high organic content and biological oxygen demand (B.O.D).

In July the dissolved oxygen concentrations in the untreated sections were less than 50% of saturation, and at night these values might be considerably lower. The high percentage cover of the water surface, by floating-leaved plants, would limit the exchange of oxygen in the atmosphere and in the water. The herbicide sections had several shallow riffles, free of floating-leaved vegetation, in which the water would have been aerated.

After the herbicide treatment, although the decaying Ranunculus

might have increased the B.O.D., the algae (which were predominant by this time) were still healthy and photosynthesising. The algae in the untreated sections were not very healthy, with a low proportion of green tissue, probably due to the low light availability. Decay of this algae, in the untreated sections, would have added to the B.O.D.

The major limitation of the Mouse Water as an experimental site was the difference in physical and floral characteristics between the upstream and downstream halves of the site. The experimental design segregated the treatments into these halves. This limited the interpretation of results because of the need to distinguish treatment effects from the upstream-downstream differences.

In view of the effects of the herbicide up to 400-500m downstream of the herbicide sections, an interspersed experimental design, with the treatment replicates separated into blocks, would only have been of value if the gap between the blocks had been very long.

The highly effective action of the herbicide, on the susceptible Ranunculus at least, resulted from the high concentrations of diquat, (up to 1.8mg l^{-1}) and its long persistence (over an hour),occurring in the treated sections.

The dosage of 1 litre Midstream per 100m^2 of water, was greater than necessary for the control of susceptible species, such as Ranunculus, but was not sufficient to have a major effect on moderately susceptible species, like P.natans. It is unlikely that an increase in dosage would have had much effect on the P.natans which had predominantly floating leaves at the time of treatment.

Some loss of leaves from submerged P.natans in the riffles,was observed after herbicide treatment. Thus, an earlier application of diquat-alginate onto predominantly submerged plants, before the floating-leaved canopy could develop, might increase the kill of P.natans and S.emersum.

Neither P.natans nor S.emersum have the densely packed, small-leaved morphologies shown by susceptible plants, such as Elodea and Ranunculus. These growth forms easily trap, and are penetrated by strings, or tadpoles, of diquat-alginate. Direct application of Midstream onto, or into, submerged beds of P.natans and S.emersum, might ensure a better distribution of the diquat-alginate in the weed beds, than can be obtained by a surface application.

4.7 Conclusions of the Individual River Trials

R.Petteril

1) The cutting treatment was highly specific to the Ranunculus beds and so had little effect of the total amount of vegetation, dominated by Cladophora, in the river. The cut was fairly late, in July, and there was little regrowth.

2) The diquat-alginate was very effective on the Ranunculus, with phytotoxic symptoms developing within eight days of treatment. The effects of the herbicide occurred a long way downstream of the application, and a half-dose rate would have been suitable in the shallow stream. Very high concentrations of diquat residues occurred briefly, (max = 4.64mg l^{-1}) and the total availance values were high.

3) The water chemistry and macroinvertebrate populations did not appear to be directly effected by the herbicide treatment.

4) Macrophyte removal by either method may have reduced the habitat available for some macroinvertebrates but the data were highly variable.

R.Windrush

5) The cut in June cleared a central channel in the river. Some regrowth was evident by July.

6) The herbicide appeared to have little effect. Low concentrations of diquat residues were recorded, resulting in low availance values. Reasons for this poor performance of the herbicidewere attibuted to:

   High calcium concentrations of the river water

   Presence of several plant species of limited susceptibility to diquat-alginate

   High turbidity of the water

7) There was no evidence that the water chemistry was affected by the management methods. Some taxa of macroinvertebrates may have been reduced by the removal of macrophyte habitats by cutting. Data were highly variable.

R.Coln

8) The method of cutting Ranunculus beds was so localised and specific that its effects could not be distinguished, by the the sampling method, from the changes in biomass occurring in the untreated sections.

9) Despite a late application, after the Mayfly hatch, at the end of June, the herbicide appeared to be effective in removing Ranunculus. The biomass data were highly variable so that the reductions in biomass caused by the herbicide, were not significant. Old, silt-covered stems were left on the river bottom and some of these showed a very small amount of regrowth by the end of the season.

10) Neither the water chemistry, nor the populations of macroinvertebrates appeared to be affected by the herbicide treatment.

Mouse Water

11) The diquat-alginate was very effective in removing beds of the susceptible Ranunculus. The availance values of the diquat residues were high, explaining the downstream effect of the herbicide treatment on Ranunculus plants. Other,less susceptible species, such as Potamogeton natans, Sparganium emersum and Cladophora, only showed very localised symptoms of herbicide damage.

12) The water chemistry did not appear to be affected by the herbicide treatment, but differences in the characteristics of the two halves of the site were probably more important. These differences within the site, limited the interpretation of the results.

CHAPTER FIVE

FIELD TRIALS OF WEED CUTTING AND THE USE OF DIQUAT-ALGINATE IN RIVERS

II. COMPARISON OF WEED CONTROL METHODS BETWEEN RIVERS

5.1 Introduction

In Chapter Four comparisons of the efficacies and ecological effects of the weed management regimes, were considered in each river. In this chapter, comparisons between rivers are discussed.

A direct comparison of the data from each site, using an analysis of variance is not possible because the sites were not replicated. No valid conclusions could be drawn about any differences between the sites if they were not replicated. The R.Coln and R.Windrush are similar in physical characteristics and might be considered to be replicates of a eutrophic, calcareous river type (Holmes 1983). However, there were differences in their macrophyte communities, and they were cut and treated with herbicide at different times in the season.

An alternative method, for making comparisons between sites, is the use of multivariate analyses.

5.2 Multivariate analyses: Ordination and classification techniques

The floristic data obtained from the mapping of the permanent transects, were simplified to produce a plant species x sample data matrix. This matrix was analysed using the ordination technique of detrended correspondence analysis (DCA), and the related classification procedure of two-way indicator species analysis. Both of these analyses were carried out on the Glasgow University main-frame computer, using the FORTRAN multivariate analysis programmes DECORANA and TWINSPAN (Hill 1979a, 1979b).

DCA analysis is a widely used eigenvector ordination procedure which is closely related to reciprocal-averaging (RA), but correcting the axis distortion problems (the arch effect and compression of axis ends) of the RA procedures (Gauch 1982). DCA compares favourably with principal components analysis and polar ordinations (Gauch et al. 1977) and is considered to be an improvement on RA (Gauch et al. 1981).

Once the ordination of the data has been produced, it is possible to correlate the distribution of the samples on the major axes, with environmental data collected with the samples (e.g. Wright et al. 1984).

TWINSPAN analysis provides anhierarchical divisive classification of the data matrix (Hill et al. 1975, Gauch 1982). TWINSPAN classifies both samples and species and constructs an ordered, two-way table which expresses succinctly the relationships of samples and species within the data set. TWINSPAN also identifies "indicator species" which differentiate the sample-groupings at each level of division. These indicators can be used, if required, to construct a key to the sample classification so that new samples can be classified without the need to reclassify all samples.

A comparison of TWINSPAN with several alternative hierarchical classifications (both agglomerative and divisive) was made by Gauch and Whittaker (1981), who concluded that TWINSPAN is usually the best general purpose classification procedure for ecological data. The procedure is gaining widespread acceptance for freshwater community ecological studies (Holmes 1983, Wright et al. 1984, Murphy et al. 1987).

5.3 Methods for the Multivariate Analysis of the River Sites

A single data matrix, of species x samples, can be used for both the DECORANA and the TWINSPAN analyses. The abundance of a species may be taken into account. In the classification technique species at different levels of abundance are considered as separate entities ("pseudospecies"). To prevent the generation of an unduly large number of pseudospecies, a limited set of abundance values (e.g. 1-3 point scale) is needed (Hill 1979b).

The %-frequency data from the permanent transect maps were used for these analyses. These samples included a greater diversity of plant species than the biomass samples, which had been deliberately biased in favour of Ranunculus spp.

To convert the %-frequency score onto a 1-3 point scale, three divisions of the %-frequency scores, of the whole data set, were tested and the one best representing a normal distribution was chosen.

Abundance Score	1	2	3
%-frequency class	0 - 1	1.1 - 10	10.1 - 100
Number of samples	71	180	185
%-frequency class	0 - 1	1.1 - 25	25.1 - 100
Number of samples	71	277	88
%-frequency class	0 - 5	5.1 - 25	25.1 - 100
Number of samples	194	154	88

The three point abundance score would distinguish between scarce (1), common (2), and abundant (3) species within a sample.

The total number of samples (436, one per section per sampling time), was considered to be too large for the analysis. With only twenty species, or groups, of plants identified, the analysis would be unbalanced with so many samples. The number of samples was reduced by using the mean score, per sampling time, of the treatment replicates. The mean scores for the two (or three in the case of the cut and untreated sections of the R.Windrush) replicates were determined as follows:

Mean of two scores

Replicate 1	Replicate 2	Mean score for treatment
0	1	1
0	2	1
0	3	2
1	1	1
1	2	Sum of %-frequency
		13 1
		13 2
1	3	2
2	2	2
2	3	Sum of %-frequency
		50 2
		50 3
3	3	3

Mean of three scores

Mean score of three Reps.			Mean score for treatment
	$\bar{x}$	1	1
1	$\bar{x}$	2.3	2
2.3	$\bar{x}$		3

The procedures involved in running the DECORANA and TWINSPAN programmes are described by Hill (1979a, 1979b). The downweighting option was used for both analyses. This means that rare speices are downweighted in proportion to their frequency within the data matrix, to avoid the tendency of individual samples with rare speices to distort the ordination (Hill 1979a).

The eigenvalues are a representation of the total variance accounted for by each axis. As is usual for this procedure, the eigen values were higher for the first two DCA axes of the ordination, than for Axes 3 and 4.

Axis	1	2	3	4
Eigenvalues	0.525	0.189	0.083	0.052

This difference between the pairs of axes, indicating that Axes 1 and 2 account for most of the variance in the ordination, justifies the use of the DCA axes 1 and 2 as the main ordination framework (Hill 1979a).

As well as plotting the ordination on various combinations of axes, the DECORANA programme produces a list of samples, or species,

with their co-ordinates on the four axes. These axis units are standard deviations of species turnover (Gauch 1982), a measure of the natural length of the gradient expressed by each axis. A short axis length would indicate a degree of homogeneity of the data set.

Other parameters, such as environmental data for each sample (in this case the mean values from the treatment replicates per sampling time), can be correlated with the ordinates for each axis. From these correlations some of the environmental influences which may have determined the species distribution between samples, may be identified. This correlation of individual samples with environmental data, is different from the types of analysis carried out by Wright et al. (1984), in which environmental data were correlated with sample classes.

The DECORANA programme does provide a species ordination, but only the sample ordination will be discussed because it is the comparison of samples that is of interest.

In addition to downweighting, an option was used on the TWINSPAN programme which permitted pseudospecies to be identified as indicators.

5.4 Results of the Multivariate Analyses of the River Sites

The sample ordination on axes 1 and 2, produced by the DECORANA programme, is illustrated in Fig. 56a. The rivers from which the samples came have been indicated and it is evident that the ordination on the first two axes, has separated the samples on the floristic composition of each river. For comparison, the ordination on axes 3 and 4 has been illustrated in Fig. 56b. The river samples are less discrete.

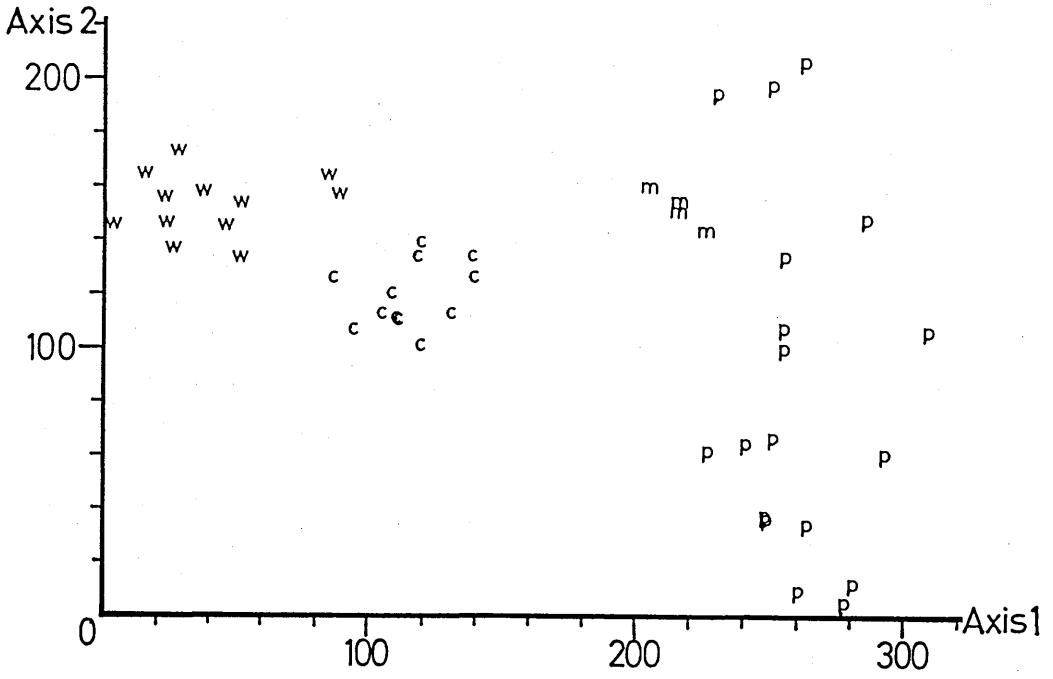
The TWINSPAN analysis has been expressed as a dendrogram in Fig. 57. The hierarchical sub-divisions of the data set to level three are shown, with the indicator or preferential pseudospecies.

The species x sample data, as expressed in an ordered two-way table are presented in Fig. 58. The values in the table are the species abundance scores.

The ordination on the first two axes is shown again in Fig. 59 with the level 2 TWINSPAN classes superimposed, A-D. Polygons to define the classes have been drawn, with the level 3 sub-divisions.

DECORANA ordination of the vegetation data from the river samples

Fig. 56a Samples plotted on Axes 1 and 2



p = R.Petteril w = R.Windrush c = R.Coln m = Mouse Water

Fig. 56b Samples plotted on Axes 3 and 4

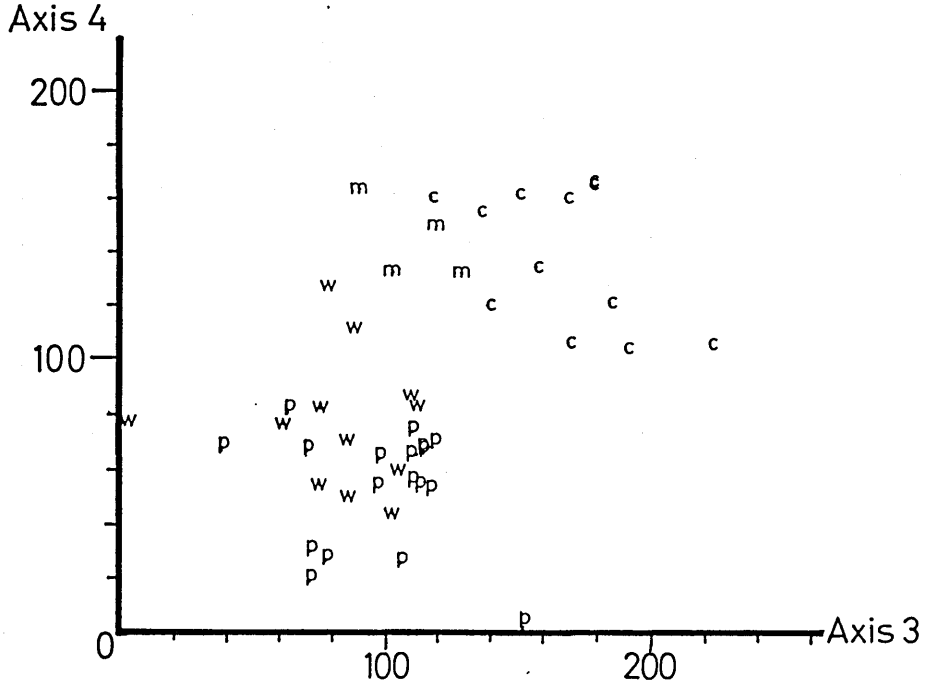


Fig. 57

Dendrogram illustrating the TWINSPAN classification

Key to species in Fig. 58
RANS 1 = Indicator pseudospecies
Rans 1 = preferential pseedospecies

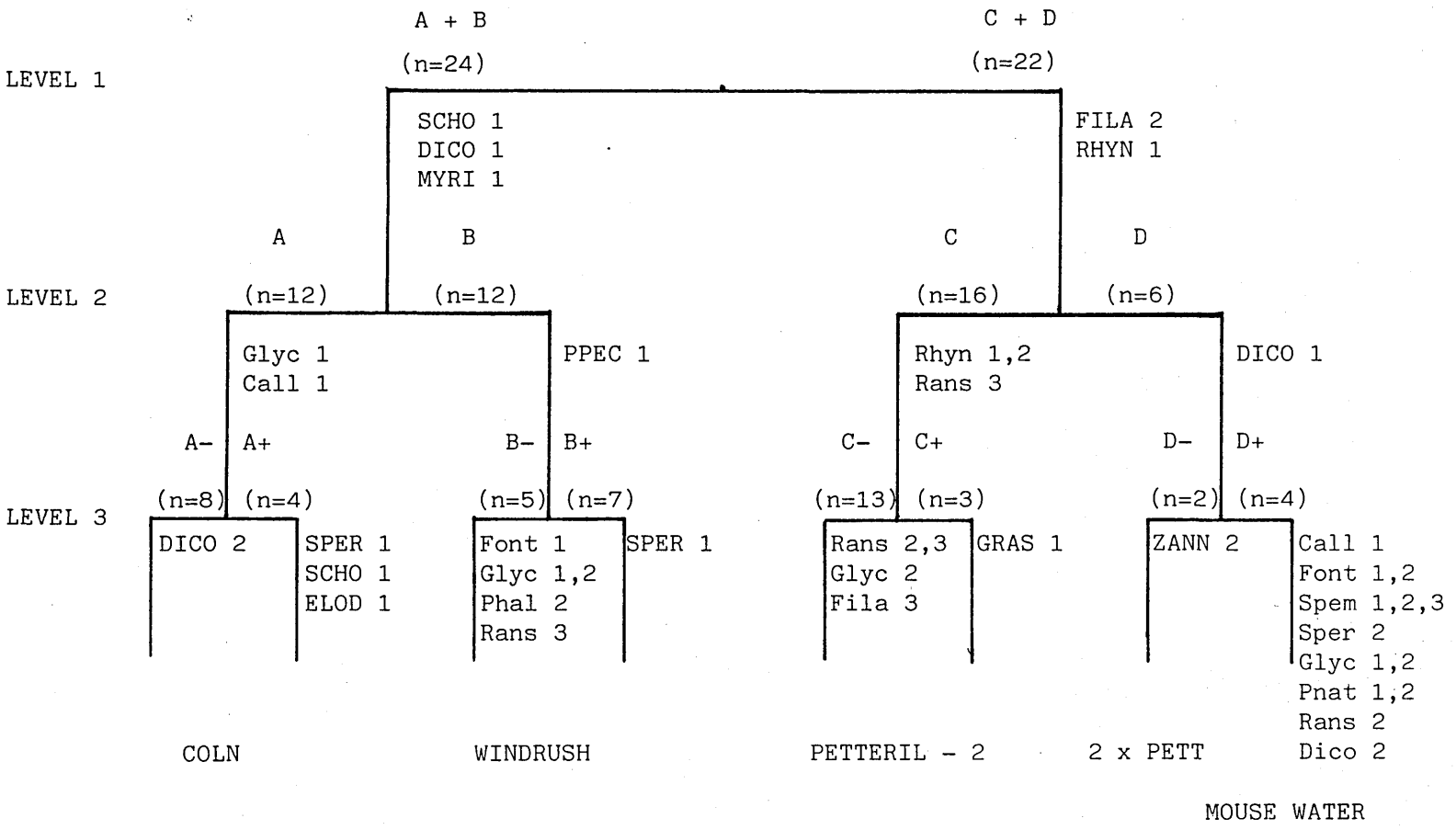
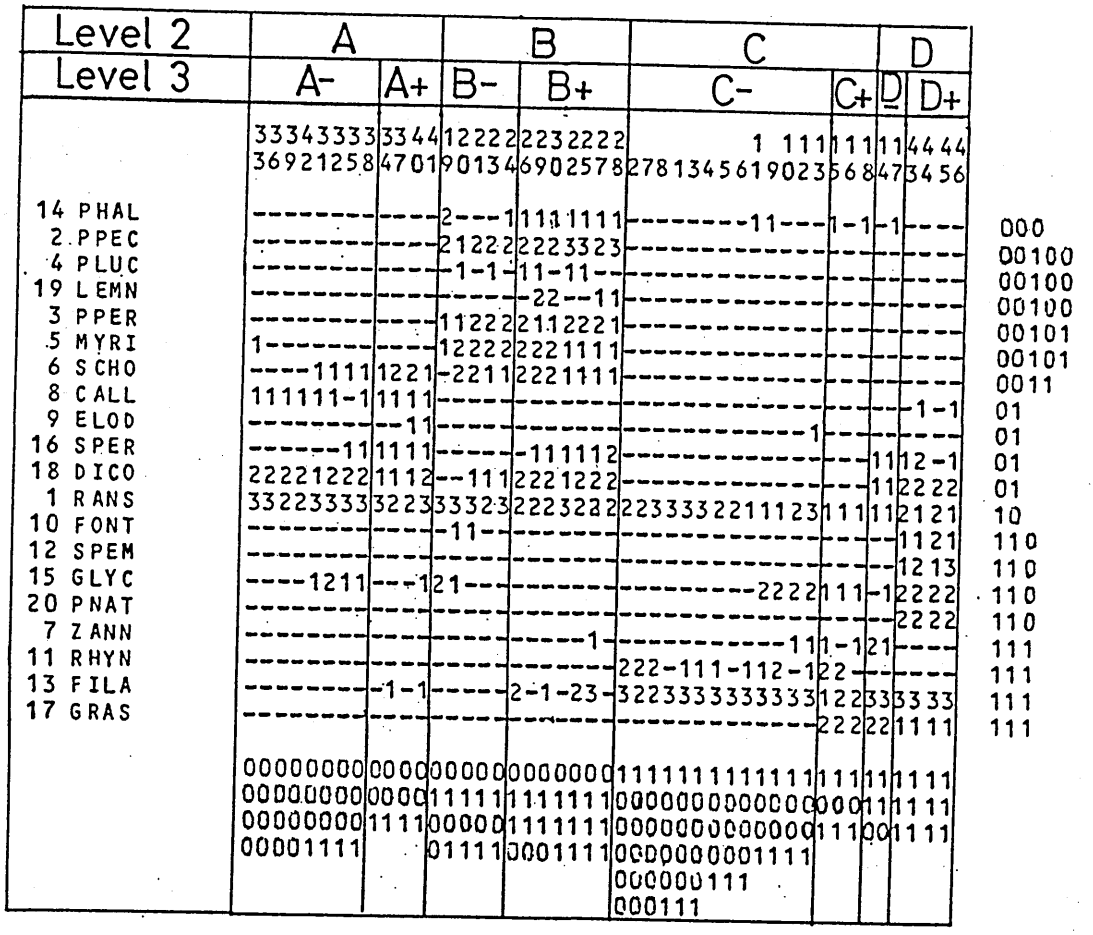


Fig. 58

Results of the TWINSPAN analysis expressed in an ordered two-way table, with the divisions of levels 2 and 3 indicated



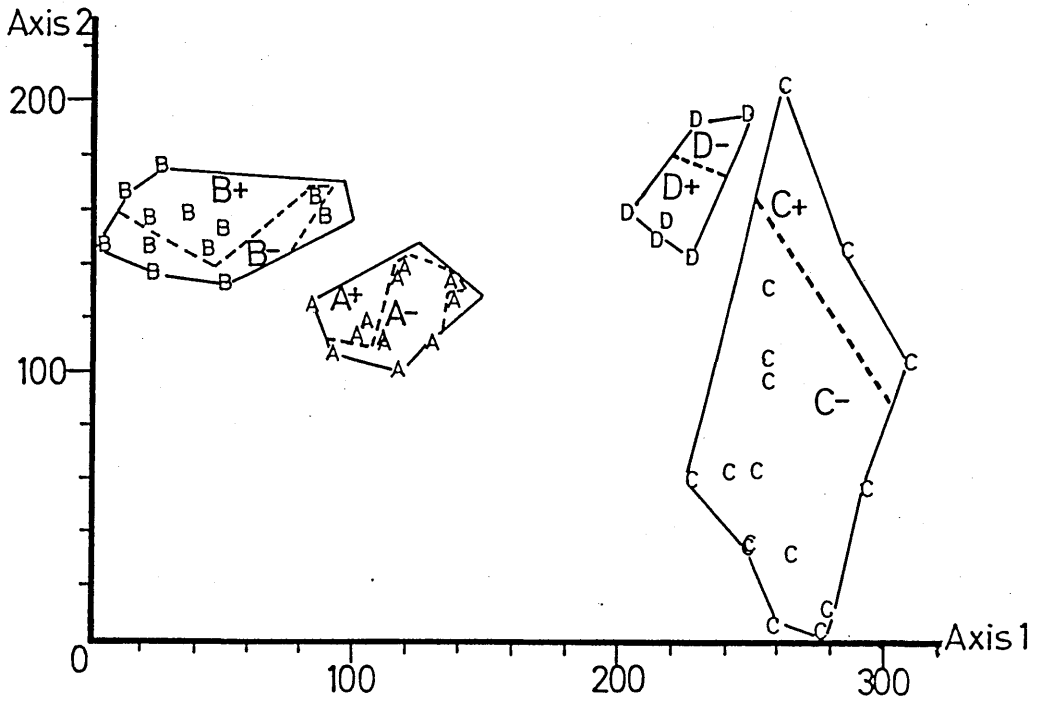
PHAL	Phalaris arundinacea
PPEC	Potamogeton pectinatus
PLUC	P. lucens
LEMN	Lemna spp.
PPER	Potamogeton perfoliatus
MYRI	Myriophyllum spicatum
SCHO	Schoenplectus lacustris
CALL	Callitriche spp.
ELOD	Elodea canadensis
SPER	Sparganium erectum
DICO	Dicotoledon emergents
RANS	Ranunculus spp.
FONT	Fontinalis antipyretica
SPEM	Sparganium emersum
GLYC	Glyceria maxima
PNAT	Potamogeton natans
ZANN	Zannichellia palustris
RHYN	Rhynchostegium riparioides
FILA	Filamentous algae
GRAS	Emergent grasses

ORDER OF SAMPLES

33 COLN	1U:	36 COLN	2U:	39 COLN	3U			
34 COLN	2H:	37 COLN	3H:	40 COLN	4H			
24 WIND	2U:	26 WIND	3C:	29 WIND	4C			
2 PETT	1A:	7 PETT	2A:	8 PETT	2U			
11 PETT	4B:	9 PETT	4H:	10 PETT	4A			
14 PETT	5H:	17 PETT	5C:	43 MOUS	1H:			
42 COLN	4U	31 COLN	1H:	32 COLN	1C			
41 COLN	4C	19 WIND	1H:	20 WIND	1C			
30 WIND	4U	22 WIND	2H:	25 WIND	3H			
1 PETT	1H	3 PETT	1B:	4 PETT	1C			
12 PETT	4C	13 PETT	4U:	15 PETT	5A			
44 MOUS	1U	45 MOUS	2H:	46 MOUS	2U			
		35 COLN	2C:	38 COLN	3C			
		21 WIND	1U:	23 WIND	2C			
		27 WIND	3U:	28 WIND	4H			
		5 PETT	1U:	6 PETT	2H			
		16 PETT	5B:	18 PETT	5U			

Fig. 59

DECORANA ordination of vegetation samples from the rivers with the TWINSPAN classes at Level 2 superimposed



A B C D Level 2 TWINSPAN classes

A- A+ B- B+ C- C+ D- D+ Level 3 TWINSPAN classes

This combination of the results of the ordination and classification analyses is useful in demonstrating the relationships between the classes produced by TWINSPAN (e.g. Daniels 1978, Wright et al. 1984).

In these analyses both methods neatly divide up the samples according to their rivers of origin; the only exception being that at level 2 of the classification, two of the R.Petteril samples have been grouped with the Mouse Water samples.

In Fig. 60a & b two factors, which could not be quantified for correlation with the axes ordinates, have been illustrated on the ordination, the management regime and the sampling time respectively. Neither of these factors show gradients or patterns over all of the data set, but within the sample groups for each river, some trends are apparent, particularly in the time of sampling.

A variety of environmental data was correlated with the axes ordinates, Table 13. An example of the distribution of one of these environmental variables (nitrate), on the ordination is given in Fig. 61.

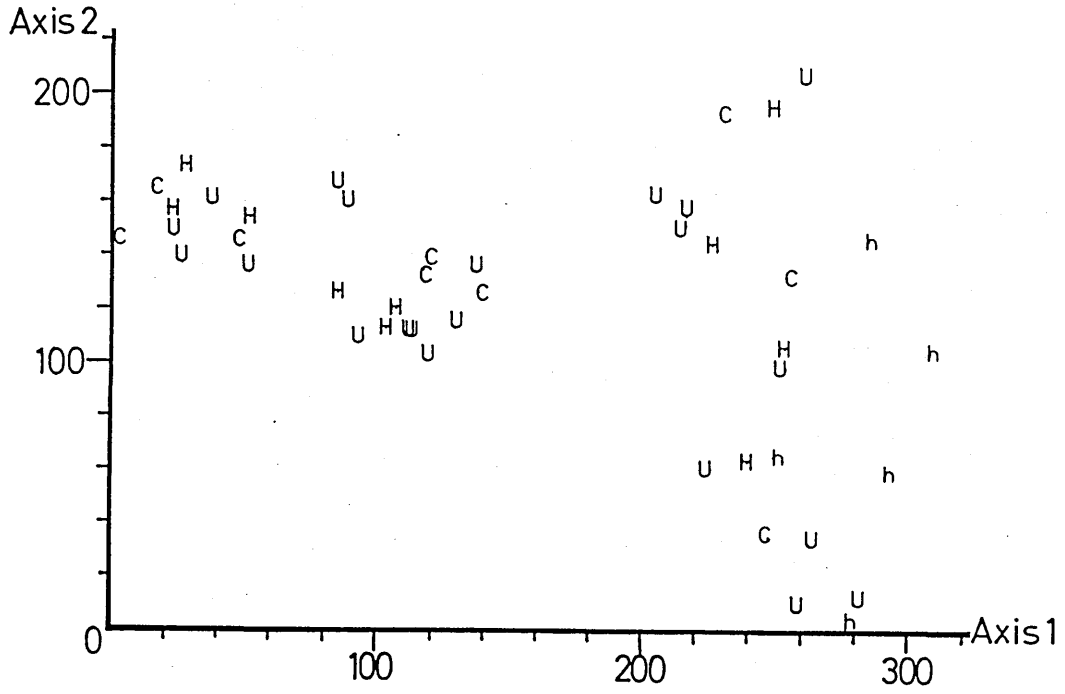
Nitrate and calcium concentrations, and depth were very highly correlated to Axis 1. Dissolved oxygen, conductivity and calcium concentration were very highly correlated to Axis 2. Only dissolved oxygen and pH were correlated to Axis 3, but Axis 4 was very highly correlated to pH, total water hardness and phophorus content.

Some of these relationships were intrinsic in the choice of sites, (e.g. water hardness and calcium concentration). The correlation of more than one factor to an axis may be coincidental (e.g. water depth and calcium concentration), but other factors may tend to be related in British rivers (e.g. chalk streams rich in calcium flowing through agricultural land with nitrate fertilizer run-off). Some of the environmental factors have been correlated, for the whole data set, to indicate which factors may be related, or are coincidentally correlated to the same ordination axes (Table 14).

Two quantitative aspects of the flora have been correlated with the axes. The percentage of emergents out of the total %-frequency data, and the percentage ratio of emergents:submerged aquatic plants, were calculated in an attempt to distinguish the early and late plant communities. The proportion of the channels covered by emergents increased throughout the summer (see vegetation maps, Appendix 5).

Fig. 60a

DECORANA ordination with the management regime applied to each sample indicated



H Direct herbicide treatment

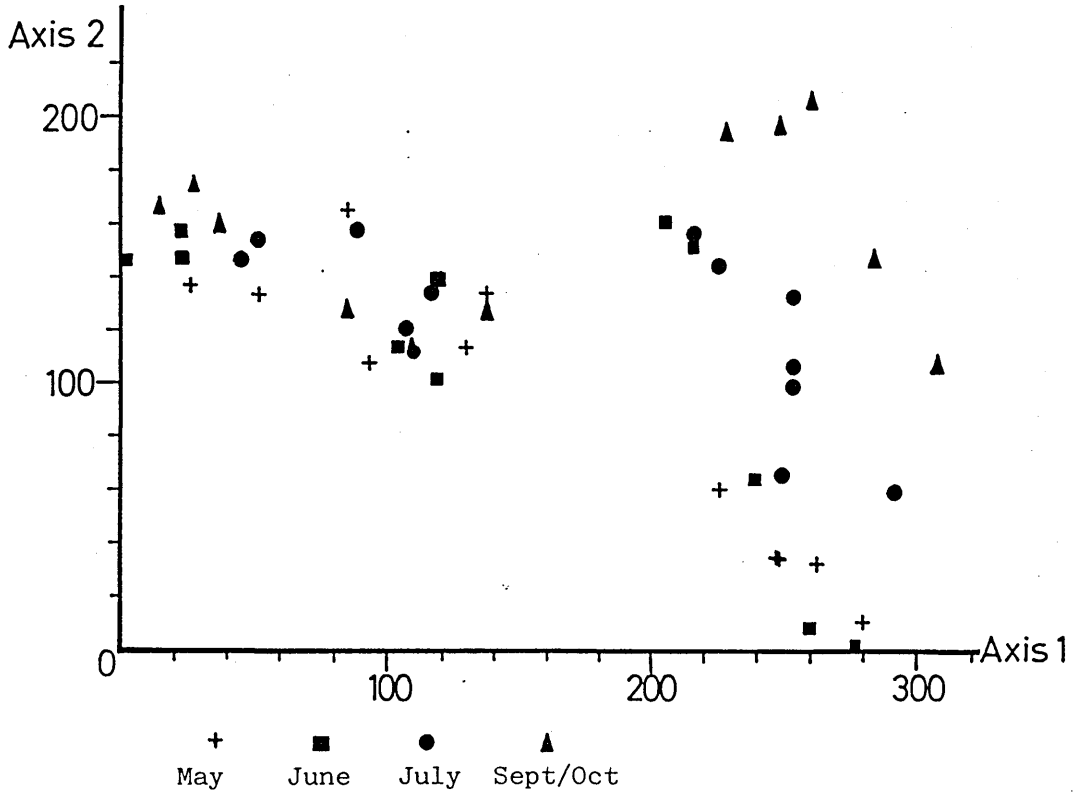
h Herbicide drift

C Cut

U Untreated

Fig. 60b

DECORANA ordination with the month of sampling indicated



+ May

■ June

● July

▲ Sept/Oct

Table 13

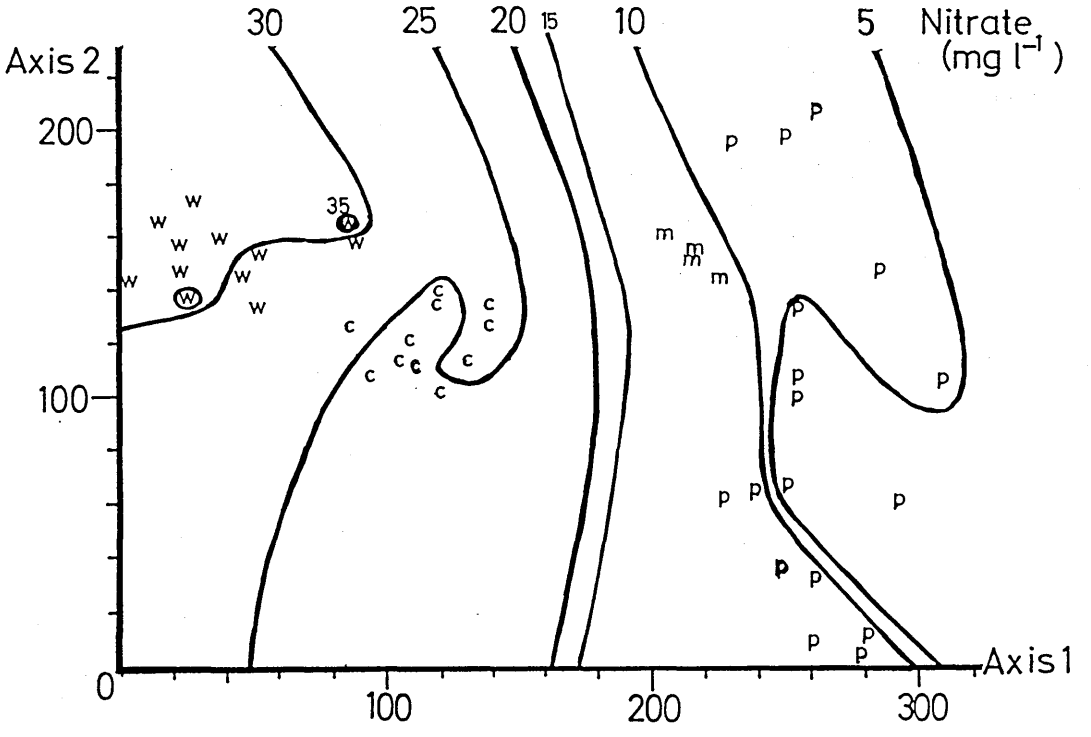
Correlation coefficients for the ordination axes and various environmental variables

	Axis 1	Axis 2	Axis 3	Axis 4	(d.f.)
Depth	0.827 ***	0.460 **	0.015	0.432 **	44
Surface water velocity	0.119	0.198	0.055	0.089	44
Mean water velocity	0.215	0.204	0.104	0.179	44
Dissolved oxygen	0.454 **	0.576 ***	0.413 **	0.261	44
pH	0.312 *	0.090	0.514 ***	0.495 ***	44
Calcium ion conc.	0.878 ***	0.762 ***	0.119	0.122	38
Total water hardness	0.304	0.393 **	0.154	0.634 ***	32
Conductivity	0.156	0.579 ***	0.164	0.292	38
Nitrate ion conc.	0.950 ***	0.327 *	0.182	0.310 *	38
Reactive phosphorus	0.285	0.378 *	0.247	0.528 ***	38
Emergent/submerged %-frequency	0.162	0.565 ***	0.036	0.125	44
%-frequency of emergents	0.161	0.631 ***	0.079	0.036	44
%-frequency bare substrate	0.041	0.108	0.336 *	0.034	44
Diversity indices of macroinvertebrates	0.618 *	0.0491	0.170	0.567 *	14

d.f.= degrees of freedom. *** = significant at 0.1%
** = " at 1.0%
* = " at 5.0%

Fig. 61

DECORANA ordination of the river vegetation samples with isopleths for different nitrate concentrations



p = R.Petteril w = R.Windrush c = R.Coln m = Mouse Water

Table 14

Examples of some of the environmental variables which were correlated for all the data from the river trials

Water depth	pH	-0.407	**
"	Dissolved oxygen	-0.448	**
"	Mean water velocity	0.110	
Conductivity	Total water hardness	0.841	***
"	Calcium ion conc.	0.228	
"	Reactive phosphorus	-0.526	**
"	Nitrate ion conc.	0.044	
Calcium ion conc.	Nitrate ion conc.	0.836	***
"	Reactive phosphorus	-0.430	**
"	Total water hardness	0.503	***
Nitrate ion conc.	Reactive phosphorus	-0.184	
Dissolved oxygen	pH	0.556	***

These factors were intended as a quantitative illustration of the sampling time. The very high correlations of these factors with Axis 2 matches the vertical separation of the sampling times for the R.Petteril and R.Windrush (Fig. 60b).

The significance of the correlations of macroinvertebrate diversity indices with the ordination axes, was limited by the few values available for the invertebrate data.

5.5 Discussion of the Use of Multivariate Analyses on the Results of the River Trials

For these field trials multivariate analyses of the data were of limited value. This was because the differences in the floristic composition of the samples from different sites, was much greater than the differences between times or treatments within sites.

The fact that cut or herbicide treated samples were not clearly distinguished from the others, at least showed that the management regimes had not drastically altered the river floras. For example, if an extremely efficient and destructive clearance method was used (e.g. dredging, acrolein), then the treated samples from a variety of sites would be similarly depleted of plants, and would be grouped together in the ordination.

Although these results indicated that the cut and herbicide treatments did not alter the floral compositions of the rivers sufficiently to be noticeable on the ordination or classification, it must be noted that these analyses were based upon simplified abundance scores. Major changes in the river flora would have been needed to distinguish between pre- and post-treatment samples, within a site.

An important point that these techniques have shown, is that the floristic compositions of the rivers in terms of the species present, were not obviously altered by the management regimes, at least in the short-term.

The value of these analytical techniques may be greatest in the long-term assessment of sites, particularly those subject to repeated management regimes, (e.g. Wade 1981). Gradual changes in flora over time, or between similar sites under different management regimes, might be demonstrated well by these techniques. Similarly, the re-establishment of native plant communities could be objectively analysed in sites in which a dominant exotic had been controlled.

Separate ordinations could be carried out for each site, if enough data is available, possibly using the sub-samples of each section. These ordinations might distinguish between treatments within a site, more clearly, but there would be little point in such analyses, when the data have already been analysed quantitatively.

The use of DCA ordination and TWINSPAN classification on samples taken from vegetation subject to different management regimes in the Union Canal, has been discussed by Murphy et al. (1987). This work will be discussed in more detail in Chapter Six. The analyses showed that even samples taken from similar habitats were not clearly distinguished by their management regimes.

The correlation of environmental data with the sample ordination did not provide information relating to the management regimes applied to any samples. These correlations indicate which environmental factors may be involved in determining the floral composition of the samples. Since the rivers of origin of the samples, were well distinguished by Axis 1, environmental variables strongly correlated to that axis are likely to differ between rivers and may be involved in determining the floral composition of the sites.

Whenever correlations are considered it is important that causal relationships between two correlated factors are not implied, unless some justification is produced.

5.6 Discussion of the River Trials: Comparisons between sites

5.6.1 Comparison of untreated sections

The DECORANA and TWINSPAN analyses have illustrated the degree of dissimilarity between the sites. The correlations between the environmental data and the sample ordination would provide a basis for explaining the floristic differences between the sites. Such an exercise would be interesting but is not necessary for this discussion especially since the process has been carried out quite adequately for three of the sites by Holmes (1983).

One comparison of the untreated sections which has relevance to the application of weed control methods, is the timing of maximum plant biomass. The available information is limited by the timing and frequency of site visits.

Maximum plant biomass in the R.Windrush was recorded in the first visit in May, whilst the Ranunculus was still flowering. In the R.Coln and the R.Petteril the maximum biomass was recorded in July, about a month after the Ranunculus had been flowering. A similar relationship, of a month's delay, between the time of peak flowering and maximum biomass,was observed by Dawson (1976), and was principally due to the loss of the weak, buoyant flowering stems.

The early maximum biomass in the R.Windrush may have been possible if there had been a large overwinter inoculum of plants remaining from the previous autumn (Westlake and Dawson 1986). The overwinter water discharges in chalk streams do not tend to show the high extremes found in rivers which are supplied by direct run-off from resistant rocks. If the growing conditions were favourable early in the spring, the plant biomass in the R.Windrush could increase very quickly if a large inoculum of vegetation had not been washed-out during the winter.

The decline in biomass in the R.Windrush after May could have resulted from loss of the weak flowering stems of Ranunculus, and from the competitive shading of aggressive vegetation, such as Potamogeton pectinatus growing across the water surface. A similar decline from a peak biomass at the first sampling time in the Mouse Water, was also likely to be the result of competitive shading from floating-leaved plants (e.g. P.natans). Self shading of Ranunculus in the R.Coln and R.Petteril was likely eventually but there were no other aggressive

species growing at the water surface in these rivers.

A knowledge of the timing of maximal plant biomass in rivers is important in assessing the correct time to apply weed control methods, both in terms of pre-empting maximum flooding risks and in order to achieve greatest efficiency in removing plants and preventing regrowth.

5.6.2 Comparison of the cutting treatments

Of the three cutting treatments, the only site in which a significant regrowth of vegetation after cutting was observed was in the R.Windrush. This was the earliest of the cutting treatments and the removal of surface vegetation removed the shading limitation which had prevented further growth in the untreated sections. According to these results, the regrowth in the cut sections was not sufficient to equal or exeed the biomass of macrophytes in the untreated sections, but this might have occurred in August or September, when no samples were collected. If the cut had been made earlier in the season, it is likely that the regrowth would have been sufficient to reach the pre-cut biomass.

If the timing of the first cut in the R.Windrush had been influenced by the same factors as are used in the R.Frome (e.g. the estimation of flood risk and avoidance of the Mayfly hatch) (Westlake and Dawson 1982), the cut might have been made in May rather than mid June. Regrowth from a cut that early might well have been sufficient to impose the same flood risk and require a second cut. In fact if the biomass in May was not causing a major flooding risk, a cut would have been unnecessary because the biomass decreased afterwards.

It is not surprising that there was no regrowth seen in the R.Petteril in the post-cut sample because it was collected only seven days after the cutting treatment. Biomass samples should have been collected in August or early September to have allowed a better assessment of regrowth.

The cutting procedures in the R.Coln had little effect on the overall biomass of vegetation, and it was not possible to judge whether Ranunculus beds which had been cut showed a significant regrowth. The specific cutting of weed beds creating a nuisance to fly-fishing was suitable in a short length of river which did not have a flooding risk, but the highly labour intensive method would be

uneconomic in larger areas of river.

The weed cut in the R.Windrush was the only one not to be species specific. The removal of Ranunculus from the R.Petteril had little effect on the overall amount of vegetation because of the large quantity of filamentous algae remaining. The ecological effects of the non-specific removal of vegetation from the R.Windrush would have been minimised, however, by the presence of the plants left uncut at the edge of the channel.

5.6.3 Comparison of the herbicide treatments

The diquat-alginate was most effective in removing Ranunculus in the R.Petteril and the Mouse Water. In both of these streams lower dose rates of herbicide could have been used, which might have reduced the downstream effects, probably caused by the high concentrations of the diquat residues.

The availance values of the diquat residues in these rivers, (estimated over 40 minutes) were 2-4 times greater than those in the R.Coln and 4-7 times greater than in the R.Windrush. The concentration of calcium in the R.Petteril at the time of treatment 62mg l^{-1}, was the lowest of all the four rivers. The calcium content of the Mouse Water, 81mg l^{-1}, was higher than anticipated, but reflects the limestone origin of the water source. The total water hardness and conductivity of this river were the highest of all the rivers, suggesting the presence of high concentrations of ions, such as magnesium or sodium, which did not appear to have much effect in reducing the activity of the herbicide.

The herbicide did not appear to have any effect in reducing the quantity of macrophytes in the R.Windrush. An immediate assumption might be that the high calcium concentration of the water, 107mg l^{-1} at the time of treatment, caused the poor performance of the herbicide. A significant reduction in the damage score (4 d.a.t.) was seen in cultures with 100 mg l^{-1} compared to 50mg l^{-1} calcium (Fig. 11).

However, the calcium concentration in the R.Coln, at the time of the herbicide application, was even higher than in the R.Windrush, at 120mg l^{-1}, and the diquat-alginate appeared to be effective there.

It is likely that a combination of other factors, in addition to the high calcium concentration, were responsible for the failure of the herbicide in the R.Windrush, some of which were discussed in Chapter Four.

Factors which may have contributed to the poor performance of diquat-alginate in the R.Windrush:

1) The high turbidity of the water, resulting in the adsorption and inactivation of diquat residues in solution. Layers of marl and sediments covering macrophytes and forming a barrier inactivating diquat on the plant surfaces prior to uptake into the tissues.

2) The R.Windrush, especially the downstream sections, was the deepest of the rivers treated.

Deep water does not affect the efficacy of diquat-alginate in static water because the strings of gel fall through the water column to the vegetation at the bottom. The overall concentration of diquat in solution will be less in deeper water, but this will not be important if the diquat-alginate is well distributed over the target plants, (Clayton and Tanner 1983).

In flowing water the depth is more important because the tadpoles of diquat-alginate will be carried further downstream, the greater the vertical distance between the water surface and the vegetation. Thus, for a given water velocity, one might expect a greater downstream displacement of the herbicide's effects, from the point of application, in deeper rivers.

In turbid rivers the water depth will be important because the greater the distance the herbicide has to travel from the water surface to the vegetation, the greater the risk that the diquat will be adsorbed and inactivated on the suspended material, and so the effective concentration of the herbicide would be reduced (e.g. Bowmer 1982a). The formulation of diquat in the alginate carrier should reduce this problem of the interception of diquat in the water column, because the blob of alginate would shield the diquat.

However, in turbid waters the surfaces of many of the plants will be covered in sediments which would have settled out of suspension, in the low water velocities in the macrophyte beds. The diquat might be inactivated before reaching the plant tissues, by this layer of sediments sandwiched between the alginate and macrophyte surface.

This problem will be particularly acute if the plant surfaces are covered with marl, which commonly occurs in calcareous waters. The adsorption of diquat from solution by suspended material will reduce the concentration of residues detected in the water. This might explain the low diquat concentrations detected in the R.Windrush.

3) Only 100m lengths of river were treated in the R.Windrush. Any edge effects, such as the downstream displacement of the effects of the herbicide, will be proportionally greater than in a large plot.

A shorter treated length would also result (for a given water velocity) in a shorter period during which the passage of the pulse of diquat residues, from upstream of any point, would occur over that point. In the conditions of the R.Windrush where the residues would have a short persistence time, the difference in diquat exposure period that would be caused between 100m and 200m sections, would be small. The availance of the herbicide might be significantly increased if residues from several kilometres of treated river were passing downstream over an area of river. This subject will be discussed further in Section 7.7.2.

4) The presence, and increasing dominance throughout the summer, of plant species which are only moderately susceptible to diquat-alginate, will have reduced the efficacy of the herbicide over all vegetation.

5) Another environmental factor which was exceptional in the R.Windrush compared to the other sites, was the high concentration of nitrate. At present, there is no experimental evidence that nitrate concentrations are related to a poor performance of diquat-alginate.

In field trials Midstream has occasionally failed to control susceptible species under apparently ideal conditions. A common factor in these cases was that the water was fairly enriched (Barrett, Spencer-Jones pers. comm.) It is possible that the concentrations of nutrients may not directly affect the herbicide's activity, but under these conditions the plants may be coated with a layer of bacteria, or microscopic epiphytes (e.g. Bowmer 1982b). This layer, like the marl and sediment coatings, would provide an adsorptive barrier to the diquat, between the alginate and the epidermis of the plant.

The activity of the herbicide in the R.Coln was surprising, after the failure in the R.Windrush. The calcium concentration was higher in the R.Coln, the Midstream was applied late, to large mature weed beds, and there was some turbidity in the water.
The maximum diquat residue concentration from the downstream section

was similar to that found in the Mouse Water, but the period of persistence, and hence the availance, was less. The residues detected in the upstream section were similar to those from the R.Windrush.

Just as the failure of the herbicide in the R.Windrush might be attributed to a combination of unfavourable environmental factors, so the successful weed control in the R.Coln might have resulted from a combination of slightly more favourable conditions:

1) The turbidity at time of treatment was not as severe in the R.Coln, the bottom was always visible in the deepest areas.

2) The R.Coln was shallower than the R.Windrush.

3) Sections of 200m were treated.

4) Susceptible Ranunculus plants dominated.

5) Nitrate (and phosphorus) concentrations were lower at the time of treatment in the R.Coln than in the R.Windrush.

6) The water temperature at time of treatment was 4°C higher in the R.Coln than in the R.Windrush.

Plant species susceptibility

In both the R.Petteril and the Mouse Water the severe effects of the herbicide on the Ranunculus did not appear to destabilise the stream habitat, because the proportion of Ranunculus, compared to less susceptible species, was low. If a total clearance of vegetation had been the objective of the management, in either river, the herbicide could only have been considered partially successful.

Filamentous algae are notoriously difficult to control because mechanical methods, other than dredging, tend not to be very efficient and manual removal costs are twice those for other macrophytes (Cave 1981). The only chemical product currently available in the U.K. which has good algicidal properties, is terbutryne, but the long exposure periods needed for its action restrict its use to static, or very slowly moving water.

Even if the biomass of filamentous algae can be reduced by a weed control method, recolonisation and regrowth can be very rapid. This is especially a problem in flowing waters, where propagules can be carried into a cleared area from upstream. Growth of these propagules will be rapid in the light conditions provided by the removal of other macrophytes or older algal mats.

It cannot be assumed from these trials that diquat-alginate does not have any effect upon filamentous algae because biomass estimates of these plants were not made. The cover of algae did not appear to be reduced in the R.Petteril, but some Cladophora did look brown and unhealthy after the herbicide application. Careful comparisons of treated and untreated sections would be needed to assess the effects of a weed control method on Cladophora because this alga may have two maxima of biomass. One is in early summer and the other is in the early autumn, with a natural die-back in mid summer, which could be confused with the effects of an early treatment (Whitton 1970).

The possible reasons for the lack of susceptibility of some plant species to diquat may be summarised:

1) Plant morphologies and growth habits which do not favour the entrapment of alginate tadpoles or the presentation of large surface areas for diquat uptake (e.g. Sparganium emersum).

2) Thick epidermal tissues, especially on floating or emergent leaves, which may limit the uptake of diquat.

3) Differences in physiology which are not as severely affected by diquat's action, or which can adapt to them. The uptake of the herbicide might also be reduced in some plants because of differences in physiology.

4) Plants with very rapid recolonisation and regrowth may appear to be resistant to a herbicide.

5) Some plants may provide a more favourable substrate for periphyton, which form an adsorptive barrier to the herbicide.

5.6.4 Comparisons of the ecological effects of the management regimes between sites

Physico-chemical characteristics

There was no evidence from these trials that the water chemistry in any of the rivers was grossly affected by the decay or removal of submerged vegetation, following cutting or herbicide use.

Only gross effects could have been detected by the sampling regimes used in these trials. More frequent sampling would have been needed to detect subtle changes in concentrations of nutrients or dissolved gases. Night-time measurements of dissolved oxygen concentrations, or specific B.O.D. methods, would have been necessary to assess the effects that the herbicide-induced plant decay might have had in the oxygen dynamics of the rivers.

No changes in water depth or mean velocity, at the transects in the treated sections, could be distinguished from the seasonal fluctuations occurring in the untreated sections.

The effects of the decay or loss of vegetation on the water chemistry and hydrology, will have been minimised in these trials by several factors:

1) Small areas of river treated, with high throughputs of water. Any release of nutrients or additional consumption of oxygen would be rapidly diluted by fresh water from untreated areas upstream.

2) The retention of vegetation (e.g. non-susceptible species, plants unaffected by the cuts), which would buffer effects by continued photosynthesis and/or utilisation of released nutrients.

3) Large background fluctuations making it difficult to distinguish treatment effects, and reducing their potential importance. Fluctuations in nutrient concentrations and B.O.D. may be sudden and irregular depending upon catchment run-off, fertilizer and animal manure inputs.

Macroinvertebrate communities

No attempt has been made to compare the macroinvertebrate communities from the untreated sections of the three rivers from which samples were analysed. The macroinvertebrate communities could have been analysed with the DECORANA programme using a similar taxon x samples data matrix, to that used for the macrophytes. Correlations with environmental data might indicate which factors influence the size and composition of macroinvertebrate communities in these sites.

An alternative approach would be to apply these data to an existing macroinvertebrate classification, so that the sites could be related to a large number of other rivers throughout the U.K. A suitable classification of rivers according to their macroinvertebrate communities, has been developed from a large-scale survey of 268 sites on 41 river systems (Wright et al. 1984). Samples identifed only to family, not necessarily to species, could be classified using the dichotomous key derived from the TWINSPAN analysis (Furse et al. 1984).

Use of these classifications would be limited by the restriction of sampling in these trials to the Ranunculus beds and the immediate benthos. The river classification system, and most biological water quality scoring systems (e.g. Armitage et al. 1983) depend upon a species, or family, list being prepared from all microhabitats within a site. Similarly the species diversity indices calculated in these trials cannot be directly compared to other work, not only because of the limited habitats sampled, but also because of the identification of animals not to species but usually only to order. The factors, such as the level of identification, which influence the values of the Shannon diversity index, have been discussed by Hughes (1978).

The numbers of taxa identified were similar in the three rivers, (R.Petteril, R.Coln, R.Windrush) but the relative abundances of these taxa differ. For example, chironomids were much more numerous in the R.Petteril than in either of the southern rivers. Seasonal fluctuations, particularly those associated with changes in life-cycles of populations of taxa common to all sites, would not be synchronised between the populations in the three rivers. Thus, it is not possible to directly compare the populations occurring in the three sites. Only gross differences in numbers between treated and untreated sections can be compared.

Taxa which appeared to be reduced in numbers by the management regimes, but which increased or remained unchanged in the untreated sections, have been listed for each river in Table 15. Taxa which showed a correlation between numbers of animals and plant biomass, have also been indicated.

Although the indirect ecological effects of management, in reducing the plant biomass, may have resulted in the loss of animals from some taxa, this relationship cannot be assumed except for taxa known to prefer the sampled macrophytes and immediate benthos as a substrate. These data cannot indicate the population dynamics of the whole treated sections because of the specificity of the sampling areas. Thus, it is not possible to describe the effects of the treatments upon taxa which may be able to adapt to other microhabitats in the treated sections, or which can migrate to untreated areas of the river (e.g animals which can move from Ranunculus to Cladophora in the R.Petteril, or animals which could escape from the cutting process in the centre of the channel in the R.Windrush to the uncut margins).

The changes in macroinvertebrate numbers in the treated sections were rarely significantly different from changes in the untreated sections because of the large variation in the samples.

Changes in the macroinvertebrate populations after weed management, were closely associated to the severity of the reduction in plant biomass, being most evident in the herbicide sections of the R.Petteril and the cut sections of the R.Windrush.

Animals living in the benthos immediately below the Ranunculus were likely to be affected by the loss of plants, because the silt which had accumulated in the weed beds would have been washed away, when exposed to the water flow. Detritus might have increased as herbicide-treated plants decayed, providing an enriched food supply for animals inhabiting the exposed substrate.

Table 15

Macroinvertebrate taxa showing different population changes in treated and untreated sections

	Reductions in treated sections	Significant change compared to untreated sections	Correlation with plant biomass
R.Petteril			
Chironomidae	X Herb. & cut	X Herb.	X(Total biomass)
Simuliidae			X
Ephemeroptera	X Herb. & cut	X Herb	X
Coleoptera			X
Trichoptera			X (-algae)
Hydracarina	X Herb.	X Herb.	X
R.Windrush			
Coleoptera	X Cut		
Trichoptera	X Cut		X
Hydracarina	X Cut		
Malacostroca	X Cut		
R.Coln			
Chironomidae			X
Coleoptera			X
Hydracarina			X

	Increases in treated sections
R.Petteril	
Oligochates	X Herb.
R.Coln	
Malacostroca	X Herb.

5.7 Discussion of the Methods Used in the River Trials

The analysis and interpretation of the data collected from these river trials have been restricted by the frequency and number of samples that it was possible to collect.

The limitation of the number of treatment replicates to two, or three, per site meant that the ANOVA's of data taken from a single sampling time had few degrees of freedom. Large variance ratios would have been needed for differences between treatments to have been significant.

A larger number of treatment replicates would have improved the estimation of errors between treatments, but might have also increased the variation within treatments, unless very similar replicates could be found. It was the high variability within treatments which prevented some of the differences between treatments from being significant (e.g. post-treatment herbicide sections in R.Coln).

Increasing the number of sub-samples per replicate would not directly affect the ANOVA (because mean of sub-samples were used to avoid pseudoreplication). If the variation around the replicate means could be reduced, by increasing the number of sub-samples, then a greater confidence could be placed in the interpretation of differences between treatment replicates.

Macrophyte data

The collection of plant biomass samples was restricted to Ranunculus beds which had been present prior to treatment. This restriction was necessary in rivers with a very patchy distribution of macrophytes, to ensure that similar samples were collected from each section. An enormous number of sub-samples, or much larger sampling units, would have been needed to provide accurate estimates of the macrophyte biomass in the whole sections. The data collected in these trials cannot be directly compared with macrophyte biomass estimates of rivers, which have been collected per unit area of substrate from the whole channel (e.g. Westlake et al. 1972), because of the bias towards the Ranunculus beds.

Comparisons between sampling times of data collected for the %-frequency of macrophyte cover are more reliable than those for biomass, because exactly the same area was being assessed, minimising the sampling error. This method for assessing the quantity of vegetation present, depends upon the selection of transects which

show mimimal variation between them prior to treatment.

Cover estimates are of limited value in assessing plant biomass because large changes in biomass, by removal of vegetation from the water column, may be undetected if the substrate is still covered by a residual layer of stems (e.g. R.Coln post-herbicide). A reduction in the macrophyte cover of the water surface may be important for some types of management (e.g. fly fisheries) but it is often the biomass, or volume of submerged plants that needs to be reduced to allow efficient water flow (Dawson 1976).

More frequent sampling would have been valuable in providing a better background picture of the seasonal variations of macrophyte growth in the untreated sections, against which to compare the effects of the treatments.

Ecological effects of the treatments

To ensure that any effects of the management regimes on the water chemistry would be detected, samples would need to be taken at regular intervals before and after treatment, so that any significant divergence from the seasonal, and diurnal, cycles could be identified.

Automatic data loggers placed in treated and untreated sections would be ideal for the recording of parameters such as temperature,pH and dissolved oxygen (e.g. Thyssen 1982). Such equipment would need to be regularly checked and calibrated, hidden from curious animals and people, and the electrodes protected from damage by floating objects.

The macroinvertebrate samples collected using the Lambourn sampler, were too large and time-consuming to count, to allow the collection of many sub-samples. More reliable and a larger amount of information on the macroinvertebrate populations could have been gained by a greater number of smaller samples. For example, the percentage frequency of taxa in a large number of samples could be assessed more quickly than counting numbers of individuals. Such samples could either be limited to a single habitat (e.g. on the Ranunculus only) or all the microhabitats in the rivers could be included. Non-destructive samples would be preferable for sites which were to be repeatedly sampled. With practice and familiarity of sites, identification of taxa in the field would greatly reduce the post-sampling laboratory work. Identification to genera would also increase the information gathered.

5.8 Conclusions of the Comparisons of River Trials

1) Multivariate analyses of the macrophyte cover estimates, were carried out, using ordination and classification techniques. The four rivers were clearly distinguished by their floral compositions, and these could be correlated to environmental factors, such as calcium and nitrate concentrations of the river water. The effects of management regimes were not distinguished.

2) These multivariate analysis techniques could be of value in comparing the long-term effects of management on the floral, or faunal composition of rivers. Comparisons of a large number of initially similar sites, after management, would also be possible.

Comparisons of the results of the river trials

3) Regrowth after cutting was limited if the cut was late, in July, or if a cover of competitive plants was left uncut. Regrowth was evident after early, non-selective cuts.

4) The herbicide was most effective in rivers with moderately low calcium concentrations and low turbidity. The concentrations of diquat residues were high in shallow streams, and very high availance values were related to severe downstream effects.

5) The herbicide appeared to be effective in the R.Coln, but not the R.Windrush, despite their similar calcium concentrations. A combination of factors (e.g. turbidity, presence of less-susceptible species, depth of water, length of treated sections, water temperature, high nutirent concentrations) may have been less favourable in the R.Windrush than in the R.Coln, resulting in the difference in the efficacy of the herbicide between these rivers.

6) The ecological effects of the treatments in all of the trials would have been minimised because of the small areas of river treated. These areas would be buffered by the inflow of fresh water and immigration of macroinvertebrates from untreated areas upstream. The presence of macrophytes not affected by the management methods in the treated sections,would also buffer and reduce any ecological effects that a particular treatment may have had.

CHAPTER SIX

FIELD TRIALS OF WEED CUTTING AND THE USE OF DIQUAT-ALGINATE IN A CANAL

6.1 Introduction

6.1.1 The canal system in the British Isles

The canal network in the British Isles was chiefly developed between 1760 and 1820 to satisfy the increased transport requirements for raw materials and manufactured products, created by the Industrial Revolution (Hall 1985). The commercial importance of the canals was eclipsed by the development of the railways, and 800km of the original 3700km of canal were lost or abandoned.

In 1962, 2500km of the canal network, by this time under the nationalised management of the British Waterways Board (B.W.B.), were classified, under the 1962 Transport Act, into three groups:

Commercial	548km
Cruising	1743km
Remainder	815km

(Hyde 1977)

The commercial canals, still serving their original purpose, are principally limited to the large ship canals, which because of their depth and frequent use do not tend to support much,if any, vegetation.

Although, like rivers, the other canals serve a variety of functions (e.g. the movement of water, industrial abstraction), amenity use is becoming increasingly important. This not only applies to the obvious use of Cruising canals for hired and private pleasure-boat traffic, but also to the Remainder canals. Only about half of these canals are navigable, but they must be kept in a safe condition, and are popular for fishing, canoeing, walking, etc. The management of the Cruising and Remainder canals has to be adapted to best suit all of these demands (Slaytor 1976).

6.1.2 Canal management

Cruising and Remainder canals are typically 8-16m wide and up to 3m deep, constructed to a standard trapezoidal cross-section (Hyde 1977). These canals are considered to provide a unique, slow-flowing freshwater habitat, which although man-made might be regarded as semi-natural since they are not completely and constantly managed (Slaytor 1976).

A survey in 1976 indicated that there were weed problems in approximately 10% of Cruising canals and 35% of Remainder canals. Where specified, over half of these weed problems were caused by submerged and filamentous algae (Murphy et al. 1980).

There are two opposing problems with macrophytes in Cruising and Remainder canals. One problem is the excessive growth of submerged and emergent vegetation in waterways which have little, or no, boat traffic. Such growth may constitute a nuisance to navigation or fishing, or may lead to the progressive infilling of the channel.

At the other end of the scale of boat traffic in popular canals, there can be problems in maintaining any vegetation. Physical damage from propellers and wave action, and shading in muddy water kept turbid by boat movements, can so reduce macrophytes that fisheries can no longer be maintained (Murphy and Eaton 1983). Approximately 20% of Cruising and Remainder canals have excessive macrophyte growth, and 20% have insufficient vegetation (Murphy et al. 1982).

Management techniques in canals with excessive weeds have been fairly diverse. For example, in 1976 about 44% of the total length of canals with weed problems were managed. Of this management 20% was by manual removal, 65% by mechanical removal, 8% by dredging and 6% involved the use of herbicides (Murphy et al. 1980).

A variety of aquatic herbicides have been used routinely, or in trials, throughout the British canal system. Some of the references to published material discussing the use of herbicides in canals, are listed in Table 16. Direct comparisons of mechanical and chemical management methods are discussed in two of these references (Eaton et al. 1981, Murphy and Eaton 1981).

Table 16

The use of aquatic herbicides in canals

1966-1970	Diquat Diquat and paraquat	Routine use in the Lancaster Canal on submerged and emergent weeds	Greenwood (1974)
1976	Cyanatryn Dalapon Diquat	Routine use in 6% of managed canals	Murphy et al. (1980)
1975-1978	Cyanatryn Terbutryne	Trials to investigate efficacy and ecological effects	Hanbury et al. (1981) Murphy et al. (1981)
1978-1979	Dichlobenil + terbutryne Dalapon/Glyphosate	Trials in comparison with mechanical clearance	Eaton et al. (1981) Murphy and Eaton (1981)
1981 1982	Terbutryne Diquat-alginate	Trials in the Union Canal	Murphy et al. (1987)

6.1.3 The Union Canal

The Union Canal was opened to navigation in 1822 to link Edinburgh and the Forth and Clyde Canal at Falkirk, in central Scotland. The canal is now of Remainder waterways status, having been closed to navigation in 1965. Parts of it are now culverted under motorways or dropped-bridges. There is a low amount of pleasure-boat traffic, on parts of the canal which are still navigable. The canal is lock-free for much of its length and the rate and direction of water flow is a function of feeder-stream inflow, rate of water abstraction for industrial use, and wind velocity and direction. The main feeder from the R.Almond enters the canal in the National Grid square NT1070. Water flows east and west from this point at a rate probably averaging 30m hr^{-1} (Murphy et al. 1987).

The canal is essentially unpolluted and with its slow flow, nutrient sediments, and shallow, clear water provides a highly favourable habitat for abundant macrophyte growth.

As a slow flowing, lowland, meso-eutrophic water, the Union Canal is an unusual habitat in Scotland, is one of the northern-most such habitats in the U.K. and so has considerable conservation value. This has been confirmed by biological surveys of several stretches of the canal, indicating a diverse community of aquatic macrophytes, invertebrates and higher animals (e.g. Sheldon 1976). Conservation in canals throughout the British Isles is being treated with increasing seriousness by B.W.B., and management procedures are being planned accordingly in particularly valuable or sensitive sections (Hanbury 1986).

6.1.4 Management in the Union Canal

Even though large portions of the canal are not used for boat traffic, management of aquatic vegetation had been necessary to maintain sports fisheries, and water flow to abstraction points. The death and decay of plants, particularly Lemna mats which can be blown into thick piles, has lead to a public nuisance, with complaints of foul smells and unsightly appearances.

Sites often have to be managed for conservation, to prevent the succession to less valuable emergent dominated communities, or to remove aggressive species, such as Elodea canadensis or filamentous algae, which can reduce floral diversity and interest.

In 1976 the major plant groups identified as causing weed problems in the Union Canal were free-floating (e.g. Lemna spp.), floating-leaved (e.g. Potamogeton natans), and submerged macrophytes (e.g. Elodea canadensis) (Sheldon 1976).

Management in the Union Canal has largely been carried out using reciprocating-cutter boats, which can cut and remove vegetation from the canal. A weed-scoop attachment can clear mats of Lemna or previously cut vegetation.

Since 1981 a series of herbicide trials, with terbutryne and diquat-alginate, has been conducted to assess the potential of chemical weed control as a supplement, or replacement, to weed cutting in the canal. Single or repeated cutting trials were carried out in 1977, 1978 and 1983, on various stretches of the canal.

The results of all of these trials, including those in 1984 described in this chapter, have been analysed using multivariate techniques, to assess the effects on the structure, abundance and interrelations of aquatic macrophytes and macroinvertebrate communities in the canal (Murphy et al. 1987).

The aims of the trials, described here, on the Union Canal in 1984 were the same as those for the river trials:

1) To directly compare the efficacies of a mechanical cutting weed control method, with the use of diquat-alginate.
2) To assess the ecological effects of each method.

The study of the canal was included in this project as a system in which regular management may be needed, and as a slow flowing watercourse to provide a contrast with the rivers.

6.2 Methods used in the Union Canal Trial

6.2.1 Experimental design

The experimental design used in the Union Canal was the segregated distribution of treatments illustrated in Fig. 25a. This design was used because the channel appeared to be uniform throughout the site, and because it was most convenient for the B.W.B. cutting schedule. The cut sections were adjacent to the B.W.B. yard from which the cutting boat was launched.

Statistically this design was unsatisfactory because some of the advantages gained in using treatment replication were lost in the segregation of the treatment replicates together. However, the use of treatment replicates rather than a single, large, sub-sampled section for each treatment, does ensure that differences which might arise between the two applications of the same treatment, will be included in the error estimate. Thus, in an extreme example, if the herbicide applied to each replicate came from separate containers, one of which had been stored in strong light and had photochemically lost some of its diquat, the variability in the treatment applied will be included in the analysis of the replicates.

Strictly an ANOVA should not be applied to data from this design, and to do so is to commit Pseudoreplication (Hurlbert 1984). The segregation of the treatments means that differences observed between treatments may include the effects of non-demonic intrusion, and upstream-downstream differences in the canal.

An ANOVA was applied to the biomass data,rather than a series of 't' tests, but attention has been paid to the limitations in interpreting the results.

6.2.2 The weed control treatments

Cutting

The two 200m cut sections and the 100m of intervening channel were cut on 2.6.84 using a Wilder Water Warrior weed cutting boat. The boat was fitted with a U-shaped reciprocating cutter bar, and made two return journeys to cover the whole width of the canal. The cut material was subsequently removed using a lift-rake attached to the boat.

Herbicide application

Midstream was applied to the two 200m sections on the same day as the cutting treatments were made. The herbicide was applied at a rate of 100 l ha^{-1} following the procedures described in Section 4.4.1. The canal is about 14m wide and the Midstream, which was applied from the towpath, could be projected over about 10m, so that most of the channel was directly sprayed.

An extra untreated section was surveyed 200m downstream of the herbicide sections. This was added in case the flow was reversed during the period of the herbicide application, which might have resulted in diquat residues reaching the upstream untreated sections. The data from this section were not included in the statistical analyses, but have been illustrated where appropriate.

6.2.3 The ecological monitoring of the Union Canal

A pre-treatment survey was carried out on 1.6.84.
Post-treatment surveys were carried out one and four months later.

Plant biomass

Samples of vegetation were collected using the replicated grapnel sampling technique of Murphy *et al.* (1981). Ten samples were collected from each section, from random points along the towpath. The vegetation was sorted into species and after a 30 second spin-dry were dried at 60°C to a constant weight. The data have been recorded here in units of grammes dry weight per grapnel (g $grapnel^{-1}$).

The surface cover of *Lemna* spp. was estimated from photographs taken of each section. The channel was divided into three longitudinal zones, near-side (towpath), mid and offside, and each zone was

given a score of the scale:

Score	% Cover
0	0
1	1 - 10
2	11 - 50
3	51 - 100

The mean score for the three zones was calculated per section.

Macrophyte-associated fauna

The macroinvertebrates collected in the grapnel samples were removed from the vegetation by repeated washing and picking out by hand using 355 um mesh nets.

Only taxa which could be immediately recognised were distinguished, and random samples of these groups were collected for identification to family, genus or species level, as appropriate.

It would have been too time-consuming to have counted all of the individual representatives of each taxon, so a simplified scoring method was used:

Abundance score	Number of individuals in a taxon
0	0
1	1
2	2 - 5
3	6 - 20
4	21 - 100
5	100+

The mean scores of the ten samples were calculated per section.

Water chemistry

Field data and water samples were collected and analysed, as described in Section 4.4.2, for the following parameters:

Air and water temperature

pH

Dissolved oxygen concentration

Conductivity

Calcium ion concentration

Total water hardness

Nitrate ion concentration

Reactive phosphorus

Photosynthetically active radiation

Diquat residues

Water samples were collected and analysed for diquat residues, as described in Section 4.4.3. Samples were taken from the mid-length and downstream ends of the two herbicide sections, before and at intervals after the herbicide application. An additional sample was collected from the downstream untreated section, 60 minutes after the downstream herbicide application.

6.3 Results of the Union Canal Trial

6.3.1 Macrophytes

Observations

Despite the apparent uniformity of the canal channel throughout the site, there were some differences in the initial dominant species between the sections. The herbicide sections, although having a small amount of Elodea canadensis, were dominated by Potamogeton natans, with extensive coverings of Lemna minor and L.gibba. The cut sections also had P.natans but the Elodea formed a much greater cover of the canal bottom. The two upstream and the single downstream untreated sections had the greatest diversity of macrophytes, with Elodea and P.natans plus P.crispus, Callitriche spp. and much Lemna.

By July, 47 days after both treatments, the Lemna cover in the herbicide and untreated sections was about 75-100%. The cutting procedure had not affected the Lemna but the removal of the cut weed using the rake attachment on the cutting boat, may have removed some Lemna because the cut sections had only about 50% cover.

No Elodea was found in the herbicide sections after treatment. Although the P.natans had not been obviously reduced, some leafless stems were visible, and many floating leaves which had been directly hit by the Midstream were badly damaged. Emergent plants on the nearside bank, which had been directly hit, showed the classic diquat burns seen on terrestrial plants. Sparganium erectum plants on the offside, which had not been reached by the spray, showed phytotoxicity symptoms, such as leaf chlorosis, at the water level, suggesting that they had been affected by diquat in solution.

In July, the Elodea in the cut sections was uniformly shorter than in the untreated sections, but the area of canal bottom covered was not reduced from the pre-cut condition. Some Elodea was still found in the downstream untreated section, but it was less abundant than before the treatments.

By mid October, the Elodea, P.crispus and Callitriche spp. in the upstream untreated sections were still healthy and were approaching the water surface. The surface cover of Lemna had been reduced by wind action, so that in sheltered areas mats of several centimetres thickness had accumulated.

There was no Elodea evident in the herbicide sections, but P.natans was still dominant. The Elodea in the cut sections had densely regrown to fill at least half of the height of the water column and the plants were still of uniform height. The condition of these plants remained good but there were signs of deterioration of older stems. These stems would eventually die-back to leave the strongest shoots with dormant buds to remain over-winter.

There was little vegetation in the downstream untreated section, although whether this was the effect of herbicide contamination, or the shading effect of the Lemna cover was not evident.

Biomass

The biomass data have been analysed in three categories:

1) Submerged macrophytes only (not including algae)
2) Submerged macrophytes + P.natans (not inc. algae)
3) Total submerged and floating macrophytes (i.e. including P.natans, Lemna, algae, but not emergents)

The biomass data were analysed using a split-plot ANOVA and a $\log_e$ (X x 100 +1) transformation (as described in Section 3.2.3).

When the submerged macrophyte only data were analysed for all three sampling times, none of the factors or interactions were significant (Fig. 62a). If the data from only the June and July sampling times were analysed both factors (Treatment $F_{2,2}$= 18.08, Time $F_{1,3}$ = 26.02) were significant as well as the time * treatment interaction ($F_{2,3}$= 29.89). The biomass in the herbicide sections did

Fig. 62

Biomass of macrophytes in the Union Canal

Fig. 62a. Submerged macrophytes only (-algae)

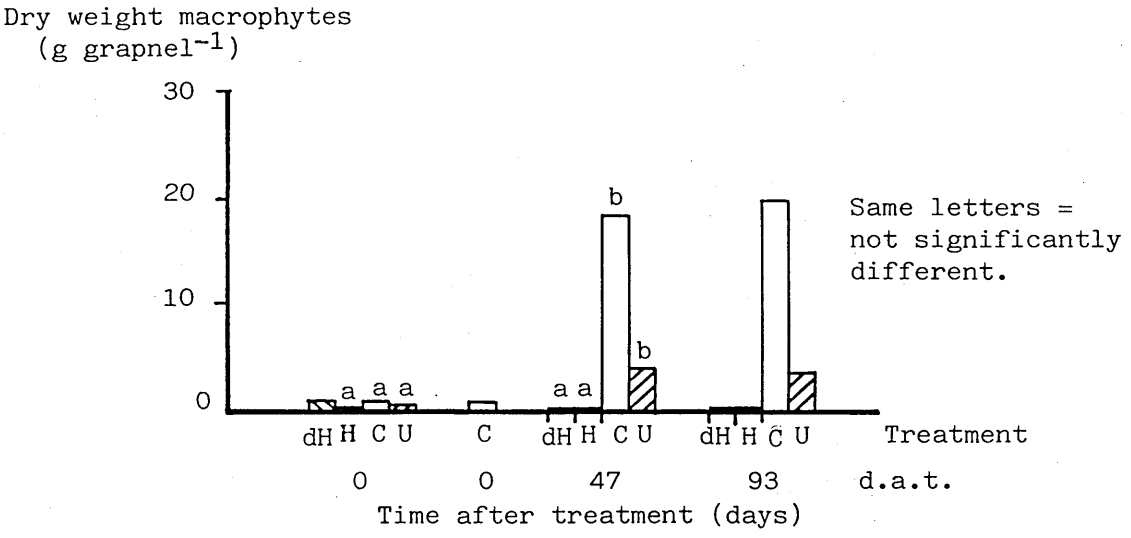
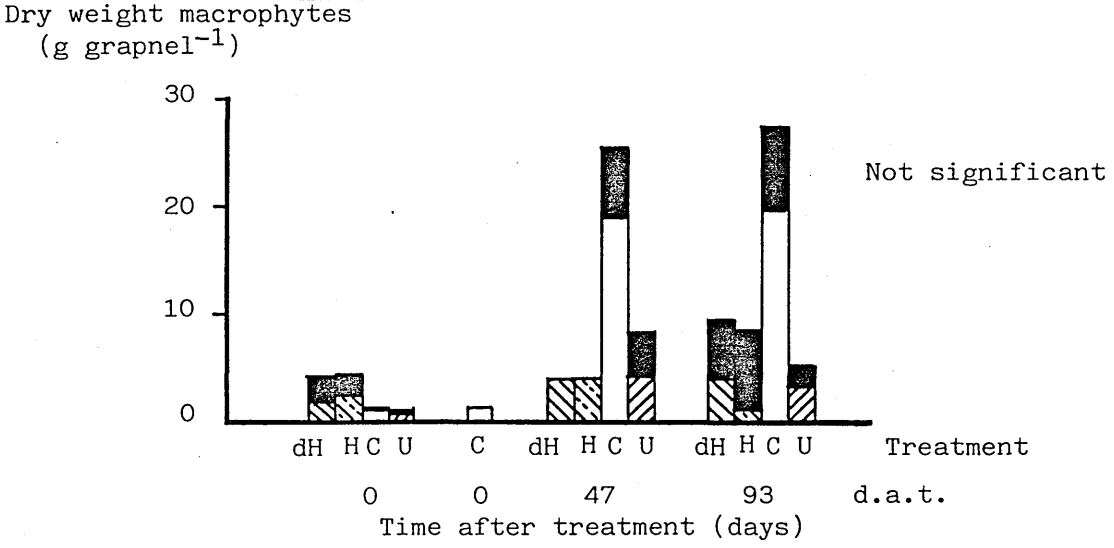


Fig. 62b. Total macrophytes



dH = section downstream of herbicide application UC1

H = Herbicide UC2 UC3

C = Cut UC4 UC5

U = Untreated UC6 UC7

Lemna and algae

not change significantly between the sampling times but there were significant increases in biomass in the cut and untreated sections. There was little difference in biomass between the July and October samples.

When the analysis was repeated to include P.natans there were no significant factors if all three sampling times were analysed, but the time factor was significant ($F_{1,3}$= 12.44) if data from only the June and July samples were analysed. The biomass of these plants was similar in the herbicide and untreated sections in July(Fig. 62b).

Inclusion of all submerged or floating macrophytes in the analyses (Fig. 62b) resulted in the time factor being significant ($F_{1,3}$= 7.44) whether two or three sampling dates were included.

Data from the downstream untreated section were not included in the ANOVA,nor were the samples taken from the cut sections immediately after cutting. These results have been included in the histograms. The biomasses in the downstream control sections were similar to the values from the herbicide sections. The samples taken just after the cut had similar biomasses to the pre-cut samples.

The data for the percentage change in biomass (between the pre- and post-treatment samples), did not need to be transformed. There were no significant factors or interactions when the submerged macrophytes only, data were analysed. These data were similar for the July and October samples (Table 17a) but even if a one-way analysis of variance was used on the July data only, the differences between the treatments were still not significant. The reason for this can be seen in Table 17b which shows the percentage biomass changes for each section. There were large differences between the treatment replicates, especially the low values of the upstream untreated section.

If P.natans (Table 17c) or all the other submerged or floating macrophytes were included in the percentage biomass change analyses, the only significant factor was between the blocks. The blocks were not physically separate, but the change in biomass after treatment was significantly greater in the downstream replicates of each treatment.

The results for the scores of Lemna cover are illustrated in Fig. 63. The post-treatment cover in the herbicide and untreated sections was almost 100% but this decreased by October. The post-cut cover was only about 60% and this cover was maintained into October.

Table 17

Percentage change in biomass of macrophytes in the Union Canal compared to the pre-treatment values

Table 17a Submerged macrophytes only, means per treatment

	July	October
Herbicide	13	50
Cut	2132	2347
Untreated	732	577

Table 17b Submerged macrophytes only, values for each section

		July	October	
Herbicide	d/s	3.3	0	
	u/s	22.2	100.0	---High?
Cut	d/s	2627.9	3186.8	
	u/s	1636.7	1507.7	
Untreated	d/s	1150.0	1141.4	
	u/s	313.3	13.3	---Low?

Table 17c Submerged macrophytes and Potamogeton natans

		July	October
Herbicide	d/s	2828	836
	u/s	95	10
Cut	d/s	2429	2941
	u/s	1621	1457
Untreated	d/s	1317	1174
	u/s	313	13

Fig. 63

Percentage of maximum score for Lemna cover of the Union Canal

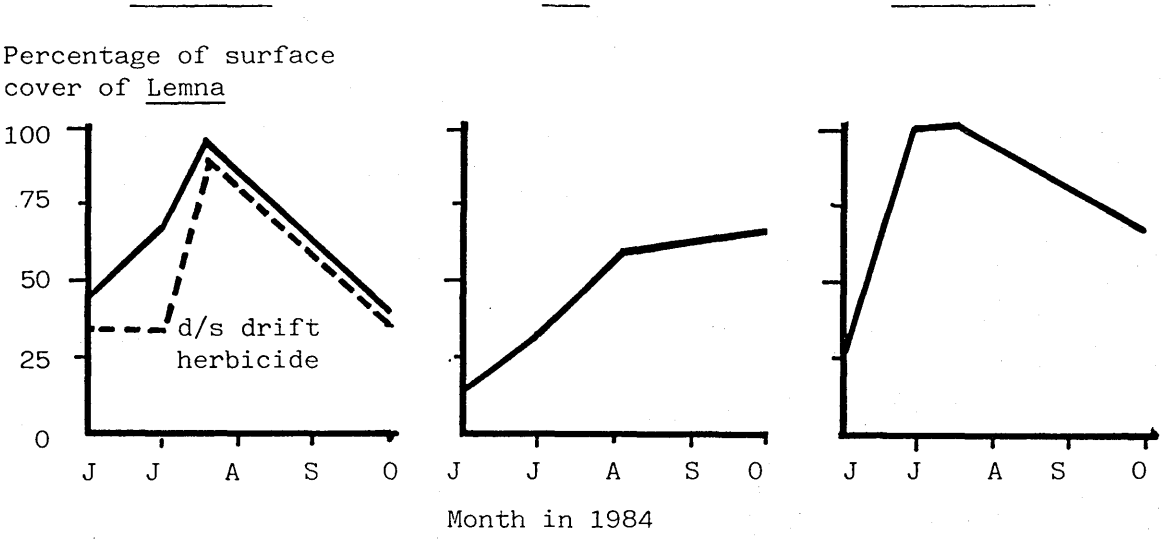
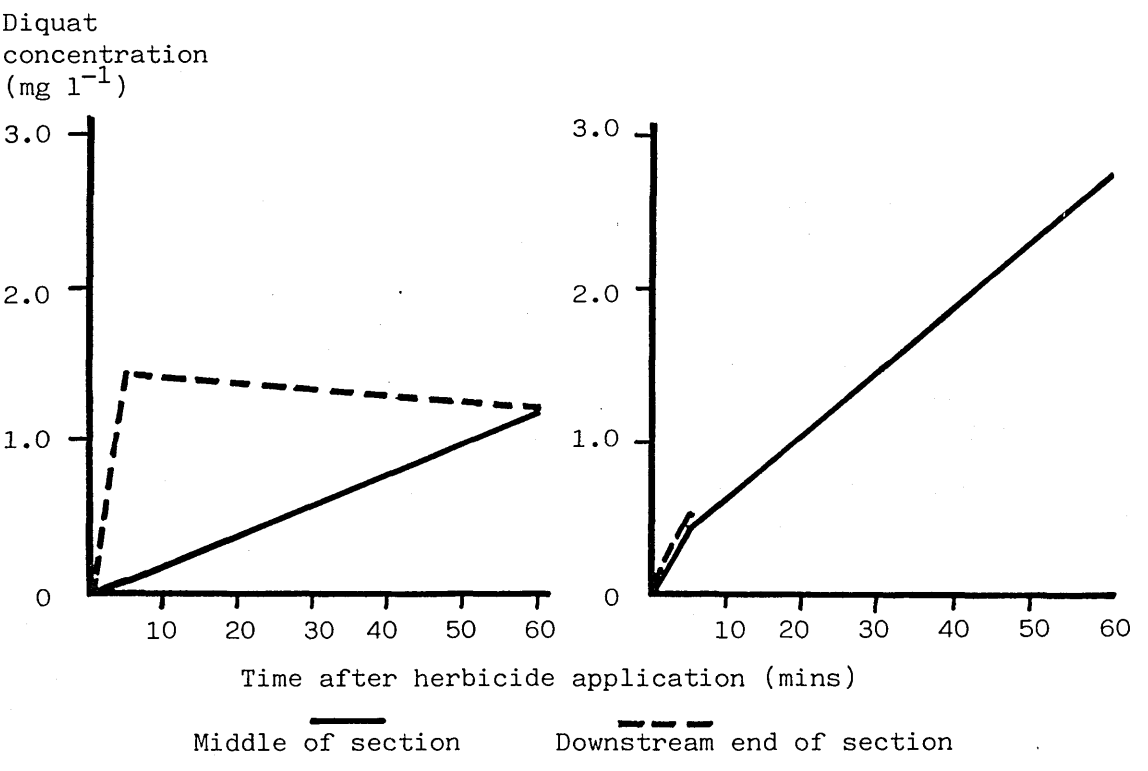


Fig. 64

Diquat residues from the Union Canal



Availance (mg l^{-1}.min)

	40 min	60 min
Mid section	13.7	33.82
End section	51.4	76.22

	40 min	60 min
Mid section	42.11	88.55

6.3.2 Diquat residues

The results of the diquat residue analysis are shown in Fig. 64. There was no diquat present in the water samples collected from the upstream ends of either section or prior to treatment. At the downstream end of the downstream section the diquat concentration was greater than 1.0mg l^{-1} within five minutes of the start of the herbicide application. The concentration decreased slightly during the following 55 minutes, but was still over 1.0mg l^{-1} by one hour after treatment. Samples taken from the middle of the section contained little diquat within 5 minutes of treatment but after 60 minutes, the concentration was the same as at the downstream end of the section.

Diquat residues in the upstream herbicide section were similar in the middle and downstream end of the section, 0.5mg l^{-1}. The sample from the downstream end, collected 60 minutes after the herbicide application, was lost but the concentration in the middle of the section was very high, nearly 3.0mg l^{-1}. The availance for the mid-length of the upstream section was similar to the value for the downstream end of the other herbicide section. Further samples would have been useful for indicating the total period over which diquat residues remained in the herbicide sections.

A low concentration of diquat was detected in the untreated section downstream of the herbicide sections, 60 minutes after the application of the Midstream to the downstream herbicide section. This indicates that diquat drifted downstream of the points of application, but it is not possible to tell whether a phytotoxic availance was achieved.

6.3.3 Water Chemistry

The mean values of water chemistry parameters from all sections per sampling time are given in Table 18. The data for dissolved oxygen, pH and reactive phosphorus have been plotted for each section in Fig. 65a-c.

Prior to the treatments there was a marked gradient of increasing dissolved oxygen concentrations and pH, going upstream in the canal. Dissolved oxygen concentrations and pH were lowest in July and usually in sections with a high percentage cover of Lemna.

Table 18

Mean water chemistry data from the Union Canal

Means of 7 sections, ( )= standard error

Date	1.6.84	19.7.84	19.10.84
Start time	11:05	09:30	11:50
Water temperature (°C)	15.7 (0.2)	17.8 (0.2)	9.0 (0.1)
Dissolved oxygen (% saturation)	150.8 (12.3)	56.2 (4.7)	95.0 (3.1)
pH	7.97 (0.1)	7.22 (0.03)	7.46 (0.03)
Conductivity (u mohs cm^{-1})	844.3 (2.2)	756.1 (18.6)	458.6 (10.3)
Total water hardness (mg l^{-1} $CaCO_3$)	328 (4.0)	307 (4.5)	187 (3.76)
Calcium ion conc. (mg l^{-1} Ca^{++})	82.3 (2.3)	52.0 (0.7)	61.1 (0.6)
Nitrate ion conc. (mg l^{-1} NO_3^-)	12.6 (1.0)	7.84 (0.4)	25.8 (0.5)
Reactive phosphorus (mg l^{-1} P)	0.291 (0.004)	0.181 (0.004)	0.181 (0.007)

Fig. 65

Water chemistry data from the Union Canal

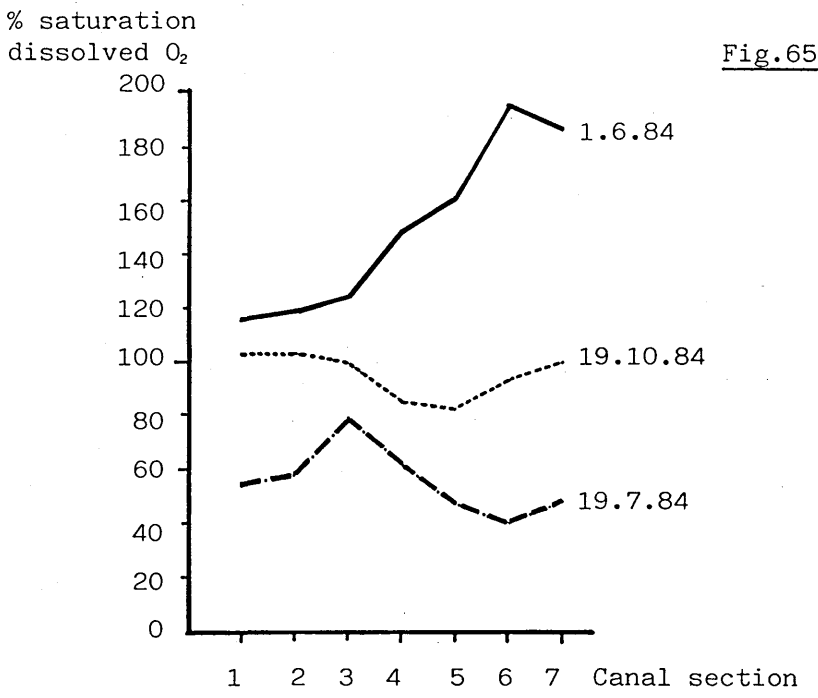


Fig.65a Dissolved oxygen

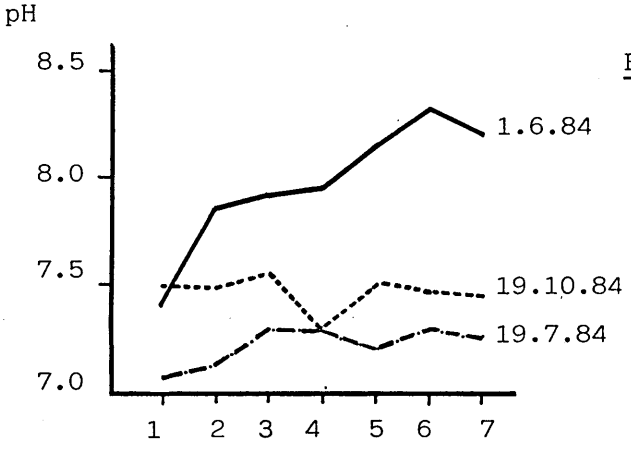


Fig. 65b pH

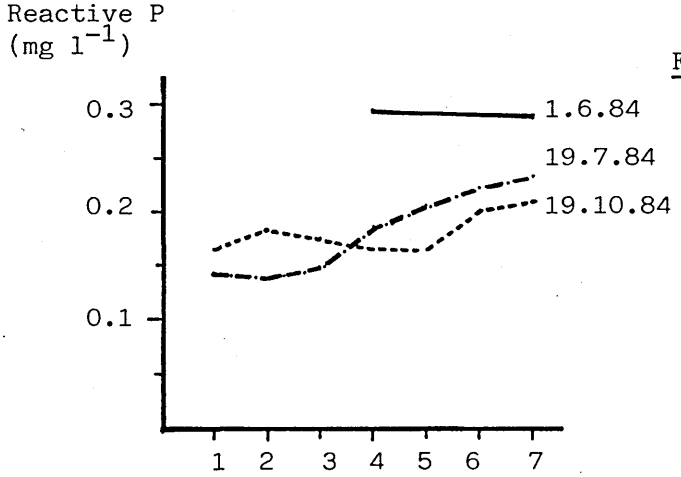


Fig. 65c Reactive phosphorus

1 Downstream of herbicide
2 d/s herbicide
3 u/s herbicide
4 d/s cut
5 u/s cut
6 d/s untreated
7 u/s untreated

Phosphorus (and to a lesser extent nitrate) concentrations showed an increasing gradient going upstream in the canal, in all three sampling times. Nitrate and calcium concentrations, total water hardness and conductivity (like pH and dissolved oxygen) showed large variations between the sampling dates.

6.3.4 Light (P.A.R.) transmission

Extinction coefficients were calculated for the post-treatment sampling times (Table 19) but these data do not give a fair representation of the water conditions. There was considerable variation between P.A.R. measurements, even from the same sections, and many of the extinction coefficients were very high. If undisturbed the water appeared to be very clear, with the bottom easily visible, if not covered in vegetation. P.A.R. measurements which had to be taken through a Lemna mat have been indicated. Even when this could be avoided, P.A.R. readings at the bottom were often partly shaded by the emergent vegetation (all measurements being taken within reach of the bank), and surrounding submerged macrophyte beds.

The canal water samples were the only ones, of all the field trials, to have detectable concentrations of chlorophyll a, indicating the presence of phytoplankton. These algae would have reduced the amount of P.A.R. penetrating the water column.

6.3.5 Macroinvertebrate communities

A list of the species, and other taxa, identified in the samples from the Union Canal, is presented in Appendix 8. Mean abundance scores, per treatment, were calculated for some of the major taxa. These means were then converted to percentages of the maximum possible abundance score (5), and the results are illustrated in Fig. 66. The most noticeable difference between treatments was the increase in abundance of the molluscs (Sphaerium corneum and Physa fontinalis) after treatment, in the cut sections, compared to the herbicide and untreated sections. There were dips in abundance of leeches following treatments, in comparison with untreated sections (Glossiphonidae, and Erpobdella octoculata).

The species diversity indices, based upon the abundance scores for each taxon, not the numbers of individuals, are given in Table 20.

Table 19

Light extinction coefficients in the Union Canal

Section		19.7.84	19.10.84
Herbicide	d/s	4.011	3.175
	u/s	2.075	3.174
Cut	d/s	2.198	5.26
	u/s	1.543	2.443
Untreated	d/s	2.869	-
	u/s	2.666	3.886

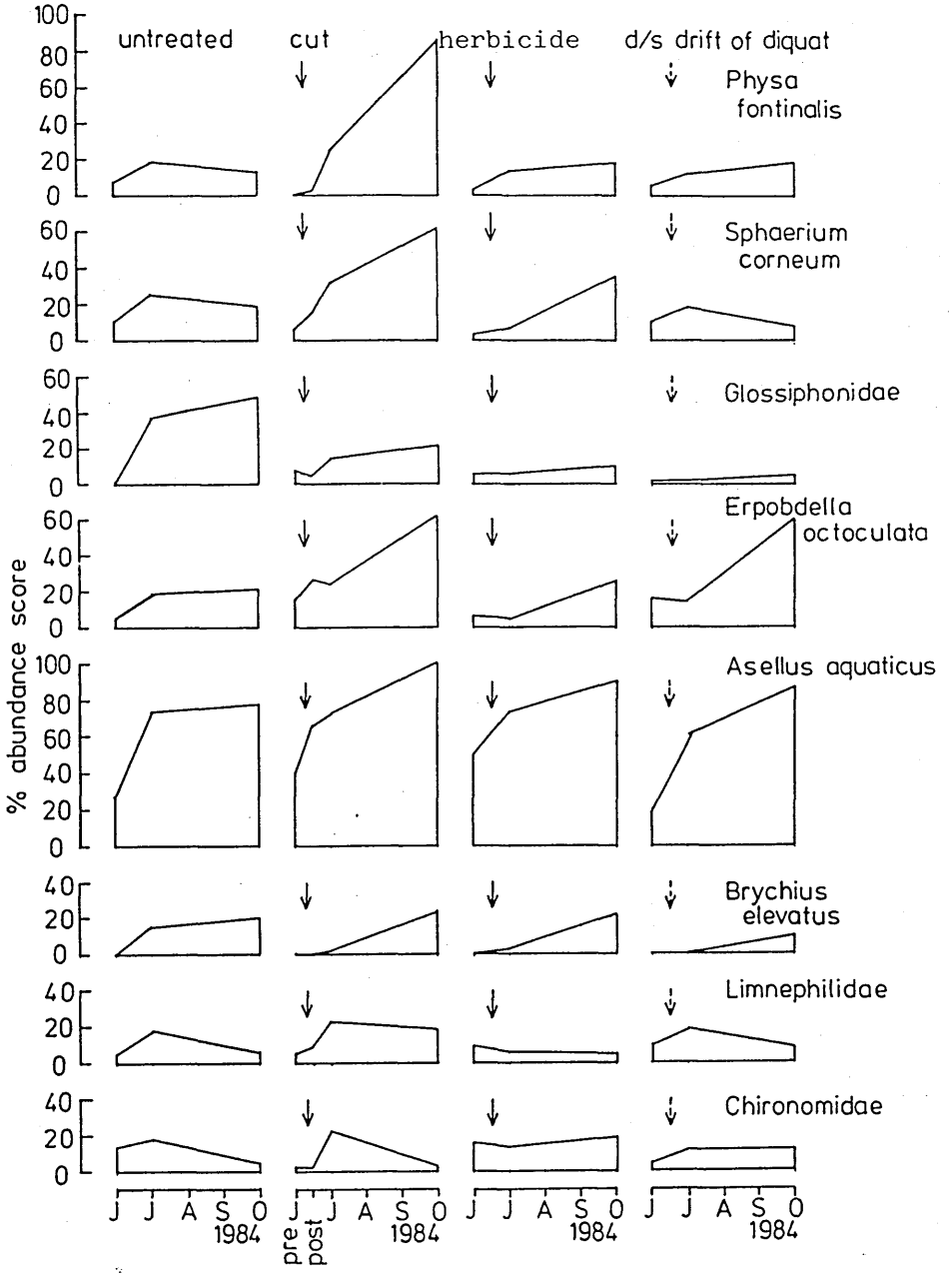
Table 20

Shannon-Weaver diversity indices for the macroinvertebrates in the Union Canal

	1.6.84	2.6.84	19.7.84	19.10.84
Herbicide	2.049		1.921	2.253
Cut	1.904	1.929	2.215	2.208
Untreated	2.158		2.186	2.178

Fig. 66

Macroinvertebrate data from the Union Canal



6.4 Discussion of the Union Canal Trial

6.4.1 Macrophytes

Although the pre-treatment biomass results did not significantly differ between the treatments, there did appear to be differences in the floral composition. There was a greater proportion of floating and floating-leaved macrophytes in the herbicide sections, compared with a prevalence of submerged plants in the cut and untreated sections.

These differences in macrophyte community composition had not been evident when the site was first visited. The disadvantages of the segregated experimental design were aggravated by these pre-treatment dissimilarities.

Untreated sections

The two untreated sections at the upstream end of the site had similar biomasses of macrophytes in June but considerable differences developed between the replicates during the summer. The diversity of flora remained similar in both sections but the biomass of all species (except Lemna) remained low in the upstream section throughout the season.

This discrepancy between the untreated replicates may have arisen because there appeared to be a moderately high loading of suspended solids, which limited the light penetration into the upstream section. This was the only section in the site in which the water was noticeably turbid. The source of the suspended silt was probably a point 400m upstream of the site, where sediments from dredging operations further upstream were being dumped. Suspended sediments may have drifted downstream from this dump as far as the upstream untreated section, but did not reach the downstream section in the same quantities.

The differences in biomass between these two replicates will have contributed to the high error terms in the ANOVA. The possibility that the macrophyte production of the downstream replicate was also limited by shading from the suspended material, reduces the value of these sections as control sections against which to compare the effects of the other treatments.

The canal water was flowing in the downstream direction at the time of the herbicide application. This resulted in the extra 'untreated' section, downstream of the herbicide treatments, being affected by the downstream drift of the diquat. Diquat residues were found to have reached this section within an hour of the applications of Midstream, but the availance of the herbicide in this section could not be estimated.

The biomass of submerged macrophytes remained very low in this section after the herbicide treatments, despite having had a pre-treatment biomass greater than most of the other areas in the site. The slow flow of water and large gap below the herbicide sections, make it unlikely that the diquat-alginate would have drifted downstream from the points of application. The reduction in submerged macrophytes may have resulted from the downstream drift of diquat in solution. The floating and floating-leaved species did not appear to be affected by the diquat drift.

Cutting treatment

The cutting had the immediate effect of reducing the biomass of submerged vegetation in the downstream cut section, but no such reduction was apparent in the upstream section. The biomass of Potamogeton natans in the upstream section had increased from the pre-cut value, immediately after the cut. The immediately post-cut samples were collected before the cut material had been removed by the rake attachment on the Wilder boat. Thus, if cut material had not drifted out of the section, more vegetation might have been caught and pulled out of the water on the grapnel than if the plants were still rooted. This does not explain the differences between the sections unless the cut material drifted out of the downstream section but not the upstream one.

The biomass of submerged vegetation was similar in the two cut sections in July. The biomass did not change between July and October and was higher than in either the herbicide or untreated sections.

From a comparison of post-treatment biomass data, it appeared that in comparison with the untreated sections, the cutting treatment considerably stimulated the growth of the submerged macrophytes, (principally the dominant Elodea canadensis). A stimulation of the regrowth of submerged species after cutting, has been observed in

canals by Eaton et al. (1981), and Eaton and Freeman (1982), and was similar to the regrowth of Ranunculus, reported to occur after cutting in chalk streams (Dawson 1976, Ham et al. 1982).

However, as a result of the segregated experimental design and the unexpected interference of suspended materials in the untreated sections, it cannot be assumed that if left unmanaged the biomass of submerged plants in the cut sections would have remained at less than a quarter of the biomass which resulted after the cut. What can be assumed is that regrowth of Elodea did occur after cutting, and that more than one cut per season would have been required to maintain the pre-cut biomass.

Another factor which would have allowed the development of a greater biomass of submerged plants in the cut sections, compared to the other treatments, was the lower percentage cover of the water surface by Lemna and P.natans. The growth of P.natans in the cut sections was no greater after cutting than in the untreated sections.

The cover of Lemna was always less in the cut sections than in either of the other treatments. The cutting process may have dispersed the Lemna and some will have been removed in the rake, with the cut material. Lemna can recolonise suitable areas, and grow so quickly that it is unlikely that the cutting process alone was responsible for reducing the cover of Lemna 47 days later, in comparison with the other sections.

It is likely that the Lemna cover in the cut sections was prevented from reaching 100% because these sections were the only ones orientated in the direction of the prevailing Westerly winds. The Lemna would have been blown out of the cut sections, against the slow water flow (Duffield and Edwards 1981). This dispersal of Lemna would reduce the shading of the submerged plants allowing faster growth than in the other well-shaded sections.

Herbicide treatments

The availance estimates for the diquat residues, up to 40 minutes after treatment, were comparable to those found in the Mouse Water and the R.Petteril. Because of the long retention time of treated water in the herbicide sections of the canal, the total availance of diquat was probably much greater than in the rivers.

By July the biomass of submerged vegetation in the herbicide sections had been reduced from the pre-treatment value and was

significantly less than in the untreated sections. By October some regrowth had occurred in one section, but no submerged vegetation was found at all in the downstream section.

More P.natans occurred in the upstream herbicide replicate than in any other section in the site, and there was little change in its biomass after the herbicide treatment. The biomass of P.natans in the downstream section increased after the herbicide application and in July there was not a significant difference between the total biomass of vegetation in the herbicide and untreated sections.

A few P.natans stems which had lost their floating leaves were observed in the herbicide sections in July, but apart from the localised burn marks on a few floating leaves directly hit by the Midstream, most plants were apparently unaffected.

The high percentage cover of the water surface by Lemna and floating P.natans leaves would have shaded submerged plants. Thus the herbicide may not have been solely responsible for the low biomass of submerged macrophtyes during the summer, competitive shading by other species may have also contributed.

The untreated sections had a complete cover of Lemna by July but still had a significantly greater biomass of submerged vegetation than the herbicide treated sections. This suggests that the herbicide may have reduced the biomass of submerged plants before the Lemna and P.natans cover developed. The shading in the untreated sections may have limited growth of submerged species but only after an initial period of growth between the time of the pre-treatment survey and the establishment of the Lemna mats.

Reasons for the poor susceptibility of P.natans to diquat-alginate have been discussed in Sections 4.6.4 and 5.6.3. The problem of achieving an efficient dispersal of the diquat-alginate onto P.natans plants is more acute in static or slow-flowing water, because the alginate strings, not caught on surface leaves,will fall straight through the water column and will not be carried horizontally by water currents onto the P.natans stems. The gel is only likely to stick onto submerged leaves which provide a sufficient horizontal surface area, to intercept the descending alginate strings. The small surface area of submerged P.natans plants exposed to the high concentrations of diquat in solution, may have contributed to the lack of damage to this species.

Lemna spp. are highly susceptible to diquat, and are used for very sensitive bioassays for the herbicide (e.g. O'Brien and Prendeville 1978). Only a small proportion of Lemna will have been directly hit by the jet of Midstream from the sprayer, but the concentrations of diquat in the water would have been sufficient to kill most of the Lemna present at the time of treatment.

A difficulty in trying to manage Lemna is that recolonisation and regrowth can be very rapid, especially if fronds can be blown or drift into the cleared sections, from untreated areas. Thus, it is possible that the diquat would have immediately killed much of the Lemna but that the population had recovered, and grown, within the 47 days before the July sample.

The degree of sucess of these management regimes will depend upon the reasons for which the management was required. Lemna and P.natans do not restrict the passage of water along the canal (except where Lemna blocks culverts), because these plants occupy only a small proportion of the channel volume. Submerged species, such as Elodea, fill a large proportion of the channel volume and by reducing water velocity in the weed beds, encourage the accumulation of silt, reducing the cross-sectional area of the channel even further.

Thus, if the maintainance of a deep channel through which water could easily flow, had been the primary objective of the canal management, then the herbicide, and not the cutting, treatment was successful. The high percentage cover of the water surface in the herbicide sections would help to prevent the regrowth of submerged plants, by shading. The cutting treatment appeared to have the opposite effect, with the substantial growth of Elodea presenting a major obstruction to water flow.

If the intention of the canal management had been to clear the vegetation from the water surface, to facilitate fishing and boating, then the herbicide treatment would not have been satisfactory. The cover of the water surface appeared to be reduced in the cut sections but it would be necessary to repeat these trials with the treatments interspersed, or at least swapped around throughout the site, to be certain of distinguishing between treatment effects and the differences between the macrophyte communities occurring along the canal.

6.4.2 The ecological effects of the management regimes

Water chemistry

There was no evidence that the water chemistry of the canal was directly influenced by the management regimes. Differences occurring between sections, for most parameters, showed an upstream-downstream gradient, which was evident in the pre-treatment samples. Reasons for such gradients, or for the often substantial differences in parameters between sampling times, are not obvious but are likely to depend upon the amount and types of water input along the canal. As well as being provided with water from the feeder-stream, a canal can receive water from industrial effluents, groundwater and a variety of drainage sources,which may vary substantially in water quality (Hyde 1977).

Some of the water chemistry parameters, such as dissolved oxygen and pH, may have been influenced by the amounts and type of vegetation in the sections. High biomasses of submerged vegetation tend to supersaturate the water with dissolved oxygen, during the day, but water underneath a layer of floating vegetation is usually oxygen depleted. This depletion results from the lack of a clear water-air interface at which reaeration may occur. The need for reaeration is particularly important if there is an oxygen demand by the sediments or by submerged vegetation which is dying as a result of severe shading. This sort of relationship may be evident from the dissolved oxygen measurements in July, especially if a slight downstream displacement of the effect is assumed as a result of water flow.

Thus, even if the water chemistry has been influenced by the composition of the vegetation, it is still uncertain as to how much the management treatments were involved in determining the macrophyte communities. The effects of the management on the water chemistry are likely to have been indirect effects, if they existed at all.

Macroinvertebrate communities

As with the water chemistry it is not possible, from these data, to ascribe any differences in the macroinvertebrate communities of any sections of the canal, to the direct influence of the cut or herbicide application. Some taxa appear to have been surprisingly little affected by the differences in the macrophyte communities in the various sections. The numbers of animals in other taxa, such as the molluscs, appear to be related to the biomass of submerged vegetation,

which these animals would use as a substrate.

The distribution of Glossiphidae, which are most numerous in the untreated sections, could be related to the disturbance of the canal, by cutting or the use of the herbicide, but it is not possible to distinguish such a relationship from a difference in the distribution of the animals occurring along the length of the canal, regardless of the treatment.

The important points are that no taxa have been lost as a result of either treatment and that the diversity of invertebrates increased slightly in all treatments during the summer.

6.4.3 The sampling methods used in the Union Canal

The collection of macrophyte samples from canals using a grapnel is quick, convenient and, because is does not require the user to leave the towpath, is relatively safe. Murphy (1980) provided a synopsis of the work which led to the calculation of calibration constants for converting biomass results per grapnel to dry standing crop values per m^2. These calibration constants were calculated using data collected from a variety of macrophyte communities and canal sizes, and this diversity of samples was given as a justification for using a single calibration constant in most canals.

However, when samples are collected with different macrophyte species compositions, it becomes evident that some plants are caught and retained more efficiently by the grapnel than others. Most of the submerged species, such as Elodea canadensis, Potamogeton crispus, and Callitriche spp., easily become tangled in the grapnel and the stems break free from adjacent vegetation. Mats of filamentous algae tend to be so tangled that they have a high tensile strength, and large areas of surrounding mat may be collected in addition to the material with which the grapnel made direct contact.

Other species with thin, strap-like leaves or stems, such as P.natans or Sparganium emersum, may be caught on the grapnel but will be easily pulled from between the prongs by the strong rooted stems, unless they become tangled in other vegetation on the grapnel.

Sampling by grapnel may provide an accurate estimate of the biomass of small-leaved, bushy, submerged macrophytes like Elodea, but there will tend to be a bias towards an overestimation of mats of filamentous algae. Smooth, thin stemmed, or leaved, species may tend to be underestimated in grapnel samples,unless the samples have a large amount of other vegetation in which these species are caught. Thus, the estimation of biomass of species like P.natans will vary in accuracy depending upon the composition of the macrophyte community sampled.

The sampling of Lemna by grapnels is also unsatisfactory, because the amount of Lemna collected as a grapnel is pulled up from the water surface, will depend upon the amount of vegetation on the grapnel on which the Lemna will be caught. For example, there will be little Lemna collected on an empty grapnel, but a grapnel covered in filamentous algae will trap a large amount of Lemna.

The bias in the sampling of different macrophytes by grapnel, will also affect the macroinvertebrate samples. Macroinvertebrates associated with plant species which are easily caught and removed by the grapnel, will be trapped in the vegetation and may be brought to the water surface with relatively little loss. Animals associated with plants which may slip through the grapnel, are likely to be dislodged and lost during the removal of the grapnel. Thus, in addition to the low efficiency of sampling of the host plant, the sampling efficiency of macroinvertebrates associated with thin-leaved and stemmed plants will be even lower.

Some sediments may be collected with the vegetation in the grapnel samples this is a poor method of sampling benthic invert-ebrates. The amount of sediments retained in grapnel samples will depend upon the retention of the macrophyte species in the sample. Thus, the proportions of invertebrates in a grapnel sample, from the benthos or associated with the macrophytes, will depend upon the type of macrophytes in the sample.

6.4.4 Comparisons of the canal and river trials

One of the major differences between the canal and river systems studied, was the water velocity. This not only affects the rate of dispersal of the diquat residues from the treated sections, but will also affect the impact that the plants have on the water chemistry.

The period of exposure to diquat residues in the canal was likely to have been considerably longer than the hour over which samples were taken. Thus, despite the lower concentrations of diquat in the canal (resulting from the greater depth of water in the treated areas), the availance of the heribicide was likely to have been similar, if not larger, than in the over-dosed R.Petteril and Mouse Water. The diquat may not have drifted as far downstream before being adsorbed, in the canal as compared with the swiftly flowing rivers, but the availance of these residues would have been greater. Thus, although the placement of the diquat-alginate might be more localised in the canal, as in static water, the dispersal of soluble residues of the herbicide would be greater than in static water and more persistent than in fast-flowing water.

The severity of any impacts that the vegetation (e.g. Lemna cover), removal of vegetation (e.g. after a cut), or the decay of vegetation (e.g. from herbicide-induced death) may have on the water chemistry, would be more localised but greater in the canal, compared to a river which has a swift mixing and dilution of treated with untreated water. Static, or slow-flow, water conditions are also more favourable, for the growth of macrophytes with floating leaves, than swiftly-flowing rivers. The presence of these plants, and the lack of shallow riffles, would reduce the opportunites for reaeration of water in which the concentrations of dissolved oxygen had been depleated, by the removal of photosynthesising vegetation, especially if the biological oxygen demand is increased by the presence of herbicide-treated plants decaying in situ.

The differences in water velocity, between canals and rivers, will also affect the species composition of the macroinvertebrate communities. In the canal these communities are likely to be of an intermediate nature, between those animals adapted to (and often requiring for feeding) a flow of water, and those intolerant of water movement. In term of the effects of macrophyte management, it might be suggested that animals in a fast-flowing river, with a stony

substrate, may adapt to living on that substrate,after the removal of vegetation, more easily than macroinvertebrates displaced from the vegetation onto a soft, silty substrate, as found in conditions of slow flow. The water current in rivers would also lead to a more efficient and rapid recolonisation, by drift from upstream, of cleared areas, than would be expected in static or slow-flowing systems.

An observed difference between the biomass of macrophytes in the canal and the rivers, was the absence of a reduction in biomass in the canal, in October. There is usually a large increase in the discharge of water in rivers in the autumn (e.g. Dawson 1976). This tends to wash-out much of the moribund vegetation which has collected in the late summer, after flowering. This will explain the often large reductions in plant biomass recorded in the rivers between July and Octber.

This wash-out does not occur, or at least not to the same extent, in the canal, in which discharge is regulated. As in lakes, dead vegetation in the canal will eventually fall to the bottom, and add to the organic content of the benthic sediments.

6.5 Conclusions of the Canal Trial

1) The cut and removal of submerged plants in the Union Canal caused an initial reduction in biomass but within a month there had been a vigorous regrowth of the dominant Elodea canadensis. The surface cover of Lemna was less in the cut sections than in the other treatments, where growth of submerged plants may have been limited by the light reductions caused by the Lemna mats. Lemna may have been removed from the cut sections when the cut weed was lifted out. Otherwise the prevailing winds may have blown the Lemna out of the cut sections.

2) The biomass of submerged plants was low after treatment in the herbicide sections. The floating and floating-leaved species were little affected and these plants may have limited submerged plant growth by shading.

3) The availance values of diquat residues in the herbicide sections were high, due to the long retention time of the treated water. There did appear to be some downstream drift of the diquat and its effects.

4) There were greater differences in the water chemistry between sections than had been observed in the rivers. Differences in the concentrations of dissolved oxygen were more likely to be related to the cover of Lemna than to the management method applied to a section.

5) The numbers of Mollusca appeared to be related to the amount of submerged vegetation, and so were highest in the regrowth of Elodea in the cut sections. There were no marked reductions in numbers of any taxa, which could be related to treatment.

6) The results of this trial had to be interpreted with caution for two reasons:

   Lack of segregation of the replicates of each treatment.

   Possible species bias in the method of plant and macroinvertebrate sampling, using a grapnel.

CHAPTER SEVEN

THE USE OF LARGE-SCALE RECIRCULATING CHANNELS TO INVESTIGATE THE ACTIVITY OF DIQUAT-ALGINATE IN FLOWING WATER

7.1 Introduction

7.1.1 The use of artificial channels for experiments in flowing water

Extrapolating the conclusions of experiments, directly from laboratory responses to field conditions, is usually a dubious procedure (Barrett 1981a). This is especially true when working with diquat-alginate because its activity in the field appears to be closely related to the physical properties of the viscous formulation. These effects are lost when very small quantities of the herbicide are used in laboratory-scale experiments.

In order to overcome these difficulties, whilst experimentally determining the causes of any ecological effects of diquat-alginate use, which may have been observed in the field, an intermediate system is needed. Such a system should permit a degree of control over the variable(s) under investigation, but would need to be on a large enough scale to simulate some of the interacting factors found in a river ecosystem. This scale should be sufficiently large to allow the expression of the physical characteristics of the diquat-alginate formulation.

The most suitable intermediate channel system would have facilities for manipulating some of the physical characteristics, (such as water depth, flow rate, substrate type) and it would be possible to establish specific macrophyte populations, and to some extent, macroinvertebrate communities. Other factors, such as climatic conditions and the development of the biota, would not be artificially determined, so that the system retained some relation to field conditions.

There are two types of artificial channel which could fulfil the requirements of such an intermediate system:

1) Throughflow channel

2) Recirculating channel

The advantages and disadvantages of each type are listed by Ladle et al. (1981). The chief difficulty of maintaining a throughflow system is the provision of a large and consistent water supply. Such channels have been developed to study the growth of Ranunculus fluitans (Eichenberger and Weilenmann 1982), and to study the efficacy of herbicide/adjuvant mixtures in flowing water (Getsinger and Westerdahl 1984). In both of these examples only the macrophytes, no other part of the ecosystem, were under observation. If the effects of a herbicide application on water chemistry and non-target organisms are to be observed from the treatment of several weed beds, either a very long channel is needed, or a means of prolonging the retention time of the water over the treated plants, so that the effects become cumulative each time the water passes over the treated plants. This points to the use of recirculating channels, which have the advantages of requiring only a small water supply, and of being compact.

The recirculation of herbicide treated water might be considered as a disadvantage in terms of trying to simulate a throughflowing river. Instead the results of a treatment in a recirculating channel should be regarded as the cumulative effects found at the downstream end of a treated length of river, over which all the treated water from upstream has passed.

The recirculating channels belonging to the Freshwater Biological Association (F.B.A.) were available for this project. It was originally intended that three channels would be used for simultaneous herbicide, cut and untreated treatments. Only two channels could be used,so only experiments comparing the effects of a diquat-alginate treatment with an untreated control, were carried out.

7.1.2 A description of the Waterston Experimental Recirculating Channels (W.R.E.C.s)

The F.B.A. maintains three recirculating experimental channels at an outstation at Waterston (N.G.R. SY742953). The first channel was constructed in 1974 and was described by Ladle et al. (1977). Two more were built in 1980 which were slightly longer, and had other modifications, such as a lowering of the outfall of the recirculation pump. Although all three channels are of essentially the same design, the differences in details between the prototype and the two later channels prevent them from being regarded as three identical replicates.

Only the two identical channels were used in this work, and for this reason the following detailed description may be found to differ slightly from the earlier publications which refer to the original channel.

The channels are constructed from fibre-glass reinforced plastic sections, which are bolted together in a race-track shape, with a trapezoidal cross-section (Fig. 67, Plate 4). For these experiments the channels were approximately half-filled with a gravel substrate, with a 1:250 gradient. The channels are partially buried in the gravel of a disused watercress bed and the surrounding groundwater, which is at a constant 10°C, stabilises the temperature of the water in the channels.

Dimensions

The dimensions of the channels are shown in Table 21. Some of the volume and area measurements differ between the channels because of differences in the depths of gravel or water. These depths are the mean values of sixty readings taken from all around each channel.

Sampling was restricted to the six straight sections on each side of the channels (indicated in Fig. 67), where the water flow was most uniform. To reduce algal growth in the unsampled areas the covers were placed over the corner sections.

Inflow pumps

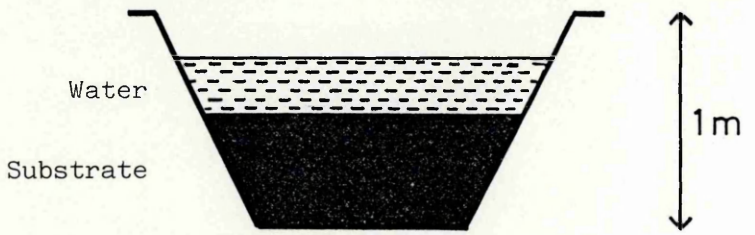
Water is extracted by two electric pumps from a chalk aquifer borehole, which was originally 54.8m deep. The maximum capacities of

Plate 4

Longitudinal view of channel 3 prior to macrophyte establishment in September 1984, (channel 2 in background, right)

Fig. 67

Cross-section and plan of a recirculating channel



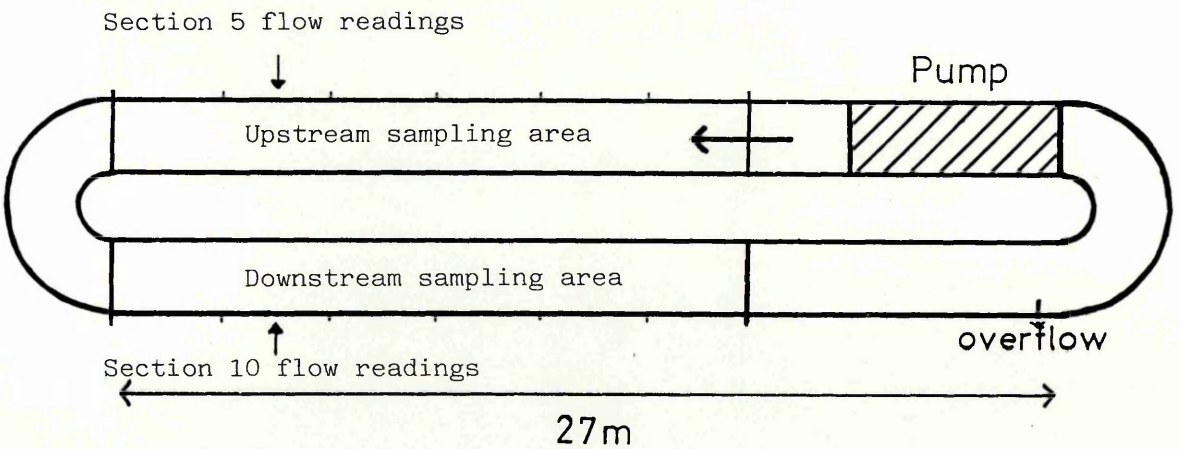


Table 21.

Dimensions of the recirculating channels.

	Channel 2 Untreated		Channel 3 Treated
Total length		67.4m	
Width at rim		2.02m	
Width at base		1.0m	
Width at water surface	1.73m		1.69m
Width at gravel surface	1.53m		1.48m
Depth of channel		1.0m	
Depth of gravel	0.53m		0.48m
Depth of water	0.26m		0.31m
Gravel surface area	93.8m^2		90.8m^2
Sampled area of gravel per section	32.5m^2		31.5m^2
Sampled area of gravel per channel	65.0m^2		63.0m^2
Water surface area	114.8m^2		112.3m^2
Submerged surface areas suitable for algal colonisation (not covered) Channel sides	30.6m^2		35.5m^2
Total (gravel surface + sides)	109.9m^2		112.2m^2
Total channel volume to rim		101.3m^3	
Volume of channel occupied by gravel	40.8m^3		36.7m^3
Volume of water above gravel surface	28.3m^3		27.9m^3

these pumps are:

small $7m^3\ hr^{-1}$

large $30m^3\ hr^{-1}$

During normal usage the small pump was used to provide $2.25-2.5m^3\ hr^{-1}$ to each channel. The $30m^3\ hr^{-1}$ pump was only used when chemicals were being diluted out of the channels.

Water is lost through overflow notches (Fig. 67), and flows away under the channels to join the Waterston stream, which was built as a branch of the R.Piddle to feed the watercress beds. The water depth in the channels can be regulated by connecting outflow pipes of adjustable height.

The chemical composition of the borehole water, described by Ladle et al. (1981), is almost constant and is thought to be similar to many natural springs (Ladle et al. 1977).

Recirculation pump

The water in each channel is recirculated by an Archimedian screw pump, powered by a seven horse-power electric engine. These motors are geared to allow a choice of three pump speeds. At the slowest rate filamentous algal blooms can cause severe problems. It was thought that it would be difficult to anchor newly transplanted vegetation securely in the fastest flow, so the middle pump speed was used.

It is difficult to give 'average' values of the water velocity for the channels, especially when they contain obstructions such as beds of submerged macrophytes. A comprehensive set of water depth and velocity measurements were made at points around each channel, prior to planting with vegetation, using an Ott propeller flow meter. Boulders were placed on the bends to disperse the flow more evenly along the downstream sampling section.

7.1.3 A review of work in the Waterston recirculating channels

The channels have been intensively studied since their construction. Most of the initial work was concerned with the effects of recirculation and flow on the water chemistry, under various controlled conditions, and with the colonisation of the substrate by bacteria, algae and macroinvertebrates.

Studies of the water chemistry and bacterial populations, made in the absence and presence of a gravel substrate, have shown that the substratum exerts an important stabilising influence on the concentrations of dissolved elements, particularly calcium, reactive phosphorus and silica (Ladle *et al.* 1977).

The colonisation and population dynamics of algae in the light or dark, and in variable conditions of water velocity and retention time, have been investigated. These population dynamics have been related to changes in water chemistry (Marker and Casey 1982, Marker *et al.* 1984) and to the invertebrate grazer populations (Ladle *et al.* 1981).

The colonisation and ecology of macroinvertebrates has been described both in terms of the whole community, in which Chironomidae and Ephemeroptera play an important role (Ladle *et al.* 1980), and in terms of the growth and production of specific groups. Examples of this autecological approach were studies of:

- the larvae of the net-spinning caddis, *Polycentropus flavomaculatus* (Bass *et al.* 1982)
- the productivity of five species of Ephemeroptera larvae (Welton *et al.* 1982a)
- the larval growth and production of three species of Chironomidae (Ladle *et al.* 1984, Ladle *et al.* 1985, Pinder 1985)

The specialised conditions of the channels, in which the substrate can be replaced, has made it possible to test and compare methods for estimating macroinvertebrate colonisation (Welton *et al.* 1982b).

All of the work carried out in the original channel during the first three years of its use, has been reviewed for water chemistry and algae by Marker and Casey (1983), and for macroinvertebrates by Ladle and Welton (1984).

The channels have also been used as a system in which models of physico-chemical processes, such as calcite precipitation and the the co-precipitation of phosphates (House et al. 1986b) and gaseous exchange (Shelley et al. 1987, Fox et al. 1987), have been tested.

This is not an exhaustive review, a list of at least 36 publications, relevant to the work in the channels, was compiled in September 1986.

Although some of the work discussed has involved some environmental manipulation (i.e. the addition of a substrate, removal of covers, the variation of recirculation velocity or water turnover), the organisms studied were not introduced but were allowed to colonise naturally.

In 1983-84 experiments were carried out to investigate the stocking capacities of the channels with introduced salmon and trout.

The present work was the first, in these channels, in which macrophytes have been introduced and studied. Without previous work of this nature to refer to, the initial period of the study was spent in attempting to provide conditions in which the plants could become established and grow.

Prior to, and during, the herbicide trials, various physical characteristics of the channels were assessed, so that the behaviour of the diquat residues could be predicted and related to observations of residue dispersal in rivers. The following two sections 7.2 and 7.3 will deal with these assessments.

The channels are distinguished by numbers 1-3. The original channel is channel 1. In these experiments channel 2 was the control and channel 3 was treated with herbicide.

7.2 Estimation of the Water Recirculation Time

7.2.1 Methods for estimating circulation time

A variety of methods have been developed for measuring the discharge of water in natural and artificial channels. These can be divided into two categories:

Gauging weirs

Velocity-area methods

The discharge itself is not important, in estimating the circulation time of a constant volume of water in a channel, but the same methods of measuring water velocity which would fall into the second category can be used. The three types of method for measuring water velocity are:

	Examples
Floats	Surface-, subsurface-, captive floats, floating rods
Current meters	Propeller-, cup meters, pitot tubes
Chemical tracers	Dyes (e.g. fluorescein, potassium permanganate, Congo red), radio-isotopes, salt-velocity or dilution

Most of these methods have been reviewed by Linford (1961), King and Brater (1963) and Pilgrim and Summersby (1966).

Prior to the herbicide treatments, estimates of circulation time were derived from current meter measurements but later, accurate estimates were made in channel 2, employing methods from each of the three types of velocity measurement.

Float

The time taken for an orange to float around the channel, after its release just downstream of the pump, was recorded ten times. The orange passed undamaged through the pump in a consistent time, ($\bar{x}$ = 14.2 seconds, S.E. = 0.3 seconds, n = 10).

Oranges provide a simple, easily-observed, semi-buoyant float which is unaffected by wind (Pilgrim and Summersby 1966, Dawson and Robinson 1984).

Current meter

Measurements of water velocity were made with an Ott propeller meter at sections 5 and 10 of the channel (Fig. 67). Thirteen

measurements were made at 12.5cm intervals across the channel at each point, just below the water surface and at 0.6 x depth (mean velocity for the channel). There were no significant differences between the water velocities measured at sections 5 and 10, results from the former point are considered. The circulation time was calculated by dividing the mean channel length of 61.4m by the velocity.

Chemical tracers

The circulation time was also estimated by timing the passage of chemicals, added to the water for other experiments. The method used for timing the peaks of water conductivity, after the addition of concentrated salt solutions, is described by Shelley et al. (1987). This paper also describes the method used for estimating the time between the troughs of reduced oxygen concentration after the addition of sodium sulphite. The mean time from eight of these salt and sodium sulphite additions was calculated.

7.2.2 Results and discussion of the circulation time estimates

The results for the three methods are shown in Table 22, 't' tests were used to compare the estimates of circulation time.

The circulation times of the surface water,estimated using the current meter and the orange,were not significantly different. The small standard error for the float method, suggests that under these conditions, it was a reliable method for assessing surface water velocity.

The results of the tracer method and the 0.6 x depth current meter measurements were not significantly different, suggesting that the use of tracers gives an estimate of mean water velocity.

A comparison of the current meter results from the two depths showed a significant difference. The greater variance in the velocities at 0.6 x depth may have been due to the disruption of flow caused by the remaining Ranunculus and filamentous algae, near the bottom of the channel.

Estimates of mean velocity can be made from surface measurements by a conversion of:

mean velocity = 0.85 x surface velocity

(Linford 1961, Vennard 1963).

This relationship was tested and there was no significant difference between the circulation time estimated from this conversion of surface measurements,and from those made at 0.6 x depth.

The presence of Ranunculus beds in the channels on 9.8.85 probably explains the greater variation in the results from this date, but the circulation times of the two channels were not significantly different. There were also no significant differences between the two dates of measurement, in channel 2.

Table 22

Estimates of circulation time

	Mean (sec)	S.E. (sec)	n
Channel 2 26.9.85			
Surface flow measurements	173.4	4.9	13
Orange circulation time	162.1	3.6	10
0.6 x depth flow measurements	218.6	10.6	13
Tracer circulation time	226.0	7.6	8
Surface flow x 0.85	204.0	5.8	13
Channel 2 9.8.85			
Surface flow measurements	183.4	11.9	11
0.6 x depth flow measurements	207.6	14.3	11
Channel 3 9.8.85			
Surface flow measurements	192.8	3.1	11
0.6 x depth flow measurements	240.9	12.4	11

7.3 The Dilution of Solutes from the Recirculating Channels

7.3.1 Methods for diluting diquat residues out of the channels

To obtain permission from Wessex Water Authority to discharge diquat-treated water from the channel into the Waterston stream, estimates of the time and amount of diquat to be released had to be submitted. Methods for minimising the discharge were also requested, so it was proposed that the channel outflow would be directed through two pipes onto an area of grass adjacent to the channel. The water would have to percolate through grass and soil, which should adsorb most of the diquat, before entering the Waterston stream. The subsequent condition of the grass would indicate the residual toxicity of the outflow.

To minimise the retention of diquat residues recirculating in the channel, and so to more closely simulate a through-flow system, such as a river, the diquat needed to be removed from the channel as quickly as possible. There were two options as to how to achieve this:

1) After the required exposure period, to pump all the treated water out of the channel and refill with fresh water
2) To immediately and rapidly dilute out the treated water with the maximum input of fresh water

The first option would have been very artificial and risked damaging the plants by exposure to air, during the time that it would take to drain and refill the channel. The vegetation would be in contact with water containing the maximum concentration of diquat for the two hours that it would take to drain the water above the gravel.

Using the $30m^3\ hr^{-1}$ inflow pump, the second option appeared to be more realistic, simulating the input of fresh water from upstream in a river. Although at an inflow rate of $30m^3\ hr^{-1}$ the volume of the channel (approximately $50m^3$) would take less than two hours to fill, the continuous mixing of the fresh inflow and the treated water would result in an exponential dilution over a longer period.

To provide Wessex W.A. with the required information about discharge, and a basis for relating the channel work to rivers, advantage was taken of the regulated nature of the system, and models and tests of the herbicide dilution were made.

It was also intended that by making and testing a model for the dilution of an inert chemical, a comparison with the diquat residue results would show differences in behaviour due to the release of diquat from the alginate and the uptake by plants.

7.3.2 A theoretical model for dilution from a recirculating channel

The recirculation of the water in the channel is not itself involved in the dilution of treated water, except that it ensures efficient mixing with the fresh water inflow. The important properties of the channel, which can be manipulated or measured are:

V = total volume of water in the channel

f = flow in/out of the channel, volume per unit time

C_o= concentration of the inert solute at time 0

C_t= concentration of the inert solute at time t

where C_o and C_t are excess concentrations of the solute above the background value.

An equation can be derived to describe the concentration of the inert solute at any time t :

$$\frac{C_t}{C_o} = e^{-\left(\frac{f\ t}{V}\right)}$$

Assumptions relating to the use of this equation:

1) The volume of water can be measured and is either constant or any variation can be quantified per unit time.

2) Flow rates in and out of the channel are equal. This is assumed to be the case because there appeared to be little leakage and loss of water level when the channel was left without in/out-flow. The inflow could be measured regularly on a meter on the $30m^3\ hr^{-1}$ pump, and any variation in inflow rate could be included in the model.

3) The concentration of the solute at time t is estimated from samples assumed to be taken from fully mixed water.

4) Unless the system can be shown to be fully mixed immediately, the initial concentration of the solute (C_o) has to be estimated from the known amount of solute added to a known volume of water.

If a constant background concentration of the solute can be assumed, then the model can be simplified by taking the natural logarithm of the expression. This gives a linear equation which can be manipulated far more easily:

$$\text{Log}_e \left(\frac{C_t}{C_o}\right) = -\frac{f}{V} \cdot t \quad = \quad \text{Log}_e C_t = \text{Log}_e C_o - \frac{f}{V} \cdot t$$

By plotting Log_e (excess solute concentration) against time, a linear regression could be used to fit a straight line with an equation of this form. A statistical test can then be used to compare the linear regression of the data with a linear regression of values derived from the model.

A test described by Mead and Curnow (1983) determines whether there is a significant increase in the residual error of a single regression of all the data, compared with the sum of the residual errors of the two individual regressions.

		Degrees of freedom
Residual variation of data	Rd	nd - 2
Residual variation of model	Rm	nm - 2
Sum of residual variations	Rd + Rm	(nd+nm)-4
Residual variation about a single line	R	(nd+nm)-2

$$F_{df\ [2,(nd+nm-4)]} = \frac{[R - (Rd + Rm)]\ /2}{\frac{Rd + Rm}{(nd+nm-4)}}$$

This test only compares the relationship between the overall regressions and does not distinguish whether a significant difference is due to dissimilar gradients or y intercept. Tests to distinguish these differences are possible but are not necessary here.

Having derived an equation of the form:

$$\text{Log}_e\ C_t = \text{Log}_e\ C_o - \frac{f}{V} \cdot t$$

the value of C_o can be compared with that estimated from:

$$\frac{\text{Weight of solute added}}{\text{Known volume of water}}$$

Similarly, if the inflow rate has been measured, an estimate of the water volume can be calculated from the coefficient of t :

$$\frac{f}{V} = \frac{\text{inflow rate}}{\text{volume}}$$

and this may be compared with the known volume.

All linear regressions were calculated using MINITAB on the University of Glasgow's mainframe computer.

7.3.3 Choice of solutes for testing the dilution model

An inert tracer was required to test the model and to simulate the conditions for the herbicide application. Several such tracers have been used, especially in flow and discharge measurements. They consist of either:

1) Small additions of a material not normally present in the water, e.g. dyes (Waldermeyer 1958), elements such as lithium (Agg et al. 1961), or radio-isotopes (Pilgrim and Watson 1965).

or 2) Additions of common substances in sufficiently large quantities to elevate the concentration well above the background levels, e.g. potassium or sodium (Linford 1961).

Substances in the first category are usually expensive to buy, require complicated or unusual methods of analysis, or may have adverse effects on the biota.

Sodium chloride was chosen for these experiments because salt is cheap, quick and easy to analyse, and being a major constituent of natural waters would not be toxic, except in unnecessarily high concentrations.

The quantitative analysis of sodium

Sodium was analysed by atomic absorption using a Varian 375 atomic absorption apparatus. To avoid interference by potassium, 15ml of potassium chloride solution were added to 10ml of sample. This mixture was made up to 100ml with distilled water. Standard solutions with 0, 0.5, 1.0 and 1.5mg l^{-1} sodium were also produced and a calibration curve of the atomic absorption readings against concentration was plotted, from which the concentration of sodium in a sample could be read.

7.3.4 Estimation of the channel volume

Methods

An estimation of total water volume was made in each channel when the in- and out- flow of water was stopped but the recirculation was continued. A salt solution was made up from cooking salt, 750g in channel 3 and 850g in channel 2, in exactly 4 litres of water. Samples of these solutions were serially diluted and analysed to accurately calculate the total amount of sodium added (Na Add). Samples of the channel water were analysed prior to the salt addition to give the background sodium concentration of the channel (Na_o), and further samples were removed at intervals after the salt addition (Na_t).

The concentrated salt solutions were added just upstream of the pump to ensure good mixing, over the circulation period of about three minutes. After 24 hours the 30m^3 hr^{-1} inflow pump was used to dilute the salt out as quickly as possible.

The total volume of water in the channel was calculated from:

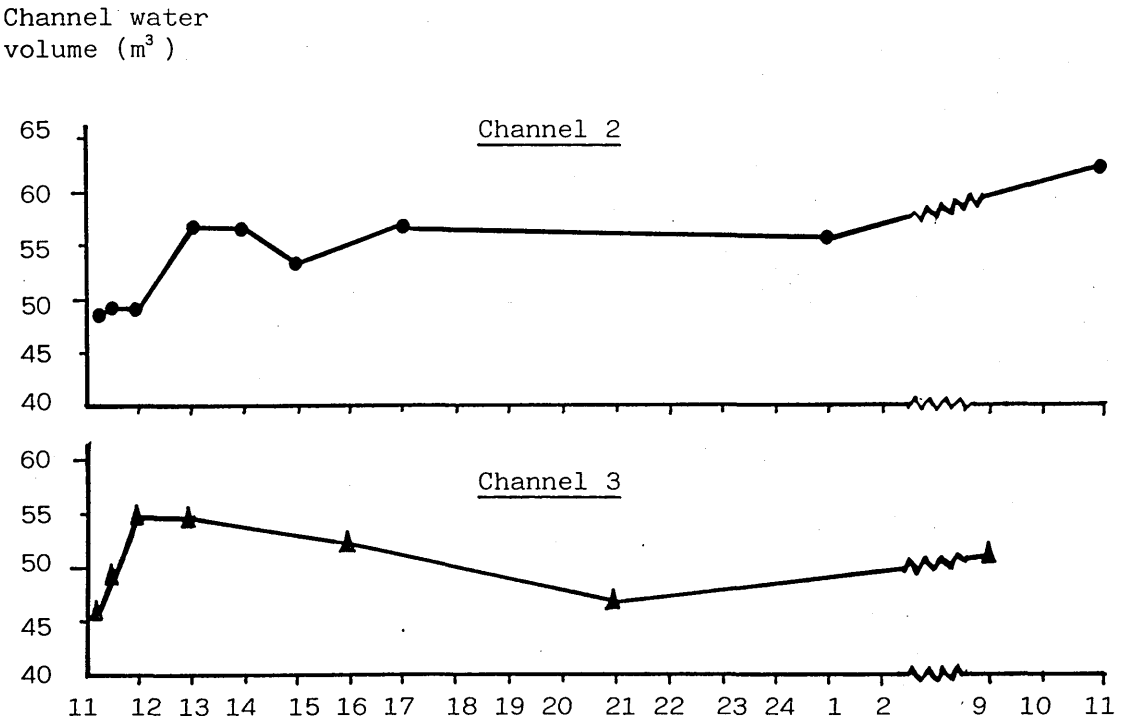
$$\text{Volume (litres)} = \frac{\text{Na Add (mg)}}{Na_t - Na_o \quad (\text{mg } l^{-1})}$$

Results and discussion of the channel volume estimates

The channel volume estimates over 24 hours are shown in Fig. 68. The low initial estimates of volume, within one hour in channel 2 and 30 minutes in channel 3, were probably a result of incomplete mixing of the salt solution into the total water volume, including the interstitial water in the gravel. With recirculation, the mixing of the salt in the water above the gravel, was seen to be fairly rapid, within 10 minutes (Shelley et al. 1987).

Fig. 68

Estimates of the volumes of the recirculating channels over 24 hours



Both experiments started at 11:00

Estimates of the time taken for complete mixing suggest that this is a fairly rapid process taking no longer than an hour (Marker and Casey 1982). Attempts have been made to measure the rate of penetration of salt into the gravel. Salt was detected by a conductivity electrode at 20cm depth in the gravel only 20 minutes after the addition of salt to the water above the gravel (Shelley et al. 1987). Penetration to the bottom of the gravel, which may contain a considerable accumulation of detritus, may take longer than 20 minutes but it would be difficult to measure this accurately without a major disturbance of the system which is being measured.

Assuming that one hour is an adequate mixing period, the mean volumes of the channels are:

$$\text{Channel 2} = 56.9m^3$$
$$\text{Channel 3} = 51.3m^3$$

There was no obvious pattern to the fluctuations in sodium concentration (and hence volume estimate) over 24 hours. The loss of water volume (most apparent in channel 3) during the day may have been the result of evaporation, the effect of which has not been estimated. The increase in volume overnight, may have been due to the condensation of water from the atmosphere, although no actual precipitation occurred during these experiments.

These fluctuations should be interpreted with some caution because the sodium concentration estimates were derived from single, unreplicated water samples. As will be seen, accurate determinations of the total water volume and complete mixing time are difficult to achieve, but are important for modelling chemical processes in the channels.

7.3.5 Testing the dilution model using sodium chloride

Three attempts were made to test the dilution model in channel 3:

1) Prior to the herbicide trials, but under the expected conditions, using the $30m^3\ hr^{-1}$ pump. The salt solution was added whilst there was continuous in- and out-flow. This experiment provided the basis for the predictions of the discharge of diquat, which were sent to Wessex W.A.

2) Repeat of 1) after the herbicide trials, with an exact simulation of the conditions at the time of treatment in the second trial. This experiment provided a direct comparison of the behaviour of an inert solute with that of diquat.

3) Prior to the herbicide trials, salt was added to a closed channel (no in-/out-flow) and was recirculated for 24 hours (volume estimation). The dilution of salt out of the fully mixed channel, using the $30m^3$ hr^{-1} pump, was recorded.

The salt additions for each experiment, in exactly 4 litres of water were:

	Weight of cooking salt (g NaCl)	Total sodium (from measured concentration of salt solution) (mg Na)
1)	1000	334000
2)	750	282500
3)	750	262200

The additions of the salt solutions and the sampling of the water, were carried out as for the volume estimates, except that replicate pairs of water samples were taken. The water samples were collected from the mid-width of the channel near the outflow, in 30ml glass vials which had been washed with chromic acid and were rinsed with channel water before each collection. The vials were kept in the dark at 4°C, and were analysed for sodium within 72 hours of collection.

Results and discussion of the sodium dilution experiments

Experiment 1)

The results of this experiment are plotted, without and with a Log_e transformation, in Fig. 69a, 69b. The excess sodium concentrations have been calculated assuming that the background concentration of 8.0mg l^{-1} sodium, from the T_o samples, was correct throughout the experiment. The linear regression of the Log_e plot of the data gives the equation:

$$y = 1.86 - 0.686\ x \qquad (a)$$

$$(Log_e\ C_t = Log_e\ C_o - \frac{f}{V}.\ t)$$

Fig. 69

Results of the sodium dilution in Experiment 1

Fig. 69a. Untransformed data assuming BG = 8.0mg l^{-1}

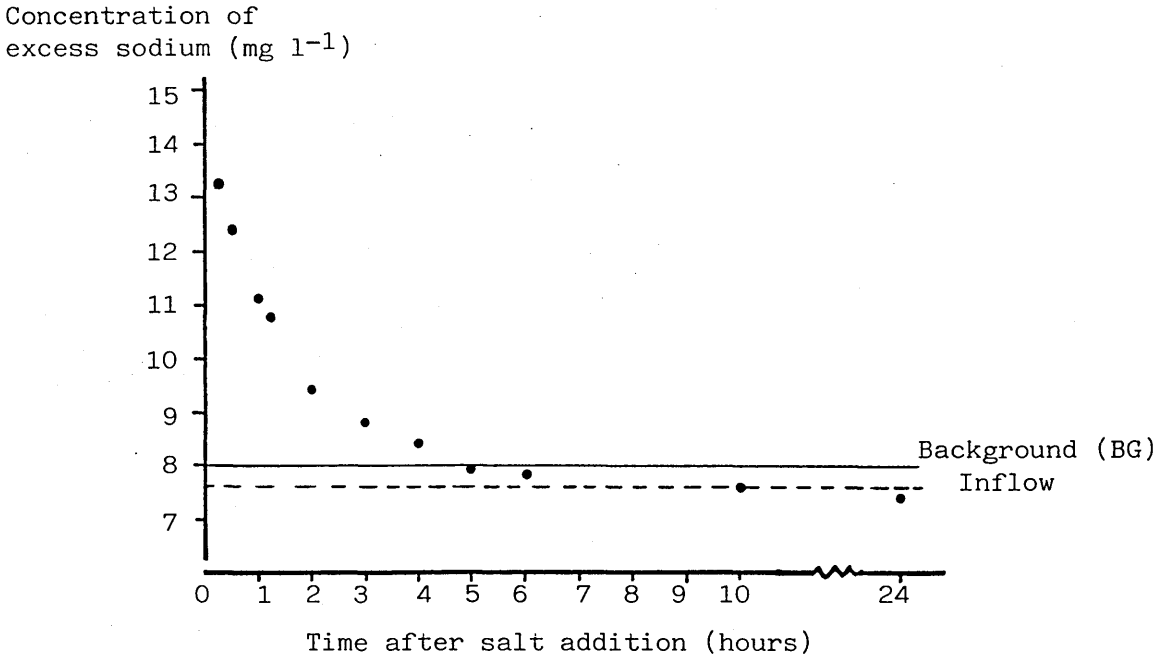
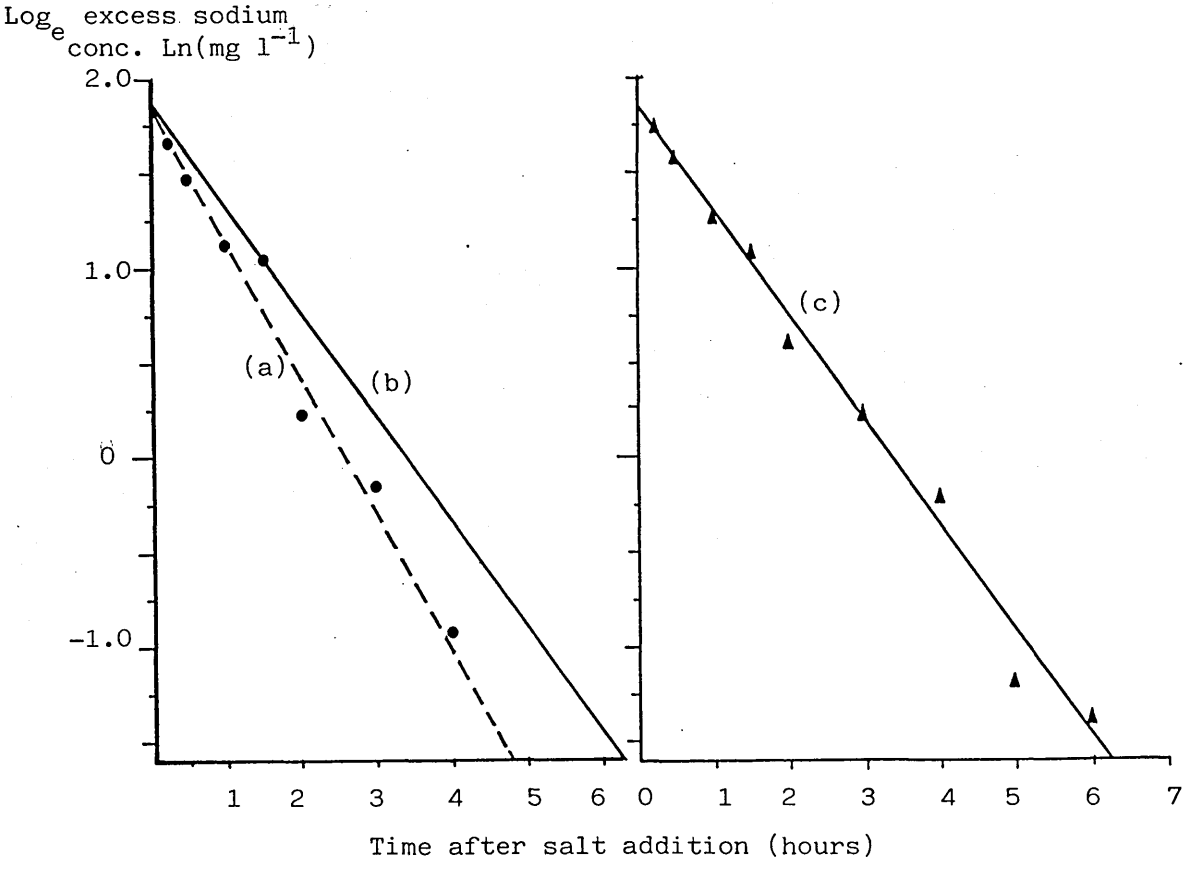


Fig. 69b

Log_e transformed data BG = 8.0mg l^{-1}

Fig. 69c

Log_e transformed data BG = 7.6mg l^{-1}



See text for regression equations (a), (b), (c).

From this equation C_o = 6.42mg l^{-1} sodium. Knowing that the total input of sodium was 334000mg, the volume of water in the channel can be estimated from C_o as V = 52.0m^3. This value compares well with results of the volume estimate from Section 7.3.4 of V = 51.3m^3.

However, when the dilution model was regressed using V = 51m^3 and C_o = 6.42mg l^{-1}, the regression equation of the model:

$$y = 1.86 - 0.565\ x \qquad (b)$$

was significantly different from the regression equation of the experiment results (a) (Fig. 69b).

The volume of water predicted from the regression of the results (a) (calculated from the coefficient of t, knowing that the inflow f = 28.8m^3 hr^{-1}) was V = 42m^3. Using this volume in the model, exactly the same regression equation was derived as for the results of the experiments (a).

Thus, there appears to be an incongruity between the model and the experimental results, relating to the water volume.

It was observed that after 5 hours, the sodium concentration in the channel tended to be less than the assumed background value (Fig. 69a). A sample of the inflow water taken 15 minutes after the salt addition, had a sodium concentration of only 7.6mg l^{-1}. If this value were used as the background concentration of sodium, the results of the experiment, (in terms of excess sodium, Fig. 69a) would have been underestimated by 0.4mg l^{-1}. When the corrected results were regressed (Fig. 69c):

$$y = 1.86 - 0.561\ x \qquad (c)$$

the estimate of C_o was still 6.42mg l^{-1}, but V was calculated from this equation as 51.3m^3. There was no significant difference between the regression of the corrected reults (c) and the model (b).

It is not clear why the initial concentration at T_o was higher than the background concentration of sodium occurring in the inflow.

Experiment 2)

The results of Experiment 2) are plotted in Fig. 70a, 70b. The excess sodium concentrations from 15 minutes after the salt addition were regressed:

$$y = 1.58 - 0.542\ x \qquad (d)$$

The sodium concentrations recorded after only 5 and 10 minutes, were likely to have been higher than expected because there would have only been mixing in a limited volume of water.

Knowing that the inflow rate during this experiment was $f = 27.78 m^3\ hr^{-1}$, the volume of water could be estimated from the regression of the results (d) as $V = 51.2 m^3$. This agrees well with the previous volume estimates.

Using the C_o value of $4.85 mg\ l^{-1}$ from the regression of the result (d), and $V = 51 m^3$ in the model, there was no significant difference between the regression of the results and of the model (Fig. 70b):

$$y = 1.58 - 0.544\ x \qquad (e)$$

The results of this experiment indicated that under the conditions which prevailed at the time of the herbicide trial, the model could be used to describe the dilution of salt out of the channel. The value of the model for prediction of solute dilution would depend upon a prior knowledge of the values of V and C_o.

These experiments have confirmed the volume estimate of $V = 51 m^3$. The estimation of C_o depends upon accurate measurements of sodium concentrations immediately after the salt additions, when there will have been very limited mixing, or upon accurate estimates of the total input of sodium.

In Experiment 1) the value of C_o predicted from the estimated input of sodium and water volume, was close to the value of C_o extrapolated from the regression of the results. In Experiment 2) if C_o was predicted from the water volume and estimated input of sodium, the resulting value, $C_o = 5.51 mg\ l^{-1}$, when used in the model gave a significantly different regression from the model using C_o derived from the results (e). It appears that the estimation of the input of sodium in Experiment 2) was overestimated by approximately 34000mg or 13%.

Fig. 70

Results of the sodium dilution in Experiment 2

Fig. 70a. Untransformed data for sodium dilution

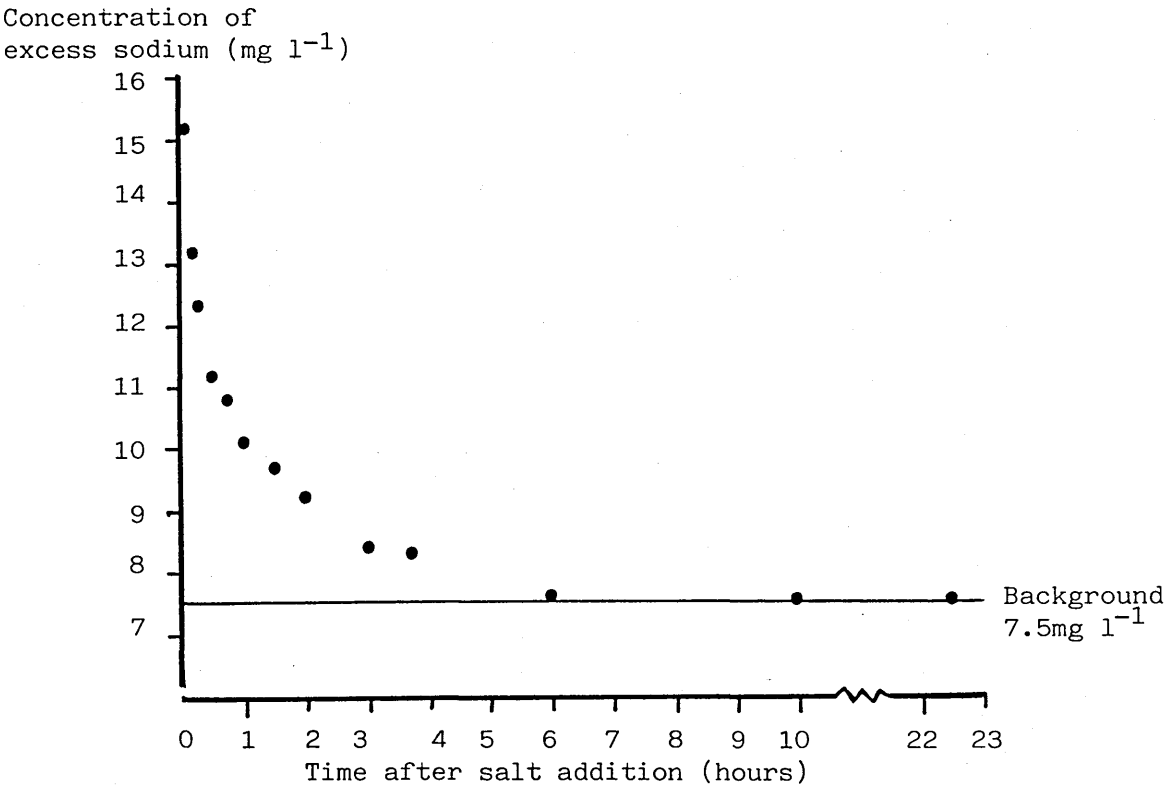
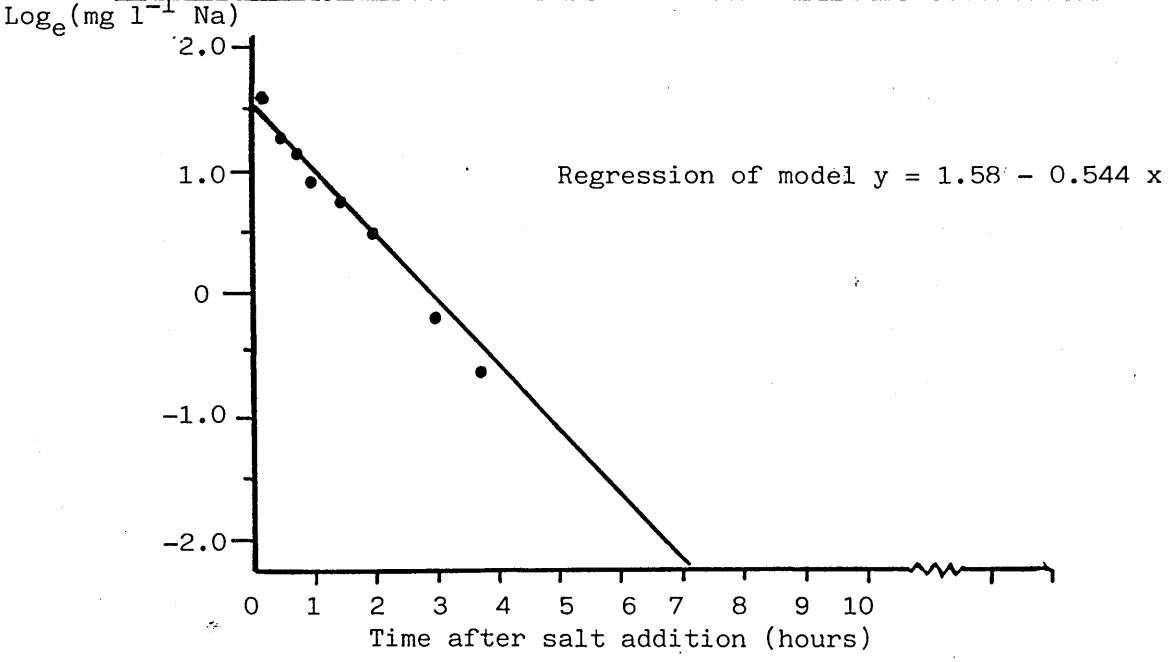


Fig. 70b Log_e transformed data with the regression of the model



It was hoped that Experiment 3) would help to resolve the problem of the discrepancies arising in the prediction or measurement of C_o . The salt had been recirculating in the channel for 24 hours prior to the dilution experiment, so that an accurate estimate of C_o could be made from a water sample collected just before turning on the $30m^3\ hr^{-1}$ pump.

Experiment 3)

The results of Experiment 3) are presented in Fig. 71a, 71b. The sodium concentrations 15 and 30 minutes after the salt addition did not appear to fit the curve of the excess sodium concentrations found after 45 minutes (Fig. 71a). A linear regression was only applied to the data collected later than 45 minutes after the salt addition:

$$y = 1.57 - 0.560\ x \qquad (f)$$

The value of C_o extrapolated from this equation was $4.81mg\ l^{-1}$. The water volume predicted from equation (f), (knowing that the inflow rate $f = 28.54m^3\ hr^{-1}$), was $V = 49.4m^3$, which was close to the previous estimates. Using the extrapolated value of C_o ($4.81mg\ l^{-1}$) and $V = 51m^3$ in the model, its regression was not significantly different from the regression of the experimental results:

$$y = 1.57 - 0.560\ x$$

The C_o value estimated from the mean of the two samples taken prior to the salt addition was $5.4\ mg\ l^{-1}$, which was larger than the value of C_o extrapolated from the regression (f). One of the samples, however, had a C_o value of $4.8mg\ l^{-1}$.

The C_o value predicted from the estimate of the sodium input and the water volume, was $5.11mg\ l^{-1}$. As in Experiment 2), this C_o value was larger than that extrapolated from the results and may have arisen because of an overestimation of the sodium input. The same weight of salt was added in Experiments 2) and 3) but the estimated inputs of sodium (282500mg and 262200mg) were different. The C_o values extrapolated from the regression of the dilution results, ($4.85mg\ l^{-1}$and $4.81mg\ l^{-1}$) were similar, suggesting that an error in the estimations of the total sodium inputs may have occurred.

The data from Experiment 3) were derived from single water samples, so that there could be no estimate of error on the results. The conditions in the channel immediately after the start of the sudden inflow from the $30m^3$ hr-1 pump, would have been rather unstable,

Fig. 71

Results of the sodium dilution in Experiment 3

Fig. 71a. Untransformed data for the sodium dilution

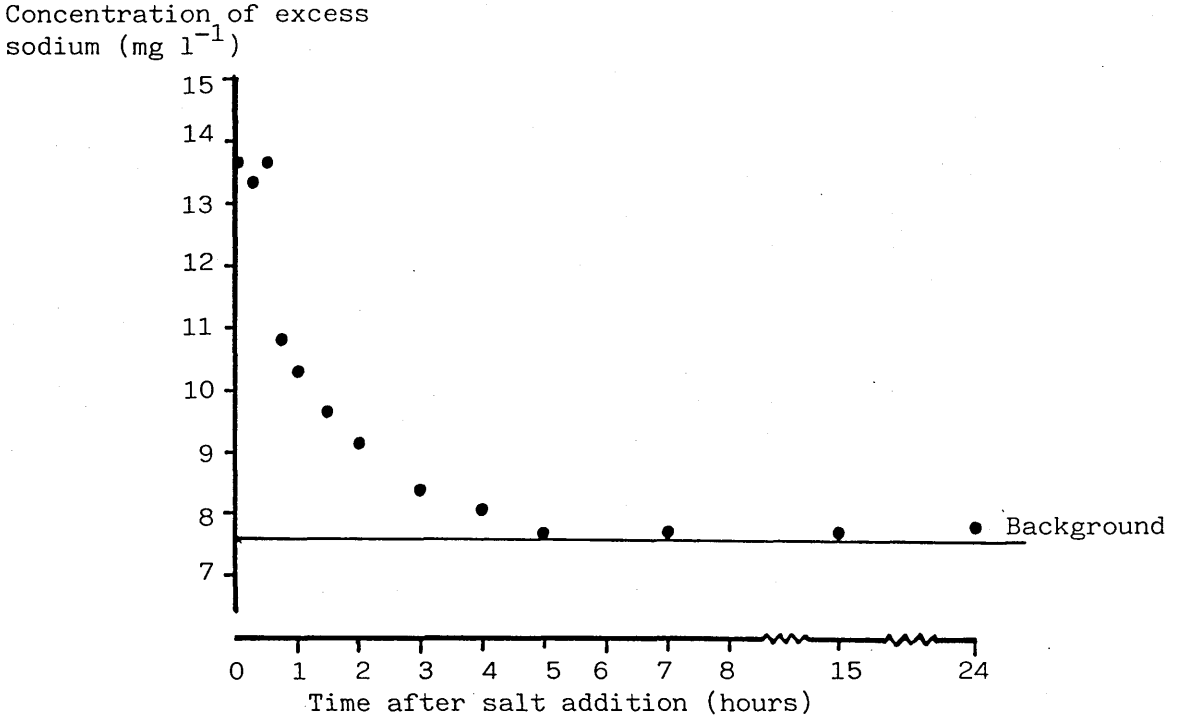
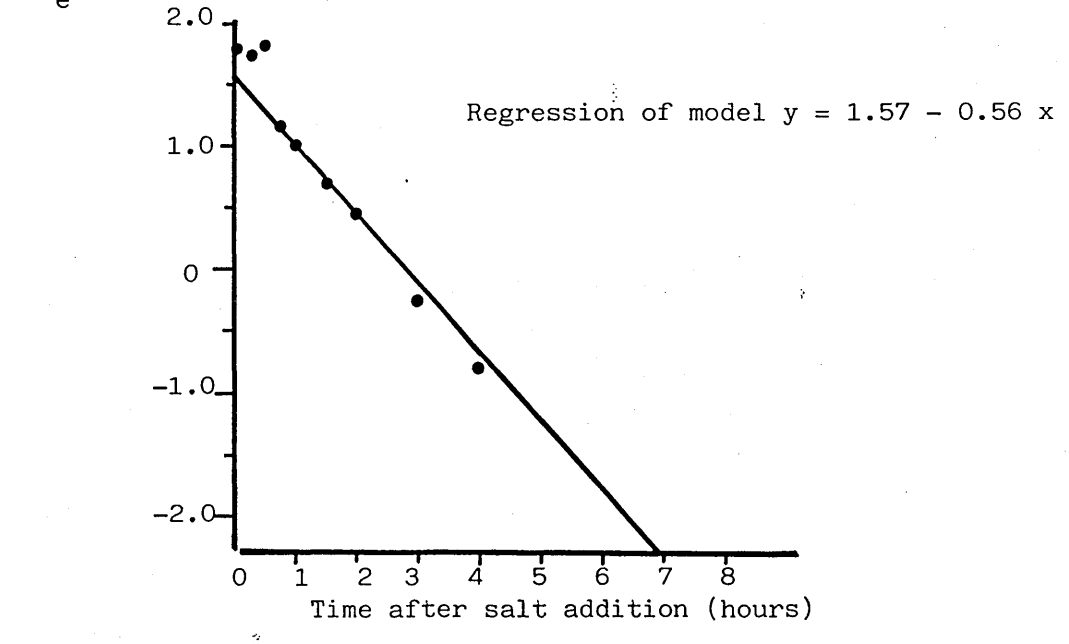


Fig. 71b. Log_e transformed data with the regression of the model



until the fresh inflow had fully mixed with the salt-treated water. The water level in the channel had fallen by about 2cm over the 24 hours without inflow, and there was an immediate rise of about 5cm as soon as the inflow had started, before the outflow had become balanced. This disruption might account for the apparently spurious sodium concentrations in the samples taken 15 and 30 minutes after the start of the inflow, although on this basis, lower values might have been expected.

Conclusions from the salt dilution experiments

1) When the C_o value extrapolated from the regression of experimental results is used, the dilution model:

$$\text{Log}_e\ C_t = \text{Log}_e\ C_o - \frac{f}{V} \cdot t$$

fits the experimental results very well.

2) The total water volume of channel 3 was confirmed by these experiments to be $51m^3$.

3) It is important to have accurate measurements of the background sodium concentration, with regular samples taken from the inflow. With the addition of a substance which is not normally present in the water, such as a herbicide, this is not a problem.

4) The predictive value of the model is limited by the accurate prediction of certain quantities. In these experiments the volume prediction appeared to be fairly accurate but the prediction of C_o was more difficult.

Even if the total input of sodium could be measured accurately resulting in an accurate estimate of C_o, until complete mixing in all of the channel occurs, there will be initially a higher concentration of sodium and a greater rate of loss of sodium than expected. This would be because the initial effective volume (V eff), would be smaller than the eventual, fully mixed, total volume (V tot). For the same inflow rate, the initial flushing of water through the channel would be faster from the smaller volume of water:

$$\frac{f}{V\ eff} > \frac{f}{V\ tot}$$

Thus, the value of C_o extrapolated from the dilution curve when the total volume is mixed, will be lower than the actual value of C_o.

The effects of the two volumes (V eff and V tot) on the dilution rates have been simplified in the following illustration:

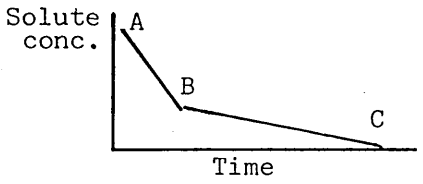


Fig. 72

Two dilution rates in a poorly mixed channel

The initial dilution rate (A-B) is when the effective volume of water, is that which is immediately mixed with the salt addition, above the gravel. The eventual dilution rate (B-C) is when the total volume of water in the channel is involved.

These dilution rates will gradually replace each other as mixing occurs throughout the channel not, as illustrated, in a sudden change. These two dilution rates could only be distinguished if the dynamics of the water mixing and exchange in the gravel, were fully understood, both initially and throughout the dilution process.

Thus, the proposed dilution model shows what might be expected to happen in a channel filled only with well mixed water. Otherwise, the model gives a simplified view of what happens in a gravel-filled channel once the sodium is assumed to have penetrated the whole of the water volume, and assuming that the exchange rate of the fresh water from the inflow, is the same in the gravel as it is at the surface of the gravel.

7.3.6 Using the dilution model to predict the behaviour of diquat residues

Using the dilution model and some water discharge readings for the R.Piddle (provided by Wessex W.A.), the maximum possible concentration of diquat that might enter the R.Piddle, from the channel discharge, was calculated. The water discharge of the Waterston stream, downstream of the input from the recirculating channels, was estimated from measurements of the cross-sectional area of the stream and of the water velocity at 0.6 x depth.

The predicted maximum concentrations of diquat, occurring in the channels, Waterston stream and in the R.Piddle, for both of the herbicide trials, are listed in Table 23.

Table 23

The maximum concentrations of diquat predicted to occur during the herbicide trials in the recirculating channels

	Trial 1	Trial 2
Dose rate of Midstream (containing 100g diquat l^{-1}) (litres per $100m^2$)	0.5	1.0
Area of channel to be treated (m^2)	31.5	31.5
Amount of diquat added (mg)	15750.0	31500.0
Volume of water in channel (m^3)	50.0	50.0
Initial concentration of diquat in channel (assumed fully mixed) (mg l^{-1})	0.315	0.630
Concentration of diquat in channel (mg l^{-1}) x minutes after application		
x		
30	0.233	0.467
60	0.173	0.346
120	0.095	0.190
240	0.028	0.104
360	0.009	0.060
Discharge of water in Waterston stream (l sec^{-1})	135.0	135.0
Maximum possible concentration of diquat in Waterston stream (mg l^{-1})	0.019	0.039
Discharge of R.Piddle (downstream of Waterston stream inflow) (l sec^{-1})	240.0	240.0
Maximum possible concentration of diquat in R.Piddle (mg l^{-1})	0.011	0.022

Maximum permissible concentration of diquat in water to be used for irrigation = 0.02mg l (MAFF 1985).

These estimates do not take into account the loss of diquat from solution by:

target plants
channel substrate
grass and soil at the outflow
plants and substrate in the 500m length of the Waterston stream

It is unlikely that any of the diquat residues would reach the R.Piddle.

7.4 Preparation of the recirculating channels for the diquat-alginate trials

7.4.1 The establishment and over-wintering of the channels

Although both channels were available for use from September 1984, for economic reasons only channel 3 was recirculated over the winter. Channel 2 was left full of static water. It was intended that by planting one channel in the autumn, any difficulties experienced in maintaining productive plants could be overcome, well before the herbicide trials in the summer of 1985.

Substrate

The substrate in the channels was of 40mm diameter flints from a terrestrial source, which were similar to the stones found in chalk streams. This substrate was too coarse for the secure rooting of transplanted Ranunculus. Shallow trenches, about 30cm wide by 50cm long, were dug in a uniform pattern in the straight sections of the channels, and these were filled with finer, flint gravel chips of 10mm diameter.

Vegetation

Rooted stems of Ranunculus penicillatus var. calcareus were collected from the R.Frome (N.G.R. SY868868) and Bere Stream (N.G.R. SY863958). The latter site has been intensively studied, (Dawson 1976) and the plants were short-stemmed and well rooted, characteristic of a swift, shallow stream of 5-10cm deep. The R.Frome is considerably deeper, 60-70cm deep, and the plants from this river had stems of 50-100cm in length.

After draining for one minute,samples of Ranunculus were roughly weighed out in a bucket suspended on a spring-balance, about 150g from R.Frome and 50g from Bere Stream. The roots were embedded in the upstream ends of the patches of fine gravel, in a random pattern around the channel. Samples of mixed origin were used in case either of the biotypes should fail to establish, and so that the growth of the two types could be compared.

Invertebrate control

Healthy and stable populations of macroinvertebrates were required in the channels, because these populations were to be studied in the herbicide trials. However, certain herbivorous species can

become so successful that their consumption of vegetation can limit plant growth (e.g. Gammarus sp. Eichenberger and Weilenmann 1982). To insure against this problem, 200 Bullheads (Cottus gobio) were electro-fished from a local stream and were added to the channel.

Nutrient supply and stability

An input of approximately $3m^3$ of water per hour from the bore-hole, was maintained so that there was a constant replenishment of the nutrients required for plant growth. A summary of the chemical composition of the bore-hole water was recorded by Ladle et al.(1977).

Some chlorosis of Ranunculus plants had been observed during work in a concrete recirculating channel at Waterston. It was possible that this was due to the presence of galvanized metal, which may have caused elevated concentrations of zinc, which can be toxic to plants. Galvanized bolts at the outflow were removed and the safety grid, (which stops floating objects from entering the pump) was immersed in concentrated sulphuric acid for 24 hours, to remove most of the zinc.

To ensure that iron, which was thought to be in fairly low concentrations in the bore-hole water, did not become limiting, a supplement of iron was dripped into the channel. A solution of ferric chloride ($FeCl_3$) and E.D.T.A. (ratio 6.5:1), was dripped at a known rate, depending on the rate of water flowing into the channel, from a 25l carboy. Some chlorosis was still observed after a month when an arbitrary dose of 25µg of iron, per litre of water entering the channel, had been supplied. The symptoms disappeared after the rate of iron supplement had been doubled to 50µg l^{-1}. It was important that not too much E.D.T.A. was added as this might have removed other necessary ions, such as phosphates.

7.4.2 Over-winter growth rates of Ranunculus

Approximate over-winter growth rates were calculated using the fresh weights of whole beds of Ranunculus and a growth rate equation:

$$\text{Growth rate } K_w = \frac{\text{Log}_e W_2 - \text{Log}_e W_1}{t}$$

W_1 = initial biomass
W_2 = final biomass
t = observation time (Eichenberger and Weilenmann 1982)

The initial weights W_1 were measured on 27.9.84 prior to the transplantations into the channel. The final weights W_2

were measured on 12.3.85, when the transplants to channel 2 were made. The mean overall growth rate, per week, for all beds was:

0.114 (S.E. 0.0017, n=46)

There was a significant difference ('t' test) in the growth rates between the plants from the two sites of origin. A greater growth rate was shown by the plants from the Bere Stream:

	Growth rate ($week^{-1}$)	S.E.	n
Bere Stream	0.122	0.0026	12
R.Frome	0.111	0.0019	34

These growth rates compare favourably with those found for the over-winter growth of Ranunculus fluitans in the artificial channels of Eichenberger (1983).

7.4.3 Preparation of the channels for the herbicide trials

In March 1985 channel 2, which had been left unplanted, was recirculated with fresh water for five days, and was then prepared as outlined in Section 7.4.1. All of the Ranunculus beds from the over-wintered channel 3 were carefully uprooted and half of them were randomly distributed around channel 2. The remaining beds were replaced in channel 3, so that all plants had been equally disturbed.

The remaining spaces in the channels were filled with 500g fresh weighed beds of Ranunculus penicillatus var. calcareus collected from the R.Piddle (N.G.R. SY753948) 800m downstream of the confluence with the Waterston stream.

It was noted that the Ranunculus from the R.Piddle started flowering about a month before the plants from the R.Frome or Bere Stream. This was probably not an effect of transplantation to the channels, but relates to the observations made by Dawson (1980) that there are differential times of flowering in the R.Piddle, being progressively later going downstream.

7.4.4 Growth rates of fresh and over-wintered plants: March-May

The approximate growth rates per week, during the spring, were calculated from the fresh weights of the Ranunculus beds at the time of transplant on 12.3.85, and on 28.5.85 when the pre-treatment samples were collected for the first herbicide trial.

There was no significant difference between the growth rates of the Ranunculus in the two channels. There were very significant differences ('t' test) between the growth rates of the over-wintered plants from the R.Frome and Bere Stream, and those collected from the R.Piddle in March:

	Mean growth rate ($week^{-1}$)	S.E.	n
Over-wintered plants	-0.008	0.0085	9
March transplants	0.098	0.0076	11

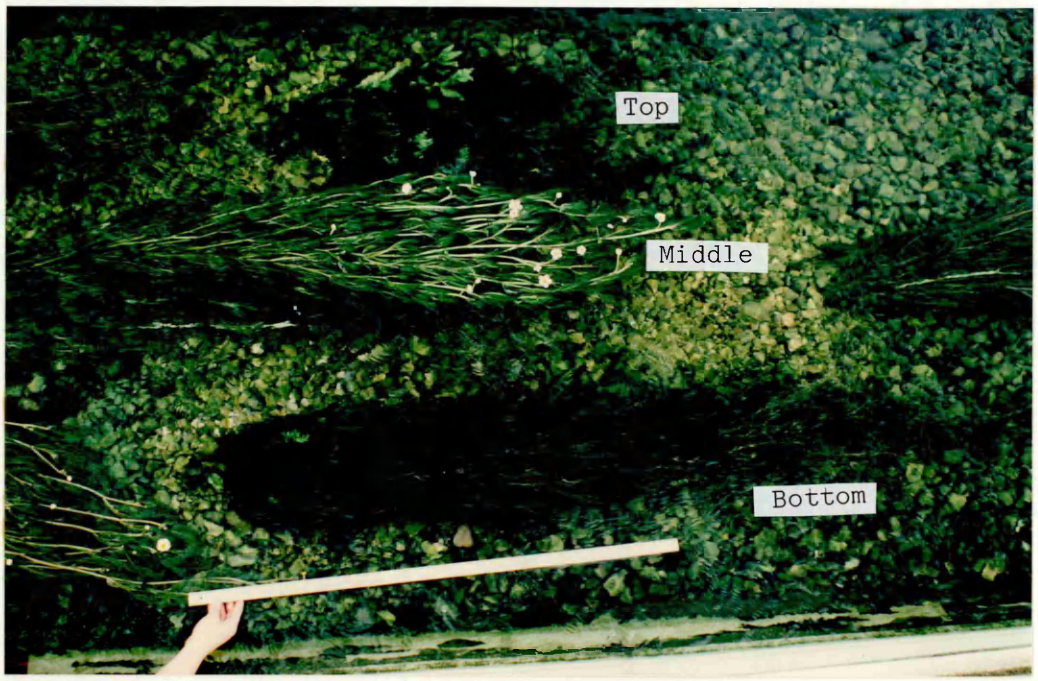
The growth rate of the over-wintered plants was lower between March and May than it had been between September and March, despite the advantage of the spring growth season. The negative growth rate for the over-wintered plants indicates a net loss of biomass rather than growth.

These observations will be considered further in the general discussion at the end of the chapter (Section 7.8.1).

The three types of Ranunculus beds are shown in Plate 5.

Plate 5

Ranunculus beds in a recirculating channel, April 1985



Top: Small, over-wintered bed from Bere Stream (with some Veronica)

Middle: Flowering bed from R.Piddle, March transplant

Bottom: Over-wintered bed from R.Frome

1 metre rule

7.5 Diquat-alginate in the Recirculating Channels: Trial 1

7.5.1 Methods: Herbicide application

Only the upstream half (Fig. 67) of channel 3 was directly treated with diquat-alginate. The downstream half was regarded as having received an indirect application of diquat released into solution.

An application rate of 0.5l of Midstream per $100m^2$ of water surface, as recommended for water less than 30cm deep, was used. Over the $31.5m^2$ area to be treated, this required only 0.157l of Midstream. This volume would have taken only 6.7 seconds to deliver from the knapsack sprayer, which would have meant covering the 20m length of channel at a fair pace! Even at a more controlled delivery rate, with only about 35% of the channel area being covered by Ranunculus, a large proportion of the Midstream would land directly on the substrate and there could be no guarantee that every bed would be treated.

Consequently it was decided that the Midstream would be applied from a syringe, directly onto each Ranunculus bed. At the proposed rate of application for the area, each of the twenty beds to be treated required 7.9ml of Midstream. The total application of diquat was 15750mg (Table 23).

Samples of the treated water were collected for the quantitative analysis of diquat residues. However, the polypropylene bottles used, had contaminated lids. This lead to spurious results so that the analysis had to be abandoned.

The two outflow pipes were attached to the channel and the $30m^3\ hr^{-1}$ pump was started thirty minutes before the herbicide was applied. The Midstream was applied to the twenty Ranunculus beds over a period of three minutes, to allow the water to completely recirculate and mix. After 24 hours the large inflow pump was switched over from channel 3 to the untreated channel 2. The reduced inflow, from the small pump was resumed in channel 3. The $30m^3\ hr^{-1}$ inflow to channel 2 was left for 24 hours, after which the inflow from the small pump was resumed.

7.5.2 Methods: Ecological monitoring

Ranunculus mapping

The beds of Ranunculus were mapped, prior to the herbicide treatment, by plotting the cover in 0.5m x 0.25m rectangles. It was found that the method was not sensitive enough to detect plant growth over the few weeks of monitoring. It was also observed that cover was not a reliable indicator of changes in the quantity of Ranunculus present. This was because after the removal or loss of part of a bed, the remaining stems usually spread out to cover most of the exposed substrate. This discrepancy between biomass and cover estimates of changes in amounts of vegetation, has also been observed in natural streams (Dawson 1976).

Mapping at the beginning of the trial, of the upstream halves of the channels,produced estimates of the initial Ranunculus cover of:

Channel 2 (untreated)	39%
Channel 3 (treated)	34%

Ranunculus biomass

The distribution of the Ranunculus beds meant that any method of sampling by area, such as with the Lambourn sampler, would have required many random samples to produce consistent results. Removal of whole beds would have not only considerably limited the number of samples possible, but might have caused a greater disruption to the system than the herbicide itself.

As a compromise, half of a bed was removed as a sampling unit, the longitudinal division of area being visually estimated. Any smaller fraction of a bed would have been difficult to estimate.

Five half beds were sampled for each half-channel section, at each harvesting time. It was ensured that each set of five samples included at least two of the over-wintered beds and two of the March transplants from the R.Piddle. Within these limitations, the beds were chosen randomly.

The Ranunculus was cleaned of macro-algae and was sorted into healthy and decayed (i.e. brown, flaccid tissue) portions. These were dried separately at 110°C to a constant weight.

Testing the accuracy of the half-bed sampling method

At the end of Trial 1, two collections were made of five beds from each sampling section, in which both halves of each bed were removed. The first half was sampled as outlined above, and then the rest of the bed was collected, so that the total biomass of the bed could be found from the sum of the two halves. From this total, the "expected half-biomass" for each bed could be calculated, and a comparison could be made with the "observed half-biomass" of the first half of the bed sampled.

A simple 't' test comparison of the observed vs. expected half-bed biomasses was made for each set of five samples, because it was necessary to show whether the sampling method affected these means which were calculated for each section. Thus, if within a set of five samples some of the observed halves were larger than expected and some were smaller, if overall these cancelled out, then the sampling method would not significantly affect the results. If there was a systematic bias (e.g. a regular overestimation of half a bed) then this would be indicated by a significant difference between the observed and expected half-bed biomasses. In such a case, the sampling method would have to be altered, or an allowance made for the quantified bias.

If it had been necessary to assess the deviation of the observed, from the expected, half-bed biomass for each bed sampled, rather than for the mean of a set of five samples, then another statistical test (e.g. Chi square) would have been required.

Losses of Ranunculus

Periodically the Ranunculus which had accumulated on the safety grid in front of the pump, and at the outflow catchment, from each channel, was collected, dried and weighed. This lost material could not be compared between sampling times because the period between collections was not necessarily the same, but comparisons between channels were possible.

The biomass of benthic algae and moss

At regular intervals five samples of benthic moss and algae were collected from each sampling section using a 0.0625m^2 quadrat, placed at random on the substrate. The samples were sorted and dried at 110°C to a constant weight.

Macroinvertebrate communities

The macroinvertebrates associated with the Ranunculus were sampled by removing similarly sized and positioned apical pieces of Ranunculus stems. The samples were quickly placed into small plastic bags, that were partly filled with water. By performing such a manoeuvre rapidly the losses of macroinvertebrates from the stems were minimised and were assumed to be reasonably constant for each sample. The method was devised and tested in rivers by Williams (1981).

Using a 211µm sieve the macroinvertebrates were washed and picked off the plant material. The Ranunculus was dried at 110°C and weighed. The macroinvertebrates were preserved in 70% alcohol, divided into major taxa and all individuals were counted. Random sub-samples of individuals from all taxa were identified to species. The data was recorded as number of individuals of a taxon per gramme dry weight of Ranunculus.

Water chemistry

Water samples were taken, initially daily and then at 2-3 day intervals after treatment, from both channels, and the inflow. Calcium ion concentrations and alkalinity were immediately determined in the laboratory at Waterston, using the standard methods of sulphuric acid and E.D.T.A. titrations respectively. Phosphate and nitrate concentrations were determined in the analytical chemistry laboratory, and periodically a full ion analysis was performed, including sodium, magnesium, and potassium. The standard methods of Casey and Newton (1973) were used throughout.

Readings of temperature and pH were occasionally taken in both channels, using a mercury and glass thermometer and a Radiometer glass electrode respectively. The digital pH meter had no facility for compensating for temperature,so that it was calibrated at the channel temperature using buffers which had been left in bottles in the channel water.

7.5.3 Statistical analysis of the data from the channel experiments

The importance of replication has already been discussed, in relation to the field trials (Section 4.2.1). The limitation to two recirculating channels meant that replication of the herbicide treatment and of the untreated controls, was not possible. As discussed, the analysis of variance cannot be validly used without treatment replication. Instead comparisons between the channels, the upstream and downstream sections, and between sampling times were made using 't' tests, using MINITAB.

The use of 't' tests based upon the sub-samples from each channel section only indicates whether a significant difference occurs between two populations, without treatment replication it is not valid to assume that these differences are caused by the treatments. For example, if a significant difference were found between the two channels for plant biomass, at some time after the herbicide application, it could not be assumed that the herbicide was responsible, because there would be no means of testing whether the difference between the channels was due to anything other than random variation.

To use the sub-samples for each channel section as treatment replicates in an ANOVA would be to commit Pseudoreplication (Hurlbert 1984), unless one could justify regarding each sub-sample as an individually treated experimental unit. It might be possible to regard each whole Ranunculus bed as an experimental unit, since the herbicide treatments were applied to each bed. However, it would still not be possible to mix treated and untreated 'replicates' because diquat-alginate might be carried from a treated to an untreated bed. Thus, the experimental units would not be independent. If the treatment replicates had to be segregated between the channels, because of this lack of independence, the problem of pseudoreplication would still exist. This lack of independence between the Ranunculus beds is more evident if mobile parameters, such as water chemistry or macroinvertebrate populations, are considered.

Accepting that these experiments must comprise unreplicated treatments, any conclusions drawn about differences between the treated and untreated channel, must be tentative. Even if the channels show no differences prior to treatment and then develop some afterwards, it is not valid to assume that the differences have arisen

as a direct result of the treatments. Such conclusions could only be drawn if the experimental units were identical at the time of treatment and would have remained so except for the effect of the treatment (Hurlbert 1984). In biology, and especially ecology, there are very few circumstances in which that assumption can be made. This is especially true of invertebrate populations, which may show great changes over short periods of time, the timing of which may vary between populations or environments.

7.5.4 Results of Trial 1

Testing the half-bed sampling method

There were no significant differences between the observed and expected half-bed biomasses for any of the eight sets of five-bed samples which were tested, Table 24. By the end of the trial, Ranunculus beds of all states and sizes would have been included in these tests, suggesting that the sampling method was suitable throughout the trial, and that there was no bias with particularly large or small beds.

Table 24

The observed and expected half-bed biomass data used to test the sampling method

		Observed		Expected	
		$\bar{x}$	(S.E.)	$\bar{x}$	(S.E.)
18.6.85					
Channel 2	u/s	74.82	(21.5)	70.47	(21.32)
	d/s	53.26	(18.72)	50.82	(14.61)
Channel 3	u/s	63.46	(29.23)	56.64	(24.50)
	d/s	39.86	(11.98)	39.45	(10.61)
24.6.85					
Channel 2	u/s	73.50	(14.75)	75.0	(4.02)
	d/s	47.82	(11.88)	52.45	(12.13)
Channel 3	u/s	17.68	(5.51)	16.51	(5.11)
	d/s	69.06	(7.08)	66.41	(7.66)

Ranunculus biomass

The decayed material was not distinguished at the first two sampling times.

Comparison of channel 2 vs. channel 3

Comparisons of the half-bed biomasses were made between the channels, pooling the upstream and downstream data, for each sampling time, using 't' tests. Both the full biomass data, Fig. 73a, and the percentage of decayed material in each sample, Fig. 73b, were tested.

There were no significant differences between the channels for the full Ranunculus biomass, before or after treatment.

There was a significantly greater percentage of decayed material in the channel 3 samples on both post-treatment sampling dates.

Comparison of upstream and downstream sections of the channels

To assess whether the direct dose of diquat-alginate (upstream), or the indirect dose of diquat (downstream) had different effects on the Ranunculus, comparisons of the two halves of the channels were made at each sampling time. The mean biomass data and percentage decayed material in each section, have been illustrated in Fig. 74.

No significant differences were found between the upstream and downstream sections of either channel.

Comparisons of sampling times per channel

For each channel, using the pooled upstream and downstream data, (Fig. 73) comparisons were made between each sampling time, Table 25.

Channel 3

There was no significant difference between the pre- and immediately post- treatment samples. There were significant reductions in biomass by 18 and 25 days after the herbicide application, compared with the pre-treatment samples. There were no other significant changes in biomass with time in channel 3.

Channel 2

There were significant reductions in biomass with time, by the last sampling time (24.6.85) when compared with the first two sampling times (28.5.85, 14.6.85).

Fig. 73

Comparisons of Channel 2 and Channel 3 in Trial 1

Fig. 73a. Biomass of Ranunculus

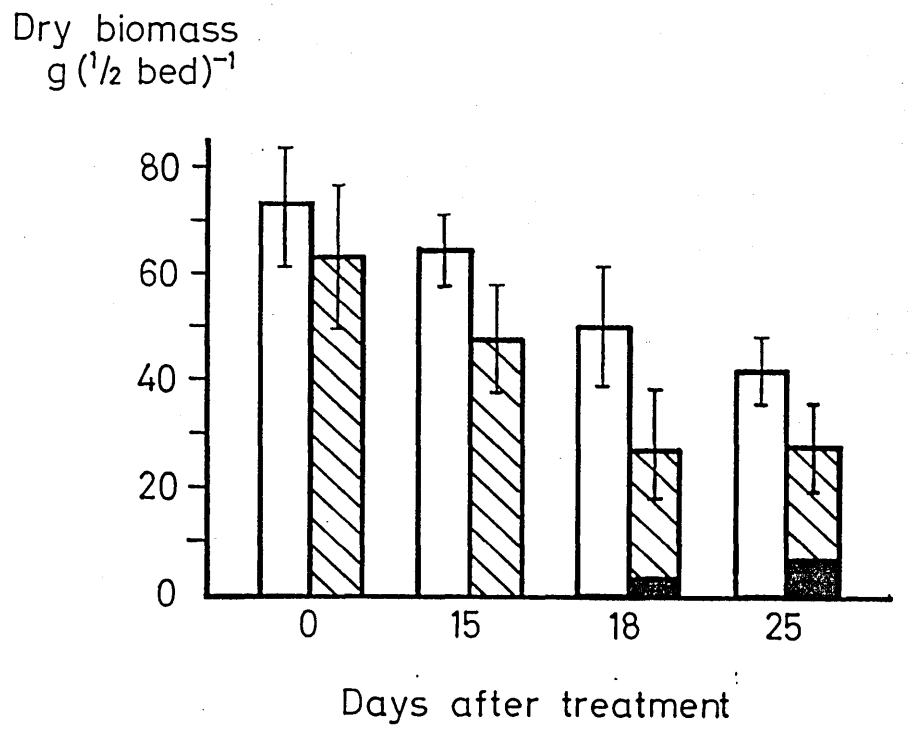


Fig. 73b. Percentage of decayed tissue

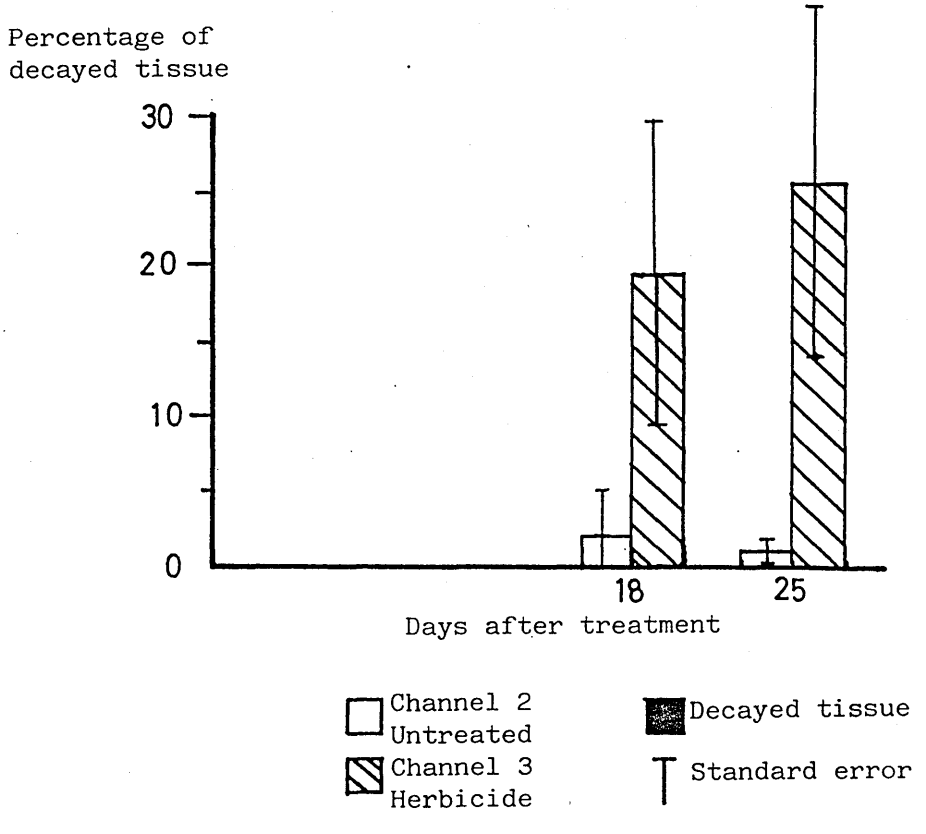


Fig. 74

Comparisons of upstream and downstream halves of the channels in Trial 1

Fig. 74a. Biomass of Ranunculus

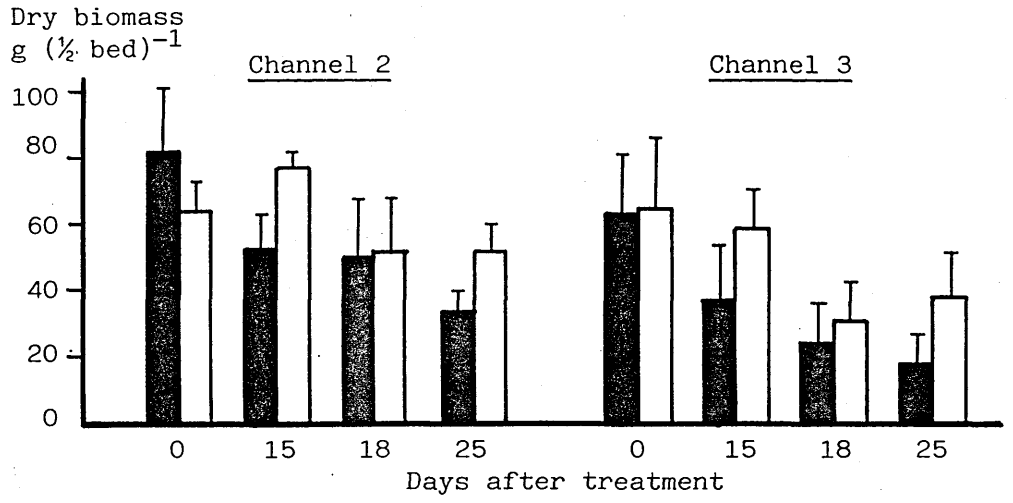


Fig. 74b. Percentage of decayed tissue

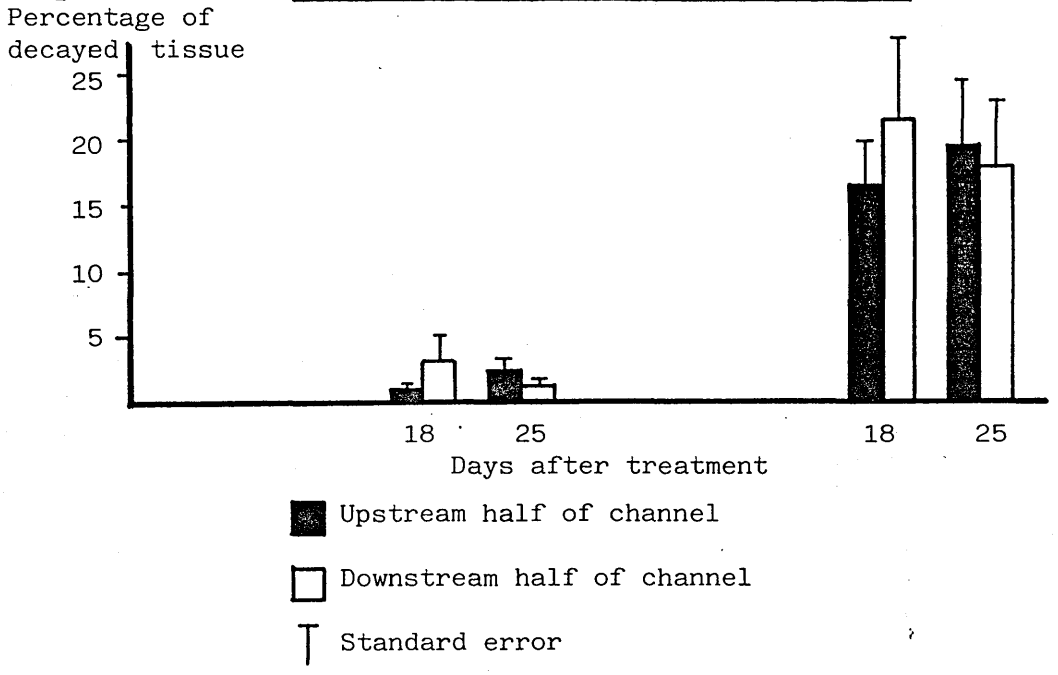


Table 25

Comparisons of the Ranunculus biomass from different sampling times in Trial 1

Sampling dates for comparison		Channel 2 (Untreated)	Channel 3 (Herbicide)
28.5.85	(pre-treatment)		
14.6.85	(15 dat)	x	x
17.6.85	(18 dat)	x	√ (3.88%)
24.6.85	(25 dat)	√ (3.15%)	√ (4.12%)
14.6.85			
17.6.85		x	x
24.6.85		√ (2.96%)	x
17.6.85			
24.6.85		x	x

x = no significant difference
( )= level of significance
dat= days after treatment

Ranunculus growth rates

The half-bed system of sampling allowed another type of comparison to be made because the change in biomass of a specific bed, between two sampling times, could be expressed as a growth rate. These growth rates could be compared for sets of beds.

The particular comparison that was the objective of this method was a comparison of plant origins. The reduction of biomass in each channel could be compared for the over-wintered and the March-transplanted beds. The data from two pairs of sampling times were pooled because no significant differences were found between them:

1st half of a bed (date)	(d.a.t.)	2nd half of that bed (date)	(d.a.t.)
28.5.85	0	17.6.85	18
14.6.85	15	24.6.85	25

Comparisons of the growth rates from channel 2 and channel 3 for beds of both origins showed a significantly more negative growth rate (i.e. greater biomass reduction) in channel 3 than in channel 2.

In both channels the growth rates of the March transplants from the R.Piddle, were significantly more negative than those of the over-wintered plants. The growth rates were not significantly different between the two channels for the R.Piddle plants. The growth rates of the over-wintered plants were very significantly more negative in channel 3, Table 26.

Table 26

Growth rates of Ranunculus beds at the beginning of Trial 1

		Channel 2		Channel 3	
	n	mean	(S.E.)	mean	(S.E.)
Pooled data	20	-0.254	(0.051)	-0.440	(0.080)
Overwintered plants	10	-0.121	(0.044)	-0.337	(0.065)
March transplant	10	-0.387	(0.069)	-0.544	(0.097)

Ranunculus losses

Broken stems of Ranunculus caught in the safety grid or at the outflow, were sampled at three times. Significance tests were not possible for the single samples, but there was always a greater percentage of the total Ranunculus biomass lost from channel 3.

Sampling date	d.a.t.	Channel 2		Channel 3	
		Biomass	% of total	Biomass	% of total
7.6.85	8	130.2	2.3	175.4	3.9
14.6.85	15	86.7	1.6	216.0	4.8
27.6.85	28	122.4	2.3	280.0	7.1

Biomasses of macro-algae and moss

A distinct succession of dominant algal species was observed in the channels in the weeks after the recirculation was started. Not all species in this succession were identified. This succession was more complex than that described by Marker and Casey (1982), because of the import of material with the Ranunculus.

The predominant filamentous alga in channel 2 was a species of Vaucheria, with some Zygnema sp.. In addition in channel 2, but not in channel 3, the Ranunculus beds, especially where they reach the water surface, were smothered by leathery crusts of blue-green algae, Phormidium autumnale, with Lyngbya kützingii.

These crusts of blue-green algae and clumps of filamentous algae growing on,or becoming caught in,the Ranunculus stems could be seen to be greatly increasing the resistance of the Ranunculus beds to the water flow. These thick algal mats were also likely to be reducing the photosynthetically active radiation reaching the Ranunculus.

Vaucheria and Cladophora were the predominant algae in channel 3 with small amounts of Anotherix and Monostroma. Although these algae were often caught on the Ranunculus, increasing the drag, the crusts of blue-green algae were absent so that the problem was not as severe as in channel 2. The effect of the filamentous algae on the Ranunculus beds is shown in Plates 6a and 6b.

The diversity of the algal populations in the two channels was apparent prior to the herbicide application and no particular change in the species composition was observed in channel 3 after treatment.

Plate 6

Filamentous algae on a Ranunculus bed from the R.Piddle in channel 2 (with 1 metre rule), June 1985

Plate 6a

Prior to removal of algae

Plate 6b

After removal of algae

Note leaf loss from Ranunculus stems after the removal of algae, similar in appearance to symptoms of diquat action.

The aquatic moss, which had been present in both channels before the start of the trial, was identified as Drepanocladus aduncus.

The biomasses of algae or moss did not significantly differ, in either channel, between the upstream and downstream sections. The mean biomasses per channel and sampling time are illustrated in Fig. 75.

The biomass of algae only differed significantly between the channels on the last sampling date (31 d.a.t.), when the biomass was greater in channel 3. The biomass of algae was significantly greater on that date in both channels, compared to all other sampling dates.

The biomass of moss was always lower in channel 3 than in channel 2, but this difference was only significant on the first sampling date (7 d.a.t.). The biomass of moss in channel 2 did not change significantly with time, but there was a significant increase in channel 3 after the first sample.

Water chemistry

Dissolved ions

The concentrations of calcium, alkalinity, magnesium, sodium, potassium and nitrates are presented in Table 27. The concentrations of these ions do not vary greatly with time, or between channels. Only the alkalinity and calcium concentrations showed a slight reduction in the channels compared to the inflow water.

The concentrations of reactive phosphorus are illustrated in Fig. 76. Single samples were collected from each channel so no statistical procedures have been attempted on the data. The phosphorus concentrations in the channels were much lower than those in the inflow. The peaks in phosphorus concentration one day after the herbicide application were due to the effect of the increased inflows (associated with the herbicide dilution), on the previous day in channel 3 and on the day of sampling in channel 2.

The phosphorus concentration in channel 3 does appear to increase to above its pre-treatment concentration, and well above that in channel 2, on 4-7 days after the herbicide application. This concentration subsequently decreases so that by 17 days after the herbicide application, the phosphorus concentration is greater in channel 2 for the first time.

Fig. 75

Biomass of algae and moss in the channels in Trial 1

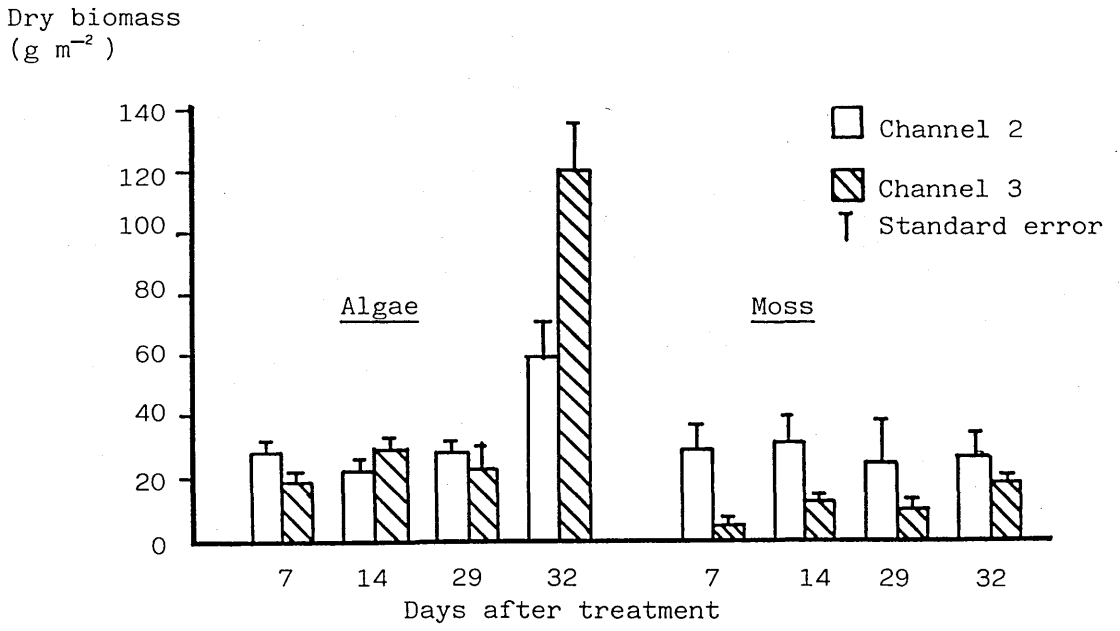


Fig. 76

Concentrations of reactive phosphorus in the inflow and channels during Trial 1

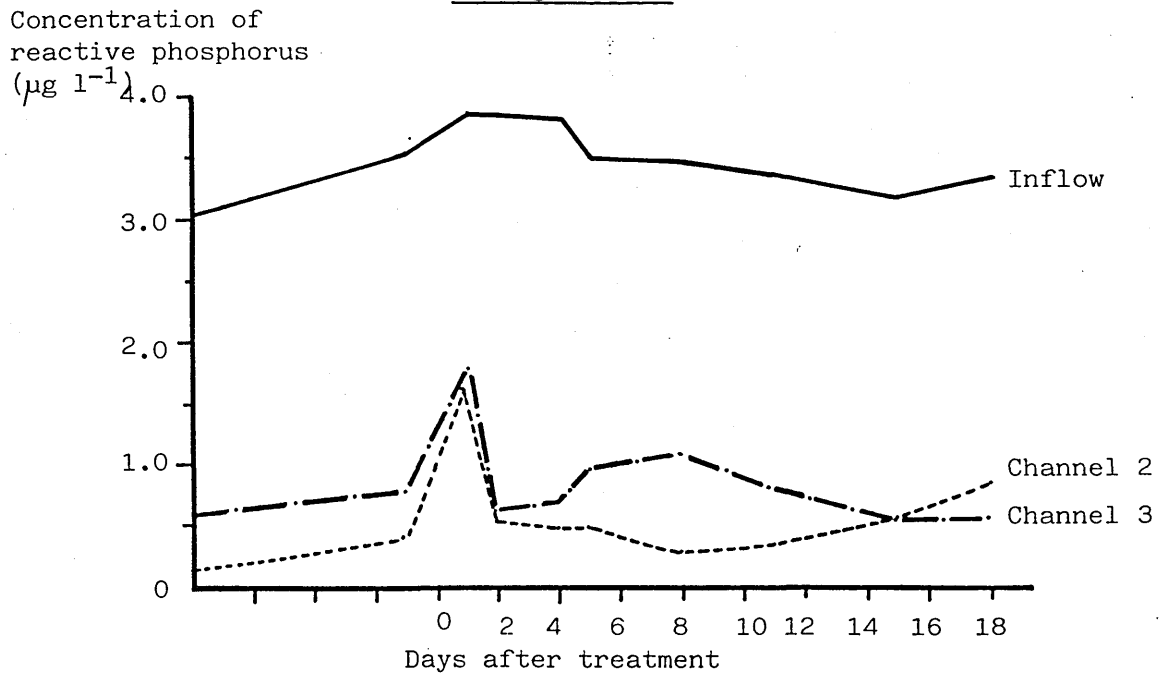


Table 27

Water chemistry data from Trial 1

Date	d.a.t.	Calcium (mg l^{-1})			Alkilinity (mg l^{-1})			Magnesium (mg l^{-1})			Sodium (mg l^{-1})			Potassium (mg l^{-1})		
		In	C2	C3	In	C2	C3	In	C2	C3	In	C2	C3	In	C2	C3
22.5		107.6	95.4	99.2	225	204	206	2.12	2.05	2.14	7.8	7.9	7.9	1.42	1.44	1.38
29.5		105.6	87.0	96.6	229	186	208				7.0	7.1	7.6	1.38	1.36	1.28
30.5	0	105.5	92.0	110.0	230	182	229									
31.5	1	107.0	104.0	107.0	233	221	232									
1.6	2	106.0	95.0	99.0	228	206	217									
3.6	4	104.5	84.0	91.0	230	180	201									
4.6	5	104.8	91.6	93.6	225	200	206	2.08	2.08	2.11	7.5	7.1	7.2	1.42	1.26	1.34
10.6	11	103.2	98.0	97.0	225	209	209	2.03	1.98	1.96	7.7	7.5	7.8	1.30	1.32	1.44
17.6	18	103.2	95.0	93.8	226	209	203									

		Nitrate (mg l^{-1})			Date	Time	Temperature °C			pH		
22.5		5.23	5.11	5.13								
29.5		5.50	4.78	5.34	28.5	12:55		13.3	13.3			
31.5	1	5.40	5.23	5.35	31.5	12:00	11.2	12.1	12.3	7.35	7.68	8.00
1.6	2	5.38	5.02	4.11	1.6	14:55		15.5	16.1	7.42	8.20	8.21
3.6	4	5.18	4.38	4.70	2.6	18:25		17.4	17.4			
4.6	5	5.07	4.74	-	3.6	10:40		13.7	13.7	7.29	8.23	8.24
7.6	8	5.47	5.40	5.25		13.45		16.4	16.5	7.31	8.27	8.31
10.6	11	5.09	4.98	4.80		16:00		17.0	17.0	7.35	8.25	8.33
14.6	15	5.33	5.13	5.07	6.6	16:08		13.2	13.4			
17.6	18	5.15	4.85	5.50	7.6	12:55		11.4	11.4	7.35	8.26	8.31
		In	C2	C3			In	C2	C3	In	C2	C3

Temperature and pH

The temperature and pH data are presented in Table 27. These data were limited but they did show that the temperature and pH of the water did not greatly differ between the two channels. There were noticeable differences between the measurements, dependent upon the prevailing weather conditions and the time of day. The pH of the inflow water did not vary between sampling times but was noticeably lower than in the channels.

Macroinvertebrate communities

A list of the macroinvertebrate species identified, found in the channels from random sub-samples, is presented in Table 28. There is no guarantee that this list is complete, but some species have been identified which were not present in the channels during their initial colonisation in 1976 (Ladle *et al*. 1980).

The mean numbers of individuals per gramme dry weight of *Ranunculus* were calculated for the most abundant taxa only.

The four most abundant taxa were:

Chironomidae
Ephemeroptera
Naididae
Simuliidae

The chironomids were predominantly in their larval stage and the small proportion of pupae did not appear to alter much over the sampling period. The Ephemeroptera were dominated by *Baetis* spp., with approximately ten times the number of *Ephemerella* spp. The simuliids were predominantly larval, although the proportion of pupae increased slightly with time.

The mean densities of macroinvertebrates on the *Ranunculus* for these four taxa have been illustrated in Fig. 77. The 95% confidence intervals have been plotted, since this appears to be the method of displaying samples variation most favoured by authors in this field (e.g. Ladle *et al*. 1980, Gunn 1985). These confidence limits were calculated on MINITAB.

Table 28

Taxa of macroinvertebrates found in the recirculating channels

	Trial 1	Trial 2
Coelenterata		
Hydra		x
Oligochaeta		
Nais bretscheri	x	x
N. variabilis	x	x
N. barbata	x	x
Hirudinea		
Piscicola geometra	x	x
Erpobdella octoculata	x	x
Hydracarina		
Hydrachnellae	x	x
Malacostroca		
Ascellus aquaticus	x	x
Gammarus pulex	x	
Ephemeroptera		
Baetis fuscatus		x
B. vernus	x	x
B. rhodani	x	x
Ephemerella ignita	x	
Coleoptera		
Elmis aenea	x	x
Trichoptera		
Rhyacophila dorsalis	x	x
Hydropsyche pellucidula		x
Drusus annulatus		x
Brachycentrus subnubilus		x
Diptera		
Simuliidae		
Simulium ornatum	x	x
S. equinum		x
S. aureum group	x	
Empididae	x	x
Ephydridae	x	x
Chironomidae		
Cricotopus bicinctus	x	x
C. trifascia	x	x
Eukiefferiella claripennis	x	
E. ilkleyensis	x	x
Orthocladius sp.	x	
Synorthocladius semivirens	x	
Chaetocldius dentiforceps group	x	
Metriocnemus hygropetricus	x	

The macroinvertebrate data were analysed using 't' tests to compare:

1) The upstream and downstream sections of each channel
2) Channel 2 vs. channel 3 at each of the 3 sampling times
3) Different sampling times per channel

A more sophisticated 't' test could also be applied to these data because the same experimental unit (i.e. channel) was being sampled at each time.

This test was applied to the Log_e transformed data, and tests the significance of the differences between the channels, in their changes of macroinvertebrate numbers with time. For example, if, between two sampling times, the numbers of an organism increase in one channel more than in the other, this test would show whether the difference in rate of increase, between the channels, is significant.

X_1 = mean density at time 1
X_2 = mean density at time 2
S_1^2 = variance at time 1
S_2^2 = variance at time 2

ch2 = data from channel 2
ch3 = data from channel 3
n_1 = number of sub-samples at time 1
n_2 = number of sub-samples at time 2

$$\frac{X_1 - X_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}} = \frac{b_{(ch2)}}{S.E._{(bch2)}}$$

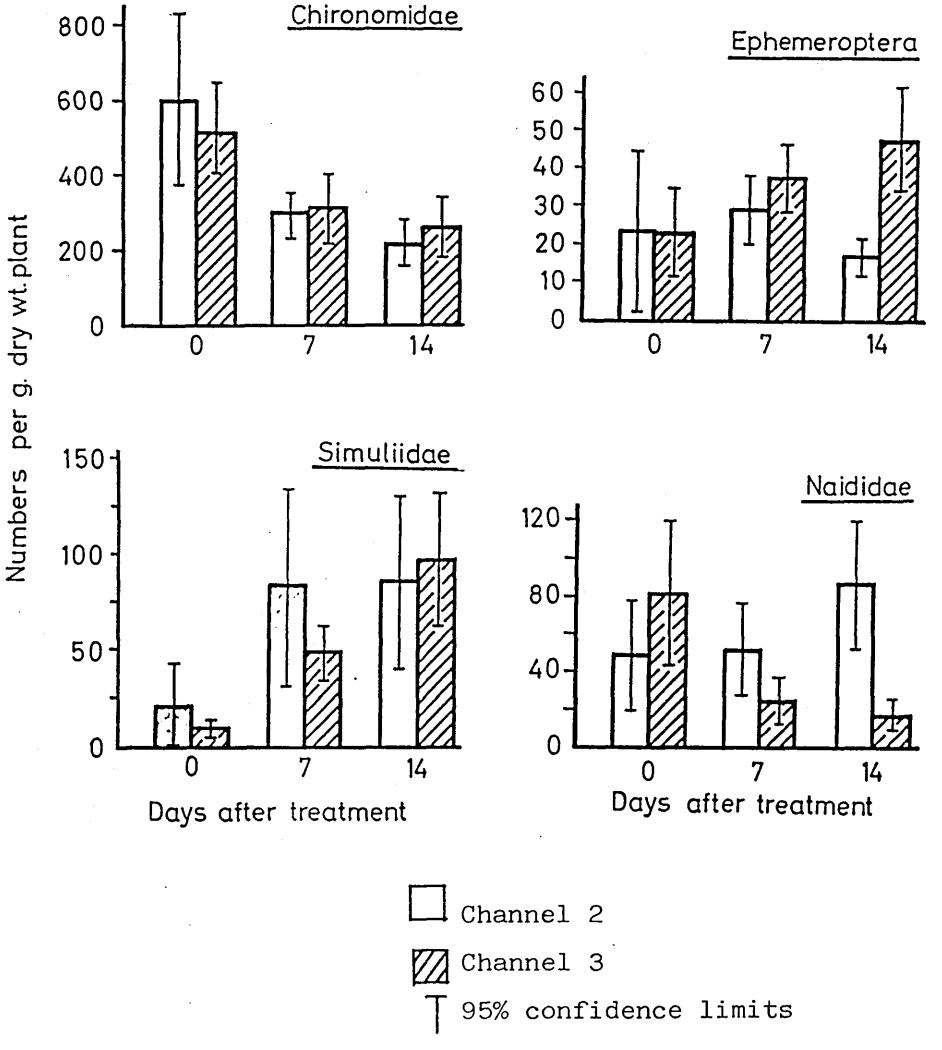
$$t \text{ (2 tailed)} = \frac{b_{(ch2)} - b_{(ch3)}}{\sqrt{S.E._{(bch2)}^2 + S.E._{(bch3)}^2}}$$

$$\text{degrees of freedom for } t = \frac{(S.E._{(bch2)}^2 + S.E._{(bch3)}^2)^2}{\frac{(S.E._{(bch2)}^2)^2}{df_{ch2}} + \frac{(S.E._{(bch3)}^2)^2}{df_{ch3}}}$$

df_{ch2} = degrees of freedom used for a 't' test comparison of X_1 and X_2 in channel 2 (from MINTAB)

Fig. 77

Macroinvertebrate data from Trial 1



There were no significant differences in the density of macro-invertebrates in the upstream and downstream sections of either channel, for any taxa, at any sampling time, with the exception of the simuliids in channel 3 at the last sampling time. There had been more simuliids in the downstream section of channel 3 throughout the trial, but this difference only became significant on 13.6.85.

Chironomidae

The density of chironomids on the Ranunculus was never significantly different between the two channels,but there was a significant reduction between the pre-treatment and the two post treatment samples.

Ephemeroptera

There was no significant difference in the density of Ephemeroptera between the two channels until the third sampling date (14 d.a.t.), when the density in channel 3 was significantly higher than in channel 2.

The density in channel 2 did not change between the first two sampling dates, but there was a significant reduction between the second and third samples. In channel 3 the density of Ephemeroptera significantly increased between the first two samples, and had increased further, but not significantly, by the third sample. The changes in density between the second and third sampling times were significantly different for the two channels.

Naididae

There were significantly fewer naids in channel 3 than in channel 2, in both of the post-treatment samples. The difference between the channels was not significant prior to treatment, when there were more naids in channel 3.

The density of naids did not significantly alter with time in channel 2,but the reduction in density after the herbicide application in channel 3 was significant.

The changes in density between the pre- and both post- treatment samples, were significantly different for the two channels.

Simuliidae

The density of simuliids in the two channels did not significantly differ at any time. There was a significant increase in density of simuliids between the first and second samples in channel 2.

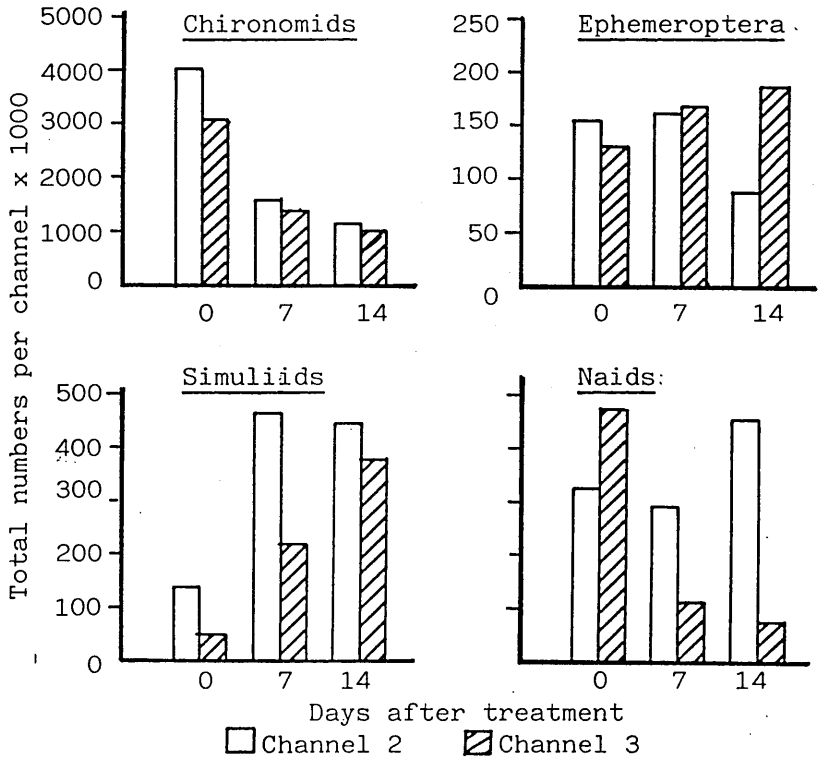
In channel 3 the density of simuliids increased significantly at each sampling time.

Between the first and third sampling times, the changes in density were significantly different for the two channels; although increasing in density in both cases, the increase in channel 3 was significantly greater.

In addition to calculating the densities of macroinvertebrates per weight of Ranunculus, the mean numbers of animals per channel were also estimated, based upon the total biomass of Ranunculus in each channel, at the time of sampling (Fig. 78). As a result of the greater biomass of Ranunculus in channel 2 at each sampling time, differences between the two channels (e.g. simuliids on 13.6.85 when the density in channel 3 was greater than in channel 2) may be reduced or reversed.

Fig. 78

Total numbers of animals estimated per channel in Trial 1



7.5.5 Discussion of Trial 1

If the Ranunculus biomass results from channel 3 are considered in isolation, the significant decrease after the herbicide treatment might be interpreted as proof of the herbicide's action. However, when the significant reduction in biomass in channel 2, and the lack of significant differences between the channels, are taken into account, the initial conclusion appears less credible.

Statistically there is no evidence that the herbicide had caused a reduction in plant biomass, that was greater than observed in untreated plants. The reductions in biomass in channel 2 were probably due to the increased drag imposed upon many Ranunculus stems by filamentous algae. This stress would have resulted in the progressive weakening, and eventually breaking and loss, of stems, removed in the fast flow of water. The flowering Ranunculus plants would have been particularly susceptible to this problem because the buoyant flowering stems would trap algae floating around the channel, and are much weaker than the vegetative stems (Dawson 1976).

The algae did not appear to cause such a severe problem in channel 3, chiefly because of the lack of encrusting blue-green alga Phormidium. The differences between the channels,in algal coverage of the Ranunculus, were not identified in the algal biomass data, because the filamentous algae were only sampled from the gravel substrate.

Significant differences between the channels were observed for the percentages of decayed Ranunculus. The low values in channel 2 supports the hypothesis that much of the loss from these plants was due to mechanical stresses. The significantly greater percentage of decayed tissue in channel 3 supports the idea that the loss of biomass was more likely to be the result of breakage and loss of necrotic tissue. Without treatment replication the herbicide cannot be assumed to be the cause of the necrosis, but much of the unhealthy tissue was near the bases of the stems where the diquat-alginate had been injected onto the beds.

Of the typical symptoms of diquat's action, chlorosis was not observed, and the leaf-loss could not be distinguished from the damage caused by the algae in channel 2 (Plate 6).

A significant difference between the channels was also observed for growth rates. Growth rate is not a particularly appropriate term since all the values were negative. The significantly greater loss rate from channel 3, might indicate that the effect of the herbicide in reducing plant biomass, was greater than the effect of the algae.

A further breakdown of the growth rate data, distinguishing between the over-wintered and March-transplanted material, showed that in both channels the latter types of plants suffered greater losses than the former ones.

The Ranunculus beds transplanted into the channels in March were likely to be more susceptible to physical damage than the over-wintered beds, because they consist of less robust tissues which had shown active growth prior to the herbicide trial (Section 7.4.4). These plants also had more of the delicate, buoyant flowering stems; the over-wintered plants tended to flower later and often underwater.

These characteristics might account for the greater damage sustained by the March-transplants both from the mechanical stresses imposed by the algae and from tissue necrosis that might have been caused by the herbicide. Younger, actively growing tissues are thought to be more susceptible to diquat-alginate (Clayton and Tanner 1982, W.R.O. 1980). This may be because the herbicidal action is expressed more quickly in plants with a rapid metabolism. Older plants may also have a greater covering of epiphytes, which may reduce the availability of the diquat to the Ranunculus.

Neither the algae nor the moss showed any symptoms of phyto-toxicity in channel 3, and there was no evidence of differences in their biomasses between the channels, after the herbicide application.

It might be suggested that the large increase in algal biomass in channel 3,in July, was the result of a release of nutrients from Ranunculus plants decaying after the herbicide treatment. There was no evidence from the water chemistry data of any such increases in nutrient loading in channel 3. The concentrations of dissolved ions were constant in both channels throughout the trial.

The densities of chironomids on the Ranunculus were remarkably similar in the two channels throughout the trial. The estimated total numbers per channel were also similar because the Ranunculus biomasses in the two channels did not greatly diverge. It would appear unlikely that the herbicide had any significant influence on these animals.

The significant increase in density of Ephemeroptera in channel 3, compared to channel 2, by 14 days after the herbicide application, was an interesting result. This might be interpreted as the increased crowding of animals onto a diminishing quantity of their preferred substrate. However, when an allowance is made for the changes in Ranunculus biomass, the total numbers of Ephemeroptera in channel 3 were still considerably greater than in channel 2, suggesting that a divergence in the population dynamics of the two channels is a more likely explanation of these observations.

In contrast, the density and total numbers of naid worms in channel 3 were significantly less than in channel 2, seven days after treatment. Some change may have occurred in channel 3 which has made the Ranunculus a less favourable habitat.

The densities of simuliids increased in both channels, slightly earlier in channel 2, but significantly more, after 14 days, in channel 3.

With the possible exception of the naid worms, these data indicate that the application of the diquat-alginate did not adversely effect the taxa which accounted for the majority of the macroinvertebrates in the channels.

7.6 Diquat-alginate in the Recirculating Channels: Trial 2

7.6.1 Replanting the channels for Trial 2

Ranunculus was collected for the second trial from the R.Frome at Grey's Bridge, Dorchester (N.G.R. SY701908) on 22.7.85. Forty-five beds, of approximately 500g fresh weight, were planted in each channel, in the same positions as in Trial 1, with an extra five beds near the outflow. These five beds were sampled on 23.7.85 and 7.8.85 so that the increase in biomass, or losses due to the transplant, could be assessed.

	Mean growth rate ($week^{-1}$)	S.E.	n
Channel 2	0.154	0.059	5
Channel 3	0.127	0.115	4

The biomasses of the Ranunculus beds and the growth rates did not significantly differ between the two channels.

Losses of Ranunculus as a direct result of transplantation were estimated from the biomass of broken stems collected one day after the transplant. These biomass have been expressed as a percentage of the total biomass of Ranunculus per channel:

Channel 2	4.2%
Channel 3	4.5%

7.6.2 Methods: Herbicide application

In Trial 2 the application rate recommended for water over 30cm deep (1.0l of Midstream per $100m^2$ of water surface) was used. Exactly the same method of application was used as in Trial 1, with 15.8ml of Midstream injected onto each bed, on 15.8.85. in channel 3.

Samples of water, 0.5 or 0.25l, were taken at regular intervals, after the application of the herbicide. These samples were taken from the downstream edge of the treated section, and from the mouth of the outflow pipes.

These samples were stored in polypropylene bottles and were frozen, along with a set of standard diquat solutions, until they could be analysed. The diquat residues were determined by the direct spectrophotometric method, at 309nm, as described in Section 4.4.3. The analysis was carried out using 4cm glass cells in a Beckman DU-8 spectrophotometer. Two replicate water samples were collected at most sampling times, and the mean of three readings per sample was calculated. The diquat residue results are discussed in Section 7.7.

7.6.3 Methods: Ecological monitoring

Ranunculus mapping and biomass

The pre-treatment mapping of the Ranunculus beds showed that the vegetation covered a considerably smaller proportion of the substrate than in Trial 1:

Channel 2	13.3%
Channel 3	13.1%

The same method of sampling the Ranunculus biomass was used as in Trial 1, but the five beds for each sample were chosen completely at random.

The biomass of benthic algae and moss

To reduce the damage caused in Trial 1 by the filamentous algae, a regular weeding regime was established. Every 3-4 days the filamentous algae was gently removed by hand from the Ranunculus beds, and as much algae as possible was cleared from the substrate and channel sides, using a rake. A wire mesh screen was placed across the channel, just above the outflow, during this operation, to trap floating algae. This screen was removed and cleaned afterwards, and any broken Ranunculus stems caught, were collected and weighed.

Samples of algae were collected, as in Trial 1, to assess the biomass of algae and moss. These samples no longer indicated the effect of the herbicide but they did show that the weeding exercise, tedious as it was, was of value in limiting the total algae biomass.

Macroinvertebrate communities

Exactly the same method of sampling the macroinvertebrates, as described for Trial 1, was used. Samples were taken prior to treatment and 18 days after the herbicide application.

To assess whether there was a short-term effect of the herbicide application on the macroinvertebrate densities, three samples were taken from channel 3 on the day of treatment. One sample was taken prior to the herbicide application, one two hours later and one after four hours.

Water chemistry

Samples of water were collected at two or three day intervals before and after the herbicide treatment, from both channels and the inflow. Reactive phosphorus was determined immediately, and 100ml samples were frozen for nitrate determinations at a later date. Analyses for calcium and alkalinity were performed on the day of treatment only. As in Trial 1 standard methods were used throughout.

Temperature and dissolved oxygen concentrations were recorded at twenty minute intervals on Squirrel RAM data loggers. These loggers were only available for both channels for 16 days, after which the logger for channel 2 was unavailable. Measurements in channel 3 were continued for a further 20 days. It was only possible to record pH on the data logger in channel 3.

After each experiment the contents of the RAM loggers were transferred to an Apple II+ computer. Basic computer programmes were developed to apply calibration and temperature correction procedures, and to display the results in a graphical form.

Temperature was measured using platinum resistance thermometers, pH using a Radiometer glass electrode with a Beckman calomel reference electrode, and dissolved oxygen using two Mackereth-type oxygen sensors.

Further details on the construction and calibration of the oxygen electrodes are described in Appendix 9.

7.6.4 Results of Trial 2

Ranunculus biomass

Comparison of channel 2 vs. channel 3

The total biomass and percentage of decayed Ranunculus in each channel were compared for each sampling time, Fig. 79a, 79b.

Prior to the herbicide application there were no significant differences in biomass or percentage decayed material, between the two channels. By 8 days after treatment the channels did not differ significantly in total biomass, but there was a significantly greater percentage of decayed Ranunculus in channel 3 than in channel 2.

For all subsequent sampling times the total biomass was significantly lower in channel 3, and the percentage of decayed tissue was higher,than in channel 2.

Comparisons of upstream and downstream sections of the channels

There were no significant differences in Ranunculus biomass or percentage decayed material, between the two sections in channel 2.

In channel 3 there were no significant differences between the upstream and downstream halves of the channel prior to treatment, but after 8 days there was a significantly greater percentage of decayed tissue in the upstream section. By 32 days after treatment, there was a significantly lower total biomass of Ranunculus in the upstream section of channel 3 than in the downstream section (Fig. 80a, 80b).

Comparisons of sampling times per channel

With one exception, there were no significant differences between sampling times for total biomass, in channel 2 (Fig. 79a). The exception was the comparison between the samples taken 19 and 33 days after treatment. The percentage of decayed material in channel 2 became significantly greater at each sampling time,with the exceptions of the pre-treatment and 8 d.a.t.,and the 8 and 19 d.a.t. comparisons.

In channel 3 there was no significant change in the total biomass between the samples taken prior to treatment and 8 days afterwards. Otherwise, there were significant reductions in biomass with time, between samples taken at greater than one day intervals.

Fig. 79

Comparisons of Channel 2 and Channel 3 in Trial 2

Fig. 79a. Biomass of Ranunculus

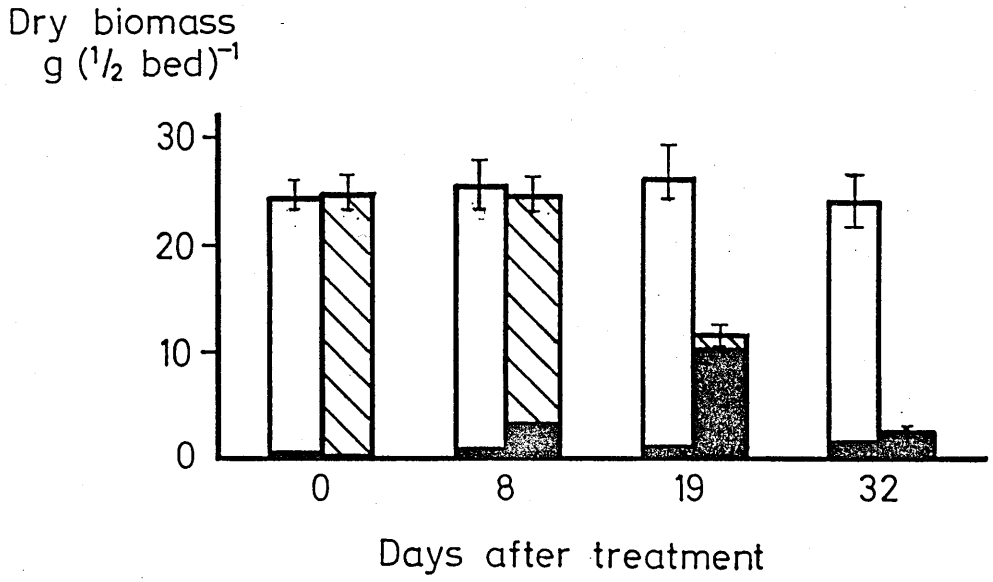


Fig. 79b. Percentage of decayed tissue

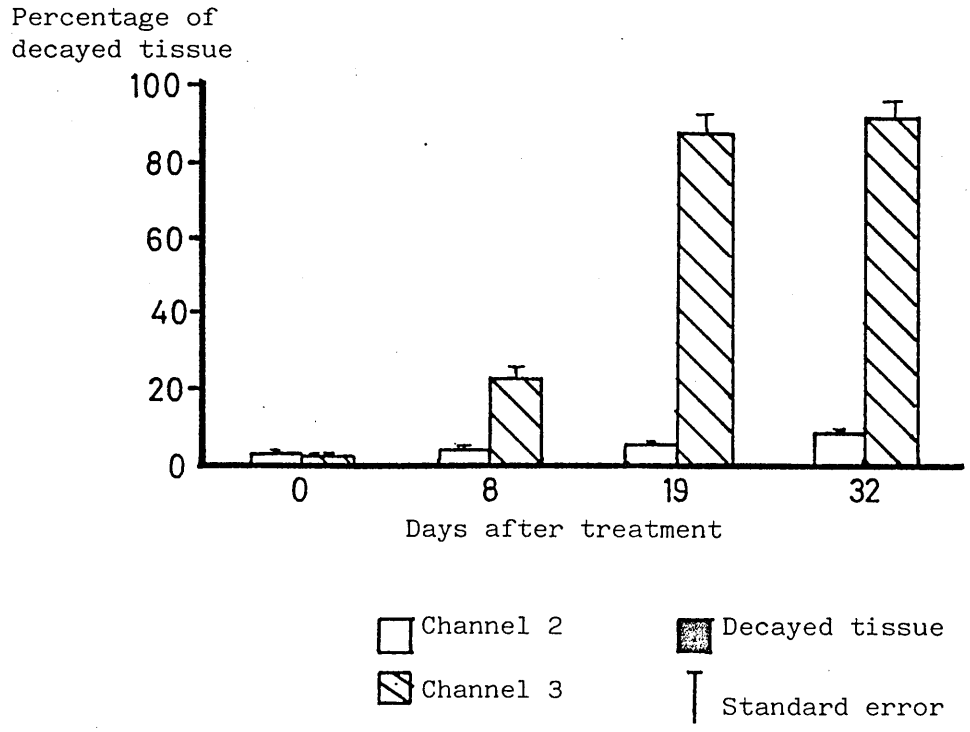


Fig. 80

Comparisons of upstream and downstream halves of hte channels in Trial 2

Fig. 80a. Biomass of Ranunculus

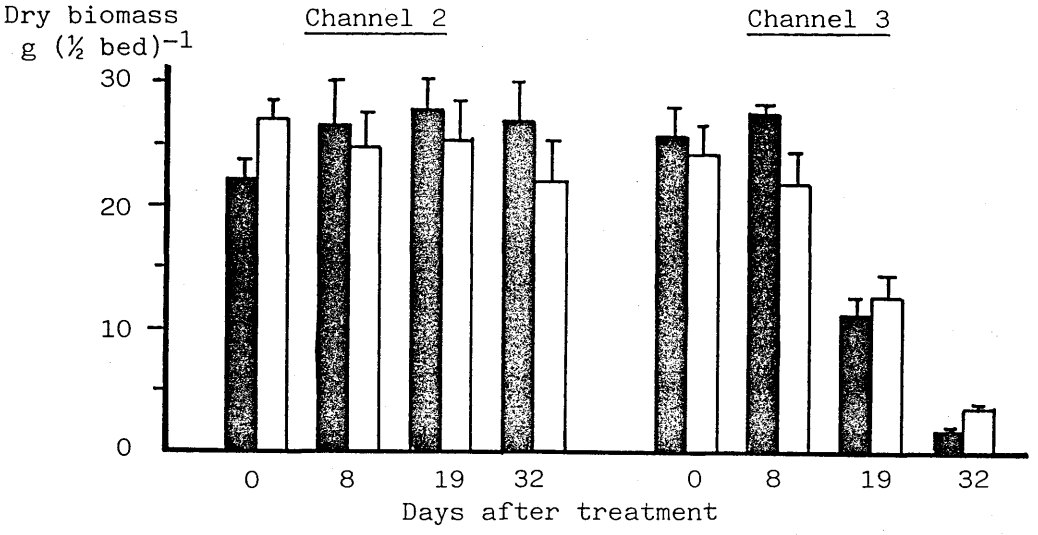
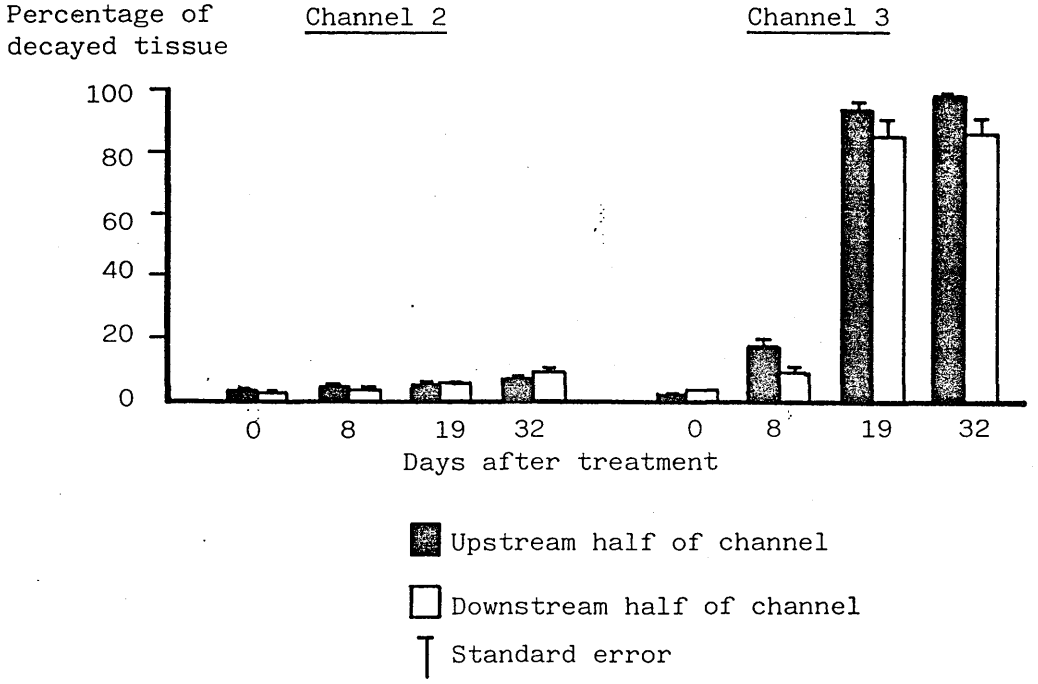


Fig. 80b. Percentage of decayed tissue



The percentage of decayed material significantly increased at each sampling time until 32 days after treatment. There was no significant change in this parameter between 19 and 32 days after treatment, and by 33 d.a.t. the percentage of decayed material had decreased.

Ranunculus losses

The percentages of the total biomass of Ranunculus in the channels which were collected off the safety screen, the algal-cleaning screen, and from the outflow, are shown in Fig. 81.

The actual biomass of Ranunculus lost from channel 2 was always greater than from channel 3, but when considered as a percentage of the total biomass present, there was usually little difference between the channels. Greater losses occurred from channel 3 on 3.9.85 and 17.9.85, which might correspond to the large reductions in total biomass prior to these dates.

Biomasses of macro-algae and moss

The blue-green algae which had caused problems in channel 2 in Trial 1, had disappeared by Trial 2, and the populations of filamentous algae were similar in both channels. By August a species of Zygnema was dominant, having partly replaced the Cladophora and Vaucheria which had been dominant earlier. The moss Drepanocladus aduncus was still present.

The mean biomasses of the algae and moss in each channel are presented in Table 29. There were no significant differences between the two halves of the channels.

Progressive changes in biomass cannot be followed because of the regular cleaning procedures. The algal biomass was always greater in channel 3, and this difference was significant in all but the first, samples.

Water chemistry

Dissolved ions

In view of the stability of the alkalinity and concentrations of magnesium, sodium and potassium, these ions were not quantified in Trial 2. The calcium concentrations prior to the herbicide application are listed in Table 30, along with the nitrate concentrations. Nitrate concentrations did not appear to vary much with time

Fig. 81

Losses of Ranunculus in relation to the total biomass in Trial 2

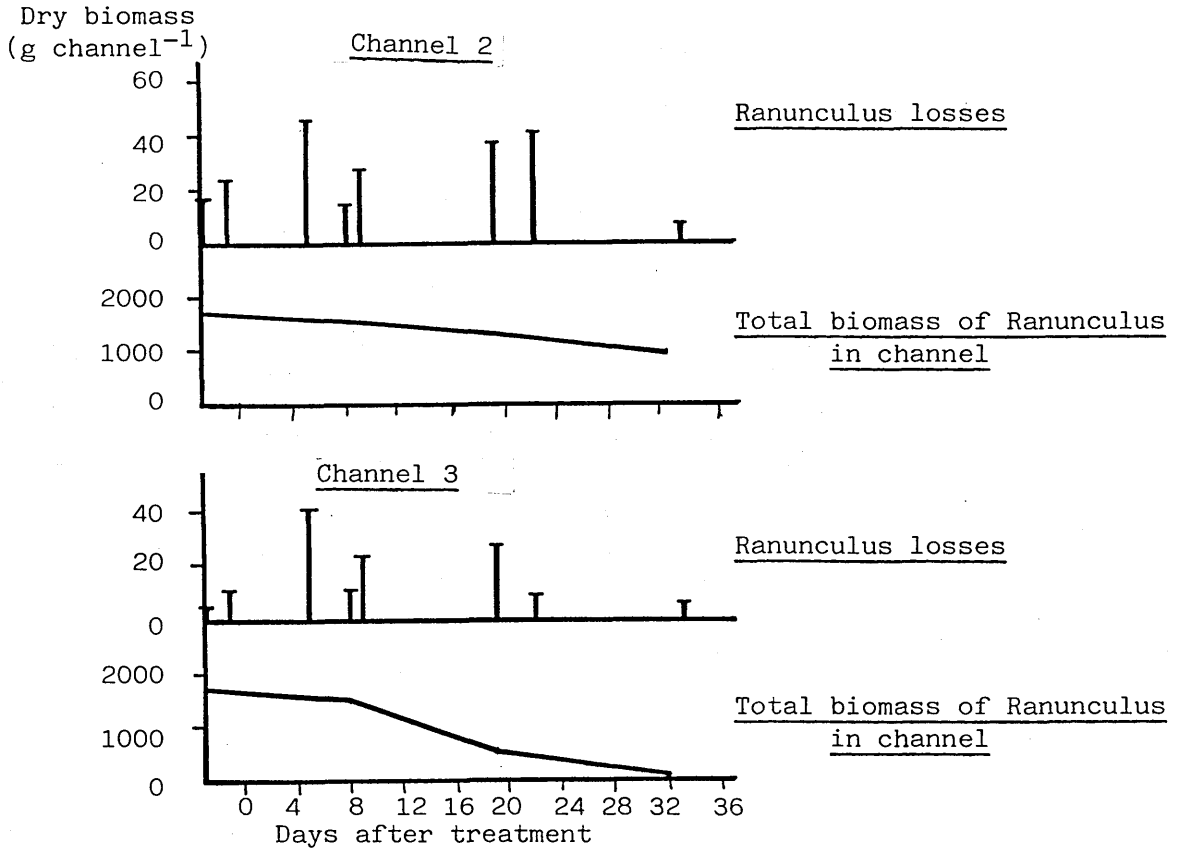
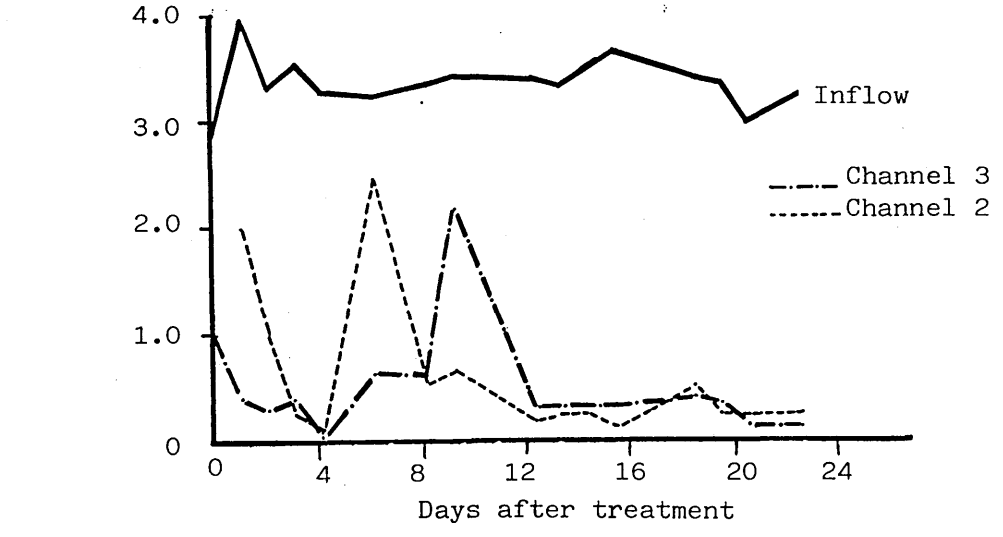


Fig. 82



Concentrations of reactive phosphorus in Trial 2

Table 29

Biomass of algae in the channels in Trial 2

Date	d.a.w.		Channel 2		Channel 3	
5.8.85	0		44.32	(8.16)	64.16	(6.4)
13.8.85	1		60.48	(6.88)	101.92	(14.72)
19.8.85	5		64.96	(11.36)	108.64	(14.72)
21.8.85	1		26.56	(4.48)	44.48	(9.12)
2.9.85	9		75.36	(7.52)	105.12	(5.6)
16.9.85	10		91.68	(8.0)	176.64	(11.84)
20.9.85	14		86.4	(7.04)		
27.9.85	21		128.16	(12.64)		
2.10.85	27		129.12	(6.08)		
		Sides	110.88	(16.16)		

Values = mean of 10 samples ( ) = Standard error

d.a.w. days after algal weeding

Table 30

Dissolved ions in Trial 2

	Nitrate (mg l^{-1})			Calcium (mg l^{-1})		
	In	C2	C3	In	C2	C3
13.8.85	5.09	4.88	5.08	101	72	73
15.8.85	5.18	4.68	5.06	101	84	102
16.8.85	5.38	5.50	5.17			
17.8.85	5.17	5.05	4.65			
18.8.85	5.50	5.07	4.68			
19.8.85	5.28	4.89	4.64			
21.8.85	5.34	5.12	5.06			
23.8.85	5.72	5.23	5.22			
24.8.85	5.56	4.78	5.05			
27.8.85	5.62	5.00	5.07			
30.8.85	5.68	4.85	5.00			
2.9.85	5.18	4.58	4.84			
4.9.85	4.95	4.60	4.69			
6.9.85	4.96	4.75	4.67			

or source.

The concentrations of reactive phosphorus are shown in Fig. 82. The pre-treatment samples became contaminated and have not been included. The high concentrations on the day of treatment in channel 3, and the succeeding day in channel 2, were the result of the increased inflow associated with the herbicide dilution.

The later peaks in the concentration of phosphorus, 6 days after the herbicide application in channel 2 and 3 days later in channel 3, were not related to changes in the inflow. Apart from these peaks, the reactive phosphorus concentrations in the two channels were similar.

Temperature, pH and dissolved oxygen

The data for the temperature, pH and percentage saturation of dissolved oxygen are presented in Fig. 83a-d.

The diurnal fluctuations in all three parameters are distinct. No statistical comparison of the data from the two channels has been attempted.

The temperature values and trends were similar for the two channels. Although there might be noticeable differences between some days, depending upon the weather conditions, the daily range of temperatures did not alter over the five week sampling period.

pH clearly increased during the day and fell at night. The nightly minima tended to be more stable over the 5 weeks, than the daily maxima, but the diurnal range of pH remained the same over the 5 weeks.

The percentage saturation of dissolved oxygen showed similar diurnal patterns to pH. The same pattern of maximal peak heights were seen for dissolved oxygen and pH, occurring on the days with highest temperatures.

The diurnal patterns of dissolved oxygen were similar in both channels, with equal night-time minima, but slightly lower daily maxima in channel 2. As the five week period progressed the diurnal range of percentage saturation dissolved oxygen values in channel 3 did not change in size, but the maxima and minima became lower. At the start of the trial a typical range was 95-140% saturation (17.8.85), but by the end it was 80-125% saturation (13.9.85).

Fig. 83a

Water temperatures in Channel 3 during Trial 2

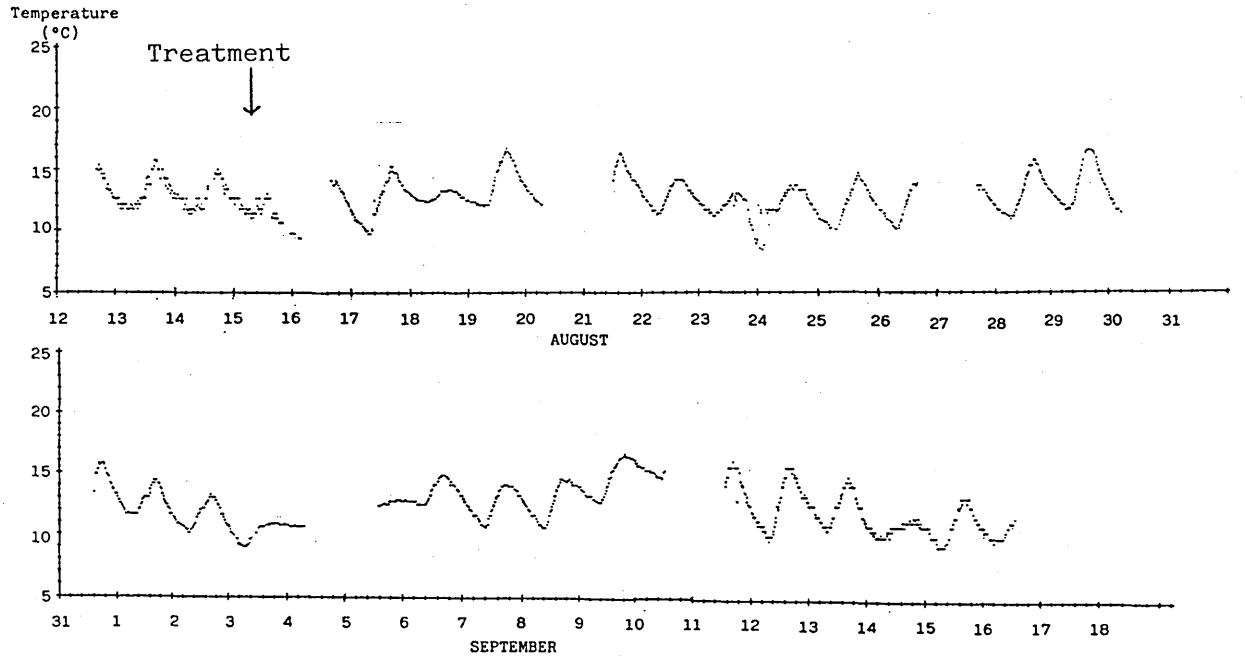


Fig. 83b

Pecentage saturation of dissolved oxygen in Channel 3 during Trial 2

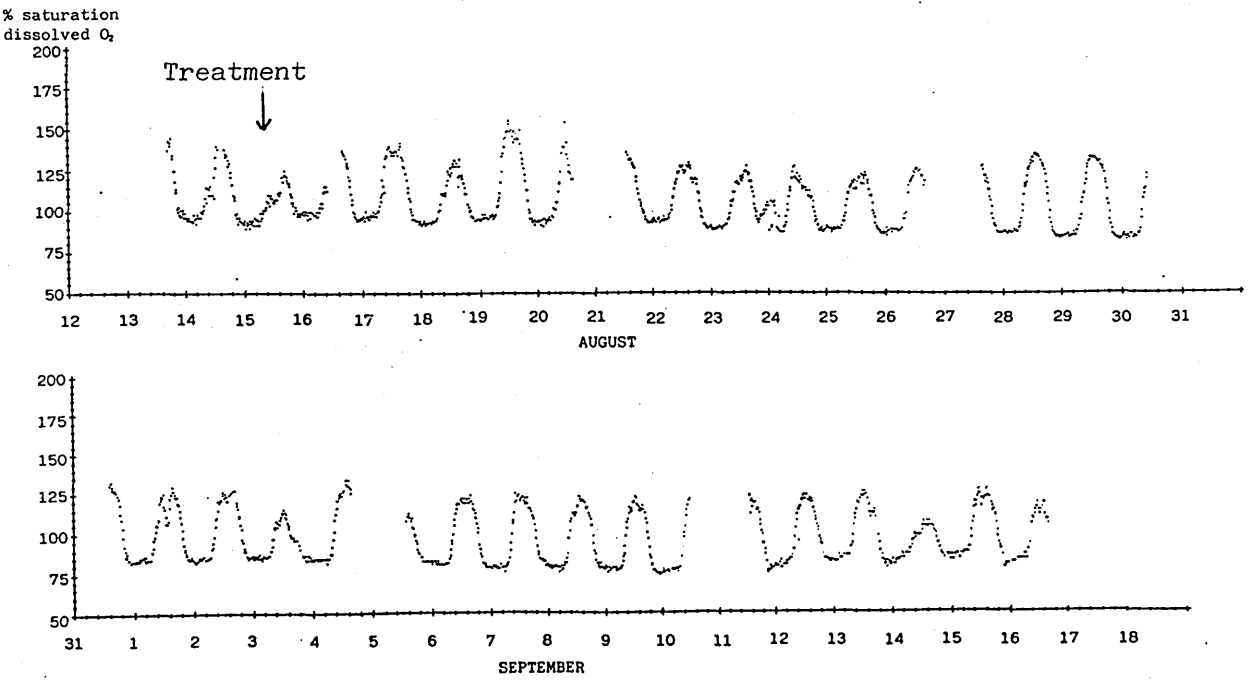


Fig. 83c

pH in Channel 3 during Trial 2

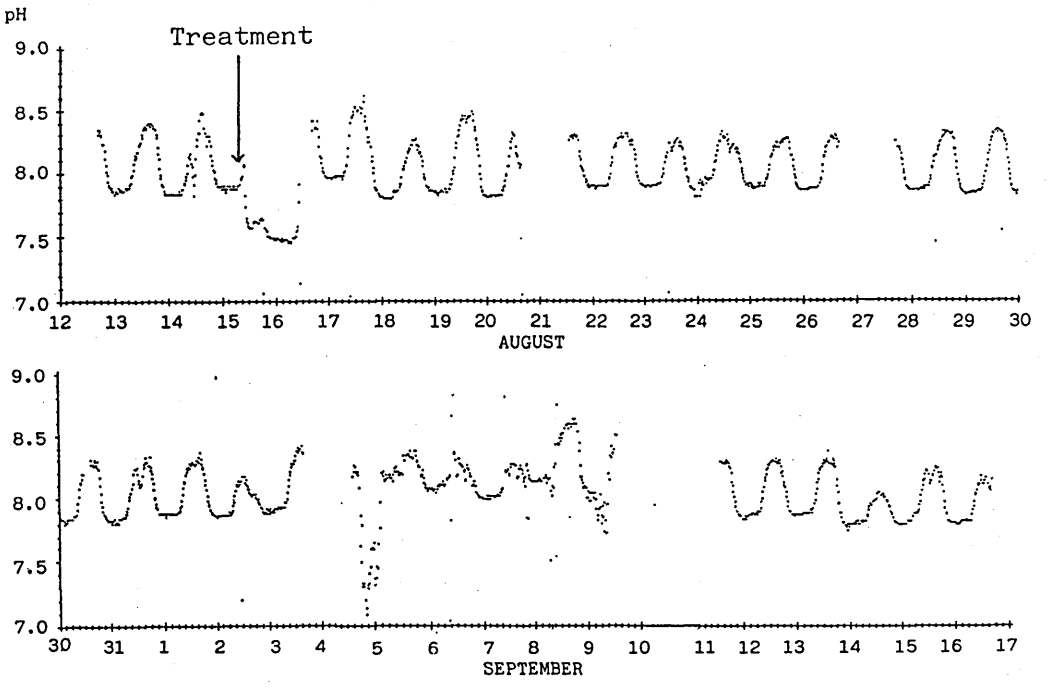
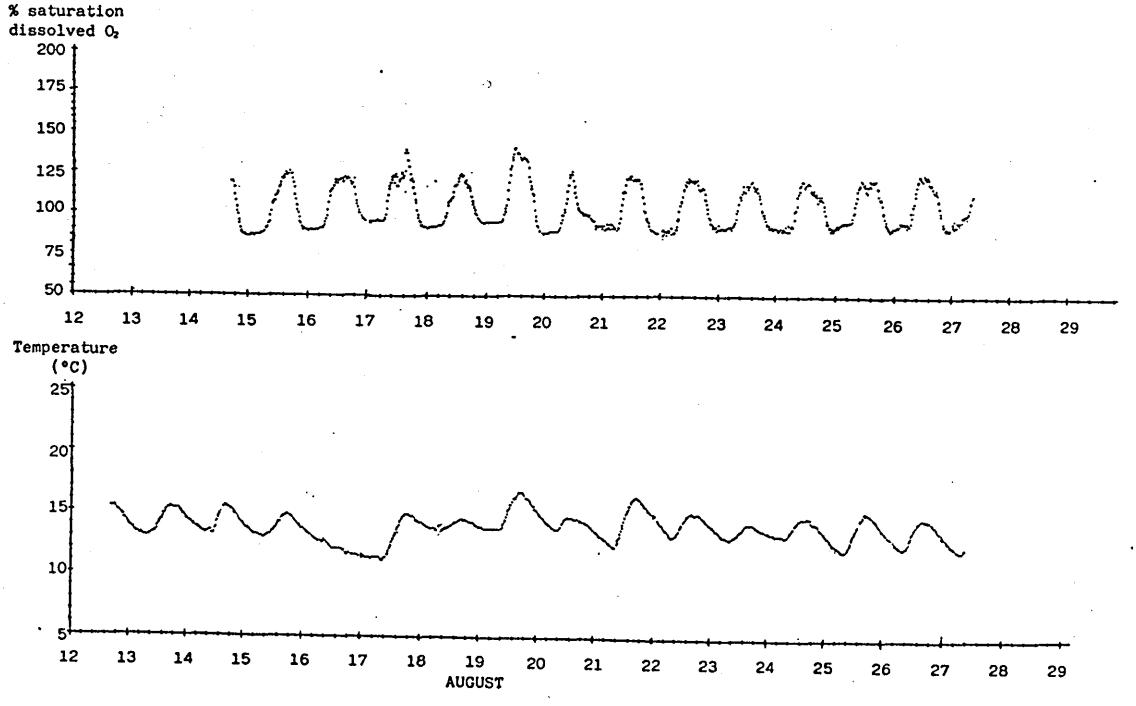


Fig. 83d

Dissolved oxygen and temperature in Channel 2 during Trial 2



Macroinvertebrate communities

The list of species present in Trial 2 (Table 28) was not very different from Trial 1. The mean numbers of animals per gramme dry weight of Ranunculus, for all taxa, are listed in Appendix 14b.

Six taxa were identified for statistical analysis:

Chirononidae
Ephemeroptera
Naididae
Simuliidae
Trichoptera
Hydra

The proportion of chironomid pupae was small throughout the trial. The Ephemeroptera were only represented by Baetis spp. and simuliid pupae were only found rarely. Data are illustrated in Fig.84.

The same statistical analyses were applied to the data from 9.8.85 and 2.9.85, as were used in Trial 1.

There were no significant differences between the upstream and downstream sections of the channels, except for the naids on the day of treatment.

Chironomidae

The densities of chironomids were significantly greater in channel 3 than in channel 2 on both sampling dates. The densities also significantly decreased in both channels between the pre- and post- treatment sampling times.

There were no significant changes in chironomid density in channel 3 during the day of the herbicide application.

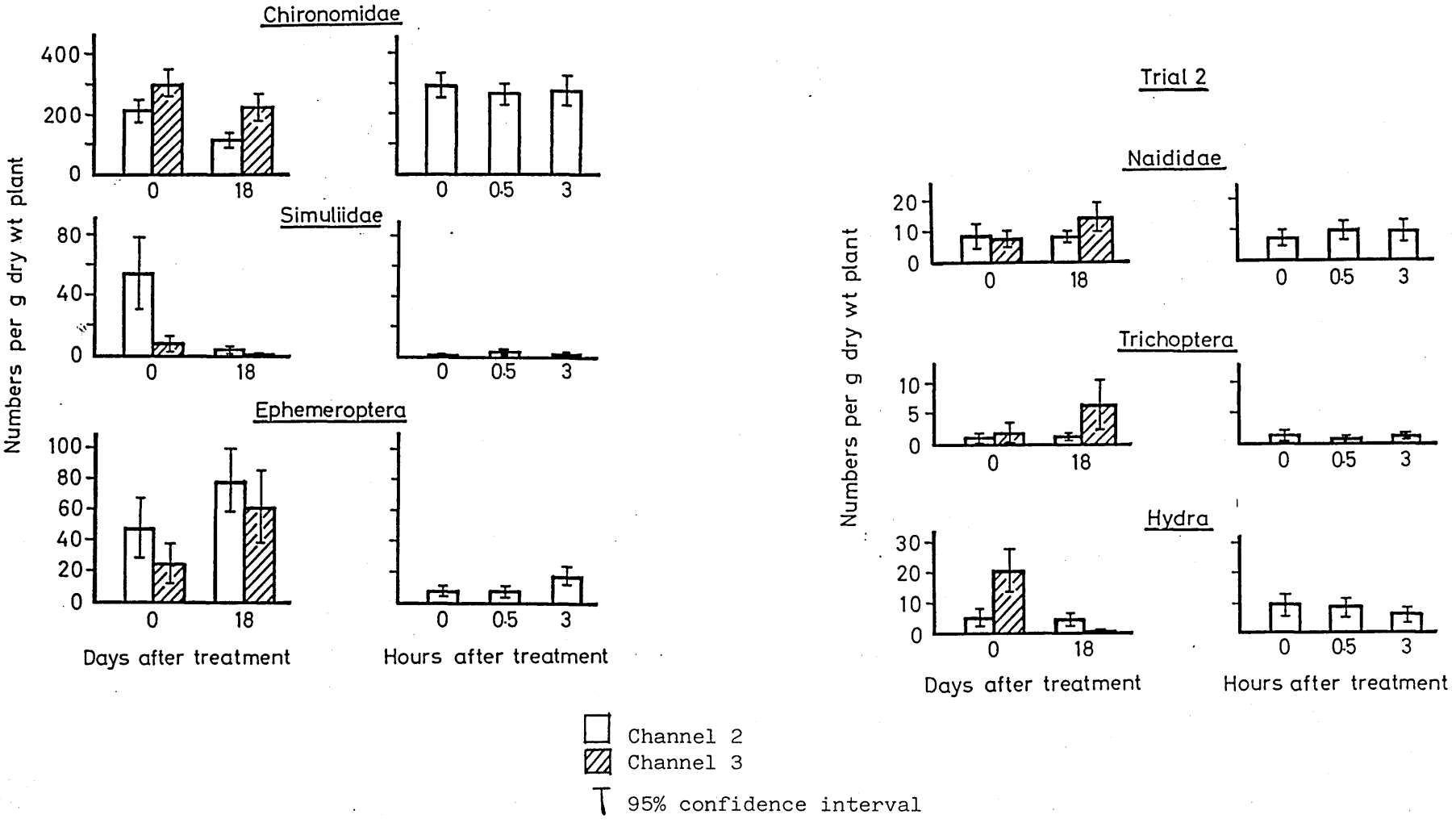
Ephemeroptera

The density of Baetis was significantly greater in channel 2 than in channel 3, prior to treatment, but the difference was no longer significant by 18 days after treatment. There were significant increases in the density of Baetis in both channels by 18 d.a.t., with a significantly greater increase in channel 3.

On the day of the herbicide application the pre-treatment density was significantly lower than on both of the other sampling occasions. The density of Baetis had significantly increased by 4 hours after treatment.

Fig. 84

Macroinvertebrate data from Trial 2



Naididae

Prior to treatment there was no significant difference in the density of naids between the channels, but by 18 days after treatment, there was a significantly greater density in channel 3.

The density of naids in channel 3 did not significantly change on the day of the herbicide application, and these densities did not differ significantly from the sample taken on 9.8.85.

Simuliidae

There were significantly lower densities of simuliids in channel 3 than in channel 2, on both sampling dates. The densities of simuliids in both channels significantly decreased between sampling dates. The density of simuliids did not change during the day of the herbicide application, but the pre-treatment density was lower than that in channel 3 on 9.8.85.

Trichoptera

Prior to treatment the density of Trichoptera did not differ significantly between the channels, but by 18 days after treatment there was a significantly greater density in channel 3 than in channel 2. The density of Trichoptera did not alter with time in channel 2, but increased significantly in channel 3. The densities did not change on the day of treatment and were not significantly different from the density in channel 3 on 9.8.85.

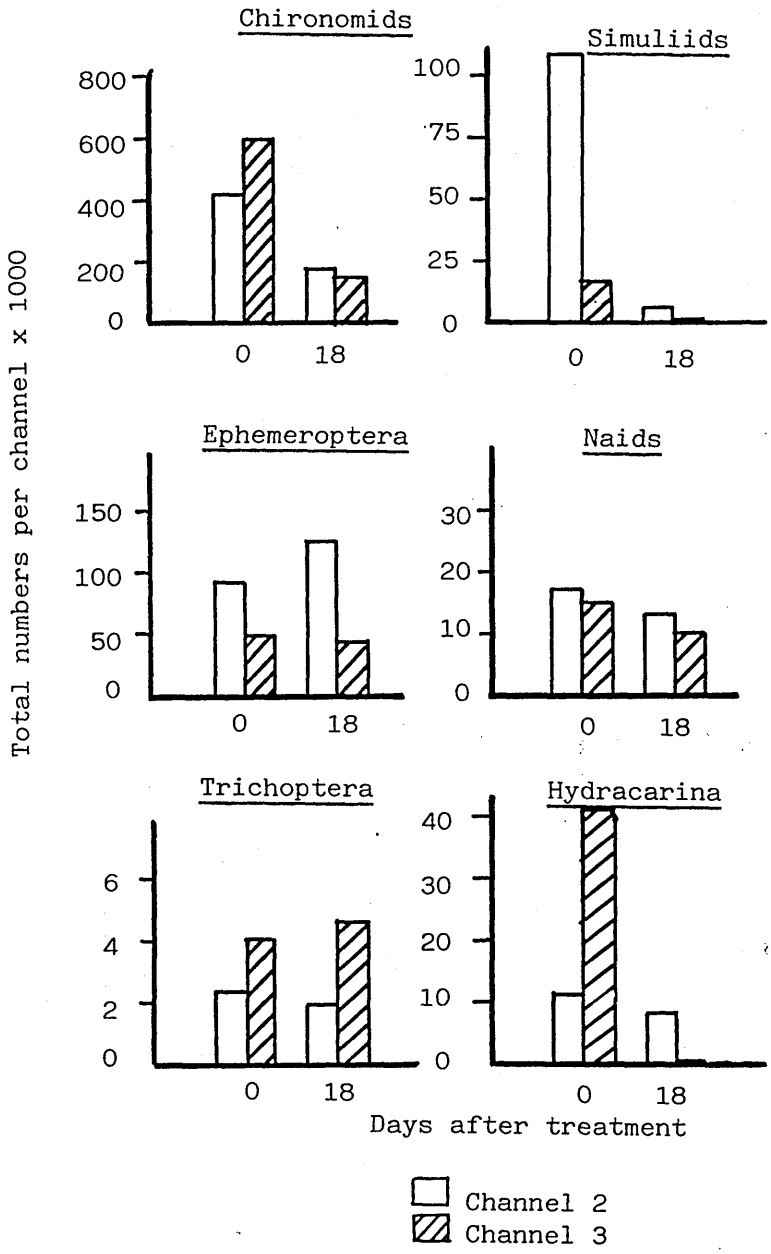
Hydra

The density of hydra was significantly greater in channel 3 than channel 2 prior to treatment,but by 18 d.a.t. the density in channel 3 was significantly less. The difference in the changes in density with time, between the two channels, was significant. The density of hydra did not significantly alter on the day of the herbicide application, but these densities were significantly lower than those from 9.8.85, and were significantly higher than those from 2.9.85.

The mean total numbers of macroinvertebrates estimated per channel are shown in Fig. 85. The large decline in the biomass of Ranunculus in channel 3, between the sampling dates, caused major changes in some of the relationships between the pre- and post-treatment densities, in channel 3 (e.g. Trichoptera, Baetis, naids), and between the post-treatment densities in channel 2 compared to channel 3 (e.g. chironomids, naids).

Fig. 85

Total numbers of macroinvertebrates estimated per channel in Trial 2



7.6.5 Discussion of Trial 2

The growth rates of the Ranunculus in the channels during the two weeks prior to Trial 2, were higher than the over-winter values, indicating that the transplants were in a healthy condition.

Unlike Trial 1, there was little doubt that in Trial 2 there was some factor in channel 3 causing a significant reduction in the biomass of Ranunculus, not present in channel 2. Without the evidence of treatment replication, it can only be suggested that the herbicide was responsible.

The biomass of Ranunculus in channel 2 remains fairly constant, with only a slight decline by the end of the trial. The percentage of decayed material increased significantly with time indicating that the conditions in the channel may not have been ideal for the long-term growth of Ranunculus.

The percentage of decayed tissue in channel 3 was significantly greater than its pre-treatment level, or than in channel 2, within eight days of the herbicide application. At that time the percentage of decayed material was greater in the upstream half of channel 3, than in the downstream section. This suggests that the direct application of diquat-alginate had a faster herbicidal effect than the indirect dose of diquat in solution. By 32 days after treatment there was a significantly smaller biomass of plants in the upstream section of channel 3, compared to the downstream half.

The biomass of Ranunculus in channel 3 was significantly reduced compared to the pre-treatment value, and compared to channel 2, by 19 days after treatment. The application of the diquat-alginate into the weed beds, on the stem bases would cause a rapid loss of biomass, much of which might still be healthy, once the bases of the stems were sufficiently necrotic and weak.

The reduction in the percentage of decayed material at the end of the trial would have been because most of the decayed material will have been lost, leaving a small amount of predominantly healthy tissue, which might survive the treatment.

The removal of the algae helped to prevent the damage caused to the Ranunculus in Trial 1, but made it impossible to show whether the herbicide affected the algae. The moss did not show any phyto-toxic symptoms in channel 3.

There was no evidence of changes in the concentrations of dissolved ions in channel 3, which might have been associated with the herbicide treatment. Although the pH and dissolved oxygen data for channel 2 (for comparison) were incomplete, there did not appear to be any disturbance of the diurnal pattern of these parameters in channel 3 which could be associated with the herbicide application, or the reduction in biomass of Ranunculus.

None of the major taxa examined showed significant reductions in density on the Ranunculus immediately after the herbicide application in channel 3. It had been expected that this sampling regime might identify any taxa showing immediate susceptibility to the herbicide, or sub-lethal behavioural changes that caused the animals to move away from the Ranunculus.

The stability and low variability of these data suggested that this sampling method provided a reliable means of estimating the macroinvertebrate populations living on the Ranunculus.

The densities of chironomids declined in both channels during the trial but there was little difference in total numbers between the channels after treatment. The density of Baetis increased in both channels and although this increase was greatest in channel 3, there was little change in the total number of animals on the Ranunculus in the channel. The stable-sized population of Baetis on the Ranunculus in channel 3, was having to crowd onto the plants remaining after the herbicide treatment.

The density of naid worms in channel 3 increased after the herbicide application, but the total numbers on Ranunculus in the channel, may have decreased. Unlike Trial 1 there was no great divergence in the population numbers between the channels, after treatment.

The densities and total numbers of simuliids were quite different in the two channels prior to treatment but the populations in both channels showed large declines by early September. It is likely that some reduction in population size was occurring in both channels, but this decline had started earlier in channel3.

The total numbers of Trichoptera on Ranunculus in channel 3 were greater than in channel 2 at both sampling times, but did not greatly differ before and after treatment. The density of these animals did increase significantly more in channel 3, after treatment, than in

channel 2. This might indicate that the Trichopterans were forced to crowd together onto the remaining plants, after the herbicide had reduced the biomass of their preferred substrate.

Hydra had not been detected in Trial 1. Their presence in Trial 2 may have resulted from their introduction on the Ranunculus beds transplanted from the R.Frome, or from a sudden increase in numbers, or size of individuals (previously too small to detect), between the trials.

The density and total numbers of hydra declined slightly in channel 2, but there was a very significantly greater reduction, after treatment, in channel 3.

These data may indicate that the herbicide caused a reduction in the numbers of hydra, either by direct toxicity, or indirectly by reducing the numbers of another species, such as microcrustacea, upon which the hydra feed. There is evidence that some microcrustacea, are susceptible to the bipyridinium herbicides, (e.g. species of Daphnia, Crosby and Tucker 1966, Saunders and Cope 1966).

Microcrustacea were not sampled but in the fast flowing channels their populations might have been limited to the sheltered water within weed beds, and the hydra were more dependent upon other food sources, such as small worms.

The connection between the decline in numbers of hydra and the herbicide treatment appears less convincing however, if the density immediately prior to the herbicide application is considered. This value was intermediate between the other two samples, suggesting that the hydra population was in decline in channel 3 prior to the application of diquat-alginate.

With the exception of the hydra (which appeared to be in decline prior to the herbicide application) there were no significant reductions in density of any of the six major taxa examined, in channel 3 which were not matched in channel 2. The significantly greater increases in densities of Ephemeroptera and Trichoptera in channel 3, compared to channel 2 may have resulted from a loss of their preferred habitat.

7.7 The behaviour of diquat residues in a recirculating channel

7.7.1 Results of the diquat residue analyses from Trial 2 and a comparison with the solute dilution model

The results of the diquat residue analyses are shown in Fig. 86 and the full data are listed in Appendix 10. The residues from the upstream and downstream sampling points were similar, and the standard errors for the downstream samples were small.

There were fluctuations in the diquat concentrations of samples collected within the initial 20 minutes. It is unlikely that the herbicide was evenly mixed throughout the water body in this period. The rise in diquat concentrations between 5 and 20 minutes after application, may indicate that there was a delay in the release of diquat from the alginate, after the initial passage of the unbound herbicide into solution. The variation between the two sets of samples and the uncertainty of the mixing, make it valueless to try to quantify this release rate.

Between 2 and 3 hours after the application, for some unknown reason, the inflow rate (f) decreased, and then increased after 3 hours. For this reason the data and the model were divided into appropriate periods, for separate regression analyses.

The results of the Log_e transformations and of the linear regressions are shown in Fig. 87.

20 minutes - 2 hours after application

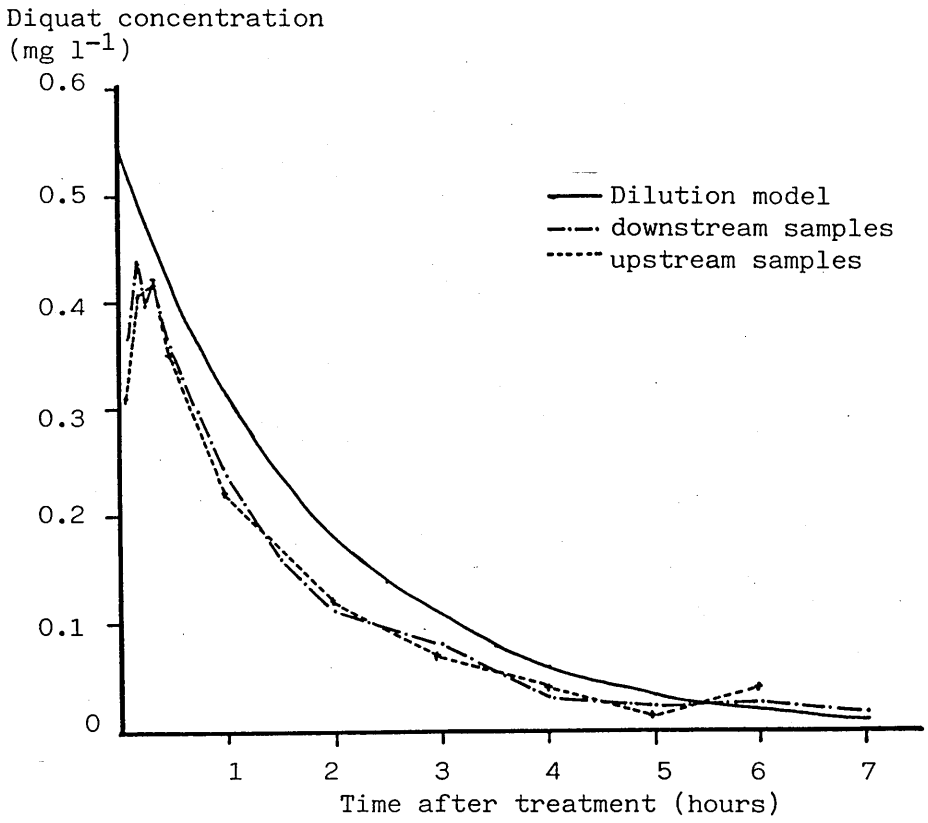
Regression of the downstream data (considered to be more reliable because of the replication) gave an extrapolation of the diquat concentrations back to the start of the application (t_o) to C_o = 0.545mg l^{-1} diquat. This value was used as C_o in the model, which was derived using the measured values of inflow (f)(Fig. 86) and the volume V= 51m^3 .

Between 20 minutes and 2 hours after application, the regressions of the downstream and upstream data were not significantly different. The regression of the model was significantly different from that of the downstream data because of the different gradients.

The significantly faster reduction in diquat concentrations over 2 hours from the downstream samples (i.e. steeper gradient), indicates that there was a greater loss of diquat from the channel water than

Fig. 86

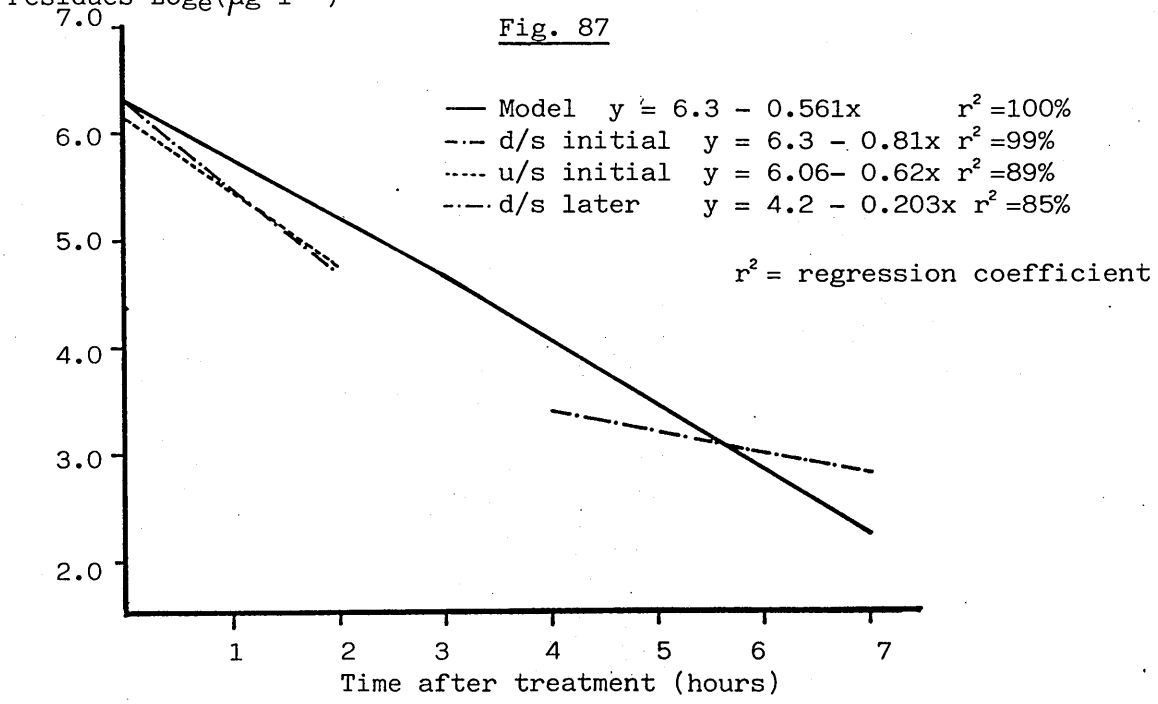
Diquat residues in Channel 3 during Trial 2



Log$_e$ diquat residues Log$_e$(µg l^{-1})

28.5 26 31.2 Inflow rates (m^3 hr^{-1})

Fig. 87



Regressions for the Log$_e$ transformed diquat residue data and the dilution model

would have been predicted just by dilution. This loss would have been due to the adsorption of diquat onto the plants, substrates and suspended materials in the water.

There was not such a great increase in the removal of diquat from the upstream samples. This may have been due to two factors:

1) The diquat released from this section had not passed over such a large area of potential adsorption sites before collection.
2) The accelerated loss of diquat by adsorption in this section was balanced by an input of diquat, slowly released from the alginate.

The downstream half of the channel (not directly treated) was an additional area of diquat adsorption but not of delayed input.

Knowing the volume of Midstream applied, it was estimated that 31750mg of diquat were added to the channel. From the extrapolated value of C_o = 0.545mg l^{-1} and the volume V= 51m^3, it would appear that only 27795mg of diquat were added. The apparent difference 3955mg, (12.5% of the expected input) would have arisen because some of the diquat would have remained bound in the alginate, whilst some will have been rapidly adsorbed and removed from detection.

Why the value of C_o extrapolated from the upstream samples was less than that from the downstream samples, was not clear.

The areas under the residue concentration vs. time curves (Fig. 86), the availance (Hartley and Graham-Bryce 1980), for this period were found by the integration of the untransformed regression equations, assuming that the data fit the type of exponential curve used in the model.

$$C_t = C_o\, e^{-\frac{f}{V}.t} \quad \text{integrates to:-} \quad \int_0^t C_t\, dt = \left[-\frac{C_o}{\frac{f}{V}}\, e^{-\frac{f}{V}.t} \right]_0^t$$

$$\text{as } t \Rightarrow \infty \quad \int_0^\infty C_t\, dt \Rightarrow \frac{C_o}{\frac{f}{V}}$$

$$\text{Let } \left[\frac{C_o}{\frac{f}{V}} \left(1 - e^{-\frac{f}{V}.t} \right) \right] = [X] \qquad \text{then } [X]_{0.33}^{2} = [X]_0^2 - [X]_0^{0.33}$$

area between 20 min and 2 hr

Availance values 20 minutes - 2 hours:

downstream samples	0.381 mg l^{-1} hr
model	0.489 mg l^{-1} hr
difference	0.108 mg l^{-1} hr

There was a 22% reduction in the availance of diquat in the downstream samples compared to the value predicted by the model.

The samples taken from 2.5 and 3 hours after the herbicide application, could not be included in the other regression analyses because of the changes in the rate of water inflow.

4-7 hours after application

The model for this time period was derived from the known gradient $\frac{f}{V}$, and a C_o value predicted by extrapolation from the last point, derived from the model, in the previous time period.

The gradient of the regression of the Log_e transformed data, had significantly decreased from the 20 minute - 2 hour time period. As a result of the slightly increased rate of inflow, the gradient of the model regression was greater than in the first time period.

These results suggest that the diquat was being lost from the channel water at a slower rate than would have been predicted by the dilution model. This might be due to a delayed release of diquat which had been bound to the alginate, providing an input of diquat into the water, in opposition to the rate of dilution.

These results and the comparisons with the dilution model show that the changes in the concentrations of diquat residues in the channel, were not simply determined by the rate of replacement by fresh water.

Diquat is not an inert chemical like the sodium in the dilution experiments. The herbicidal activity of diquat is dependent upon its uptake by plants, and its short persistence is due to rapid adsorption onto a variety of substrates. Such losses of diquat from solution would explain the initial deviations from the dilution model.

The formulation of diquat with an alginate presents further complications because of the uncertain release rate of the herbicide ions. Factors which may influence this release rate have been

considered in Section 2.2.3.

The unknown rate of release of diquat from the alginate means that estimates of the loss of diquat by processes other than dilution cannot be exact but indicate the minimum rate of loss. Only by direct comparison of the rates of residue loss from applications of diquat in solution and in alginate, could the rate of release of the herbicide from the alginate be estimated, and then be included in a model of diquat's behaviour.

7.7.2 Relating the behaviour of diquat residues in a recirculating channel to herbicide applications in rivers

In order to justify the use of the recirculating channels in the study of aquatic herbicides, it is necessary to show that the pattern of residue dispersal is not completely artificial but may simulate the results of applications to throughflowing watercourses. There are two ways in which diquat residue results from the recirculating channel may be compared with those expected in a river.

1) Availance

It may be argued that the delayed retention of diquat residues in the recirculating channel artificially increases the length of exposure of plants to the herbicide. However, the exposure period is not the only important factor in a herbicide treatment but the concentration of the chemical must also be considered. This is the reason for examining the product of these factors, the availance, and the point is illustrated in Fig. 88.

Whether there is a minimum threshold concentration or period of exposure to diquat is not certain, but it would not be difficult to examine this point in the laboratory. In terms of the availance, the prolonged exposure of the plants to diquat residues in the recirculating channel, is balanced by the exponentially decreasing concentration.

The total availance of the diquat in the channel (calculated over 7 hours from the two concentration vs. time curves indicated in the previous section) was :

$$0.713 \text{mg } l^{-1}.\text{hr} \quad \text{or} \quad 42.8 \text{mg } l^{-1}.\text{min}$$

Fig. 88

Illustration of three patterns of diquat exposure with equal availance

Avialance = $10mg^{-1}$. min

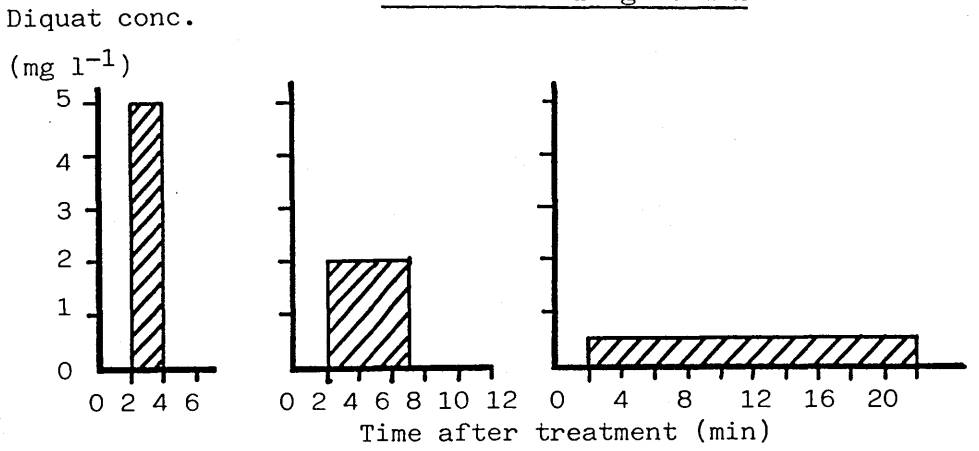
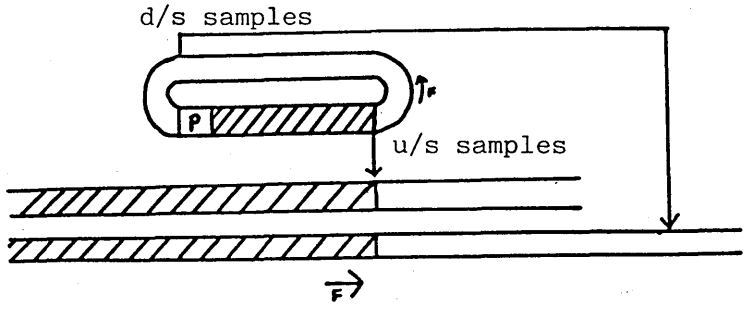


Fig. 89

The relationship between the recirculating channels and a river with regard to herbicide residue dispersal



P Recirculation pump

Directly treated area

F Flow direction

This total availance is considerably less than those recorded from the R.Petteril and the Mouse Water (Fig. 48). Comparison of availance values, over 40 minutes, for all the rivers surveyed,with the 40 minute availance from the channel, 16.84mg l^{-1}.min, indicates that the availance in the channel was similar to that ocurring in the upstream herbicide section of the R.Coln (15.25mg l^{-1}.min).

It is interesting to note that the availance of herbicides recorded in static water treatments may be several orders of magnitude greater than in flowing water. For example, for paraquat (which is likely to have similar availance properties to diquat) applied to a small lake,a total availance of 2160mg l^{-1}.min was estimated from the water residue data recorded over 8 days (Way et al. 1971).

2) Direct relation of dispersal patterns

The plants within the recirculating channel may be equated with ones in a river, a long length of which has been treated with diquat-alginate. The directly-treated plants in the upstream half of the channel would be the equivalent of plants at the downstream end of the treated length of river, which receive not only diquat-alginate, but also the diquat residues carried downstream from the whole treated length. The plants in the downstream half of the channel receive no diquat-alginate, but like plants downstream of a treated length of river, receive the diquat residues in solution (Fig. 89).

Knowing the channel length and water velocity it should be possible to estimate, from the persistence time of the residues, the equivalent length of river which would have been treated to maintain a similar passage of residues over the downstream point, to that observed in the channel.Such a calculation would be simple if an inert solute was being studied, which had been applied at a constant rate, and passed downstream at a constant rate. The passage of such a solute, under such ideal conditions is illustrated in Fig. 90a.

The downstream passage of an inert solute, such as a dye, does not remain uniform in real watercourses, even man-made ones, but the duration of the plateau (Fig. 90b) increases, while the amplitude decreases (Demint 1970). This phenomenon, caused by the non-uniform velocity of water throughout a channel, has been observed and analysed for the passage of the persistent herbicide acrolein (O'Loughlin and Bowmer 1975).

Fig. 90

A simplified illustration of the downstream dispersal of solutes

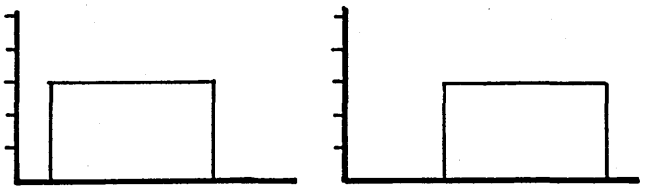


Fig. 90a

Passage of an inert solute under ideal flow conditions

Fig. 90b

Dispersal of an inert solute in normal flow conditions

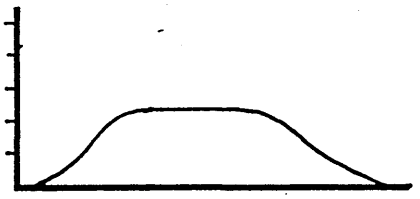
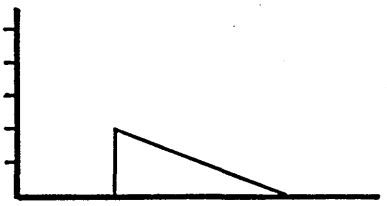


Fig. 90c

Dispersal and loss of a solute applied progressively upstream



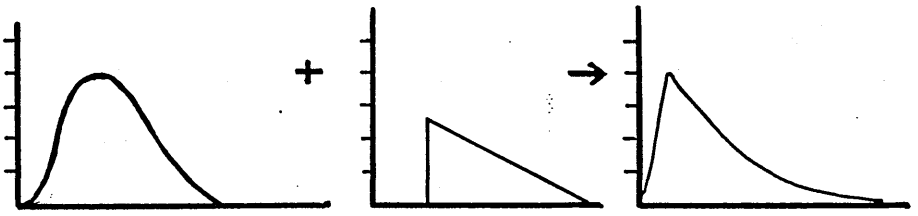


Fig. 90d. Possible combination of dispersal patterns resulting in a residue loss pattern similar to that found in the recirculating channels

The situation is further complicated if the herbicide is lost from solution, by uptake, adsoption or decay, during its passage downstream. A simple illustration of this is shown in Fig. 90c. This factor may be a function of the time that the residues have spent in solution, and of the area of unsaturated adsorptive surfaces over which they pass. These two factors are obviously related by the water velocity, but if adsorption sites are likely to become saturated, then the effect of adding the herbicide over a certain period at one site (as simulated by Demint 1970), might be different from applying the chemical to a length of river which has a greater total area of adsorption sites (as occurs in applying Midstream whilst walking along the river).

Of the factors influencing the dispersal of diquat residues considered so far, for a given length of a watercourse and conditions, it might be possible to quantify them by measurement, use of inert tracers, or testing with a solution of the herbicide.

1) Rate of application and timing of progression along the watercourse - may be controlled and measured

2) Rate and uniformity of water flow, determining the plateau shape downstream of the point of application - modelled from the passage of an inert tracer

3) Rate of uptake, adsorption or decay of the herbicide - modelled from the differences between the dispersal of an inert tracer and the herbicide applied in solution.

Factor 2) will tend to prolong the passage of diquat along a watercourse, compared with that expected from the mean water velocity. Factor 3) will tend to reduce the concentration of the herbicide during its passage downstream. These trends may result in a pattern of residue dispersal, downstream of a long treated length of watercourse, similar to the pattern observed as a result of dilution from the recirculating channel (Fig. 90d). It is evident that it would require considerable work, and knowledge of hydraulics to quantify the relationship.

The passage of residues would also be prolonged as a result of any delayed release of diquat from the alginate formulation, and a more detailed study of that process (and the conditions which affect it) would be needed to determine its importance.

7.8 Discussion of the Trials in the Recirculating Channels

7.8.1 The growth of macrophytes in the recirculating channels

This work has shown for the first time that macrophytes, such as Ranunculus penicillatus var. calcareus, can be grown in these recirculating channels, throughout the year.

Observations in other artificial channels, in which changes in flow rate, inter-species competition and herbivory did not occur, have identified seasonal changes in macrophyte growth rates. These growth rates, with minimum values in winter, are thought to be determined by the climate (Eichenberger 1983). Dawson (pers. comm.) has observed that transplants of Ranunculus, made into concrete recirculating channels at Waterston, throughout the year, have grown at approximately the same rates, regardless of the season. The water temperature of his channels was artificially kept constant, by regulating the inflow of water from a chalk aquifer. This suggests that temperature, not photoperiod is most important in determining the rate of growth of Ranunculus.

The greater over-winter growth rates of the Ranunculus plants from the R.Frome and Bere Stream, compared to those recorded in the spring, do not appear to fit this seasonal growth pattern. Ranunculus plants tend to show sigmoid growth curves both in the field (Dawson 1976) and in artificial conditions (Eichenberger 1983). In the field maximum biomass is associated with the flowering period, because of the production of weaker flowering stems, as well as the diversion of materials from vegetative growth. The subsequent decline in biomass results from mechanical damage of flowering stems, self-shading and competition from other species (e.g. Rorippa, Westlake et al. 1972).

In these artificial channels it is likely that the maximum biomass may be associated with self-shading, especially if the width of the Ranunculus beds is contained by the side-walls of the channel (Eichenberger 1983). It may be that there is an optimum maximum biomass for a Ranunculus bed, which is limited by lateral expansion, or water depth. The Waterston recirculating channels were considerably wider than any of the weed beds, but many plants had reached the water surface and so were limited in vertical expansion. The streamlining pressures of the fast water flow, may have limited the lateral

spread of the weed beds.

The over-wintered weed beds may have reached their maximum size, limited by self-shading, by March 1985 so that there could be no further growth in the spring. The plants from the R.Frome, which were larger (2000g fresh weight) than those from Bere Stream (900g) at the time of transplant, probably reached their maximum biomass prior to March 1985, and hence had a lower over-winter growth rate.

The spring growth rates of the Ranunculus transplanted from the R.Piddle were greater than of the over-wintered beds, because these small beds (500g) would not have reached their maximum size. The growth rates of the March transplants were fairly low because the beds were flowering, and the shading caused by the algal cover was increasing. The algae may have competed with the Ranunculus for nutrients, such as phosphates, as well as causing physical damage and shading.

The growth rates of the Ranunculus beds transplanted from the R.Frome prior to Trial 2 were higher than the over-winter values. The day-time water temperatures in the channels were several degrees higher in the summer (c.14°C) than in the winter (c.9°C), despite the constant temperature (10°C) of the inflow(Ladle et al. 1977). The plants would also be in a suitable state for active growth, after flowering. If left in the river the maximum biomass of Ranunculus would have been reached and further growth would have been limited by self-shading. Small beds transplanted into the channels would have been released of such constraints and could grow with the same vigour seen after a post-flowering cut (Dawson 1976).

7.8.2 Problems encountered in detecting herbicidal activity in Trial 1

The suitability of the recirculating channels for the growth of filamentous algae was particularly apparent in Trial 1. The algal problem was most severe in channel 2 because of the presence of the encrusting blue-green alga Phormidium,which was absent from channel 3.

The difference between the channels probably arose because channel 2, which was only established in March 1985, was at an earlier stage of the algal succession from static to flowing water species, than channel 3, which had been recirculating since September 1984.

In addition to the different stages of algal succession in

the channels, the populations of grazing invertebrates (which are usually responsible for limiting the blooms of certain algal species), would also have been in the early stages of development in channel 2. The eventual demise of the Phormidium bloom at the end of Trial 1, was observed to be largely due to grazing.

The discrepancies in the algal problems between the channels, might have been reduced if both channels had been established in September 1984, or if channel 2 had been allowed to recirculate for several weeks prior to the addition of the Ranunculus.

Another problem in Trial 1 was the high variation in biomass, within the channels. The variability may have masked the significance of some of the differences between the channels and sampling times. This variation resulted from the mixed origins of the Ranunculus beds, and the deliberate mixing of the types in the samples.

River of origin	n	Approximate mean fresh weights at the beginning of Trial 1
R.Frome	5	2880g
Bere Stream	4	650g
R.Piddle	11	1500g

The growth rates and percentages of decayed material, being proportional to the biomasses of individual beds, were not affected by the variation in bed sizes, and so significant differences between the channels, or the origins of the Ranunculus, could be detected.

In Trial 2 the Ranunculus beds all started at about the same size, so that within-channel variation was very low and significant differences were easily detected.

7.8.3 Water chemistry in the recirculating channels

The dense algal populations probably masked any influences that the herbicide treatment might have had on the water chemistry, in both trials. If nutrients, particularly phosphates had been released by the in situ decay of Ranunculus, killed by the herbicide, it is likely that they would have been efficiently mopped-up by the algae.

There are two reasons for the substantial reduction of phosphorus in the channels compared to the inflow water.

1) The uptake of phosphates by the Ranunculus, and particularly by the large biomass of algae, must have been considerable. At the beginning of Trial 2 the total dry biomass of Ranunculus was about 2000g and that of the algae (on the channel bottom and sides) about 6500g.

2) The coprecipitation of phosphates with calcite, in hard-waters has been observed and studied in the recirculating channels (House et al. 1986b).

A detailed study of the effects of Ranunculus and filamentous algae on the water chemistry of the channels, was carried out, immediately after the herbicide trials (Fox et al. 1987). It was calculated that only a small fraction of the phosphates were removed from the channel water by coprecipitation (a maximum of 6% of the total phosphate removed), compared with the biotic removal.

Any reductions in the concentrations of dissolved oxygen that the loss, or decay, of Ranunculus might have caused, would have been masked by the photosynthetic oxygen production of the algae.

The records of dissolved oxygen (and indirectly from the pH, the carbon dioxide) concentrations, were interesting because it was the first time that these parameters had been continuously monitored in the channels, when plants were present.

It had been suggested that there might have been very limited diurnal fluctuations of these gases because the Archimedian screw pump would cause considerable reaeration of the water. This would, for example, release super-saturated oxygen during the day and allow the uptake of atmospheric oxygen by the sub-saturated water at night.

The results of Trial 2 show that this effect was not great enough to prevent distinct diurnal variations in dissolved oxygen concentrations and pH. These observations directly led on to a series of experiments in which the reaeration coefficients of oxygen and carbon dioxide in the channels, were estimated. The chemical fluxes occurring over 24 hours in a channel supporting algae and Ranunculus, were also modelled (Shelley et al. 1987, Fox et al. 1987).

7.8.4 Macroinvertebrate communities in the recirculating channels

The macroinvertebrate samples taken on the day of the herbicide application in Trial 2, showed that the sampling method was reasonably consistent. The major potential error in the method was the risk of losing individuals from the plant stems into the water current, during the process of removing the stems from the channel. This potential error was not estimated in these experiments because comparative samples, not absolute values, were required. It was assumed that under the same conditions, the probability of these types of error occurring would be the same for all samples.

Williams (1981), in initially describing the method,tested the effects of the resultant disturbance on the numbers of animals sampled from Ranunculus plants in a river. He found that the drift of invertebrates downstream of Ranunculus beds was increased during this method of sampling, and that different taxa were more, or less, susceptible to this disturbance. The drift of chironomids only increased by 2.5 times during sampling, simuliids by 5 times and that of ephemeropterans by 6.5 times.

These taxonomic differences would be important if total numbers of individuals, or the species diversity,of samples with different taxonomic compositions,were to be compared.

The choice of a sampling method which was specifically limited to the populations of macroinvertebrates colonising Ranunculus stems was made for three reasons:

1) To minimise the problem of separating the animals from the substrate.
2) To limit the numbers and diversity of organisms collected to manageable proportions, so that a large number of easily sorted samples could be analysed, quickly.
3) Since the Ranunculus was the target plant the fate of organisms inhabiting these plants was of most interest, because the diquat concentrations would be most concentrated in this habitat.

The restriction of the habitat sampled meant that some taxa, (e.g. Mollusca and Coleoptera) were never sampled. The major limitation of the method was that the migration of animals from the Ranunculus to other habitats could not be assessed. In trying to draw conclusions about the changes in density of taxa observed, it

had to be assumed that the organisms either preferred the Ranunculus as a substrate, or were found in equal proportions in this habitat, compared to any other, at any time.

Some invertebrates may change substrate at different stages of their life-cycle, or if the substrate was decaying. Simuliids, for example, require clean plant surfaces and so prefer young, clean Ranunculus early in the year. Later, as plant surfaces become covered in epiphytes and silt, they will favour other substrates (Bass pers. comm.).

There is contradictory evidence as to whether macroinvertebrate densities, in flowing water, are greater on plants (Wright et al. 1983), or on abiotic substrates (e.g. stones, Rooke 1984). Such differences may depend upon the the nature of the watercourse studied (e.g. a deep, slow-flowing, silty river or a riffle in a stony stream), or upon the season of sampling (Whitcombe 1968). Some plant species support larger and more diverse invertebrate populations than others (Krecker 1939). This is likely to be related to the morphologies of the plants, and their exposure to water currents (Harrod 1964, Whitcombe 1968). The macroinvertebrate populations within a single weed bed may vary depending upon the tolerance, and utilisation for filter-feeding, of the water flow by the animal species. For this reason, the samples in these trials were taken from similar stems on the outside of the Ranunculus beds.

In the channels the choice of substrates was limited to the Ranunculus, filamentous algae, moss or the stony bottom. The channel walls were covered in algae or moss. Most of the macroinvertebrates on the Ranunculus would have been transplanted into the channels with the weed beds. Some species would be limited to the Ranunculus, (e.g. the trichopteran Brachycentrus subnubilus, Gunn 1985), whilst others would be able to move freely between, and adapt to, other substrates (e.g. the predatory trichopteran Rhyacophila dorsalis).

Both of the examples just mentioned, were present in Trial 2. This illustrates the limitations of identifying macroinvertebrate samples to the taxonomic level of order. The feeding habits (and often consequently the substrate preference) of invertebrate species within an order, or family, can be very diverse (e.g. Trichoptera, Hynes 1970; Chironomidae, Williams 1981).

Only by identifying the macroinvertebrate samples to species, or at least to genus, would it be possible, with a knowledge of their habitat preferences, to make firm conclusions about the population dynamics of the channels, and the response of animals to the herbicide and plant loss.

The interpretation of the macroinvertebrate data was also limited by the lack of treatment replication. This problem was more acute than for the interpretation of the Ranunculus biomass data because there may be very large, rapid changes in invertebrate populations, which are difficult to observe. Population sizes may increase suddenly after hatching,once larvae are large enough to be sampled, and sudden decreases in numbers may result from changes in life-cycle (e.g. adult emergence of flying insects) or from increased predation (Hynes 1970).

With such fluctuations in populations occurring over short periods of time, it is possible that if a slight divergence in the timing of these changes develops between the two channels, then on a given sampling date the numbers in the channels could be quite different. An extreme example of effect that this desynchronisation of populations in the two channels, could have is illustrated in Fig. 91.

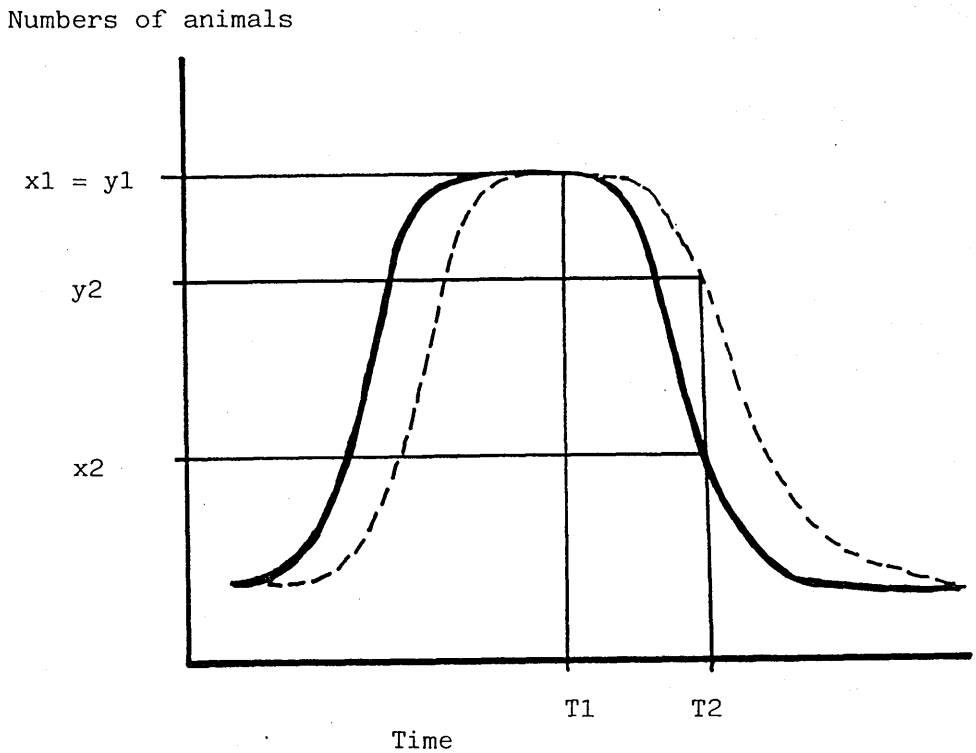
Without treatment replication, or sufficiently regular sampling to accurately follow population cycles, it is not possible to determine whether any significant differences in the macroinvertebrate populations between the channels are due to:

1) a desynchronisation of population cycles
2) indirect result of an unquantified difference between the channels (e.g. presence of a specific algal food source)
3) direct (toxicity) or indirect (loss of Ranunculus) effect of the herbicide application

As with the river and canal trials, the examination of the macroinvertebrate communities could only provide conclusive evidence that none of the taxa identified appeared to be grossly effected by the herbicide treatment. The comparison with an untreated control provides slightly more information than can be obtained from single herbicide applications, which lack reference to undisturbed population dynamics (e.g. Brooker and Edwards 1974).

Fig. 91

Simplified illustration of the possible effects on sampling of the desynchronisation of two macroinvertebrate population cycles



——— Population X

– – – Population Y

Populations appear similar at time T1

At time T2 the population Y is more numerous than population X

In addition to the provision of treatment replicates, other improvements in the macroinvertebrate sampling regimes, which would allow a broader interpretation of the results, might include:

1) Sampling all the habitats
2) Identification to genus or species, possibly only for selected, indicator species
3) More regular sampling, to provide a more complete picture of the population dynamics
4) Use of caged animal to identify direct toxicity effects

The latter method has been used in static (Way et al. 1971) and slowly flowing water (Union Canal, Murphy pers. comm.) but there would be considerable technical difficulties in trying to restrain the movement, without affecting the feeding habits, of animals living in flowing water.

7.9 Conclusions of the Trials in the Recirculating Channels

1) The physical characteristics of the channels were measured and described. These included the recirculation time (about 220 seconds) and the total water volume ($56m^3$ in channel 2, $51m^3$ in channel 3).

2) A model can be derived to describe the dilution of an inert solute out of channel 3, using the $30m^3$ pump:

$$\frac{C_t}{C_o} = e^{-\left(\frac{f\ t}{V}\right)}$$ (see 7.3.2 for key)

The model was tested, and confirmed, by diluting excess salt out of the channel.

3) Ranunculus beds were established in the channels and showed variable growth rates over-winter and during the spring. It was suggested that the maximum biomass of the beds in the channels may have been limited by self-shading.

4) In herbicide Trial 1, using a dose-rate of Midstream recommended for water less than 30cm deep, the growth of filamentous and blue-green algae caused problems. The algae smothered the Ranunculus beds in channel 2 causing mechanical damage to the surface stems, especially those on flowering beds.

5) The reductions in biomass of Ranunculus in channel 3 were not significantly greater than in channel 2. The effect of the herbicide in channel 3 was indicated by the significantly greater proportion of decayed Ranunculus.

6) The significance of the biomass results from Trial 1 was limited by the high variability of biomasses of the weed beds from different origins, within each channel.

7) In Trial 2 the beds of Ranunculus were initially of uniform size, reducing the variation within the channels. The herbicide was applied at the dose-rate for water over 30cm deep, and was very effective in removing the Ranunculus. The effects of the herbicide appeared more rapidly on plants directly treated with Midstream, but the eventual result was similar for the plants receiving only diquat in solution.

8) During the first two hours after the herbicide application, the residues of diquat were lost from the channel faster than would have been predicted by dilution alone. This would have been because of the uptake and adsorption of the diquat by plants and substrates, and because of the incomplete release from the alginate. After four hours, the loss of diquat was slower than predicted by dilution. This may have been due to the slow release of diquat ions from the alginate.

9) The dispersal of diquat residues in the recirculating channels could be related to the results which might be expected at the downstream end of a long length of river treated with Midstream. The total availance of diquat residues in the channel was similar to that found in the R.Coln.

10) The herbicide application did not appear to have any effects on the water chemistry of the channel. Diurnal fluctuations in the concentration of dissolved oxygen and pH were apparent, despite the reaeration of water at the recirculation pump.

11) No changes in the density of macroinvertebrates on the Ranunculus were seen as a direct result of the application of the herbicide. The variability of the macroinvertebrate data was much less than in the river trials, however, a much more limited habitat was being sampled.

12) Most of the changes in density of the macroinvertebrate taxa were unlikely to be related to the herbicide treatment. It was difficult to assess the effect of the removal of Ranunculus without sampling all habitats or without knowing the habitat preferences of each species. The lack of treatment replication also limited the interpretation of these data.

CHAPTER EIGHT

GENERAL CONCLUSIONS

8.1 Factors influencing management efficacy

8.1.1 Management using diquat-alginate

Several environmental factors have been identified in the course of this project as being capable of affecting the efficacy of diquat-alginate.

	Source of information		
	Lab expts	Field	Channels
Calcium concentration*	x	x	
Temperature*	x	?	
Exposure period*	x	x	x
Herbicide concentration*		x	x
Water turbidity		x	
Species susceptibility		x	
Age and condition of plants		x	x

The effects of some of these factors have been quantified in this, or previously published, work (e.g. factors asterisked). Other factors may have an indirect effect on the activity of the herbicide, usually by influencing one of the factors listed above.

Water depth
Length of watercourse treated
Water velocity
Nutrient status of water (?)

Although it is currently possible to make an informed guess as to whether diquat-alginate will be suitable as a method of macrophyte management in a given watercourse, it is recommended that trials are carried out on a small length of channel prior to full-scale treatment. This procedure not only wastes a season prior to the intended use, but there are limitations to the amount of information gained from small-scale trials.

Further laboratory experiments, particularly on factors which have not been quantified (e.g. Turbidity, age and condition of plants, nutrient status of treated water) and the correlation of the results of field trials and full-scale applications, may facilitate the production of an environmental scoring system. Potential sites could be assessed on a series of easily measured environmental factors, which would be scored on simple quantitative scales, as to the expected efficacy of a treatment of Midstream. It might even be possible to predict a suitable dose-rate which would minimise the risks of over-kill and wastage, or of inefficient control.

8.1.2 Management by weed cutting and removal

The most important factors identified as influencing the efficacy of the cutting treatments, in the field trials, were those affecting the rate of regrowth afterwards. The timing of the cut is probably most important, but the species treated and the subsequent growth conditions were also influential. The regrowth conditions were not only affected by the weather but the penetration of light to the cut stems would have been important. This could be affected by:

Water depth
Water turbidity
Competition from other species (e.g. smothering algae or shading by floating-leaved plants)

Weed removal by cutting may be very specific, which may be important in certain situations. Only the cut in the R.Windrush was sufficiently non-specific to provide a fair comparison with the general application of the herbicide. The apparent lack of susceptibility of the algae in the R.Petteril to the Midstream, resulted in the similarity of effects of the two management methods, in that river.

In rivers which are not suitable for selective cutting (e.g. are too deep for wading) diquat-alginate may, by virtue of its potential for accurate placement, be of value in the selective type of weed removal favoured in sports fisheries. The potential ecological effects of treating a long section of watercourse may also be reduced by the selective application of Midstream to only a central channel.

8.2 Investigating the ecological effects of aquatic management

8.2.1 Water chemistry

The scale of experiments intended to investigate the ecological effects of management is probably more important than for efficacy trials. This is particularly true in flowing waters where the buffering capacities of adjacent untreated areas are constantly influencing, in the downstream movement of water, any ecological changes in a managed area. Changes in water chemistry induced by the removal or decay of vegetation are more effectively diluted-out from small treated areas, the faster the inflow and mixing with fresh water.

It was hoped that the recirculation of water in the experimental channels would overcome this problem, by accumulating any effects of the herbicide on the water chemistry. The lack of evidence for such effects in the channels may well have been due to the buffering capacity of the, apparently undamaged algae.

The regularity of monitoring chemical parameters must be related to the potential effects that changes in such parameter might have on the ecosystem. A sudden, short-term increase in nutrients, released from decaying plants, might be of little importance in flowing waters, which lack the potential for blooms of phytoplankton. A severe reduction in dissolved oxygen concentrations may only need to occur briefly, once, to cause the distress, or deaths, of fish. It should be possible to predict the likelihood of this eventuality, after herbicide treatment in flowing waters, if the reaeration coefficient of the channel and the inflowing untreated water were assessed, and related to the potential oxygen demand of the susceptible species present (c.f. Brooker 1974).

8.2.2 Macroinvertebrate communities

Given the severe limitations on the time available for the sampling, identification and enumeration of macroinvertebrate samples, the sampling method used in the channel experiments produced data which were less variable than the data that could be obtained from a few Lamborn samples. The channel method was very limited in the type of habitat sampled, and the inability to predict which taxa would be successful in colonising other habitats limited the potential

for drawing conclusions regarding the indirect effects of management by macrophyte removal.

A more qualitative approach to the study of macroinvertebrates in the field trials may have been of more value; or the study of a few selected indicator species, particularly those most susceptible to changes in water chemistry and those showing greatest preference for the target plants.

The recirculating channels may have great value as a medium for studying the effects of herbicides on macroinvertebrate communities, especially if all habitats are sampled, including the use of recolonisation techniques.

Unless an efficient method of caging animals in flowing water can be developed, the use of laboratory-scale flumes would appear to be the most promising potential method for evaluating the toxicity of diquat to lotic macroinvertebrates, under realistic conditions.

As with water chemistry, the effects of management on macroinvertebrate populations may be less severe in flowing waters than in static ones. Flowing waters have potentially greater buffering capacities if any macroinvertebrates are affected by management, not only by immigration to treated areas by drift, but also because weed removal may be selectively limited to the central channel, with less risk of lateral influence than in static water. The vegetation at the edges of the channel would buffer the treated areas by providing an undisturbed source of macroinvertebrates for recolonisation of the cleared central channel.

8.3 Experimental Approaches

There are many aspects of the use of diquat that could be usefully studied in the laboratory. Factors influencing the efficacy of the herbicide could be quantified individually, or in combination. The use of flowing systems instead of static cultures, would be of great importance in the study of the effects of suspended solids and macrophyte morphology on the activity of diquat-alginate. Toxicity tests could also be carried out on lotic macroinvertebrate species.

Toxicity tests should attempt to study not only fatalities but also the potential of the herbicide to affect behaviour, at all stages

of the animals' life-cycles.

The maximum benefits of field trials are achieved only when the experimental designs include the provision of untreated controls and treatment replication. Data from all sources may be of value, even lacking these features, in identifying (even if not quantifying) conditions which appear particularly favourable, or unsuitable, for a management method.

The great potential of the recirculating channels as an intermediate medium for herbicide trials might be realised once some of the problems encountered in this project are solved. The problem of the blooms of filamentous algae might be turned to an advantage if the algicidal properties of a herbicide were to be studied. A better understanding of the reasons for the dominance of the algae in the channels should indicate means of limiting the problem. Why the algae should appear to thrive so well in the channels, compared to similar sized natural streams is uncertain. It is possible that the stability of the channels, without the cleansing influence of occasional increases in water discharge, is of importance.

If the availability of diquat-alginate,as a herbicide suitable for flowing waters, is going to result in the large-scale, or repeated treatment of watercourses with a herbicide, future research must be planned to investigate the long-term effects of such management. Such research should not only concentrate on the ecological effects of this type of management (e.g. the long-term development of plant and macro-invertebrate communities), but the possibility of saturating the adsorptive capacity of the habitat, for diquat, should be addressed.

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APPENDIX 1

Table A-1

Laboratory experiment culture medium

Macronutrients	$mg\ l^{-1}$
$K_2 HPO_4$	20
$Na\ NO_3$	25
$Mg\ SO_4 . 7H_2O$	7.5
Fe EDTA	3.85×10^{-3}
Micronutrients	
$H_3 BO_3$	0.286
$Mn\ Cl_2 . 4H_2O$	0.181
$Cu\ SO_4 . 5H_2O$	0.008
$Zn\ SO_4 . 7H_2O$	0.022
$H_2 MoO_4$	0.009

APPENDIX 2

Part of the output from a GENSTAT programme

```
GENSTAT V     MARK 4.03
(C)1980 LAWES AGRICULTURAL TRUST (ROTHAMSTED EXPERIMENTAL STATION)
ICL 1900 CONVERSION BY READING UNIVERSITY COMPUTER CENTRE

  1  'REFER' E3DOTT
  2  'UNITS' $ 288
  3  'NAMES' NCALC=C10,C50,C100,C200
  4     :    NTEMP=T9,T18
  5     :    NHERB=+HERB,-HERB
  6     :    NTIME=T3,T4,T5,T6,T7,T8
  7  'FACTORS' CALC $ NCALC
  8     :      TEMP $ NTEMP
  9     :      HERB $ NHERB
 10     :      TIME $ NTIME
 11     :      BLOCKS $ 3
 12  'GENERATE' TIME,HERB,TEMP,CALC,BLOCKS
 13  'INPUT' 2
 14  'READ' E3RC
 15  'INPUT' 1
 16  'PRINT/S' E3RC
 17  'BLOCKS' BLOCKS/(CALC.TEMP.HERB)/TIME
 18  'TREAT' CALC*TEMP*HERB*TIME+BLOCKS
 19  'ANOVA/LIMA=3, PR=00313' E3RC;
 20  RES=A; FVAL=AA
 21  'GRAPH' A;AA
 22  'RUN'
```

APPENDIX 2 (continued)

An example of the output from a GENSTAT programme with three factors

***** ANALYSIS OF VARIANCE *****

VARIATE: E3RC

SOURCE OF VARIATION	DF	SS	%S%	MS	%R
BLOCKS.CALC.TEMP.HERB STRATUM					
CALC	3	6.5929	1.63	2.1976	3.398
TEMP	1	91.6884	22.66	91.6884	141.779
HERB	1	26.5842	6.57	26.5842	41.107
CALC.TEMP	3	0.1137	0.03	0.0379	0.059
CALC.HERB	3	10.9957	2.72	3.6652	5.668
TEMP.HERB	1	4.6259	1.14	4.6259	7.153
CALC.TEMP.HERB	3	2.9957	0.74	0.9986	1.544
BLOCKS	2	0.2934	0.07	0.1467	0.227
RESIDUAL	30	19.4010	4.79	0.6467	5.183
TOTAL	47	163.2908	40.35	3.4743	77.844
BLOCKS.CALC.TEMP.HERB.TIME STRATUM					
TIME	5	138.4939	34.22	27.6988	21.997
CALC.TIME	15	3.2665	0.81	0.2178	1.745
TEMP.TIME	5	55.7023	13.77	11.1405	89.283
HERB.TIME	5	14.3064	3.54	2.8613	22.931
CALC.TEMP.TIME	15	1.7665	0.44	0.1178	0.944
CALC.HERB.TIME	15	3.3012	0.82	0.2201	1.764
TEMP.HERB.TIME	5	2.7023	0.67	0.5405	4.331
RESIDUAL	175	21.8359	5.40	0.1248	
TOTAL	240	241.3750	59.65	1.0057	
GRAND TOTAL	287	404.6658	100.00		

GRAND MEAN 0.915
TOTAL NUMBER OF OBSERVATIONS 288

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	CALC	TEMP	HERB	TIME	CALC TEMP	CALC HERB	TEMP HERB
REP	72	144	144	48	36	36	72
SED	0.1340	0.0948	0.0948	0.0721	0.1895	0.1895	0.1346

TABLE	CALC TIME	TEMP TIME	HERB TIME	CALC TEMP HERB	CALC TEMP TIME	CALC HERB TIME	TEMP HERB TIME
REP	12	24	24	18	6	6	12
SED	0.1879	0.1328	0.1328	0.2681	0.2657	0.2657	0.1879
EXCEPT WHEN COMPARING MEANS WITH SAME LEVEL(S) OF:							
CALC	0.1442						
TEMP		0.1020					
HERB			0.1020				
CALC.TEMP					0.2039		
CALC.HERB						0.2039	
TEMP.HERB							0.1441

TABLE	BLOCKS
REP	96
SED	0.1161

APPENDIX 3

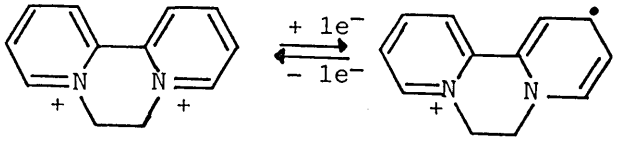
Mode of Action of Diquat

Since the original discovery of the herbicidal properties of diquat by Brian et al. (1958), there have been many publications of work which has attempted to elucidate the mode of action of the bipyridinium herbicides. There are several review papers which discuss the development of these herbicides (e.g. Akhavein and Linscott 1968, Calderbank 1968), but two of the best reviews of the early discoveries are by Dodge (1971) and Summers (1980).

A summary review is presented here, of the work in this field which is of particular relevance to the use of diquat and paraquat as aquatic herbicides. Although most initial work was carried out using terrestrial plants, because of the observations of Brian et al. (1958) that the herbicides were ineffective if applied to soils, many experiments used water cultures of plants, or tissue discs floating in water.

It had been noted as early as 1933 that some of the bipyridinium quaternary salts could be reduced, by the addition of one electron, to a stable free radical (Michaelis and Hill 1933). This reduction could be achieved chemically using zinc dust or sodium dithionite (Homer and Tomlinson 1959). This property is used in some of the qualitative and quantitative methods of analysis of these compounds (Section 4.4.3).

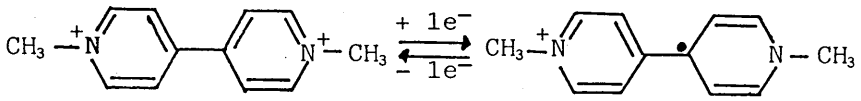
Homer et al. (1960) proposed that the ability of certain bipyridinium diquaternary salts, such as diquat and paraquat, to reversibly form stable free radicals, was related to the molecular structure of these salts. More specifically, this reaction was related to the coplanarity (flatness) of the two pyridine rings, which allowed the delocalisation of the extra electron over the whole molecule.



Oxidized Reduced

Diquat cation

• Delocalised electron (From Summers 1980)



Oxidized Reduced

Paraquat cation

• Delocalised electron (From Summers 1980)

Homer et al. (1960) also suggested that there might be a reducing system in plants with a potential of about -0.35 to -0.45 V, which could reduce the diquat and paraquat salts respectively. The important characteristics of these salts, as opposed to diquaternary salts of other bipyridines which do not show herbicidal activity, is the stability and reversibility of this reduction.

Observations by Homer et al. (1960) that the rates of herbicidal activity were significantly reduced in the dark, pointed to the involvement of the photosynthetic system in the reduction of the bipyridinium salts. An elaborate series of experiments by Mees (1960) investigated the role of the photosynthetic processes further. Leaf discs of broad bean plants, floating in herbicide solution, were readily bleached in light but only green tissue was affected. Damage occurred in the dark but only over long periods of time. The rate of kill of tissue treated in the dark, was increased by subsequently exposing the material to light. The rate of the tissue kill was proportional to the light intensity, up to a certain intensity. These observations were confirmed by Baldwin (1963), using whole tomato plants.

The effect of light, in speeding up the kill of tissue treated in the dark, was inhibited by the application of inhibitors of photosynthesis, such as monuron. The removal of carbon dioxide or the application of a respiratory inhibitor, such as potassium cyanide, had no effect on the rate of herbicidal activity in the light.

Mees (1960) concluded that the photolysis of water in photosynthesis, produced enough energy and reducing potential to reduce

diquat to its free radical. In the dark, respiration could reduce the herbicide, but the process was considerably slower.

The inhibition of photosynthesis by diquat and paraquat, has subsequently been shown in many plants, including aquatic species such as Lemna minor (Funderburk and Lawrence 1964). In some cases, the inhibition of respiration, by the herbicides, was noted (Mees 1960), but sometimes a short-term stimulation of the process was observed (Funderburk and Lawrence 1964).

The stage in the photosynthetic pathway, at which the bipyridinium cations are reduced, was pinpointed by work on isolated chloroplasts. This pathway is summarised in Fig A1 , which has been adapted from Dodge (1971) and Summers (1980).

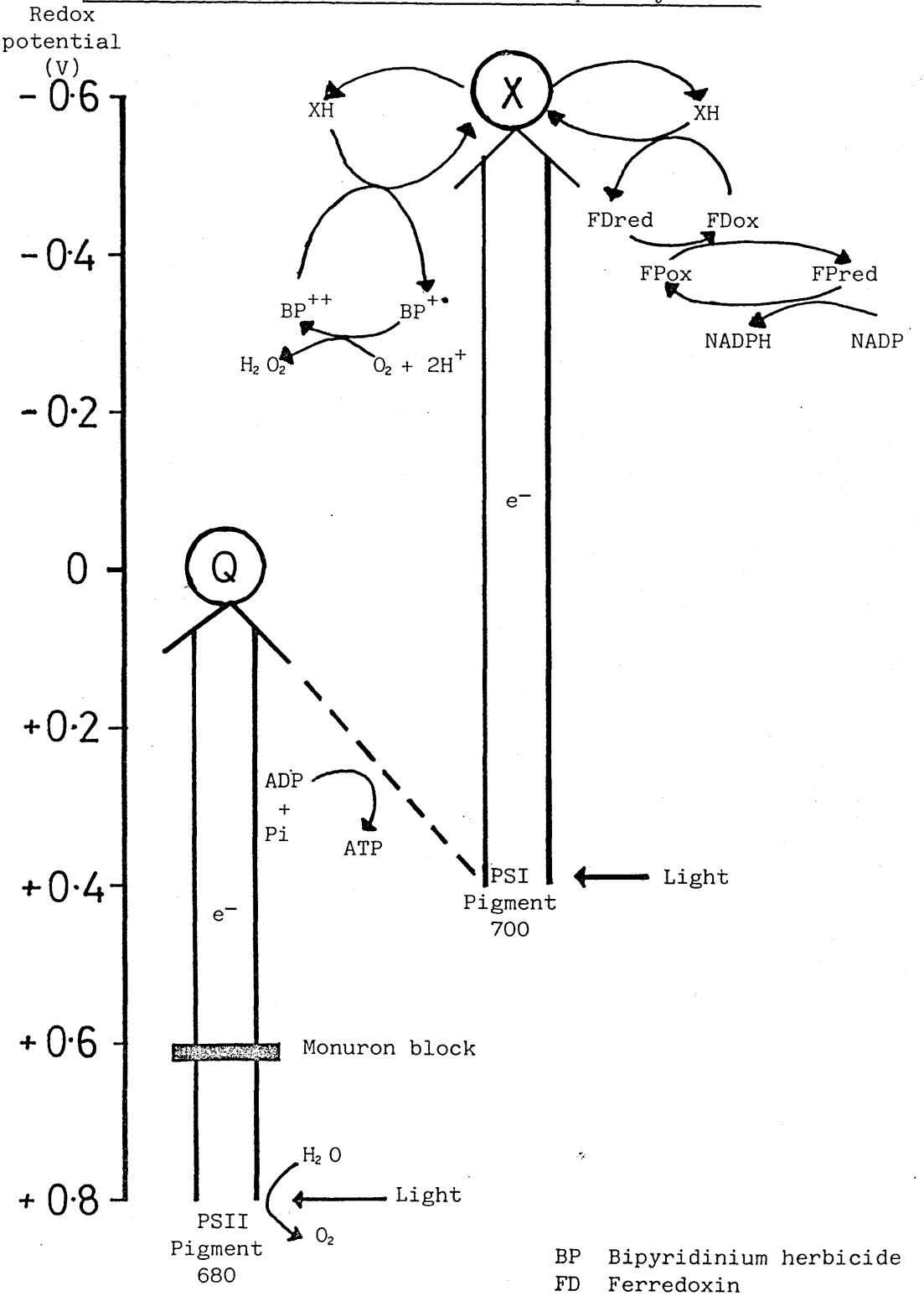
The inhibitory effect of monuron on the bipyridinium herbicides' activity, is caused by the blockage of the photosynthetic electron flow from the photolysis of water, in the chloroplast. The reduction of NADP is competitively inhibited by the bipyridinium herbicides, causing a deviation of the electron flow from Photosystem I. The bipyridinium herbicides preferentially compete for photosynthetic energy, with the naturally occurring electron acceptor, ferredoxin (Zweig et al. 1965).

The actual kill caused by the herbicides is so rapid, that the interference with photosynthesis alone, which would cause a long-term starvation of the plant, is not the primary cause of plant death. Homer et al. (1960) suggested that oxygen was essential for the activity of diquat. If treated plant material was illuminated under nitrogen, rather than air, the diquat kill was considerably reduced (Mees 1960). Mees proposed that these observations indicate that the rapid reoxidation of the reduced bipyridinium free radical, is an important part of the herbicidal mechanism.

The instantaneous reoxidation of reduced diquat in air, was shown to produce hydrogen peroxide (Davenport 1963). Hydrogen peroxide might be the toxic agent involved in oxidising lipids. Black and Mayers (1966) suggested that enough of the enzyme, catalase, which destroys hydrogen peroxide, is present in plant cells to allow detoxification. However, there appears to be little catalase in the chloroplasts (Gregory 1968),most is located in extra chloroplasmic cytoplasm. Enough hydrogen peroxide may survive inside the chloroplasts to kill the plant.

Fig. A1

Effect of bipyridinium herbicides on photosynthesis



BP Bipyridinium herbicide
FD Ferredoxin
FP Flavoprotein

(Adapted from Dodge 1971 and Summers 1980)

Slade (1966) showed that the degradation of paraquat observed when applied to maize, tomato or broad bean plants, occurred under conditions least favourable to metabolism. It was concluded that the herbicide acted as a catalyst, not as a substrate, for the processes which caused the plant death. The degradation of paraquat which was observed in these experiments, was attributed to photochemical degradation. The importance of this process has been discussed in Section 4.4.4.

The hydrogen peroxide and radicals derived from hydrogen peroxide (e.g. the superoxide and hydroxy radicals), can cause the breakdown of unsaturated fatty acids, by the abstraction of hydrogen. Chloroplast and cell membranes are chiefly composed of unsaturated fatty acids. Thus, the destruction of these lipids, by the hydrogen peroxide and free radicals, leads to the destruction of cell membranes. This damage has been observed by electron microscopy, in chloroplasts (Dodge 1971).

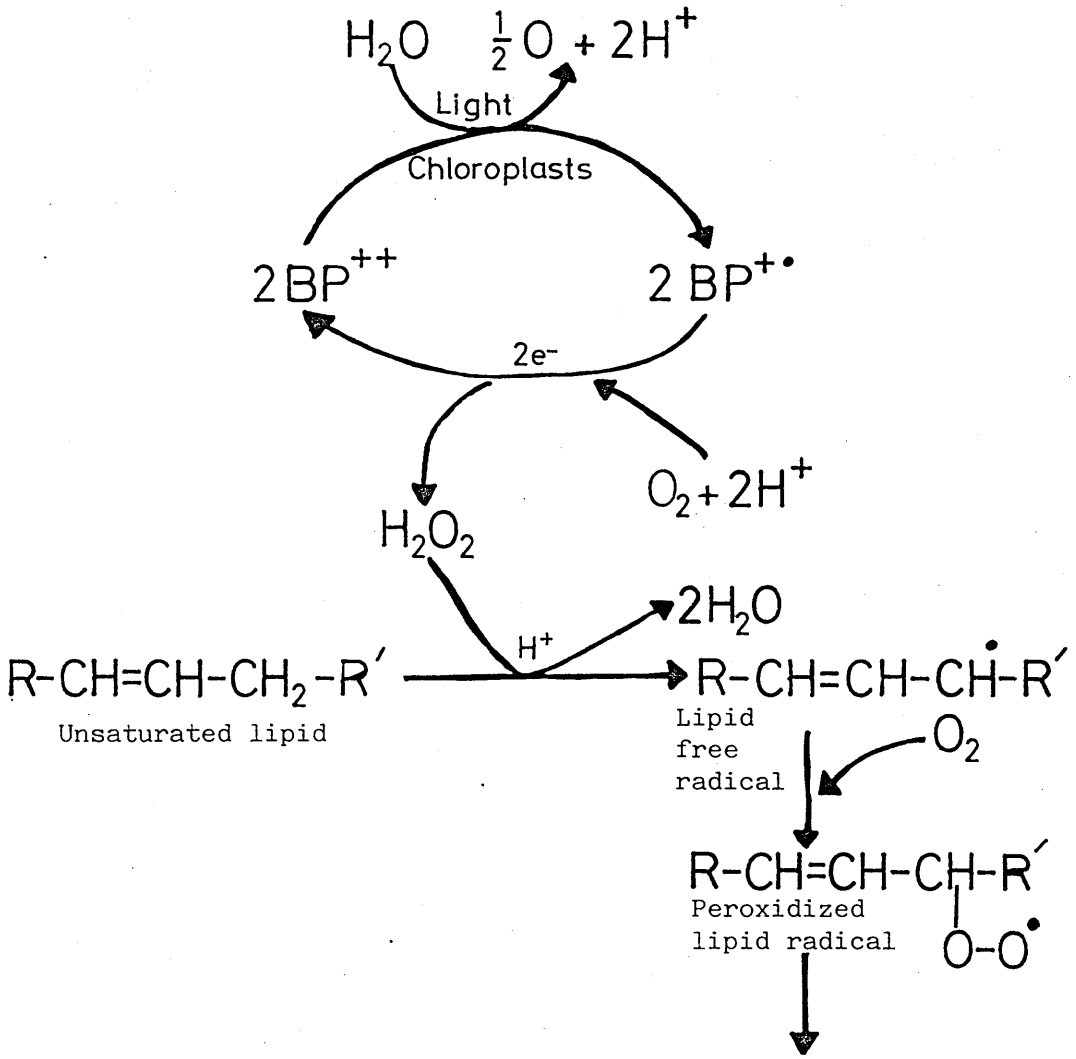
Rupture of the tonoplast would lead to the release of the vacuolar contents, such as hydrolytic enzymes, which would cause further structural damage to the cells. The bleaching of green tissues is often observed as a symptom of the activity of the bipyridinium herbicides. This results from the oxidation and destruction of the carotenoid pigments in the chloroplast. These secondary pigments usually protect the chlorophyll, which is destroyed by photooxidation, in their absence (Merkle et al. 1965).

Damage to the tonoplast would also result in the disruption of the osmotic balance of the cell (Dodge et al. 1970).

The overall effects of the bipyridinium herbicides, on the metabolism and structural integrity of cells and plant tissues, are similar to a rapid senescence. The main differences are the comparative rates of the changes in photosynthesis and membrane destruction (Dodge et al. 1970).

The mode of action of diquat is summarised in Fig. A2.

Fig. A2



BP Bipyridinium herbicide

• Electron

Further hydrogen abstraction from unsaturated lipids by peroxidized lipid radical attack

Summary of the mode of action of bipyridinium herbicides

(adapted from Dodge 1971)

APPENDIX 4

Macrophyte biomass data (g dry weight m^{-2})

4a R.Petteril

	31.5.84										8.6.84									
	P1	P6	P2	P3	P4	P5	P7	P9	P8	P10	P1	P6	P2	P3	P4	P5	P7	P9	P8	P10
Ranunculus	74	42	94	54	52	70	15	11	11	84	40	45			26				7	154
Zannichellia																				
Rhynchostegium			1				34	7	1					1					15	
Algae																			158	

	10.7.84										12.9.84									
Ranunculus	2	7	8	4	3	11	35	20	25	300		4	14		8	1		1	1	21
Zannichellia									18		28	3	0.1			0.8	0.2			
Rhynchostegium					29	5			2				1	0.7	3	1	1		0.1	
Algae	19	16	4		29		11	15	49		13	14	0.1	0.3	1	3	13	12	30	8

4b R.Windrush

	25.5.84								21.6.84							
	W1	W4	W2	W5	W7	W3	W6	W8	W1	W4	W2	W5	W7	W3	W6	W8
Ranunculus	130	312	264	292	438	381	344	281	122	86	20	67	46	230	385	24
P.pectinatus										86	20					84
P. perfoliatus						6		3	4	1		1		1		
P.lucens									0.6							
Myriophyllum												13	3		0.3	
Schoenoplectus														1		
Fontinalis																
Algae									5		3					

	25.7.84								3.10.84							
Ranunculus	20	184	10	46	136	185	168	159		28	11	59	36	6	70	37
P.pectinatus		94		7		0.9	8	28	13	108				58		
P.perfoliatus	60	25	0.1	56	33	31		0.2								
P.lucens																
Myriophyllum			19	34	1	0.2	0.7	50								24
Schoenoplectus																
Fontinalis																
Algae	42	5	105	2	21	28	1	23	4	11	59	7	2	17	14	3

4d R.Coln

	23.5.84						19.6.84					
	C1	C4	C2	C5	C3	C6	C1	C4	C2	C5	C3	C6
Ranunculus	129	38	99	145	92	72	164	89	111	109	139	134
Schoenoplectus										2		

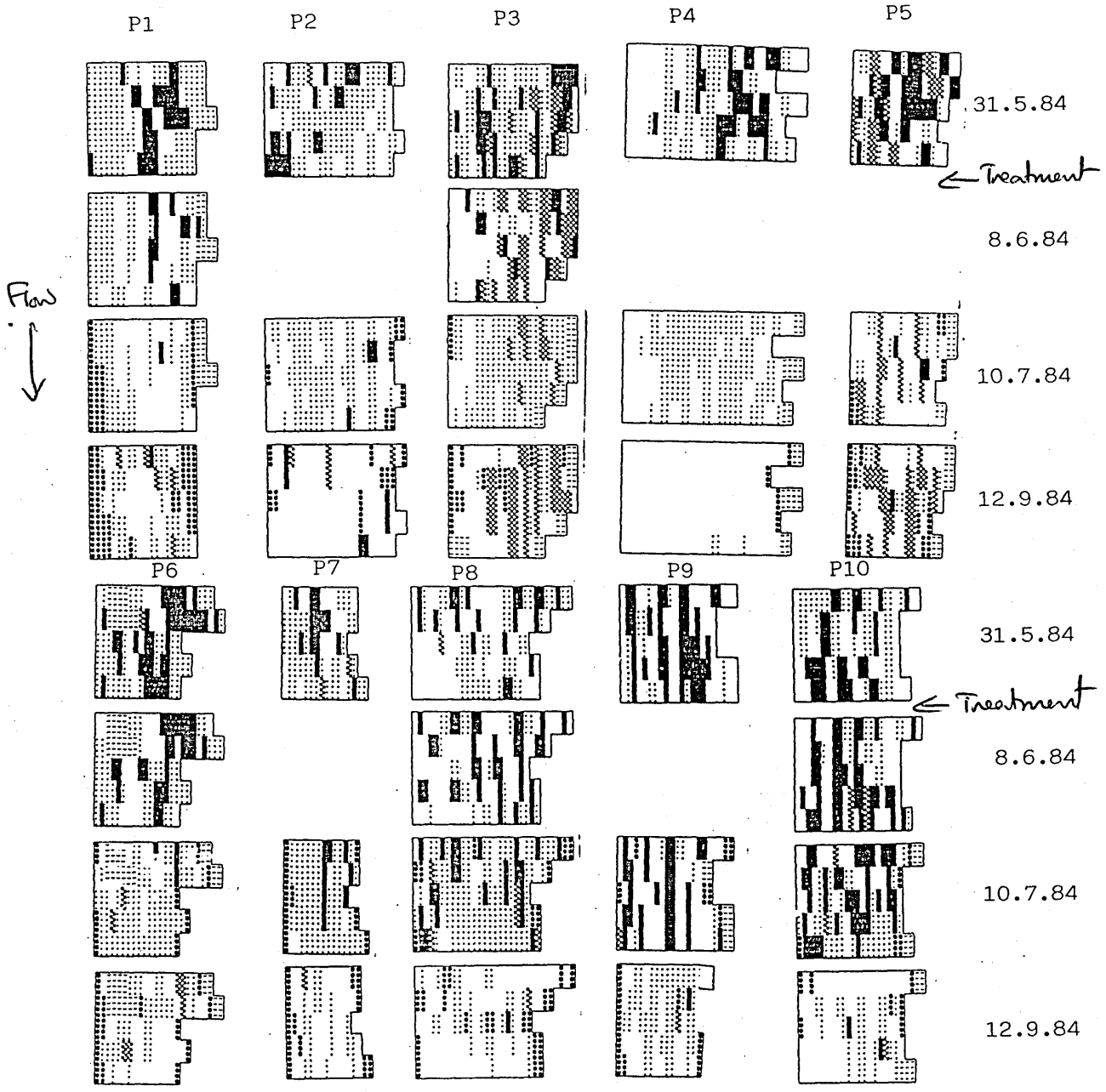
	26.7.84						2.10.84					
Ranunculus	112	29	79	172	128	220	9	11	35	55	16	143
Callitriche			0.1		1							
Elodea						0.1						
Fontinalis					0.5			0.9		2	3	
Algae	20	1	11	0.1	0.6		15	29	4	3	7	

4d . Mouse Water

	26.6.84				16.7.84			
	M1	M2	M3	M4	M1	M2	M3	M4
Ranunculus	229	186			27	3		
Callitriche			50					
Fontinalis	91	0.9	1			3		
P.natans		32	151	273		34	45	42
S.emersum		63		15		7		94
Cladophora	134	98	162	119	18	106	98	15

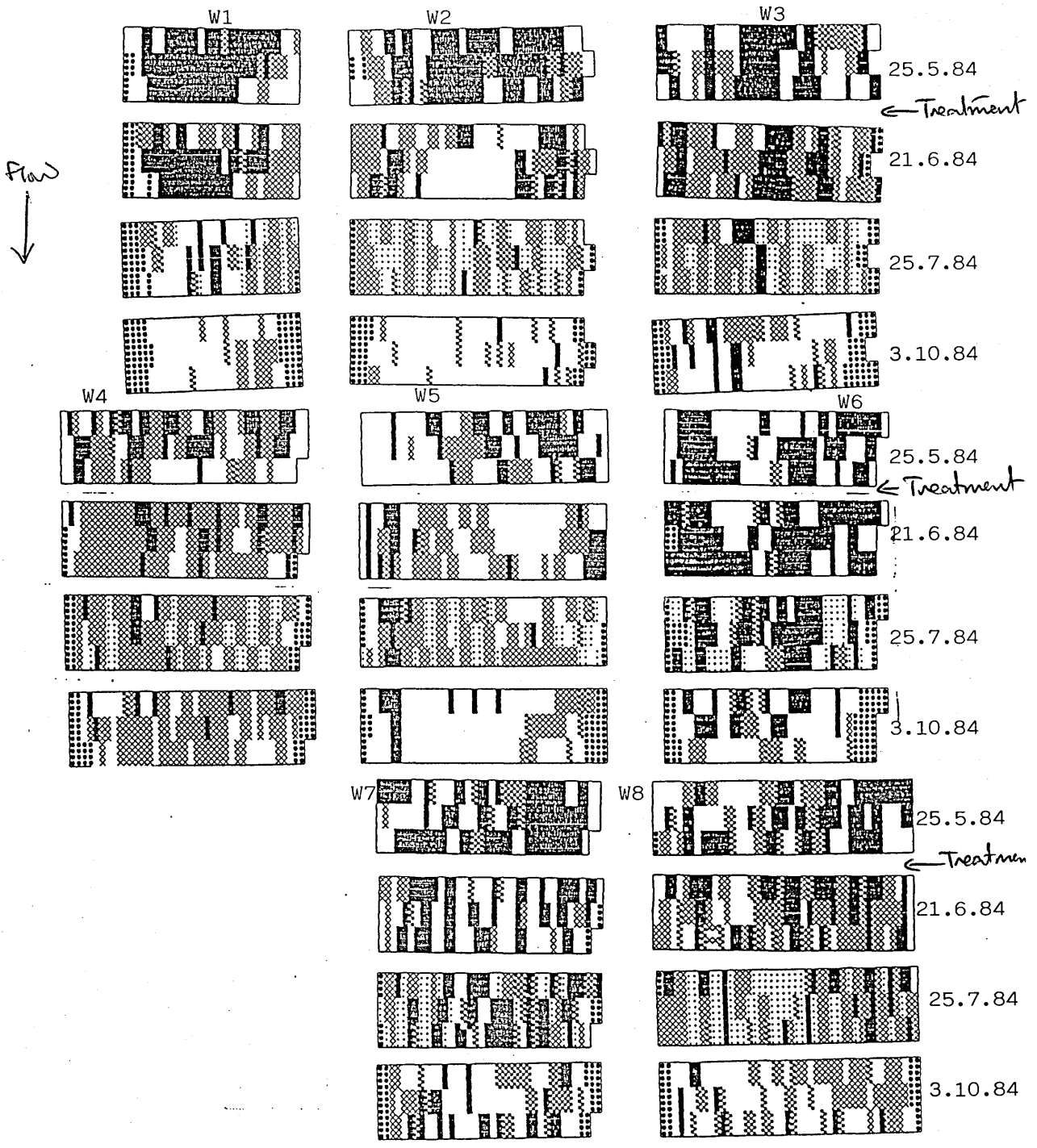
APPENDIX 5a

Complete set of vegetation maps from the R.Petteril



APPENDIX 5b

Complete set of vegetation maps from the R.Windrush



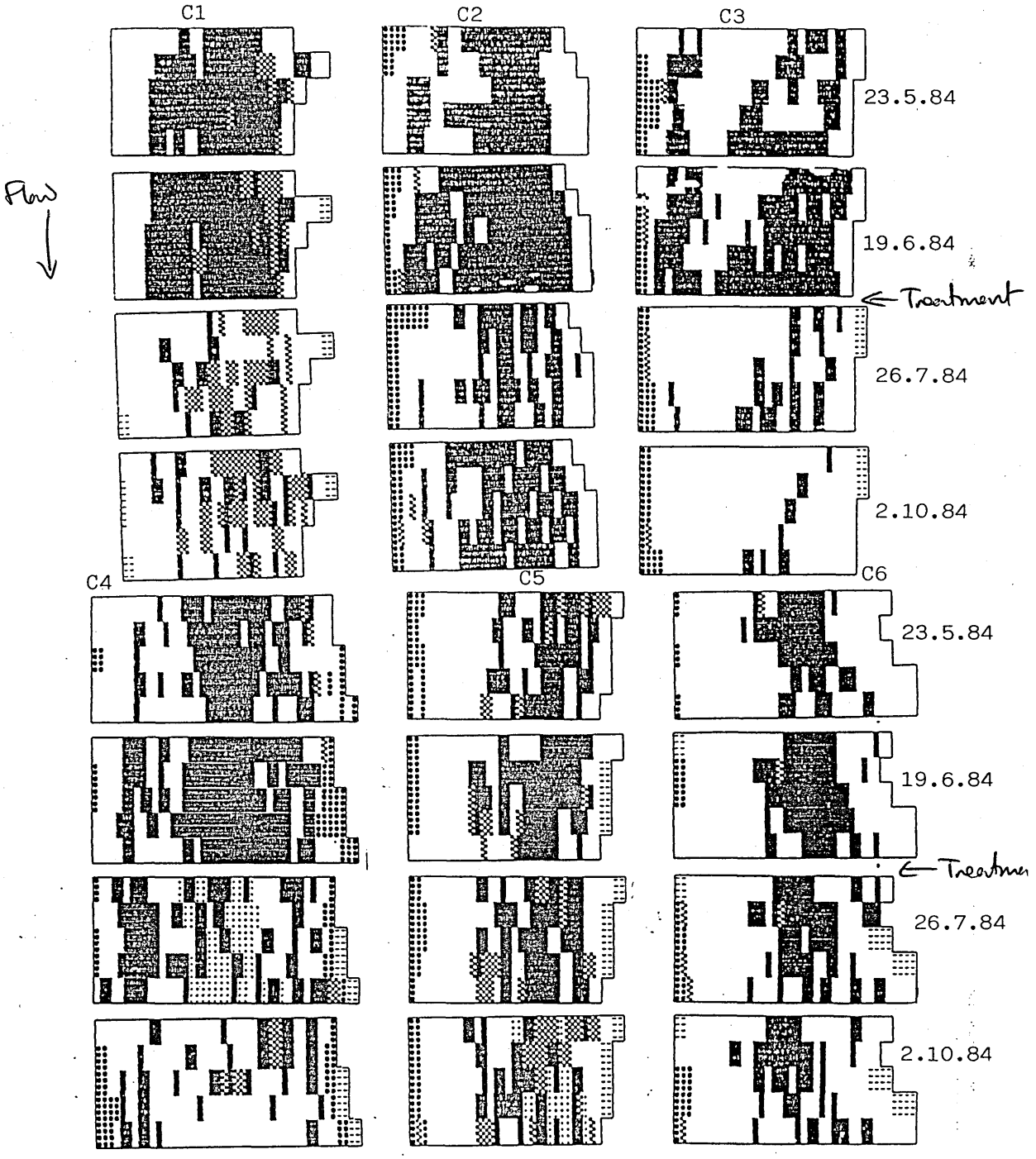
W1 W4 Herbicide

W2 W5 W7 Cut

W3 W6 W8 Untreated

APPENDIX 5c

Complete set of vegetation maps from the R.Coln



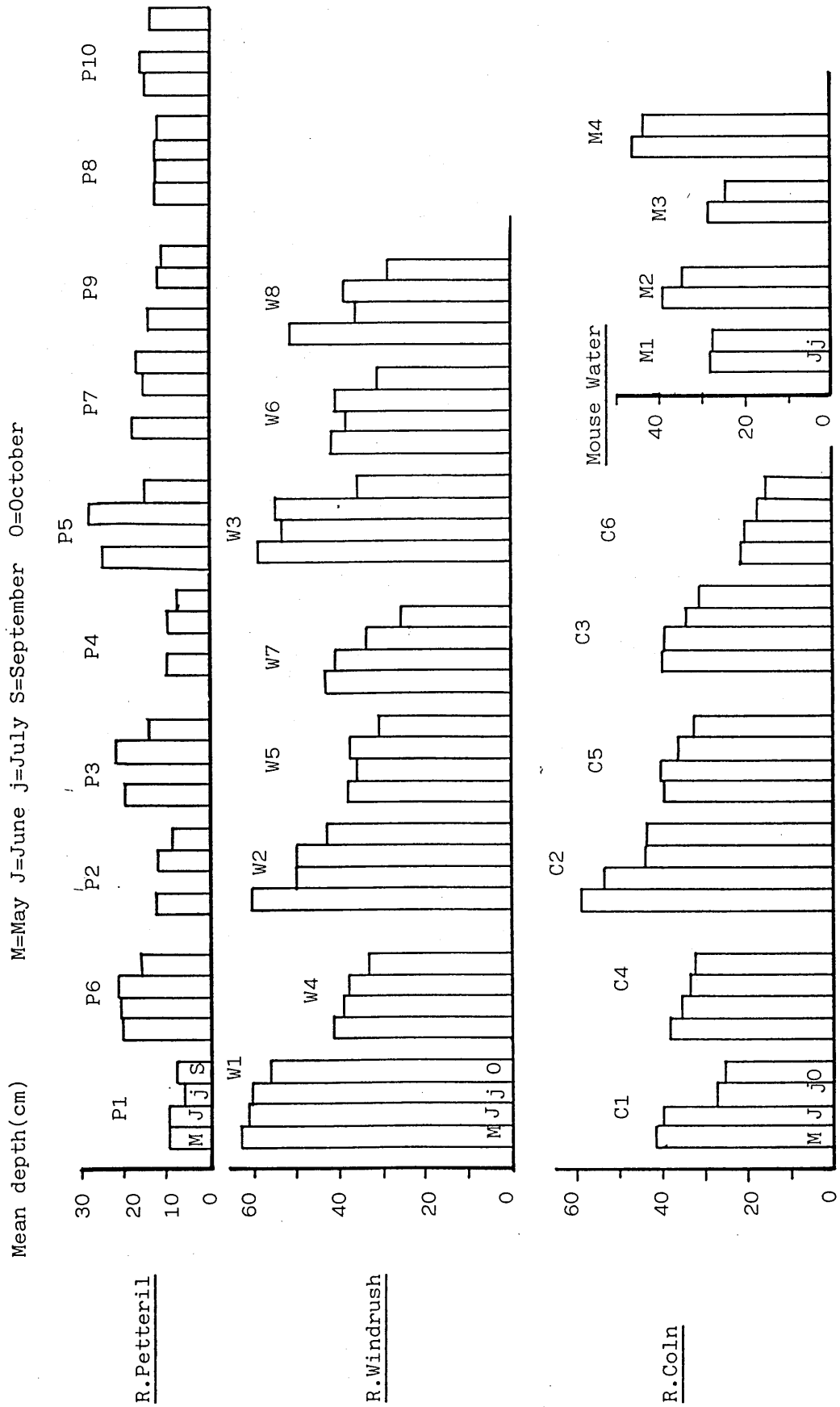
C1 C4 Herbicide

C2 C5 Cut

C3 C6 Untreated

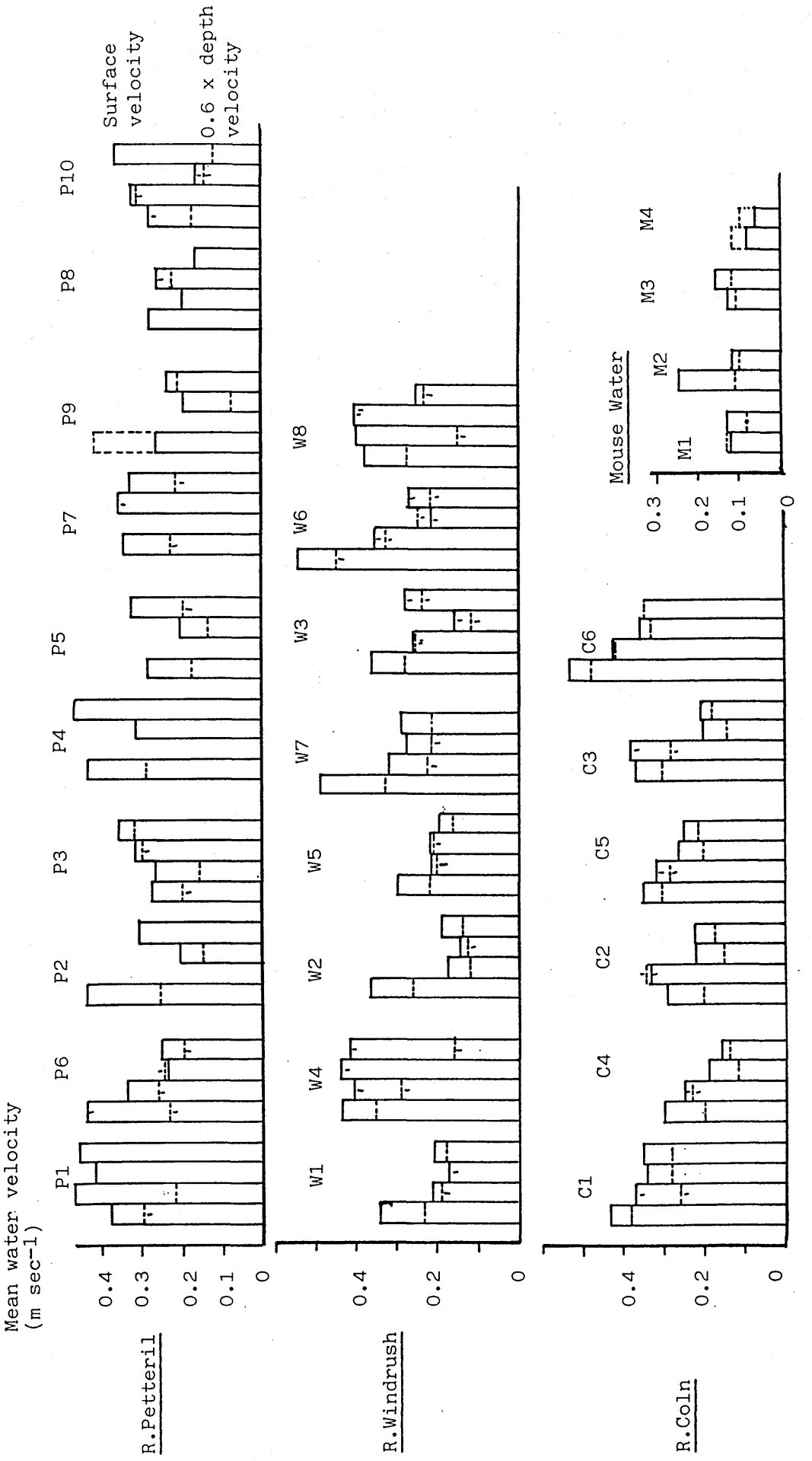
APPENDIX 6

Mean water depths per river section per sampling time



APPENDIX 6

Mean water velocities per river section per sampling time



Species list of macroinvertebrates from the rivers

	Petteril	Windrush	Coln
Gastropoda			
Valvata piscinalis			x
Potamopyrgus jenkinsi	x		x
Lymnaea peregra	x	x	x
L.stagnalis			x
Armiger crista	x		
Gyraulus albus	x		
Bivalva			
Sphaeriidae	x		
Oligochaeta			
Nais communis		x	
N.barbata		x	
Tubifex ignotus	x		
Psammoryctes barbata	x	x	x
Spirosperma ferox			x
Enchytraeidae		x	
Lumbriculus variegatus			x
Stylodrilus sp.	x		x
Hirudinea			
Theromyzon tessulatum			x
Glossiphonia complanata			x
Helobdella stagnalis	x		x
Erpobdella octoculata	x		
Hydracarina	x		
Oribatei	x		
Malacostroca			
Gammarus pulex		x	x
Ephemeroptera			
Baetis scambus/fuscatus	x	x	x
Ephemerella ignita	x		x
Ephemera danica		x	x
Caenis macrura		x	
C.rivulorum	x		x
Plecoptera			
Taeniopteryx nebulosa	x		
Amphinemura sulcicollis	x		
Leuctra geniculata	x	x	
L.fusca	x		
Coleoptera			
Haliplus sp.	x		x
Potamonectes depressus		x	
Elmis aenea	x	x	x
Esolus parallelepipedus		x	
Limnius volckmari	x		x
Oulimnius tuberculatus	x	x	x
Riolus cupreus			x
R.subviolaceus	x		x
Trichoptera			
Rhyacophila sp.		x	
Polycentropus flavomaculatus		x	
Hydropsyche pellucidula			x
H.siltalai	x	x	x
H.instabilis	x		
Hydroptila sp.	x	x	x
Ithytrichia sp.		x	x
Ceraclea dissimilis		x	x
Ylodes conspersus		x	
Athripsodes cinereus	x	x	
A.albifrons	x	x	x
A.bilineatus	x	x	

River species list continued:

	Petteril	Windrush	Coln
Trichoptera			
Lepidostoma hirtum	x	x	
Brachycentrus subnubilus		x	
Diptera			
Ceratopogomidae sp.		x	
Tipula sp.			x
Pericoma exquisita	x		
P.fallax	x		
Chironomidae	x	x	x
Tanypodinae	x		
Diamesinae	x		
Prodiamesinae	x		
Orthocladiinae	x		
Chironominae	x		
Tanytarsinae	x		
Simuliidae			
Simulium ornatum	x		x
S.wilhelmia	x	x	x
S.erythrocephalum		x	
Empididae			
Chelipoda sp.	x		
Muscidae	x		

APPENDIX 8

Species list of macroinvertebrates in the Union Canal

Gastropoda
- Lymnaea stagnalis
- Physa fontinalis
- Planorbis sp.
- Acroluxus lacustris

Bivalva
- Sphaerium corneum

Oligochaetae
- Tubificidae

Hirudinea
- Glossiphonia complanata
- Helobdella stagnalis
- Haemopsis sanguisuga
- Erpobdella octoculata

Hydracarina
- Hydrachnellae

Malacostroca
- Asellus aquaticus

Odonata
- Coenagrion sp.

Hemiptera
- Corixa sp.

Coleoptera
- Haliplidae sp.
- Brychius sp.
- Dytiscidae sp.
- Helophorus sp.

Megaloptera
- Sialis lutaria

Trichoptera
- Polycentropodidae
- Holocentropus stagnalis
- Phryganea grandis
- Limnephilidae
- Leptoceridae

Diptera
- Ceratopogonidae sp.
- Chironomidae spp.
- Chironomus spp.

Summary of the calibration of the oxygen electrodes from Shelley et al.(1987)

Oxygen

Oxygen electrodes of the Mackereth-type were constructed according to the design of Dawson and Henville (1985). The electric current, i, from such an electrode is:

$$i = N_A F A_c P [O_2]/d \quad (21)$$

where F is the Faraday constant, A_c the interfacial area of the cathode, P the membrane permeability coefficient and d the membrane thickness. The current was measured from a voltage drop, V, across a load resistance, R. The temperature dependence of i was taken into account by substituting temperature dependent functions of the form:

$$P = P_o \exp(-E_a/RT) \quad (22)$$

and

$$[O_2] = K_H P_{O_2} \quad (23)$$

where P_o and E_a are constants, K_H is the Henry's law coefficient and P_{O_2} is the partial pressure of O_2. If the temperature dependence of K_H is written in the Harned and Robinson form:

$$\ln K_H = \alpha + \beta/T + \delta T \quad (24)$$

where α, β and δ are constants, then equation (21) may be approximated by:

$$\ln V - \ln P_{O_2} = a + bT \quad (25)$$

where $a = \ln(RN_A FA_c P_o/d) + \alpha + 2(\beta - E_a/R)/273.15$

$b = \delta - (\beta - E_a/R)/(273.15)^2$

The calibration was performed in a recirculating water thermostat at temperatures of 10, 15 and 20 °C with oxygen/nitrogen compositions of 20.9, 30.0 and 40.0% by volume of oxygen. The initial calibration gave [illegible] correlation coefficient of 0.950. Recalibration after 10 weeks service gave a = 4.98 ± 0.025 and b = 0.0558 ± 0.0016 with a correlation coefficient of 0.997. Differences in the atmospheric pressure at the times of calibration caused errors in the oxygen concentration <0.1%. The significant decrease in the parameter a was assumed to be linear with time and a correction factor was applied as appropriate. The decrease might be associated with a change in surface area of the lead electrode during service (see equation (25)). The change in the parameter b was not found to be significant. A second electrode was calibrated as described above. This was only used over a period of 3 weeks and was not recalibrated. The parameters were determined as a = 5.13 ± 0.05, b = 0.0477 ± 0.0030.

In the determination of the oxygen concentration from P_{O_2}, the equilibrium oxygen concentrations were calculated according to the equation given by Benson and Krause (1980).

The initial calibration gave a= 4.99 ± 0.044 (standard deviation) b = 0.0628 ± 0.0033, with a correlation coefficient of 0.989. Recalibration after 10 weeks showed that the electrode was no longer functioning efficiently because although the a parameter was unchanged, 4.98 ± 0.14, the b parameter had changed to 0.0473 ± 0.0092. More importantly the sensor was not corresponding correctly to changes in the calibration temperature. This electrode had been used in channel 2 and because the logger did not need to be changed over the 16 day run, the electrode was calibrated using the inital conditions

APPENDIX 10

Diquat residues collected from channel 3 during Trial 2

Time after treatment	Upstream samples (mg l^{-1})	Downstream samples (mg l^{-1})	
5.0 min	0.31		
6.5 min		0.366	(0.0015)
10min	0.405	0.442	(0.0004)
15min		0.397	(0.0004)
20min	0.42	0.423	(0.013)
30min	0.35	0.359	(0.013)
45min		0.295	(0.008)
1 hour	0.22	0.237	(0.003)
1.5 hours		0.162	(0.003)
2	0.12	0.108	(0.007)
2.5		0.094	(0.0005)
3	0.07	0.079	(0.0005)
4	0.04	0.03	(0.002)
5	0.017	0.022	(0.0005)
6	0.04	0.023	(0.001)
7		0.015	(0.0005)
23	-	-	

Proceedings EWRS/AAB 7th Symposium on Aquatic Weeds, 1986

EFFECTS OF DIQUAT ALGINATE AND CUTTING ON THE SUBMERGED MACROPHYTE COMMUNITY OF A *RANUNCULUS* STREAM IN NORTHERN ENGLAND

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Summary A replicated field trial conducted in a moderately soft-water Ranunculus stream, the River Petteril (Cumbria, England) directly compared the use of an aquatic herbicide with the traditional cutting method of managing submerged macrophytes in rivers. Diquat-alginate was effective in removing submerged vascular plants but had no effect on the predominant filamentous algae. The cutting treatment checked the growth of the weed beds, which if left unmanaged showed significant increases in biomass. No changes in water chemistry were detected. Water quality and concentrations of diquat residues explained why the herbicide caused a rapid and widespread kill of macrophytes.

INTRODUCTION

Diquat-alginate is the only herbicide formulation used in the U.K. in fast flowing waters (90-1800 m/hr (Barrett 1978; Barrett & Murphy 1982). Prior to the introduction of diquat-alginate, in 1982, manual or mechanical methods were the only available means of weed control in such waters.

The study reported here was part of a series of trials conducted in a range of rivers throughout the U.K., in 1984, designed to compare quantitatively the efficacy and ecological effects of river management using chemical and mechanical methods.

METHOD AND MATERIALS

Site A 2 km length Grid Reference: (NY 498349 to NY 492367) of the River Petteril, a moderately soft-water, stony stream in Cumbria, England, was subdivided into six 100 or 200m sections. Physical and chemical attributes of the stream are given in Tables 1 and 2.

Treatments Two 200m sections were treated with the commercial formulation of diquat-alginate ("Midstream"), applied from a knapsack sprayer, on 31.5.84 at a rate of 1 litre /100m^2 water surface. Only the central 100m lengths of the sprayed sections were surveyed to minimize edge-effects. Replicated cut and untreated sections were located upstream. Immediately after spraying, water samples were collected, over 40 minutes, from the downstream end of the treated sections, and analysed for diquat residues, using a U.V. spectrophotometer to measure absorbance at 309 nm against a series of standard diquat solutions, with a blank of untreated river water. The limit of sensitivity of this method was 0.05 mg/l diquat.

Vascular macrophytes were cut and removed manually from two 100 m sections on 3.7.84. Two 100m length sections were left as untreated controls. The cut and untreated sections were placed alternately, with unsurveyed, intervening gaps of at least 100 m.

Macrophyte surveys Two replicate samples of macrophyte biomass were taken per section, using a Lambourn sampler of 0.05 m^2 sampling area (Hiley et al. 1981), from existing macrophyte beds, or where such beds had been mapped prior to treatment. The samples were sorted into species and dried at 60°C to constant weight. Permanent transects, not destructively sampled, were established in each section. The vegetation was mapped using the rectangles method of Wright et al. (1981), modified so that for each 0.5 m^2 with an equal cover of two species or substrates, this was recorded as 0.25 m^2 cover for each.

Water chemistry In each section, temperature, pH, dissolved oxygen (% saturation) and conductivity were measured electrometrically. Samples were also collected for immediate laboratory analyses. Total water hardness was estimated by a standard titration technique and [Ca^{++}] and [NO_3-N] were assessed using ion-selective electrodes. Reactive phosphorus samples, [PO_4-P] collected in boro-silicate bottles, were analysed by the standard spectrophotometric method (HMSO, 1981).

Table 1

Summary of the depth, width and flow data from the R. Petteril recorded just before the herbicide treatment

	Depth	Width	Water Velocity Surface	Subsurface
	(cm)	(m)	(m/s)	(m/s)
No. of measurements	18	6	15	7
Mean	14.7	5.2	0.31	0.25
Standard error	1.37	0.39	0.03	0.03
Minimum	5.0	4.0	0.09	0.17
Maximum	26.0	6.2	0.54	0.41

Subsurface measurements made at a depth of (0.6 x total depth).

RESULTS

Diquat residues Figure 1 shows diquat concentrations in the treated sections up to 40 minutes after the start of the herbicide applications which took eight minutes to complete. All upstream samples had no detectable traces of diquat. The diquat residues from both treated sections show an immediate peak of soluble diquat, rapidly released from the diquat-alginate, which dissipated within 10-20 minutes. In the downstream section the subsequent, lower pulse was probably due to the release of diquat more closely-bound to the alginate, and to the passage of residues caught in slower flowing water. In the upstream treated section the initial peak may have been of residues from a narrow channel of rapid flow followed by a mixing with slower-flowing water caught in the vegetation. This may explain the gradually increasing concentration detected after the initial pulse.

Macrophyte biomass and cover Results are presented for surveys carried out on 31.5.84 immediately prior to the herbicide treatment, and on 10.7.84, forty days after herbicide application and seven days after cutting treatments. A standard two-block, split-plot analysis of variance was carried out on the biomass data. Treatments were assigned to main-plot sections within an "upstream" and a "downstream" block, with times of harvest as splits within the main-plots. The means of two subsamples from each main-plot section were log e transformed to provide an adequate approximation of the data to the normal distribution. The treatment * time interaction was significant and there were no significant differences between the sites prior to treatment whether or not the biomass of algae was included in the data. Forty days after the herbicide application, the biomass of Ranunculus only from the herbicide treated sections had been significantly reduced below the pre-treatment value and was also significantly less than the untreated section's (Fig. 2). These differences were not seen with the inclusion of the algal biomass but the increase in biomass between harvests in the untreated sections was significant. The cutting treatment caused no significant changes in biomass.

The samples were dominated by two Ranunculus species, 90% of which was identified as Ranunculus penicillatus var. vertumnus C.D.K. Cook, and the rest as Ranunculus fluitans Lam. The other species found were Zannichellia palustris L., Elodea canadensis, Michx., Rhynchostegium riparioides (Hedw) C. Jens. and filamentous algae, dominated by Cladophora glomerata L. (Kutz.). Within eight days of the herbicide application, Ranunculus plants showed typical diquat toxicity symptoms (flaccid and chlorotic leaves and stems). In the transects mapped before and after treatment (Fig. 3) the water level had dropped by the second survey so that marginal submerged areas had become exposed. However, the reduction of Ranunculus is evident in the herbicide section, leaving a clear channel down the centre of the stream. Clearance is not as obvious in the cut section: the rooted remains of weed beds were still mapped if they covered the substrate.

Water chemistry In Table 2, data from 1982 (provided by the North West Water Authority) are included as baseline values. Pre-and post-treatment results in 1984, are averaged over all sections: the stability of the standard errors implies that the treatments had caused no detectable changes in water chemistry. Time of day and order of sampling, rather than the treatments, were responsible for most of the variation.

Figure 1

Diquat residues in the R. Petteril.

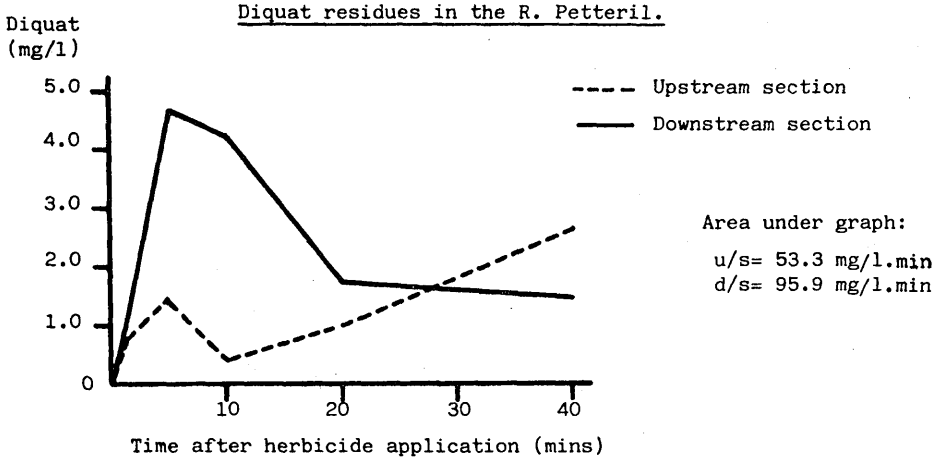


Figure 2

Vegetation maps from three permanent transects in the R.Petteril.

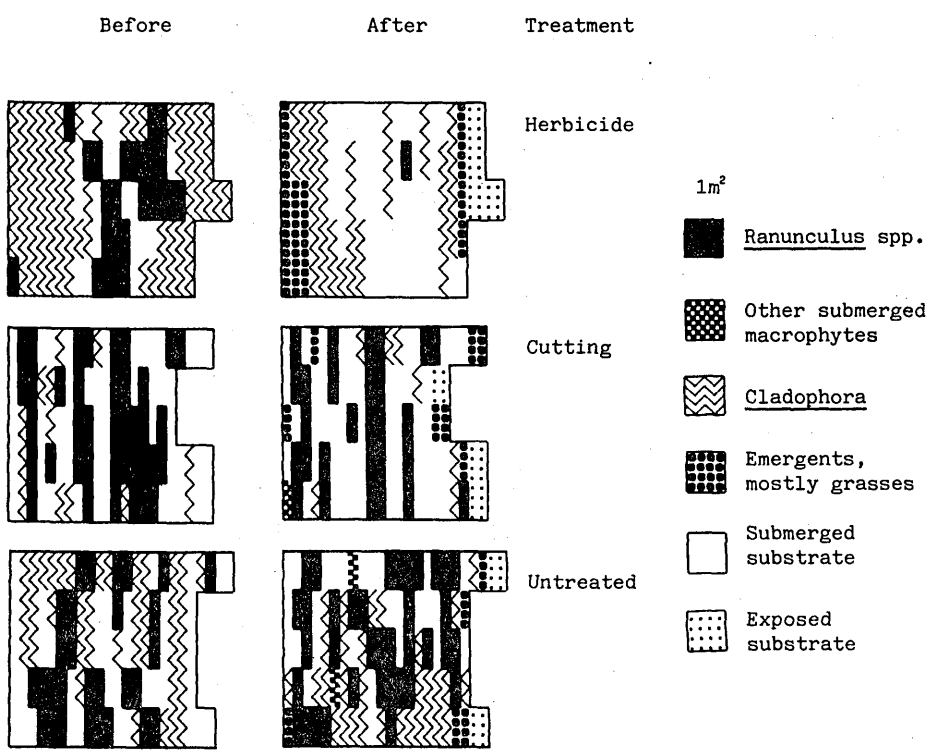


Table 2

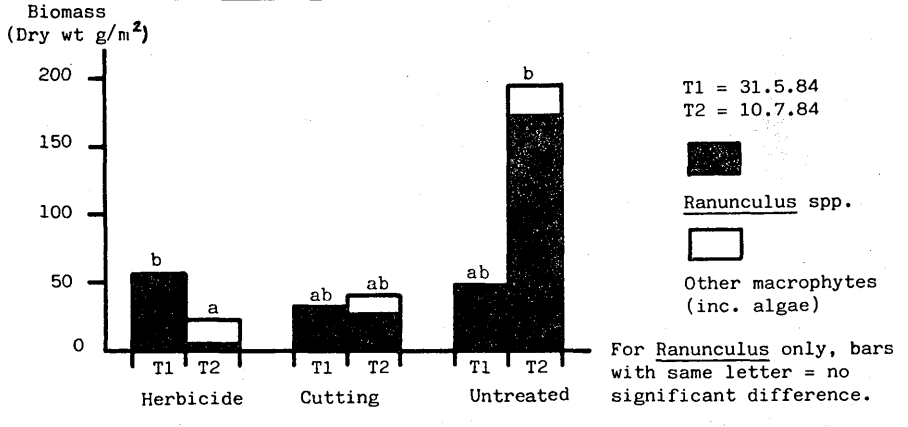
Water chemistry data from the R. Petteril

	Date 26.5.82	14.7.82	31.5.84		10.7.84	
Time	11.40	10:30	12:40$_a$		15.20	
Water temperature (°C)	12.5	14.5	17.1	(0.08)	20.2	(0.11)
Dissolved oxygen (% saturation)	131.0	124.0	165.6	(4.9)	196.7	(10.1)
pH	8.50	8.50	8.11	(0.11)	8.93	(0.04)
Conductivity (µS/cm)	n.d.	n.d.	423.8	(3.9)	436.8	(6.0)
Total hardness (mg/l $CaCO_3$)	n.d.	n.d.	164.0^c	(4.0)	226.3	(1.6)
Calcium (mg/l Ca^{++})	73.6	80.0	62.0b	(2.0)	68.8	(1.2)
Nitrate (mg/l NO_3^-)	7.53	6.44	10.2^b	(0.2)	3.10	(0.31)
Reactive phosphorus (mg/l P)	0.10	0.01	0.20bn	(0.01)	0.09	(0.01)

( ) = standard error; a = time of start of sampling; 1982: single readings from North West Water Authority records; 1984: values are means of readings from six sections, except, b = mean of 3 readings; c = mean of 2 readings.

Figure 3

Macrophyte biomass before and after treatments.



DISCUSSION

The herbicide effectively cleared a central channel in the stream, killing back Ranunculus plants so completely that the root stock did not regrow at all later in the season. There was some colonisation of the substrate thus exposed by Zannichellia palustris, which appeared in all sections later in the season.

Although Cladophora is classed as moderately susceptible to diquat-alginate the herbicide had no detectable effect on the algae, perhaps because cleared areas could be rapidly recolonised by propagules carried down from upstream sources. Algae were not effectively cleared by the cutting treatments either.

Although there was little reduction in biomass between the two surveys, weed growth was checked by cutting and did not show the great increases evident in the untreated sections. Had the cut been made earlier in the season it is likely that a substantial regrowth would have occurred, presenting more of a problem later in the season. The cutting of fairly mature plants which would have to compete with the well-established cover of Cladophora was probably responsible for the limited regrowth.

Deleterious effects of diquat treatments on water chemistry, (eg. reduction in dissolved oxygen) have been noted in lentic systems but there are relatively few data for flowing waters (Brooker & Edwards, 1975; Marshall, 1984). It is possible that some short-term effects might have occurred between the survey dates but the rapid inflow of fresh water from upstream and the presence of such large quantities of healthy algae would have diluted and minimised any changes.

In the R. Petteril it is likely that a half-dose of 0.5 litres per $100m^2$ water surface, (recommended for water less than 30cm deep) would probably be sufficient for effective weed control. The diquat residue data show that very high (albeit short-lasting) concentrations were produced, probably contributing to the rapid kill (almost complete within 8 days) which was observed.

The herbicide concentration-time combination, or "availance" (sensu Hartley & Graham-Bryce, 1980), in the downstream section was double that of the upstream section, on the available data. It is not clear why the dispersal of diquat residues was different for the two sections: they did not significantly differ in water quality, or quantity of vegetation, and flow rates were comparable. The upstream section was however narrower and deeper: a more comprehensive survey of water velocities and flow patterns might help explain the variability of the results.

With the high dose applied, more damage to the algae might have been expected, but if there was little diquat penetration of algal beds then much diquat would be lost to the water, sediment and other sinks (Simsiman et al. 1976). In swift-flowing waters a balance must be sought between the concentration of herbicide and the brief time that it spends in contact with the plants once released from the alginate. This availance will be influenced by any factors that enhance or reduce the activity of the herbicide.

Diquat efficacy is thought to be antagonised by calcium ions, and may be prevented from reaching the plant surfaces by adsorption onto

suspended matter in the water, epiphytes and aufwuchs on the plant (Bowmer, 1982). In the moderately soft-water R. Petteril, with little suspended material and clean plant surfaces, diquat may have a relatively long persistence prior to adsorption onto plant or sediment surfaces. The rapid flow combined with the apparently low uptake by the algae may explain the rapidity and severity of the herbicide's action on susceptible species and how the diquat remained active well downstream of the point of application.

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Résumé

Un essai répliqué dirigé dans la River Petteril (Cumbria, England) a permis la comparaison directe de l'utilisation d'un herbicide aquatique avec la méthode traditionnelle de coupure des macrophytes submergés dans les rivières. L'eau de la River Petteril contient 60-80 mg/L de Ca^{2+}. Le diquat-alginate s'est prouvé efficace pour supprimer les plantes vasculaires submergées mais n'a produit aucun effet sur les algues filamenteuses prédominantes. Le traitement de coupure a refréné la croissance des lits de macrophytes mais on était observé une croissance considerable si ces lits n'étaient pas traités. On n'a remarqué aucun changement chimique de l'eau. La qualité de l'eau et les concentrations des residus de diquat ont expliqué la disparition rapide des macrophytes.