INTRASPECIFIC GENETIC, MORPHOLOGICAL AND LIFE HISTORY STRUCTURING OF BROWN TROUT (*Salmo trutta*) IN A SINGLE COMPLEX CATCHMENT, THE FOYLE CATCHMENT

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ABSTRACT

Intraspecific genetic, morphological and life history structuring is evident in many taxa. Where such intraspecific structuring exists, study of the nature of the patterns displayed can reveal much about the evolutionary processes that operate during the early stages of divergence. Intraspecific structuring is particularly prevalent amongst fishes that occupy recently glaciated freshwater systems. One such species, the brown trout, *Salmo trutta*, was the subject of the work presented in this thesis.

Genetic and morphological intraspecific structuring of brown trout was examined across a single but large dendritic catchment, the River Foyle, Ireland. Structuring was examined at three spatial scales (large-scale, compared between major sub-catchments; medium-scale, compared between tributaries within sub-catchments; small-scale, compared between streams within tributaries). The two general aims of the study were to look for any structuring in either phenotype or genotype in brown trout across the catchment and, if this was found, to look for landscape or environmental gradients that might be driving such structuring.

Using a suite of 21 microsatellite markers that were chose for their ability to resolve population differences in this species elsewhere, this study identified clear and distinct genetic structuring. Brown trout collected from 28 sampling sites, resolved into 21 genetically distinct and discrete populations using a hierarchical approach implemented in STRUCTURE. The structuring was evidence across all three spatial scales. There was strong evidence of isolation by distance and isolation by environment playing a role in shaping the magnitude of the genetic differences between populations. Landscape variables which are shaped by anthropogenic impacts (urbanised area (measured as the number of houses in the catchment), proximity to farmland (measured as the distance to the nearest farm) and concentration of phosphorus in the water) showed the greatest effects in shaping the genetic population structuring (chapter 2).

In a parallel study, the morphological structuring of brown trout from across the Foyle catchment was investigated at three spatial scales. Morphology was measured as the shape of brown trout determined by Geometric Morphometric Analysis of fixed position landmarks identified on photographs of trout taken from 22 sampling sites across the catchment. Very clear, statistically significant differences in morphology (fish shape) were

evident for all the 21 sampling sites (one sampling site was removed from the analysis due to small sample size) with Canonical Variate Analysis resolving 21 discrete phenotypic groups. Morphological structuring was evident across all spatial scales (large, medium and small). Analysis showed that genetic distance and geographic distance between morphological groups was significantly correlated with morphology of populations, with morphological groups that were most divergent from each other also being most genetically distinct and geographically more distant. The effect of landscape and environmental variables driving morphology of populations was tested. In-stream substrate composition, water pH, stream order, site elevation, river gradient and the number of houses per km^2 (representative of urban area) were all found to have a significant effect on morphology of populations. However, once the effect on morphology on these environmental variables were accounted for the residual effect of genetic distance was not significant (chapter 3).

To attempt to discriminate between three alternative population genetic hypotheses for the origin of two alternative life history strategies in brown trout; freshwater residency and anadromy, the genetic structuring of brown trout was examined between life history strategy (anadromy or resident), between three sites and across two years (2013/2014) for brown trout collected from the Foyle catchment. There was no evidence of population structuring being attributed to life history strategy (that is no genetic differences between anadromous or resident trout). There was however strong and clear evidence of five genetic populations based on geographical site. Two sympatric populations were identified at each of two locations. However, both populations in each river were composed of both freshwater resident and anadromous brown trout, although the frequency of each life history strategy significantly differed between these rivers. The results of this study support the concept that partial migration in brown trout is most likely driven by a quantitative threshold trait, where the threshold trait value varies both between populations and between individuals within populations (chapter 4).

It is critical, for effective management of the relatively high economic value anadromous component of brown trout populations in a catchment, to be able to identify which tributaries are contributing most to their production. A Genetic Stock Identification (GSI) analytical framework was used to determine the tributary of origin for anadromous brown trout captured from a mixed stock within the River Faughan sub-catchment, River Foyle and to look for any evidence of straying. The results showed that three genetic populations from specific parts of the sub-catchment contributed disproportionately to the

production of anadromous brown trout. There was also evidence of straying of anadromous trout, particularly to one tributary elsewhere in the catchment (chapter 5).

Taken together this body of work has demonstrated strong genetic and morphological structuring amongst brown trout in this large dendritic catchment. Genetic structuring seems to be at its most extreme when driven by factors which could be regarded as anthropogenic. This raises questions about human effects on the process of genetic divergence. Morphological structuring was, if anything even stronger than genetic structuring. Although there was evidence of genetic divergence between populations of differing morphologies, this neutral genetic differentiation was not a significant driver of morphological variation once landscape and environmental variables, such as substrate composition, driving morphological differences were taken into account. This suggests that the environmental drivers of structuring are greater in magnitude than neutral genetic divergence. Examining genetic structuring between two common morphologies of brown trout (anadromous and freshwater resident) in more detail, revealed no genetic differentiation between life history strategies but there was evidence of differences in frequency of life history between populations. Using the genetic structuring of brown trout as a genetic baseline it was possible to determine which tributaries within the River Faughan sub-catchment produce anadromous brown trout. If some discrete populations in a catchment are contributing disproportionately to the anadromous trout population (as they are in the Foyle) there is a strong risk of over exploitation and a need for enhanced attention in the nursery areas for those populations. These results have significant implications for the management of all trout in the Foyle catchment and elsewhere.

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Authors Declaration

The material presented in this thesis is the result of original research, conducted between February 2013 and February 2017, under the supervision of Professor Colin E. Adams and Professor Paulo A. Prodöhl. This work has not been submitted, in whole or in part towards the fulfilment of any other degree. This work is based on data collected by myself, except for scale samples collected from anadromous sea trout between 2005-2008 (chapter five) and data of environmental variables within the Foyle catchment (chapters two and three), both of which was collected by the Loughs Agency. The analysis is solely my own work. Any material not of my own is acknowledged in the text.

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CHAPTER ONE: GENERAL INTRODUCTION

1.1 INTRASPECIFIC VARIATION

Evolutionary processes are ultimately the mechanisms which have given rise to the diversity of life as we know it (Weissing et al., 2011). For example, adaptive radiation was the evolutionary driving force which resulted in Darwin's finches (finches of the Galapágos Islands, Ecuador) radiating from a common ancestor and evolving beak specialisations to occupy different feeding niches (Podos & Nowicki 2004). Cichlid fish (Cichlidae) have radiated into endemic species assemblages in more than 30 African lakes with around 1000-2000 speciation events having occurred in the past 5 million years (Säisä et al., 2005). Therefore, evolutionary forces acting at the population level drive paraptric and sympatric speciation (Bush 1994). In parapatry, selection pressures drive divergence of populations which have limited contact with each other. This results in new species within a range of continuously distributed populations (Bush 1994; Polechová & Barton 2005). In contrast, speciation in sympatry occurs when a randomly mating population diverges into two or more reproductively isolated populations and is most likely to occur when the two diverging groups diverge based on adaptive traits related to resource use, such as habitat preference or morphology associated with foraging (Bush 1994; Dieckmann & Dobelli 1999). Thus, evolutionary forces, such as genetic drift, natural selection and chance mutations, which drive the earliest stages of divergence within a species give rise to intraspecific variation (Adams et al., 2016). Intraspecific variation, seen as genetic, phenotypic, behavioural, physiological variation and variation in life history strategy, is observed across multiple taxa (Metcalfe et al., 1986; Wenburg et al., 1998; Etheridge et al., 2008; Harris et al., 2015). For example, heatshock response and thermal tolerance in killfish (*Fundulus heteroclitus*) was examined across a range of latitudes. This study found a difference in critical thermal maxima and heat shock protein genes between northern and southern populations (Fangue et al., 2006).

1.2 INTRASPECIFIC GENETIC STRUCTURE

Intraspecific genetic variation can be in the form of adaptive genetic variation or neutral genetic variation (Holderegger et al., 2006). Adaptive genetic variation refers to a gene or quantitative trait which influences fitness (Holderegger et al., 2006). However, neutral genetic variation reveals more about genetic population structuring and can be used to investigate how landscape features and demographic processes shape genetic population

structuring (Holderegger et al., 2006). There is now a considerable body of evidence for intraspecific genetic population structuring (examples include the cod (*Gadus morhuna*) see Hutchinson et al., 2001; the Canadian Lynx (*Lynx canadensis*) see Rueness et al., 2003a; the Atlantic mackerel (*Scomber scombrus* L.) see Nesbø et al., 2000 and Malawi cichlid fishes see Van Oppen et al., 1997). However, the majority of examples of intraspecific structuring come from fish living in recently glaciated freshwater systems. Examples include the North American lake whitefish (*Coregonus clupeaformis*) (Bernatchez & Dodson 1991; Lu & Bernatchez 1999; Gagnaire et al., 2013), the European whitefish (*Coregonus lavaretus*) (Præbel et al., 2013; Siwertsson et al., 2013), the three-spined stickleback (*Gasterosteus aculeatus*) (Reusch et al., 2001; Defaveri et al., 2013), the Atlantic salmon (*Salmo salar*) (King et al., 2001; Primer et al., 2006; Sandlund et al., 2014), the Arctic charr (*Salvelinus alpinus*) (Danzmann et al., 1991; Brunner et al., 1998; Wilson et al., 2004) and the brown trout (*Salmo trutta*) (Crozier & Ferguson 1986; Ferguson 1989, 2007; Bernatchez et al., 1992; Carlsson et al., 1999; Massa-Gallucci et al., 2010; Swatdipong et al., 2010; Ensing et al., 2011; Stelkens et al., 2012). This work has demonstrated population structuring over several spatial scales i.e. on a large scale, such as between mountain ranges and a fine scale, such as between streams in a tributary (Schmidt et al., 2009; Stelkens et al., 2012). Linking the magnitude of genetic differentiation, spatial scale at which populations are genetically differentiated and their dispersal ability provides a link between population structuring and micro-evolutionary processes such as genetic drift, natural selection and mutation (Bohonak 1999).

There are several mechanisms which can lead to intraspecific genetic structuring which occur over varying periods of time, phylogenetic history and contemporary landscape features. Phylogenetic history of a species is one of the biggest drivers of intraspecific genetic structuring (Gaggiotti et al., 2009). Range expansion, habitat fragmentation and colonisation can all lead to increased genetic differentiation between populations (Templeton et al., 1995). For example, the uplift of the Armenian Plateau was shown to be responsible for the separation of two clades of crested newts (*Triturus karelinii*) in the near East. Genetic clustering within the identified clades was shown to be the result of alternating periods of isolation and reconnection by changing sea levels (Wielstra et al., 2010). Another example of how phylogenetic history can shape intraspecific structuring of a species is the brown trout (*Salmo trutta*) in Britain and Ireland (McKeown et al., 2010). Five lineages of brown trout (Atlantic, Danubian, *marmoratus*, Mediterranean and Adriatic) have been described with the Atlantic group splitting from eastern lineages around 700 000 years B.P

(Bernatchez 2001). More regionally, Britain and Ireland underwent several glacial and interglacial periods. The most recent glacial period started around 30 000 B.P. and reached maximum extent around 23 000 to 18 000 years B.P. (McKeown et al., 2010). There were a few ice free refugia around south and west Ireland and south England which were inhabited by anadromous brown trout (Ferguson et al., 2016). As the ice retreated during interglacial periods, anadromous brown trout expanded their range and retreated to refugia as the ice advanced (McKeown et al., 2010). This enabled opportunities for reproductive isolation following secondary contact or divergence in allopatric refugia followed by interbreeding (Ferguson 2006).

However, contemporary landscape features can also shape intraspecific genetic structuring (Holderegger et al., 2006; Dionne et al., 2008). Understanding how contemporary landscape features shape genetic population structuring within a species can provide insights into the contemporary state of a species and its evolutionary potential (Dionne et al., 2008). This has important consequences for effective management and conservation of a species (Dionne et al., 2008). In recent years, there has been an increasing number of studies which have investigated how landscape features shape population structuring (Dionne et al., 2008; Ozerov et al., 2012; Earnest et al., 2013; Lozier et al., 2013). For example, the carrying capacity of the river, stream gradients and lake proportion of anadromous salmon influenced genetic differentiation between populations of Atlantic salmon (*Salmo salar*) (Ozerov et al., 2012). Demonstrated in a study on European grayling (*Thymallus thymallus*) in Denmark, weirs were shown to be an important factor for creating genetic differentiation between populations as they obstruct upstream passage (Meldgaard et al., 2003). The landscape features which shape genetic population structuring can also be influenced by anthropogenic impacts, such as weirs, pollution and habitat fragmentation (Stelkens et al., 2012). For example, metal pollution in rivers as a result of mining practices in the South-West of England has caused low genetic diversity and population declines of brown trout (Paris et al., 2015). Populations from metal impacted rivers split from populations in clean rivers during a period of intensive mining which was dated to the Bronze age (Paris et al., 2015). Another example of a more contemporary anthropogenic impact on genetic differentiation is stocking of farmed brown trout into wild brown trout rivers (Ferguson 2016). Stocking of farmed brown trout strains could decrease the genetic potential for anadromy in wild brown trout, reduces the reproductive success of wild populations and reduce he genetic diversity of wild brown trout populations (Ferguson 2006, 2016). Thus, anthropogenic pressures often

result in habitat fragmentation which is one of the biggest threats to biodiversity (Junker et al., 2012). These pressures drive divergence of populations which can have long- and short-term negative impacts through founder effects and genetic drift. Therefore, with anthropogenic impacts being inevitable it is important to understand how humans drive the fragmentation of populations and identify a species potential for evolutionary change.

1.3 INTRASPECIFIC VARIATION IN PHENOTYPE

Like intraspecific genetic structuring, variation in phenotypes such as morphology, behaviour, life history and physiology, upon which evolutionary processes ultimately depend, is mostly continuous in nature (Skúlason & Smith 1995). Most phenotypic traits show some variation across individuals within a single species (Larsson & Forslund 1991; Adams et al., 2007; Lavergne & Molofsky 2007; Gholami et al., 2015). For example, phenotypic variation in leaf area and mass of *Pennisetum setaceum*, varies across a temperature gradient (Williams & Black 1993). Intraspecific variation in physiology can also be driven by behaviour and environmental characteristics. For example, in dominant and subordinate rainbow trout (*Salmo gairdneri*) a positive correlation was found between metabolic expenditure and food intake. However, growth rate was negatively correlated with food intake in subordinate rainbow trout (Metcalfe 1986). Intraspecific morphological variation between populations has also been studied (Pakkasmaa & Piironen 2001a). For example, in Lake Thingvallavatan, Iceland, there are four morphs of Arctic charr (*Salvelinus alpinus*) that differ in their foraging specialisations, their habitat uses and morphology (two are benthic feeding, one pelagic feeding and one piscivorous) (Snorrason et al., 1994). North Atlantic killer whale (*Orcinus orca*) populations co-existing in sympatry, also demonstrate discrete phenotypic structuring. One population is a foraging specialist and the other a foraging generalist. The two populations differ in morphology, body length, tooth count and pigmentation patterns (Foote et al., 2009).

Freshwater fish from recently glaciated freshwater systems demonstrate extensive morphological structuring (Riddell & Leggett 1981; Pon et al., 2007; Etheridge et al., 2008; Vehanen et al., 2011; Drinan et al., 2012; Garduño-Paz et al., 2012; Stelkens et al., 2012; Adams et al., 2016). Often this morphological structuring is driven by genetic and/or environmental drivers which can provide insights to micro-evolutionary processes, which ultimately lead to speciation (Adams et al., 2016). If morphological structuring is the result

of environmental drivers this is phenotypic plasticity, where morphological variation between populations is the result of differing environmental conditons experienced by the individuals themselves (Stearns 1989). For example, gill morphology of crucian carp (*Carassius carassius*) changes with oxygen levels (Sollid et al., 2003). A study found when kept in hypoxic conditions the gills protruded and the respiratory area increased. This was an adaptive reversible change in gill morphology (Sollid et al., 2003). In the Barrow and Burrishoole Rivers, Ireland, hydraulic force were found to drive differences in pectoral fin length, body depth and body length of Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) (Drinan et al., 2012). Using common garden experiments, it was found Atlantic salmon became more robust and brown trout more streamlined in fast flow conditions (Pakkasmaa & Piironen 2001b).

Morphological structuring can also be driven by neutral genetic differentiation and could result in genetic divergence and speciation (Adams et al., 2016). There have been many studies which have examined the early stages of divergent speciation in recently glaciated freshwater lakes (Ferguson & Taggart 1991; Adams et al., 2008). For example, Arctic charr (*Salvelinius alpinus*) has been studied for many years due to the morphological differences between trophic morphs (Adams et al., 2007). In Loch Rannoch, Scotland there are three sympatric (here sympatric is defined as morphs which co-exist) morphs of Arctic charr which have clearly distinct diets (Fraser et al., 2007). It has been shown that two of these morphs are genetically distinct at the mitochondrial DNA HIND-III locus with restricted gene flow between the two morphs (Adams & Huntingford 2002a). Therefore, these morphs of Arctic charr in Loch Rannoch demonstrate the early stages of evolutionary processes, phenotypic plasticity, which have resulted in divergence and possible speciation (Adams & Huntingford 2002b). Another example, of evolutionary processes driving divergence between populations that could lead to speciation is in Lough Melvin, Ireland (Ferguson 2004). There are three morphotypes of brown trout in Lough Melvin, Ferox, gillaroo and sonaghen (Ferguson 1989), which differ morphologically, genetically, in spawning area and in trophic feeding pattern (Cawdery & Ferguson 1988; Ferguson 1986; Ferguson & Taggart 1991; Prodöhl et al., 1992). This demonstrates sympatric divergence between landlocked stocks of brown trout, which has been argued to ultimately lead to speciation (Ferguson 2004).

1.4 INTRASPECIFIC LIFE HISTORY STRUCTURING

A specific and interesting case of discrete intraspecific phenotypic variation in sympatry is where the trait that is differentially expressed is a life history strategy. This is relatively common in the natural world and the most common form of this, is partial migration (Chapman et al., 2011). Partial migration occurs where some individuals in a population migrate whilst others do not (Chapman et al., 2011; Dingle & Drake 2007). It is manifested in a wide range of species for example in birds: Lapwings (*Vanellus vanellus*) (Lundberg 1988), and blue tits, (*Parus caeruleus*)) (Nilsson et al., 2006); in mammals: moose (*Alces alces*) (Ball et al. 2001) and in reptiles: green turtles (*Chelonia mydas*) (Mortimer & Carr 1987) but it is relatively common in fish species (Chapman et al., 2011). For example, for brown trout see Jonsson & Jonsson 1993; Olsson et al., 2006; Wysujack et al., 2009, for brook trout (*Salvelinus fontinalis*) see Morinville & Rasmussen 2003; Robillard et al., 2011, for rainbow trout (*Oncorhynchus mykiss*) see Olsen et al., 2006. There are several possible explanations why partial migration exists in wild populations (Chapman et al., 2011). One possible reason is competition for resources, where intraspecific competition for limited resources promotes migration in subordinate individuals (Chapman et al., 2011). For example, in European blackbirds (*Turdus merula*) juveniles and females are more likely to migrate in the winter as dominant adults and males remain resident with access to food resources (Lundberg 1984). Competition for food resources promoting partial migration can also be seen in brown trout. In transplant experiments it was shown there was a higher rate of migratory behaviour in sections of river with a high density of brown trout and low specific growth rates (Olsson et al., 2006). Another explanation is sexual conflict, where males remain resident and females migrate to avoid costly breeding (Chapman et al., 2011). Predation risk may also promote partial migration in some species where high-risk individuals migrate to avoid predation (Chapman et al., 2011).

The mechanisms driving partial migration are not fully understood (Chapman et al., 2011) and the genetic basis and evolutionary forces have not been elucidated for any species (Nichols et al., 2008). However, partial migration is either the result of genetic polymorphism, phenotypic plasticity or learned behaviour (Chapman et al., 2011). There have been several common garden experiments which have attempted to disentangle drivers of partial migration, specifically in salmonids (Wysujack et al., 2009; Chapman et al., 2011; Van Leeuwen et al., 2016). For example, examining early development of brown trout life

histories in common garden experiments it was found the life history strategy of parents influenced the migration probability of their offspring. Offspring from freshwater parents took longer to hatch from their eggs but were quicker to absorb their yolk and had higher conversion efficiencies from the egg stage to start of exogenous feeding compared to offspring from anadromous brown trout (Van Leeuwen et al., 2016). In brown trout, it has also been suggested that partial migration is the result of energy limitation in natal rivers (Ferguson et al., 2016). Therefore, brown trout which reach their asymptotic body size will mature as freshwater residents whereas those which do not will migrate to sea to reach better feeding grounds (Jonsson & Jonsson 1993). For example, using common garden experiments it was shown individuals kept on a low food availability diet tended to have lower growth rates and adopt a migratory life history (Wysujack et al., 2009). However, understanding intraspecific structuring of life history strategies provides insights into how partial migration influences the density of individuals and populations in habitats; community and ecosystem structure, as well as the early stages of evolutionary processes (Chapman et al. 2011).

1.5 WHAT CAN INTRASPECIFIC STRUCTURING TELL US?

Intraspecific structuring, whether in terms of genetics, morphology or life history strategy, can provide insights into processes which drive early evolutionary divergence and provide vital information for the effective management of a species. Understanding intraspecific genetic structuring using neutral markers can provide insights into how genetic drift, chance mutations, population bottlenecks and natural selection drives divergence between populations (Hendry & Stearns 2004; Frazer & Rusello 2013). Intraspecific genetic structuring is likely driven by either isolation by distance, isolation by environment or a combination of both. Understanding which of these environmental factors act as barriers to gene flow can not only inform our understanding of macroevolutionary processes driving divergence but provide information upon which management can act to maintain evolutionarily important genetic diversity (Stelkens et al., 2012). The environmental factors which act as barriers to gene flow can be natural, such as waterfalls, or man-made, such as weirs (Hansen et al., 2014). If anthropogenic impacts are driving genetic structuring within a species it is important to understand if this impact is detrimental and install effective management and conservation measures to protect the species. For example, it has been shown that historical anthropogenic impacts, such as medieval dams and metal pollution from the bronze age, have structured modern genetic populations of brown trout (Hansen et al., 2014; Paris et al., 2015).

Phenotypic structuring of any kind, between populations which co-exist in sympatry can also provide important insights into the processes of early evolutionary divergence, such as phenotypic plasticity (Bolnick et al., 2011). It is frequently assumed that intraspecific phenotypic structuring represents the outcome of local selection pressures operating on that population (Fraser et al., 2011). Thus, a comparison of morphological expression has the potential to illuminate the selection pressures to which that population is exposed (Garant et al., 2007). Patterns exhibited by such structuring are highly informative in that they provide insights into the evolutionary processes that have ultimately shaped morphological configurations in nature. Such insights are even more valuable where structuring has developed in a single population and is manifested as distinct intraspecific groups occupying the same ecosystem. In such systems, the observed evolutionary divergences are maintained and driven in populations of individuals exposed to broadly the same environmental conditions (such as, temperature, latitude, foraging opportunities, biotic, competition) (Bolnick et al., 2011).

For many species which display extensive intraspecific genetic and morphological structuring, resolving whether they are a polymorphic species, species complex or in fact several species have important management implications. It is important to conserve as much genetic and morphological diversity as possible to ensure the evolutionary potential of a species. For example, whether brown trout are a species complex or several species has been debated for many years. Since 1758, over 57 phenotypically different morphs of brown trout have been described as separate species (Ferguson 1989). Brown trout are now usually regarded as one polymorphic species (Klemetsen et al., 2003). However, there have been several papers which have demonstrated certain morphs of brown trout should be regarded as a separate sub-species. For example, in Lough Melvin three morphotypes of brown trout, ferox, gillaroo and sonaghen, have been described. Ferguson (2004), based on their genetic, morphological and ecological differentiation, considered these as distinct species to highlight their evolutionary importance (Ferguson 2004). Regardless of species status it is vital to maintain the evolutionary potential of brown trout by understanding and conserving evolutionary important genetic and morphological diversity.

1.6 BROWN TROUT AS A MODEL SPECIES

The Brown trout (*Salmo trutta*) is an ideal model species for investigating morphological, genetic and life history strategy structuring. Brown trout exhibit a high degree of intraspecific diversity, especially in morphological and genetic variation among populations (Frank et al., 2011). Brown trout individuals also adopt one of many different life history strategies (Klemetsen et al., 2003). Therefore, brown trout can be used as a model species to investigate the structuring of these traits across varying spatial scales, as well as, the proximate mechanisms which shape such structuring.

1.7 BROWN TROUT ECOLOGY

1.7.1 DISTRIBUTION

Brown trout are one of the most widely distributed species of freshwater fish native to Europe, North Africa and West Asia (Bernatchez 2001; Klemetsen et al., 2003). Brown trout have also been introduced to at least 24 countries, such as New Zealand, Russia and United States of America (USA), for recreational purposes (Klemetsen et al., 2003). Their geographical range is mainly determined by water temperature with an upper critical limit of 25-30^{o}C and a lower critical limit of 3-6^{o}C (Klemetsen et al., 2003). There are five major lineages (Atlantic, Danubian, *marmortaus*, Mediterranean and Adriatic) amongst brown trout, native to the Palearctic region, which evolved in geographic isolation (Bernatchez, 2001). These lineages diversified after the most recent ice-age during the postglacial retreat 15,000 to 11000 years BP (Clark et al., 2012). The recolonisation of freshwaters was almost certainly the result of postglacial invasions of the anadromous form of brown trout which have since adapted to exist in freshwater (Ferguson 2006).

1.7.2 LIFE CYCLE

A consequence of adapting to residency in freshwater after the glacial retreat, brown trout now exhibit several different life history strategies: anadromous (sea) trout, migrate to saltwater to feed; potamodromous trout migrate to freshwater lakes to feed; freshwater resident trout, breed and feed in freshwater rivers (Hendry et al., 2003; Klemesten et al., 2003; Ferguson 2006). Regardless of life history strategy, adult brown trout migrate to their natal rivers to spawn in late autumn/ early winter (Quinn and Myres 2005). Females create redds, which are nests dug in gravel areas of the river bed, using the action of their caudal

fin and lay their eggs which are externally fertilised. The eggs are covered with stones and gravel for protection, as brown trout do not exhibit nest guarding behaviours, and incubate for several months. The exact timing of the incubation period is dependent upon water temperature. Alevin then hatch in spring and have a yolk sac which they rely upon as a source of nutrition for a short period. When most of the yolk sac is consumed, the alevin will swim from the protection of the gravel and start feeding near the spawning areas developing into fry (Klemesten et al., 2003). Brown trout fry form a dominance hierarchy, as they obtain territories and defend them aggressively. The fry which are unable to compete for territories will drift downstream and are likely to die (Hutchison and Iwata 1997; Lahti et al., 2001; Klemetsen et al., 2003). As brown trout grow they require more space and resources so disperse from spawning areas further downstream and inhabit relatively fast flowing waters in their first year (0.2-0.5ms^{-1}) but as they grow larger, they switch to deeper, slower moving waters (Klemesten et al., 2003; Ferguson 2006). By their second year, juvenile trout are known as parr. At this stage, parr may migrate to lakes and become lake dwelling brown trout or deeper areas of larger rivers and become riverine brown trout. After two or more years living as parr, some of the brown trout will become sexually mature and remain as freshwater residents for their entire life time (Jonsson and Gravem 1985; Jonsson and Jonsson 1993; Klemetsen et al., 2003; Charles et al., 2004). Those that do not become sexually mature, may undergo a transformation to become a smolt (Klemetsen et al., 2003; Nichols et al., 2008). This process is a radical change in behaviour, physiology and morphology, as brown trout adapt to survive in salt water and migrate to sea (Klemetsen et al., 2003; Nielsen et al., 2003). Once at sea, anadromous brown trout will forage until they are sexually mature when they migrate back to freshwater to breed. In some cases, migratory trout may migrate back into freshwater for periods when they are not sexually mature and they may make multiple migrations to and from sea before spawning (Jonsson and Jonsson 1993; Klemesten et al., 2003).

1.7.3 ALTERNATIVE LIFE HISTORY STRATEGIES

As has been described, brown trout can adopt one of many life history strategies, with the most extreme examples being freshwater residency or anadromy (sea trout). These two life history strategies are an example of partial migration and like other species, the genetic and/or environmental factors which drive this divergence in life history strategy are unclear. One theory is that food availability is an environmental driver of partial migration (Hendry et al., 2003). It has been demonstrated through laboratory experiments when brown

trout are kept on high food availability diet a higher proportion adopt a freshwater residency life history in comparison to those kept on a low food availability diet (Wysujack et al., 2009). This could be due to low food availability being associated with low growth rate which is known to trigger migratory behaviour in brown trout (Wysujack et al., 2009). The food availability hypothesis also supports this argument, as it predicts that the relative productivity of oceans and freshwater changes with latitude. Therefore, partial migration will occur when ocean productivity is greater than that of its neighbouring freshwater habitat (Maekawa and Nakano 2002; Cucherousset et al., 2005). It has also been demonstrated that adoption of a migratory life history in brown trout is partially driven by maternal effects (Van Leeuwen et al., 2016; Ferguson et al., 2016). Through common garden experiments it was demonstrated juvenile brown trout's dominance status in territorial interactions, when kept on an intermediate food availability diet, was dependent on the life-history status of the offspring's parents. Therefore, it is likely the life history strategy of the offspring parents interacts with environmental factors and drives the migratory strategy adopted by offspring (Van Leeuwen et al., 2016).

However, it has also been shown that partial migration may depend on many other factors, such as developmental genetics and sex (Nielsen et al., 2003). There is evidence that the tendency to migrate is directly or indirectly under genetic control with the mechanism being highly heritable (Ferguson 2006). Transplant experiments have demonstrated the genetic mechanism which drives partial migration could be highly heritable as a higher proportion of offspring from parental brown trout with a freshwater residency life history strategy tended to adopt a freshwater residency life history strategy in comparison to anadromous brown trout (Jonsson and Jonsson 1993). Heritability estimates for anadromy in brown trout have not been calculated, however, there are a couple of studies which have derived an estimate for brook charr (*Salvelinus fontinalis*) and steelhead trout (*Oncorhynchus mykiss*) (Thrower et al., 2004; Thériault et al., 2007; Ferguson et al., 2016). Due to the similarity of heritability estimates for brook charr and steelhead trout, Ferguson et al., 2016 suggests the heritability for anadromy in brown trout is likely to be similar. Therefore, additive genetic variance for anadromy likely accounts for half the variability of life history among individuals in a population with environmental factors and parental effects explaining the remainder variability (Ferguson et al., 2016). Another factor which may drive partial migration in brown trout is sex, where there is a tendency for a higher proportion of females to migrate compared to males which often leads to a sex bias (Elliott, 1994; Klemesten et al., 2003; Nielsen et al., 2003; Ferguson et al., 2016). This sex bias is driven

by the costs and benefits of migration being dependent on sex. Female reproductive success is dependent on body size, as larger females produce more eggs, have greater fecundity and defend spawning sites better (Klemesten et al., 2003). Therefore, females are more likely to migrate from rivers with low food availability to the marine environment with richer feeding grounds (Klemesten et al., 2003). In contrast, the reproductive success of males is not limited by size and adopting a freshwater resident life history strategy may be advantageous as they are able to mature early and have a higher reproductive success (Ferguson 2006).

There is considerable controversy surrounding whether freshwater resident and anadromous brown trout co-existing in sympatry arise from the same gene pool or if they are in fact from separate genetic populations (Hendry et al., 2003; Charles et al., 2005; Ferguson 2006). Using neutral markers, such as microsatellites, there have been several studies which have examined whether sympatric (either breeding in sympatry or co-existing in sympatry) freshwater resident and anadromous brown trout originate from a single genetic pool or form separate populations. These studies have found varying results, with most finding sympatric anadromous and freshwater resident brown trout originate from the same gene pool (Fleming et al., 1983; Hindar et al., 1991; Cross et al., 1992; Petersson et al., 2001). However, there have been a few studies which have demonstrated genetic differentiation between sympatric anadrmous and freshwater resident brown trout (Krieg & Guyomard 1985; Skaala & Naevdal 1989). Determining the genetic structuring of life history groups of brown trout co-existing in sympatry could provide insights into the origins of partial migration in this species.

1.7.4 GENETIC STRUCTURING

Brown trout demonstrate one of the highest level of intraspecific genetic structuring of any species of vertebrate (Frank et al., 2011). For example, in a review of genetic variation among brown trout, Ferguson (1989) examined data from 116 drainages, in 12 European countries and reported 70 putative loci (encoding approximately 31 proteins), of which 54% (38 loci) were found to be polymorphic. However, only 16 % of this variation was re-found in any catchment (Ferguson 1989). One consequence of this pattern of genetic variation is that, although there is significant heterogeneity in genetic composition across the species, only a small part of this genetic variability is present at the level of the population. Thus, a large proportion of the intraspecific genetic diversity of the brown trout is represented by genetic differences between populations (Laikre 1999). There have been several studies which have examined population structuring of brown trout, either focusing on a large spatial

scale or a small spatial scale (Crozier & Ferguson 1986; Ferguson 1989, 2007; Bernatchez et al., 1992; Carlsson et al., 1999; Massa-Gallucci et al., 2010; Swatdipong et al., 2010; Ensing et al., 2011; Stelkens et al., 2012). For example, there is extensive genetic differentiation between three morphotypes of brown trout (ferox, gillaroo and sonaghen) in Lough Melvin, Ireland indicating that they are reproductively isolated (Ferguson & Taggart 1991). High levels of genetic differentiation between populations was also identified between populations of lake-run brown trout in northern Finland with each cluster of populations having different biological characteristics, such as feeding behaviour (Swatdipong et al., 2010). In the River Aare, Switzerland, large genetic variation was found between populations within a 40km stretch of river (Stelkens et al., 2012). However, there are few/ no studies which have examined population structuring of brown trout over several spatial scales.

Determining which environmental factors drive such genetic diversity is important to understand macroevolutionary processes and to provide effective management measures. The population structuring of brown trout is likely driven by isolation by distance and/or isolation by environment. There have been a few studies which have examined environmental drivers of brown trout's population structuring but not over several different spatial scales. For example, in the River Aare, Switzerland, waterway distance, number of weirs and stretches of poor habitat were shown to drive population structuring (Stelkens et al., 2012). However, in two rivers in Norway which comprised of 10 contemporary populations and three historical populations, the population structuring was driven by the effects of stocking. There was a shift in population structuring between historical and contemporary populations highlighting the impact stocking can have on wild populations (Thaulow et al., 2013). Therefore, with habitat fragmentation being one of the biggest drivers for loss in biodiversity and increasing pressures from anthropogenic impacts its vital to determine the possible environmental drivers of brown trout population structuring and if these environmental drivers are linked to anthropogenic impacts.

1.7.5 MORPHOLOGICAL STRUCTURING

Brown trout demonstrate vast phenotypic variation both between and within populations in behaviour, morphology, life-history strategy, foraging ecology, colouration, and parasitic fauna, amongst a wide range of other traits (see e.g. Ferguson & Mason 1981;

Cawdery & Ferguson 1988; Klemetsen et al.., 2003; Ferguson 2006). For example, in common garden experiments body height and fin length varied between populations of brown trout when kept in differing flow regimes (Pakkasmaa & Piironen 2001b). Brown trout can often show large phenotypic differences which have led to these morphs being described as sub-species as there is also genetic differentiation between these morphs. For example, in Lough Melvin, three morphs (Ferox, gillaroo and sonaghen) have been described which differ in colour, spot pattern, head shape, body size and exhibit food resource partitioning (Cawdery & Ferguson 1988). Fine spotted trout show large morphological differences in spotting pattern compared to brown trout in the Hardangervidda area (Skaala & Jorstad 1987). Three populations of brown trout which are found in Lough Laidon had differences body shape and gill raker length (Piggot unpublished). As a result, there was once thought to be more than 57 species of brown trout (Ferguson, 1989). Brown trout is now thought to be a "species complex", which displays a wide range of phenotypic variation with different phenotypes often occupying a range of different niches (Klemetsen et al., 2003).

Few studies have examined structuring of brown trout morphologies, over different spatial scales in the riverine environment, despite large morphological differences being identified (Stelkens et al, 2012). Therefore, unlike other species of salmonid very few studies have examined possible environmental drivers for such variation (Stelkens et al., 2012). For example, in the River Aare, Switzerland, structuring of morphologies was shown to be driven by water body size and flow regime (Stelkens et al., 2012). Understanding how environmental variables drive such structuring is important to understand phenotypic plasticity in brown trout and to identify riverine habitats which support these different morphologies.

1.7.6 THREATS AND EXPLOIATION

There are many threats to brown trout populations, the most important of these being exploitation, environmental degradation and fish movements (Laikre & Ryman 1996). Exploitation could result in a reduction or loss of genetic diversity and reduced population viability due to a reduction in population size (Klemesten et al., 2003). For example, in Finland more than 45% of the brown trout populations investigated were at threat from over exploitation (Koljonen & Kallio-Nyberg 1991). Direct degradation of brown trout habitat

can occur through dam construction and water diversions that can obstruct migration to spawning grounds. For example, migratory barriers caused the extinction of a large brown trout population in Lake Vänern (Sweden) in less than 100 years (Ros 1981). Pollution can also impact trout populations. In Finland for example approximately 40% of the brown trout populations investigated were under threat by pollution (Koljonen & Kallio-Nyberg 1991). Therefore, understanding intraspecific structuring of brown trout and how environmental factors shape such structuring is vital to mitigate against exploitation and habitat degradation.

Climate change will likely impact the frequency of both freshwater resident and anadromous brown trout life history strategies in populations (Ferguson et al., 2016). Increasing temperatures are going to change the relative productivity of both riverine and marine habitats. It has been demonstrated at lower latitudes that anadromy is unlikely due to warm seas having lower productivity, therefore, this would lead to a decrease in the frequency of anadromy (Maekawa and Nakano 2002; Cucherousset et al., 2005; Ferguson et al., 2016).

A perceived threat to brown trout populations, is the release of farm origin brown trout or hatchery-reared offspring of brown trout from other populations (Ferguson 2006). Until recently, when stocking of fertile strains of hatchery bred brown trout was banned, approximately two million farm-reared brown trout are released annually into the wild in England and Wales (Dunn 2005). The main potential impacts of stocking, are thought to be introgression of farm origin genes with locally adapted wild populations which have the potential to reduce the fitness of wild trout (McGinnity et al., 2009). Stocking of hatchery bred brown trout could also have implications for the adoption of an anadromous life history strategy, as stocking with fertile hatchery-bred brown trout could decrease the genetic potential for anadromy in wild populations (Ferguson et al., 2016). Genetic changes during domestication have reduced anadromous brown trout's ability to survive at sea and therefore, with hatchery-bred and wild brown trout successfully breeding this would suggest that a higher frequency of brown trout would adopt a freshwater resident life history strategy (Ferguson et al., 2016). Therefore, with anadromous brown trout numbers decreasing in recent decades it is important to understand intraspecific structuring of life history strategies

and how the frequency of each life history strategy may change between rivers, as well as, identifying potential environmental, physiological and behavioural drivers.

1.8 THESIS OUTLINE

The focus of this thesis was to determine how genetic, morphological, life history strategy and environmental factors drive intraspecific structuring of populations within a species and demonstrate how knowledge of these interactions can be used for effective management of a species. Using a single, large dendritic river system, the River Foyle in Ireland as a model system and the brown trout (*Salmo trutta*) as a model species, I examined intraspecific genetic and morphological structuring amongst brown trout populations. I then attempted to determine the proximate mechanisms which shape such structuring. Using life history strategy as a specific example of discrete morphological structuring in sympatry I examined whether neutral genetic differentiation. Finally, using information gained from the genetic population structuring of brown trout, I demonstrated how this can be used for effective management of the economically valuable anadromous component of brown trout populations.

Chapter Two: In this chapter I investigate the pattern of intraspecific genetic structuring and the environmental and landscape features that may drive these patterns. This question is important to an understanding of early evolutionary processes and has significant implications for effective management of populations. Therefore, in this chapter I aimed to:

- Determine the genetic structuring of brown trout at different spatial scales in a dendritic river catchment;
- Understand the role of isolation by distance in shaping genetic structuring and;
- Establish the role of isolation by environment in shaping genetic structuring of brown trout.

Chapter Three: A knowledge of intraspecific structuring of morphologies is also important for effective management practices, as well as contributing to our understanding of evolutionary processes leading to divergence between populations. Therefore, I aimed to determine:

- the structuring of morphologies of brown trout at different spatial scales;
- whether morphological structuring could be a result of underlying neutral genetic structuring;
- the role of isolation by distance in shaping morphological structuring of groups;
- how landscape features might drive the morphological structuring of brown trout and;
- which features are responsible for the absolute differences between morphological groups.

Chapter Four: Contributing to the current literature surrounding the controversy of whether the alternative life history strategies of anadromy and freshwater residency in brown trout originate from the same or separate gene pools. In this study, I aimed to determine the effect of within river genetic population structuring and geographic location on the adoption of alternative life history strategies by brown trout.

Chapter Five: Where intraspecific genetic structuring has been established, it is possible to use a Genetic Stock Identification (GSI) analytical framework to determine the population of origin of individuals from a mixed stock. In this study, I used such a framework to determine if the production of anadromous brown trout in mixed stock of a single sub-catchment is disproportionately originating from a small number of tributaries within a sub-catchment.

Chapter six: Finally, in this chapter I aimed to draw together the main conclusion and discuss their evolutionary significance as well as their broader importance for effective management of brown trout.

CHAPTER TWO: ENVIRONMENTAL VARIABLES AND ANTHROPOGENIC PRESSURES, SHAPE THE POPULATION STRUCTURING IN BROWN TROUT FROM A COMPLEX, DENDRITIC CATCHMENT.

2.1 ABSTRACT

The patterns and origins of intraspecific genetic structuring have the potential to inform our understanding of the evolutionary processes which may lead to speciation. Understanding such patterning is also crucial for the effective management of a species, through the identification of management units. This study investigated the population structuring of the brown trout (*Salmo trutta*) in a complex dendritic catchment, as well as the role of isolation by distance and isolation by environment in shaping any identified structuring. From 28 sampling sites, 21 genetic populations were identified which were separated by a river distance of between 0 km and 176km. Isolation by river distance was found to play a significant role in shaping the population structuring identified. In addition several landscape and environmental variables also significantly predicted the pairwise genetic differences between populations. These variables included: the distance to the nearest farm (km), the number of houses per km^2 in the upstream catchment and the concentration of phosphorus, which are strongly linked to anthropogenic pressures on the environment. This study is the first to show a direct anthropogenic influence on genetic structuring of salmonids in a dendritic river system. These results highlight the importance of managing identified populations and preserving the genetic diversity of brown trout, as well as mitigating against the potential anthropogenic impacts on brown trout in the Foyle catchment.

2.2 INTRODUCTION

Understanding how landscape features act as barriers to dispersal and gene flow resulting in intraspecific patterning of neutral genetic variation is fundamental for effective management and conservation of a species (Dionne et al., 2008). There are a few previous studies that have examined how landscape features shape intraspecific genetic population structuring (see Schultz et al., 2008 for lemon sharks (*Negaprion brevirostris*); Kanno et al., 2011 for brook trout (*Salvelinus fontinalis*); Earnest et al., 2013 for top minnows (*Fundulus notatus; F.olivaceus*); Lozier et al., 2013 for bumblebee (*Bombus bifarius*); Emel & Storfer, 2015 for southern torrent salamander (*Rhyacotriton variegatus*)). For example, the genetic

population structure of the American black bear (*Ursus americanus*) in the Rocky Mountains, north central U.S.A. is shaped by elevation, forest cover and roads (Short Bull et al., 2011). Another study investigated a fragmented woodland area and demonstrated that the population structuring of the roe deer (*Capreolus capreolus*) was strongly linked to woodland structures and the connectivity of the landscape (Coulon et al., 2004). Landscape features which act as barriers to dispersal/gene flow can be natural or modified by anthropogenic pressures (Stelkens et al., 2012; Hansen et al., 2014). Anthropogenic pressures, such as habitat destruction, climate change and overharvesting may drive short term change of intraspecific population structuring of a species (Dionne et al., 2008). Habitat fragmentation is considered one of the biggest threats to biodiversity, as reduced connectivity between habitats leads to inbreeding, genetic drift, erosion of genetic variation and loss of rare alleles (Stelkens et al., 2012; Hansen et al., 2014). It is important to recognise that anthropogenic impacts are inevitable but it is also imperative to understand how these impacts are driving intraspecific structuring, not only for conservation and management purposes but also to determine how humans are driving evolutionary processes within a species and the rate at which these processes are occurring (Moritz 2002).

Freshwater fish, especially post-glacial fish, make ideal model species to investigate the effect of landscape features on intraspecific genetic population structuring (McCracken et al., 2013). Freshwater species of fish have complex population structures which are greatly influenced by environmental characteristics and habitat fragmentation (Antunes et al., 2001; Leclerc et al., 2008; Kanno et al., 2011). For example, salmonids have been the focus of many studies because their complex evolutionary history and intraspecific population structuring is reflected in their extensive genetic diversity (Crozier & Ferguson, 1986).

The population structuring of salmonids are strongly influenced by stream hydrology, connectivity patterns, environmental gradients, patchiness of habitats, river structure and complexity (Neville et al., 2006). Therefore, one species of salmonid which is a useful model species to investigate the role of landscape features acting as barriers to dispersal and shaping intraspecific population structuring is the brown trout (*Salmo trutta*). Brown trout display extensive intraspecific genetic population structuring with a high degree of genetic differentiation between populations even in the absence of physical barriers to dispersal (Crozier & Ferguson, 1986; Ferguson, 1989, 2006; Bernatchez et al., 1992;

Carlsson et al., 1999; Massa-Gallucci et al., 2010; Swatdipong et al., 2010; Ensing et al., 2011). There have been many studies which have examined intraspecific genetic structuring on a large spatial scale with genetic sub-divisions most likely being the result of historical contingencies (McKeown et al., 2010). Bernatchez (2001) described five lineages of brown trout (Atlantic, Danubian, *marmoratus,* Mediterranean and Adriatic) with the Atlantic group splitting from eastern lineages around 700 000 years B.P (Bernatchez, 2001). Following this separation Britain and Ireland, the region of focus in this study, underwent several glacial and interglacial periods with the most recent glacial period starting around 30 000BP and reached maximum extent around 23 000 to 18 000 years B.P (McKeown et al., 2010). There were a few refugia along the coast of south and west Ireland and south England which remained ice free and were inhabited by anadromous brown trout (Ferguson et al., 2016). As the ice retreated during interglacial periods, anadromous brown trout expanded their range and as the ice advanced they returned to these refugia (McKeown et al., 2010). This allowed opportunities for divergence in allopatry followed by interbreeding or reproductive isolation following secondary contact (Ferguson 2006). McKeown et al. (2010) demonstrated anadromous brown trout colonising Britain and Ireland were from at least five potenital glacial refugia (McKeown et al., 2010). Therefore, most populations of brown trout in Britain and Ireland were colonised by anadromous brown trout (Ferguson et al., 2016).

There have been several studies which have examined intraspecific structuring on a micro-geographic scale (McRae 2006). These studies provide insights into the microevolutionary processes which shape instraspecific population strutcuring (Stelkens et al., 2012). However, few studies have examined intraspecific genetic population structuring over several different spatial scales (Griffiths et al., 2009). Understanding the population structure over several spatial scales is particularly important in freshwater systems where there is often asymmetric gene flow, as upstream populations converge into a single downstream population resulting in downstream populations having greater genetic variation than upstream populations (Junker et al., 2012; McCracken et al., 2013). The extent of this asymmetric gene flow depends on spatial scale and as a result brown trout can form genetically differentiated hierarchical populations over varying geographic scales (Junker et al., 2012).

There are at least three proximate landscape feature mechanisms which could result in divergence between genetic sub-groups within a species and thus intraspecific genetic structuring. These are: isolation by distance, isolation by environment or a combination of both. In the first scenario, population structuring is shaped by isolation by distance. Isolation by distance can occur when populations exchange genes at a rate which is dependent on geographic distance (Hardy & Vekemans 1999; van Strien et al., 2015). Thus, genetic differences between populations increase as geographic distance increases because genetic change at one end of the species range is not easily transmitted to the other. This genetic change could result from genetic drift, local selection pressures or random chance mutations. This would occur when habitat configuration and/ or geographic distance are restricting dispersal and gene flow within a species and this alone is shaping population structuring (Jensen et al., 2005).

In the second scenario, isolation by environment shapes the observed population structure. In this scenario, one or more environmental gradients shape population structure in the absence of isolation by distance (Wang & Bradburd 2014). Differences in landscape features may influence gene flow between populations by affecting dispersal rates between them and thus driving micro-evolutionary processes (McRae 2006). In this scenario, population structuring could arise from the following ecological processes: natural selection against immigrants adapted to different environmental conditions, sexual selection limiting the success of hybrids, reduced hybrid fitness and biased dispersal for particular environments (Wang & Bradburd 2014).

The final scenario which could shape population structuring, is that both isolation by distance and by environment is responsible for the structuring observed. Isolation by distance would be expected to have a bigger effect at larger spatial scales, because of restricted movement of genes within a population/species. Whereas, at smaller spatial scales, isolation by environment may have a stronger effect on population structuring. Environmental factors are acting as barriers to gene flow, migration and/ or dispersal which would result in multiple genetic groups. This pattern of barriers to gene flow may occur in multiple populations across the species distribution possibly resulting in considerable genetic structuring (Holderegger et al., 2006).

The aim of the work described here is to examine the pattern of genetic structuring in brown trout of a single complex river catchment and to determine proximate mechanisms that may be maintaining any pattern. Specifically, this study aims to determine:

(i) Hierarchical intraspecific genetic population structuring of brown trout at different spatial scales;
(ii) the influence of isolation by distance and isolation by environment on the genetic structuring.

2.3 METHODS

2.3.1 STUDY SITE

The River Foyle catchment, is a medium sized catchment of 4500km^2 located both in Northern Ireland and the Republic of Ireland (Niven, 2013). It is a complex, highly dendritic river catchment which comprises of many smaller sub-catchments including the Rivers Camowen, Owenreagh, Derg, Fairywater, Owenkillew, Finn, Faughan, Roe and Burndennet (Fig. 2.1). These sub-catchments drain into the River Foyle which in turn drains into a sea lough, Lough Foyle (Niven, 2013). This highly complex, dendritic system is ideal for the investigation of the influence of landscape features on hierarchical population structuring.

2.3.2 COLLECTION OF SAMPLES

Population structuring was investigated across three spatial scales (large, medium and small) to determine the effects of geographic distance and landscape features in shaping structuring (Fig. 2.2). A large spatial scale was represented by sampling locations in different sub-catchments of the Foyle catchment (Rivers Faughan, Roe, Camowen, Owenreagh, Derg, Muff and Burndennet). Tributaries in each sub-catchment were surveyed to represent population structuring on a medium spatial scale. Finally, a small spatial scale was represented by sampling sites within streams of selected tributaries surveyed on a medium scale.

Twenty-eight sites within the Foyle catchment representing the three spatial scales were electrofished between April and September in 2013/2014 (Fig 2.1; Table 2.1).

Sampling sites were selected based on information on habitat quality and abundance of juvenile brown trout routinely collected by the Loughs Agency (the cross-border government fisheries body managing the Foyle catchment). Therefore, sampling sites were primarily selected where high trout density might be expected based on previous habitat surveys. Sampling sites were electrofished over long stretches, more than 500m of river, concentrating on riffle-run habitats to collect genetic samples from mainly juvenile brown trout. At each site, an effort was made to collect the brown trout randomly to ensure that the brown trout collected from each river represented more than a few families (Hansen et al 1997). In total, 1889 brown trout were collected, anesthetised using clove oil and a non-destructive genetic sample (adipose fin clips) taken and stored in 95% ethanol. A record was also made of each fish's fork length.

River ID and Abbreviation	Location ID	Sub-Catchment	Spatial scale	Easting	Northing	N	Average Fork length (mm) with SD
Burndennet (DEN)	1	Burndennet	Large	641530.6	904685.1	17	59.40 ±7.52
Camowen (CAM)	2	Camowen	Large/medium	662460.3	870951.2	72	90.20 ±49.01
Drumnakilly (DRU)	3	Camowen	Medium/small	653773.2	873040.4	71	110.68 ±59.58
Drumnakilly A (DRA)	4	Camowen	Small	655032.3	874057.7	65	74.15 ±15.09
Drumnakilly B (DRB)	5	Camowen	Small	654245	873710.5	76	90.49 ±36.77
Granagh Burn (GRA)	6	Camowen	Medium	659846.6	872823.6	69	89.55 ±34.77
Bonds Glen (BGL)	7	Faughan	Medium	650703.4	907420.5	40	184.27 ±27.72
Burngibbagh (GIB)	8	Faughan	Medium	644497.4	912857.2	65	127.21 ±40.43
Burntollet (BUR)	9	Faughan	Medium/small	652919.5	911768.1	66	97.22 ±44.38
Burntollet A (BUA)	10	Faughan	Small	658370.5	912565.5	69	74.86 ±21.51
Burntollet B (BUB)	11	Faughan	Small	654962.9	912632	69	75.35 ±30.59
Faughan (FAU)	12	Faughan	Large/medium	657002.8	905701.6	63	95.47 ±48.61
Faughan A (FAA)	13	Faughan	Small	660556.6	900607.6	65	86.22 ±38.76
Faughan B (FAB)	14	Faughan	Small	660476.2	900491.8	65	96.08 ±35.37
Foreglen (FOR)	15	Faughan	Medium	656876.9	908861.8	65	121.65 ±40.04
Glenrandal (GLE)	16	Faughan	Medium	654296.7	904727.1	63	116.13 ±51.03
Killen burn (KIL)	17	Killen Burn	Large	622887.4	882083.9	53	207.16 ±30.32

Owenreagh (REA)	18	Owenreagh	Large/medium	632906.1	866020.7	58	103.05 ±46.04
Owenreagh A (REB)	19	Owenreagh	Small	632611.8	867336.4	65	62.29 ±20.86
Owenreagh B (REC)	20	Owenreagh	Small	638204.2	860452.6	65	94.18 ±41.85
Quiggery water (QUI)	21	Owenreagh	Medium	644305.9	858990.4	68	144.53 ±74.67
Routing Burn (ROU)	22	Owenreagh	Medium	646987.2	863690.1	71	111.57 ±51.85
Routing Burn A (RUA)	23	Owenreagh	Small	650808.4	861632.4	47	172.07 ±36.90
River Muff (MUF)	24	River Muff	Large	652304.2	918250.8	178	140.60 ±27.07
Castle (CAS)	25	Roe	Medium	671096.8	918932	64	105.50 ±37.82
Owenbeg (OWE)	26	Roe	Medium	664516.1	905941.5	66	85.93 ±41.72
Roe (ROE)	27	Roe	Large/medium	677020.9	903815.1	67	101.48 ±40.67
Roe A (ROA)	28	Roe	small	675012.1	906310.5	11	145.45 ±39.65

Table 2.1: Sampling locations (river ID, site number, sub-catchment and spatial scale (see fig 2.2)) in the coordinate system "Irish Transverse Mercator grid", sample size (N) and the average fork length (mm) ±SD of sampled brown trout.

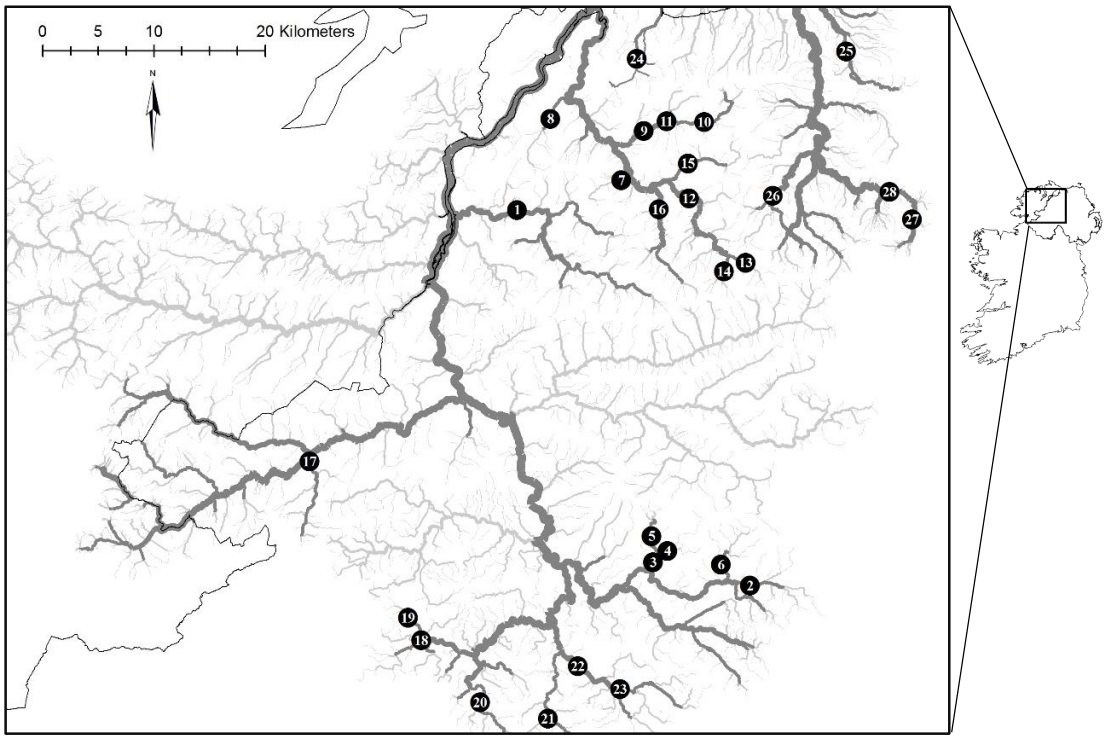


Fig. 2.1: Sampling sites for this study in the Foyle catchment. Location ID indicated on map corresponds to information in Table 2.1.

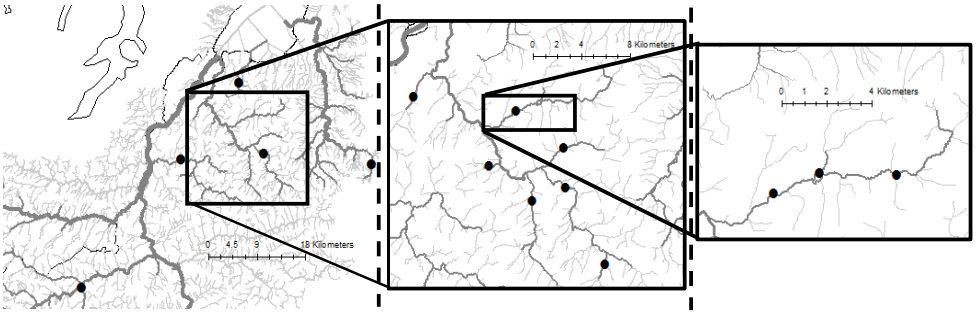


Fig. 2.2: A diagrammatic representation of the three geographic spatial scales used during this study to investigate the relationship between geographic distance and population structure. Large scale geographic (river) distances were between different sub-catchments and ranged from 52km-176km. Medium scale geographic (river) distances were between tributaries within sub-catchments and ranged from 7km-65km. Finally, small scale geographic (river) distances were between streams within tributaries and ranged from 0.3km-10km.

2.3.3 *MICROSATELLITE AMPLIFICATION*

Genomic DNA was extracted from ~20mg of tissue of each sample following promega's protocol (www.promega.com). Each fin clip was digested in a solution of 0.05ml nuclei lysis, 0.01ml 0.5M ETDA, 5.2 µl protease K and 1.3 µl RNAse A at 57^{o}C for 12-18 hours. The DNA from each digested fin clip was then extracted by adding 62 µl lysis solution to each digested sample and passing through a filter plate three times before adding 100 µl TE Buffer. The extracted DNA was added to 1xloading buffer and quantified against λHindIII size standard using a 0.8% gel, which was 1/2x10TBE, distilled water, agarose gel and 10 µl ethidium bromide. The extracted DNA was then diluted to ~5ng/µl. Following methods by Keenan et al. (2013a) two multiplex PCR reactions with 21 microsatellite markers were run (Ssa85, Oneu9ASC, Ssa416UOS, Ssa406UOS, CA054565, CA048828, CA053293, One102a,b, One108, One103, ppStr2, SsaD48, Cocl-Lav-4, BG935488, CA060177, Ssa197, MHC-I, SasaTAPA2, SsaD71, ppStr3, Ssa410UOS) (Keenan et al., 2013a) (see appendix). In addition, a sex identification marker was included in the second PCR reaction, *salmo*YF (unpublished). *Salmo*YF targeted a short polymorphic fragment within the first intron of the sdY gene, which is a male specific, Y-chromosome, sex-determining gene that is conserved in almost all salmonid species (Quéméré et al., 2014). The PCR reactions were a solution of 1 µl DNA, 2.5 µl primer mix (primers, water and Top-bio plain comb master mix) and 8 µl wax. The PCR reactions were run over 24 cycles at 95^{o}C for 45 seconds, 57^{o}C for 1.5 minutes and 70^{o}C for 1 minute, with the final extension at 60^{o}C for 30 minutes. Finally, 2 µl diluted PCR product (diluted with TE buffer) was added to 9 µl of formahyde mixed with G5600Liz size standard and denatured, the solution was then typed on an ABI3730XL 96 capillary DNA analyser and allele lengths were sized using Genemapper V4.1 (Thermofisher Scientific). SsaD48 was removed from the analysis due to inconsistences in banding patterns making it unreliable.

2.3.4 STATISTICAL ANALYSIS

A Bayesian statistical framework implemented in STRUCTURE (Pritchard et al., 2000) was run using a hierarchical approach to identify intraspecific structuring of brown trout which may have otherwise been missed (Pisa et al., 2015). This hierarchical approach initially analyses the entire dataset for genetic clusters. The identified genetic clusters, from the initial run, are then analysed independently until no further population structuring can be identified. STRUCTURE was run using 100 000 Markov Chain Monte Carlo steps after a

burn in period of 100 000, with *a priori* geographic location to prevent misclassification of individuals. Each run was performed for 1 to 10 clusters (K) with 20 iterations for each individual sample. The number of clusters identified in each run was determined using ΔK from the *ad-hoc* method by Evanno et al. (2005) implemented in STRUCTURE Harvester (Earl & vonHoldt 2012). CLUMPP (Jakobsson & Rosenberg 2007) was then used with the Greedy method to consolidate the probability of each individual belonging to each cluster from the 20 iterations used in STRUCTURE. The resulting clusters were visualised using Arc GIS V10.2 (ERSI 2010) and a population tree constructed using POPTREEW and figtree V1.4.3 (Takezaki et al., 2014; Rambaut 2007).

Summary statistics (number of individuals genotyped per locus, the number of alleles per locus, the percentage of total observed alleles per locus, allelic richness per locus, Hardy-Weinberg equilibrium (HWE) per locus and Wright's inbreeding coefficient per locus) were calculated for each identified genetic population using the R package 'diveRsity' (Keenan et al., 2013b; R Core Team 2016). Genepop on the web was used to test for linkage disequilibrium for each identified genetic population (Raymond & Rousset 1995; Rousset 2008) with Markov chain parameters comprising 1000 dememorizations, 100 batches and 1000 iterations per batch. The chance of obtaining type 1 errors was reduced by using Bonferroni correction when calculating both Hardy-Weinberg's equilibrium and linkage disequilibrium. Finally, LOSITAN workbench was used to establish if the microsatellite markers were subject to selection using a stepwise mutation model with 50 000 simulations, a confidence interval of 0.99 and a false discovery rate of 0.1 (Antao et al., 2008).

The pairwise relatedness of individuals was calculated using the R package 'related' (Pew et al., 2015; R CoreTeam 2016) to ensure that the population structure was based on mostly unrelated individuals, with more than a few families represented. Firstly, simulations were run to establish the best estimator for the analysis and to test the resolution of the dataset by visualising the differentiation between Parent-Offspring, Full-Siblings, Half-Siblings and Unrelated individuals. From the simulations, Wang's (2002) coefficient was established as the best estimator and so the pairwise relatedness of individuals was tested using the coancestry function with 500 bootstraps (Wang 2011; Pew et al., 2015). The relatedness of individuals within populations was further quantified using COLONY (Jones & Wang, 2010). Colony was used to identify the number of full-sibling families within each genetic

population and ensure each full-sibling family contained less than three individuals (Hansen et al., 1997).

The effective population size (N_e) of each population was calculated with NeEstimator v2 (Do et al., 2014) using a linkage disequilibrium random mating model which calculated N_e in the absence of temporal data. Jack-knifing confidence intervals (CIs) were used to correct for the possibility of underestimating N_e due to sampling error (Bernaś et al., 2014). N_e was also calculated using COLONY (Jones & Wang 2010; Wang 2016). Contemporary gene flow between the population clusters identified was established using the R package 'DiveRsity' (Keenan et al., 2013b; R CoreTeam 2016). This was used to determine the strength and direction of gene flow between populations. For the analysis, a threshold limit of 0.6 was set with 999 bootstraps using the D_{JOST} statistic (Sundqvist el al., 2016).

Geographic (river) and genetic distance between populations were determined to test for isolation by distance. Geographic distance was calculated using ArcGISV10.2 (ERSI 2011) and was measured as river distance (km), which was measured as the distance between two sampling locations following the watercourse, as opposed to a straight-line distance. Genetic distance between populations was established using the R package 'DiveRsity' (Keenan et al., 2013b; R CoreTeam 2016) and was calculated using D_{JOST} and F_{ST}. It is often difficult to interpret the results of F_{ST} which can lead to an underestimation of the true level of genetic differentiation between populations (Meirmans & Hedrick 2011). Once the genetic and river distances between populations were established, Mantel tests and Reduced Major Axis (RMA) regressions were performed in Isolation by Distance Web Service (IBDWS) (Jensen et al., 2005) to determine whether geographic distance could explain intraspecific genetic structuring of brown trout identified in the Foyle catchment.

The influence of environmental variables on population structuring was investigated using 31 environmental variables, collated from data collected by the Loughs Agency, representing four major categories of environmental variable type: site specific habitat characteristics; site specific water quality; geology and landscape features of the catchment upstream of each population site (Table 2.2). Genetic population sampling locations used in this study did not always match the location the Loughs Agency used to sample

environmental variables. Therefore, where possible, locations with available data on landscape variables nearest to the genetic populations sampling location were used. If this was not possible, information on environmental variables for geology and landscape features were calculated using the methods described in Table 2.2. For any genetic populations which were missing data on environmental variables for water quality and site-specific habitat characteristics an average of all populations for that variable was taken. Finally, instream substrate composition comprised seven variables which were highly correlated. Principle Component Analysis (PCA) was conducted on the seven variables to account for this and the PC scores were used in subsequent analysis.

Partial Mantel tests were used to test for correlations between genetic distance and the difference in environmental variables between pairwise genetic population sampling locations. Three genetic populations were removed from this analysis. Killen Burn B and River Muff B populations were removed as they were sympatric with Killen Burn A and River Muff A, respectively. This avoided the replication of environmental variables from each population sampling site. The genetic population River Burntollet was removed from the analysis because no environmental data were available for this population. The R package 'ecodist' was used to run Partial Mantel tests with 100 000 permutations and geographic (river) distance as a controlling variable to account for spatial autocorrelation (Goslee & Urban 2007). The pairwise 'distance' between landscape variables was calculated using Euclidean distance to create dissimilarity matrices. However, as has been shown, using these environmental distances in partial mantel tests tends to inflate Type 1 errors (Diniz-filho et al., 2013). Therefore, to further investigate how genetic distance between populations is influenced by different environmental variables, mixed models were run using the R packages 'lme4' and 'glmulti' (Bates et al., 2015; Calcagno & de Mazancourt 2010). If isolation by distance was shown to be an important driver of the genetic structuring of brown trout, then it was important to remove the effect of geographic (river) distance from any further analysis. Therefore, the absolute value residuals were used, from a reduced major axis regression, between genetic distance and geographic (river) distance to represent the genetic distance between pairwise genetic populations. Mixed models were run for each of the four categories of environmental variables (instream habitat characteristics, water quality variables, geology variables and landscape features) to investigate their effect on genetic structuring. The model which best fitted the data was selected using AICc for each category of environmental variables. The environmental variables included in each of these models

were then collated into a final model to determine which variables influenced genetic structuring. Mixed models were run using the genetic algorithm in 'glmulti' with no interactions included in each model due to the possible number of combinations of variables (often more than a billion possible models).

Variable	**Sampling methodology**	**Year(s) data collected**
Category: Water Quality		
Biological Oxygen Demand (mg/l)		2009-2014
Ammonia (mg/l)		2009-2014
Phosphorus (mg/l)		2009-2014
Suspended Solids (mg/l)		2009-2014
Dissolved oxygen (mg/l)		2009-2014
Conductivity		2009-2014
pH		2009-2014
Category: Site specific habitat characteristics		
Depth (m)	Average depth at sampling site	1998-2006
Width (m)	Average width at sampling site	1998-2006
Cover (%)	The cover provided by trees was estimated for both the right and left river bank and then averaged at each sampling site.	1998-2006
Overhang (%)	The overhang of vegetation on both the right and left river bank was estimated and then averaged at each sampling site.	1998-2006
Bedrock (%)	Percentage of sampling area containing bedrock (exposed solid rock)	1998-2006
Boulder (%)	Percentage of sampling area containing boulder (large rocks >256mm)	1998-2006

Cobble (%)	Percentage of sampling area containing cobble (loose rock 64-256mm)	1998-2006
Gravel (%)	Percentage of sampling area containing gravel (loose material 16-64mm)	1998-2006
Fines (%)	Percentage of sampling area containing fines (loose material 2-16mm)	1998-2006
Sand (%)	Percentage of sampling area containing sand (loose material <2mm)	1998-2006
Mud (%)	Percentage of sampling area containing mud	1998-2006
Category: Geology		
Stream order	Stream order was calculated using methodology explained by Horton (1945)	2002
Catchment area (km^2)	Catchment area above each sampling site was determined using the river network boundary.	2002
Elevation (m)	Calculated from height contours on either side of sampling site	2002
Stream gradient	Horizontal distance between the two nearest contour lines and dividing by the change in elevation.	2002
Number of houses per km^2 upstream of site - representative of Urban area	Number of houses upstream of sampling site divided by the catchment area above the sampling site	2002
Distance to nearest farm (km) - representative of proximity to farmland	Straight line distance from sampling site to nearest farm house	2002
Category: Landscape features		

Area of peat upstream (km^2)	Area of peat upstream of sampling site was measured using 'Drift and Quaternary editions of Geological Survey of N Ireland' maps.	2002
Area of glacial alluvium upstream (km^2)	Area of glacial alluvium upstream of sampling site was measured using 'Drift and Quaternary editions of Geological Survey of N Ireland' maps.	2002
Area of glacial sand and gravel upstream (km^2)	Area of glacial sand and gravel upstream of sampling site was measured using 'Drift and Quaternary editions of Geological Survey of N Ireland' maps.	2002
Area of Diamicton upstream (km^2)	Area of glacial boulder and clay upstream of sampling site was measured using 'Drift and Quaternary editions of Geological Survey of N Ireland' maps.	2002
Area of urban upstream (km^2)	Defined from 1:50,000 'OSNI Discoverer Series' maps. Urban area above sampling sites was calculated.	2002
Area of woodland upstream (km^2)	Defined from 1:50,000 'OSNI Discoverer Series' maps. Woodland area above sampling sites was calculated.	2002
Area of grassland upstream (km^2)	Defined from 1:50,000 'OSNI Discoverer Series' maps. Grassland area above sampling sites was calculated.	2002

Table 2.2: Environmental variables and their units from each of the four major categories used to test if landscape and environmental features influence population structure, with the year(s) the data was collected and the methodology used to collect the data. These data were collated from data collected by the Loughs Agency.

2.4 RESULTS

2.4.1 DATA QUALITY

Following the described sampling protocol, 1889 samples were collected from 28 sampling locations across the Foyle catchment. Good quality DNA with a high molecular weight was recovered from 1413 samples. Samples were deemed as being good quality if they amplified for more than 70% of the 20 microsatellite markers used.

2.4.2 POPULATION STRUCTURE

The first level of structuring defined by STRUCTURE, which included all individuals, indicated that there were five genetic clusters (Fig. 2.3.1; Fig. 2.4.1). Three genetic clusters comprised the Rivers Muff, Camowen and Owenreagh sub-catchments, while, one genetic cluster represented the Rivers Faughan, Roe, Killen Burn and Burndennet sub-catchments combined. The fifth genetic cluster represented three sampling sites in the River Burntollet which are located above an impassable waterfall.

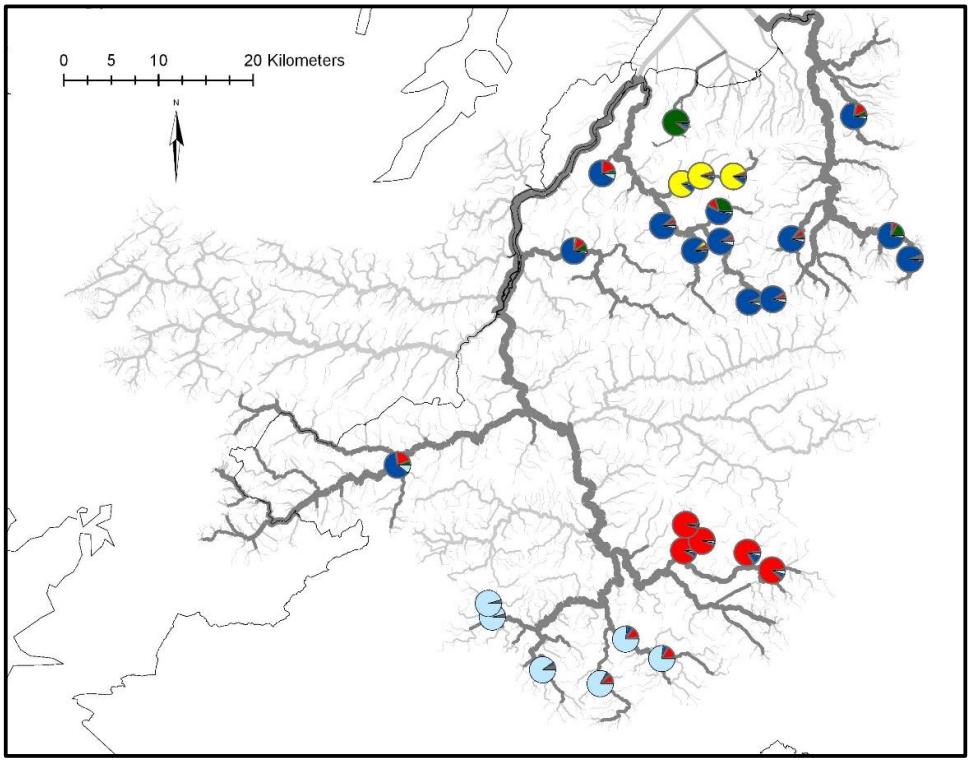


Fig. 2.3.1: Graphical representation of the first hierarchical level of population structure analysis based on 20 neutral microsatellite loci for brown trout collected from 28 sampling locations across the Foyle catchment. Each pie chart represents the proportion of individuals at each sampling location assigned to each genetic cluster.

The five identified genetic clusters were then each analysed separately in STRUCTURE revealing the second level of hierarchical (Fig. 2.3.2). The genetic cluster representing the River Muff separated into two further sympatric genetic clusters (Fig. 2.4.2). The genetic cluster representing the River Camowen sub-catchment separated into two further genetic clusters; Rivers Camowen and Drumnakilly (Fig. 2.4.3). No further structuring was identified within the River Burntollet, therefore, the three sampling locations formed a single genetic population (Fig. 2.4.8). The Owenreagh sub-catchment cluster subdivided into two genetic clusters; one cluster representing three sampling sites in the River Owenreagh and one cluster representing the Routing Burn and Quiggery Water (Fig. 2.4.9). The fifth genetic cluster from the first level of hierarchy separated into three genetic clusters; River Roe and two clusters representing a mixture of tributaries from both the River Roe, Faughan, Killen burn and Burndennet sub-catchments (Fig. 2.4.18). The first of which included the Burndennet, Killen Burn, Rivers Foreglen, Burngibbagh and Castle. The second cluster included three sampling sites in the River Faughan, Bonds Glen, Rivers Glenrandal and Owenbeg. Therefore, in the second hierarchical level nine genetic clusters and one genetic population were identified.

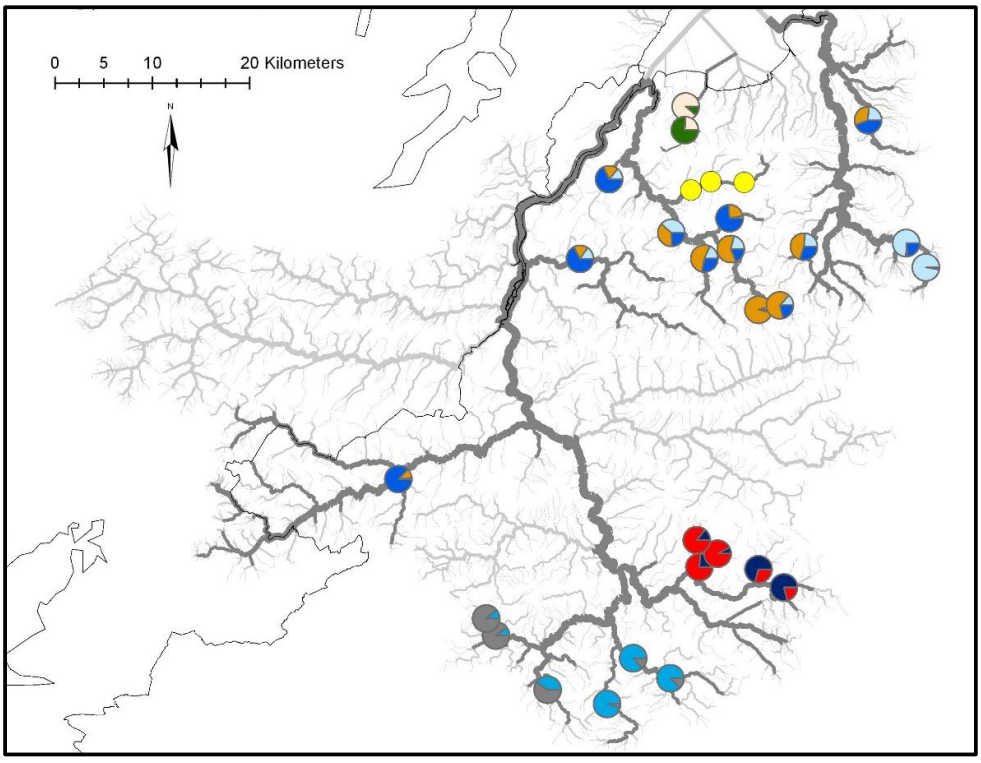


Fig. 2.3.2: Graphical representation of the second hierarchical level of population structure analysis where all five clusters from the 1st hierarchical level where analysed separately. Each pie chart represents the proportion of individuals at each sampling location assigned to each genetic cluster. Genetic populations, identified when no further sub-structuring is evident, are indicated on the map by solid colours.

The third level of hierarchical structuring (Fig. 2.3.3) revealed no further sub-structuring within the River Muff resulting in two sympatric genetic populations (Fig. 2.4.4, 2.4.5). No further sub-structuring was evident in the River Camowen sub-catchment resulting in two genetic populations, River Camowen (Fig. 2.4.6) and River Drumnakilly (Fig. 2.4.7). The Owenreagh sub-catchment formed two genetic clusters in the second hierarchical level which in the third level sub-divided further into four genetic clusters (Fig. 2.4.10 and 2.4.11). The River Roe formed a genetic population as no further sub-division was identified (Fig. 2.4.19). The genetic cluster previously identified containing sampling sites from the Killen Burn and River Foreglen amongst others subdivided into a further four genetic clusters, River Castle, Killen Burn, River Foreglen and Burndennet with River Burngibbagh (Fig. 2.4.20). The final genetic cluster, previously identified containing sampling sites in the river Faughan and River Owenbeg amongst others subdivided into two further genetic clusters, one representing samples from River Faughan A and two sampling locations in the River Faughan tributary, Bonds Glen and River Owenbeg (Fig. 2.4.21). Therefore, the third hierarchical level of structuring contained 10 genetic clusters and a total of six genetic populations (five populations identified in level two and one population identified in level one).

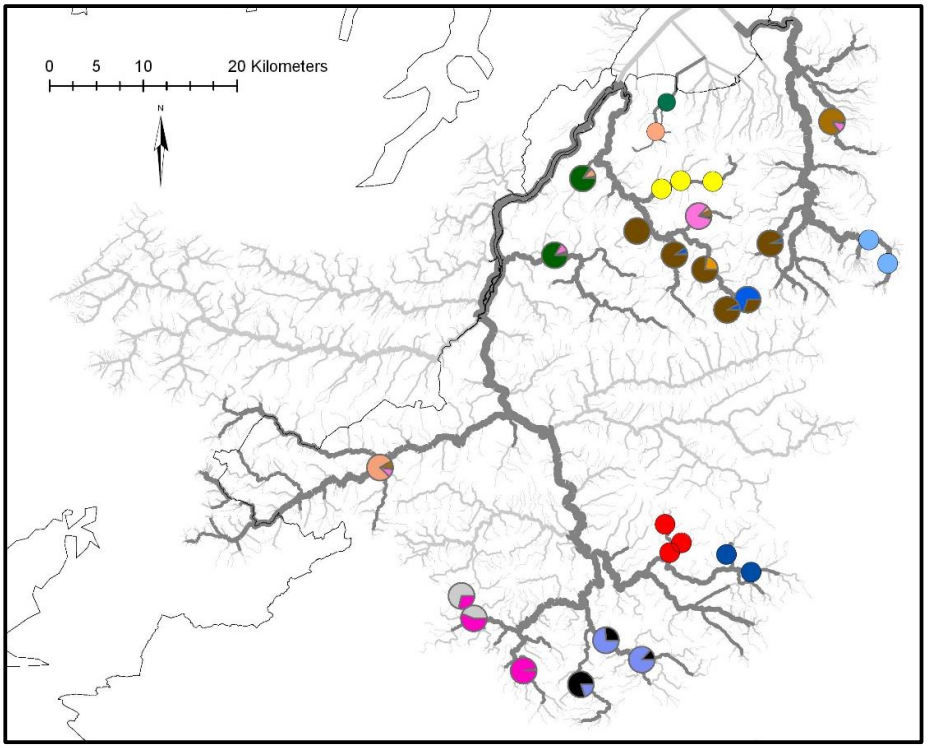


Fig. 2.3.3: Graphical representation of the third hierarchical level of population structure analysis where all clusters from the 2nd hierarchical level where analysed separately. Each pie chart represents the proportion of individuals at each sampling location assigned to each genetic cluster. Genetic populations, identified when no further sub-structuring is evident, are indicated on the map by solid colours.

The fourth level of hierarchical structuring (Fig. 2.3.4) showed further sub-structuring in the River Owenreagh sub-catchment. Two genetic clusters were identified as the River Owenreagh and River Owenreagh B (Fig. 2.4.15). All other previously identified clusters, the Routing Burn, Quiggery Water, River Owenreagh A each formed a genetic population (Fig. 2.4.12-2.4.14). Previously identified clusters representing Rivers Castle and Foreglen showed no further sub-structuring resulting in two more genetic populations (Fig. 2.4.22, 2.4.23). The genetic cluster identified in the Killen Burn sub-divided into two further sympatric genetic clusters (Fig. 2.4.26, 2.4.27). The previously identified, Burndennet/River Burngibbagh cluster formed two genetic clusters (Fig. 2.4.25). Finally, analysis of the previous cluster containing samples from Bonds Glen and River Owenbeg amongst others formed two genetic clusters. One cluster contained individuals from Rivers Faughan and Faughan B (Fig. 2.4.30), whilst the second cluster contained samples from Bonds Glen, Rivers Glenrandal and Owenbeg (Fig. 2.4.31). Therefore, the fourth hierarchical level of structuring was represented by eight genetic clusters and a total of 12 genetic populations.

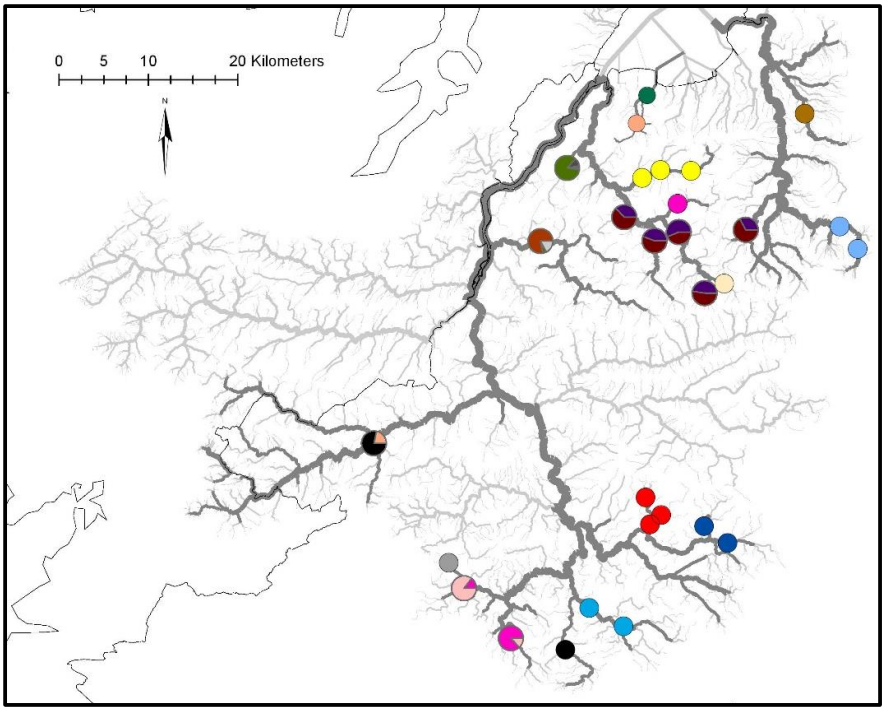


Fig. 2.3.4: Graphical representation of the fourth hierarchical level of population structure analysis where all clusters from the 3rd hierarchical level where analysed separately. Each pie chart represents the proportion of individuals at each sampling location assigned to each genetic cluster. Genetic populations, identified when no further sub-structuring is evident, are indicated on the map by solid colours

The fifth level of hierarchical structuring (Fig. 2.3.5) revealed further sub-structuring in the Bonds Glen/River Glenrandal and River Owenbeg cluster previously identified, with two further clusters (Fig. 2.4.33). Previously identified clusters in the River Owenreagh B, Owenreagh, Burngibbagh and Burndennet each formed a genetic population. The two previous clusters identified in the Killen Burn revealed no further structuring and represented two sympatric populations in the Killen Burn. Finally, the River Faughan and Faughan B showed no further structuring and represented a single genetic population. Therefore, the fifth level of hierarchical structuring was represented by two genetic clusters and a total of 19 populations.

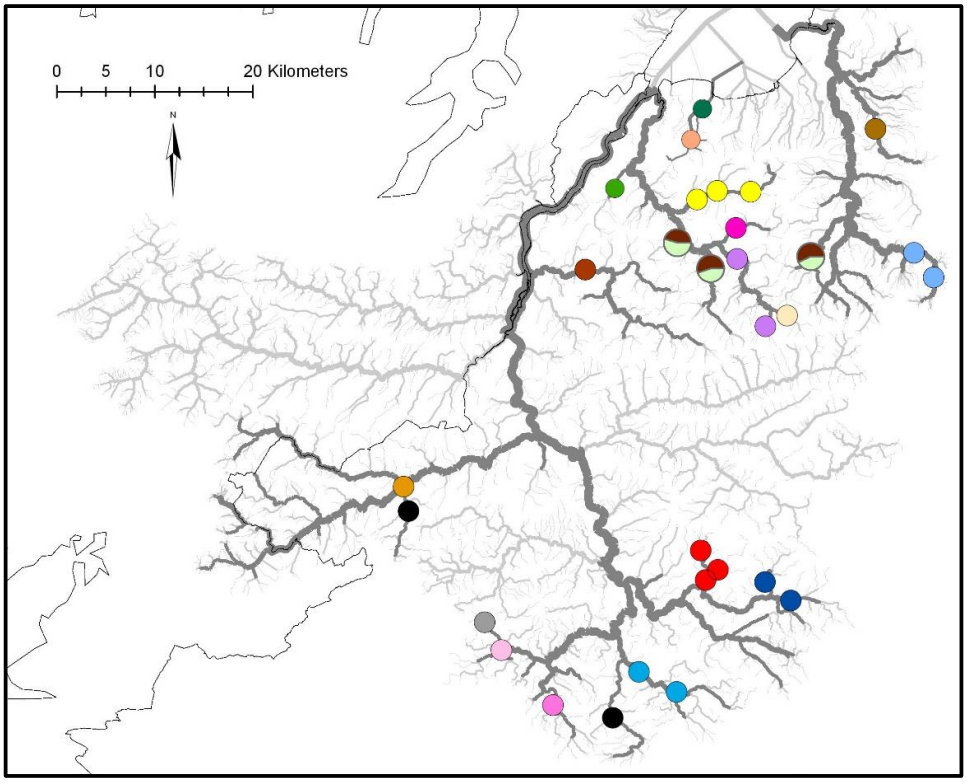


Fig. 2.3.5: Graphical representation of the fifth hierarchical level of population structure analysis where all clusters from the 4th hierarchical level where analysed separately. Each pie chart represents the proportion of individuals at each sampling location assigned to each genetic cluster. Genetic populations, identified when no further sub-structuring is evident, are indicated on the map by solid colours

The sixth level of hierarchical structuring (Fig. 2.3.6) revealed no further sub-structuring in the River Owenbeg (Fig. 2.4.35) or the River Glenrandal and Bonds Glen (Fig. 2.4.34) clusters. Therefore, 21 genetic populations were identified from a total of six hierarchical levels of structuring (Fig. 2.3.6).

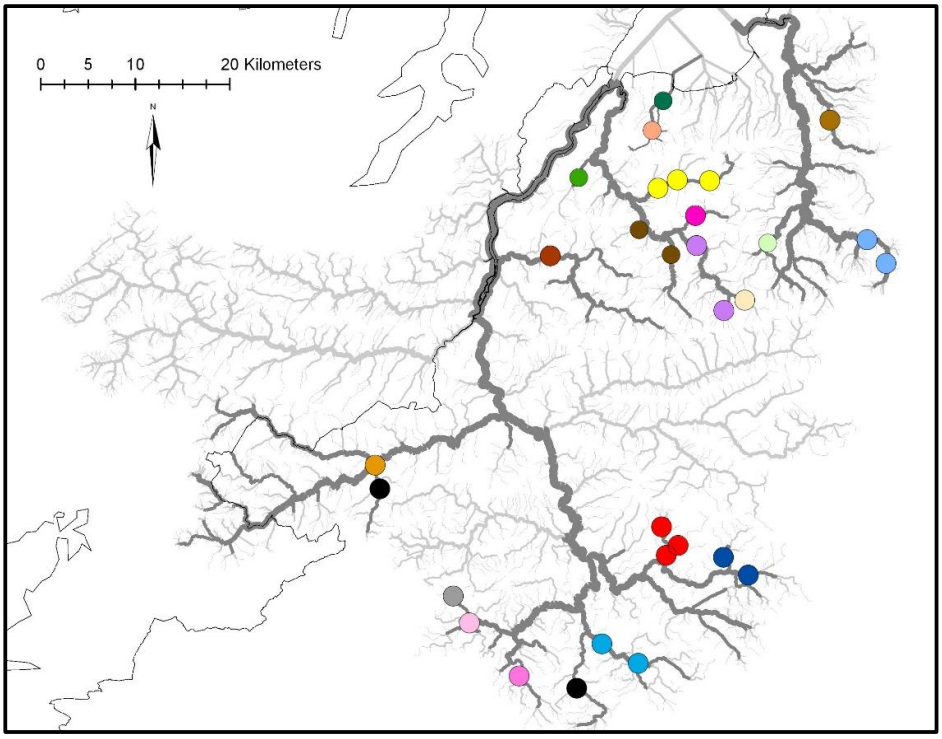


Fig. 2.3.6: Graphical representation of the sixth hierarchical level of population structure analysis where all clusters from the 5th hierarchical level where analysed separately. This is the final hierarchical level of analysis representing 21 genetic populations. Genetic populations, identified when no further sub-structuring is evident, are indicated on the map by solid colours

Overall, genetic differentiation between these 21 genetic populations ranged from 0.011 to 0.324 based on D_{JOST} and 0.008 to 0.124 based on F_{ST}, with a global differentiation of 0.138 D_{JOST}, 0.057 F_{ST} (Table 2.3). Most populations were significantly differentiated from one another (Table 2.3). The only exceptions were the pairwise comparison between Bonds Glen (BGL) and Burndennet (DEN) and Bonds Glen (BGL) and Faughan (FAU), as well as, between Burndennet (DEN) and Burngibbagh (GIB). The river Owenreagh (REA) was also not significantly differentiated from populations Owenreagh A (REB) or Owenreagh B (REC).

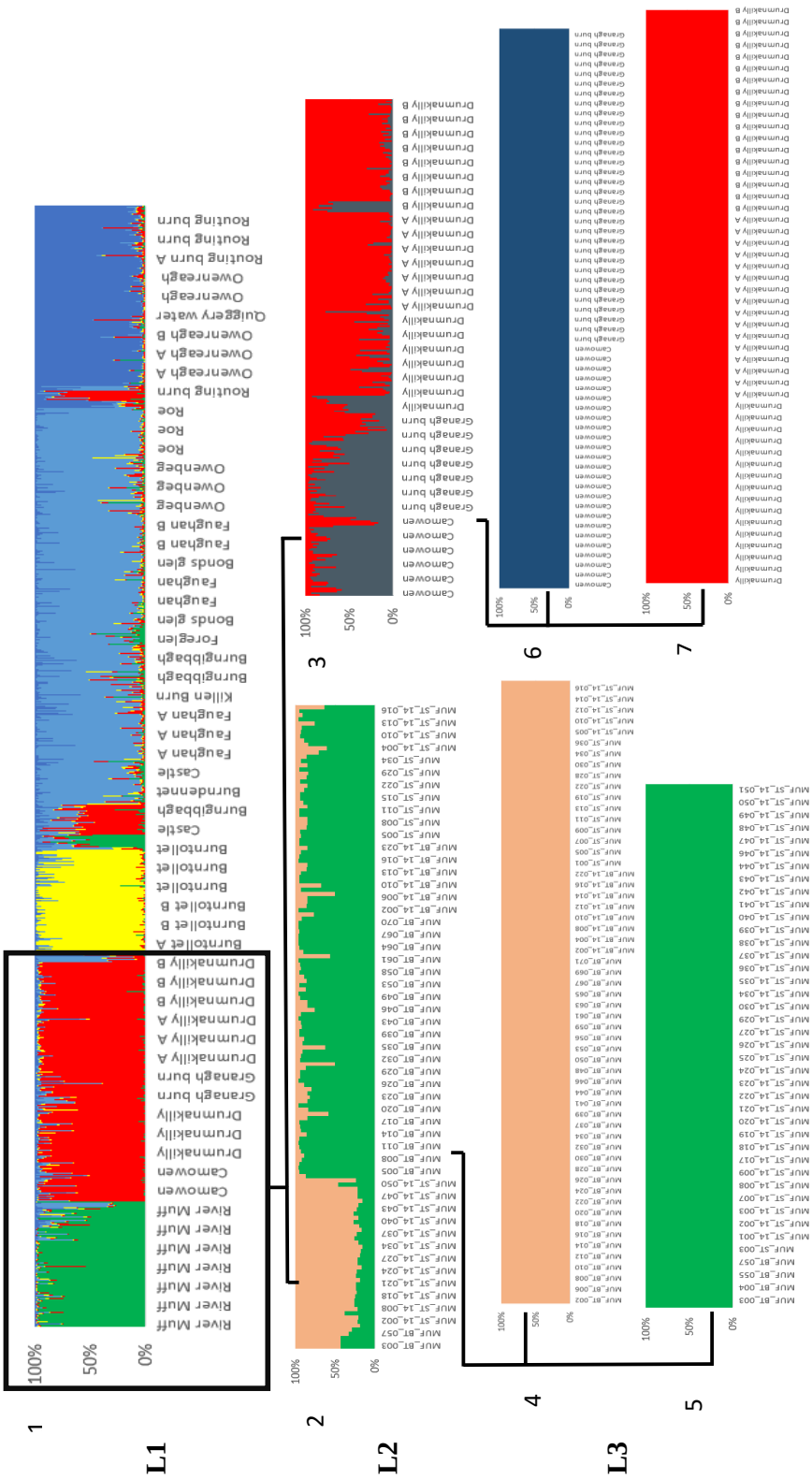


Fig. 2.4: Structure plots of hierarchical population structure of brown trout in the Foyle catchment. There are six hierarchical levels of structuring which are represented by Fig. 2.4.1-2.4.35. Solid colour structure plots indicate no further structuring and represent a population. Next to each row of bar charts are L1-L6 which represents the hierarchical level of analysis.

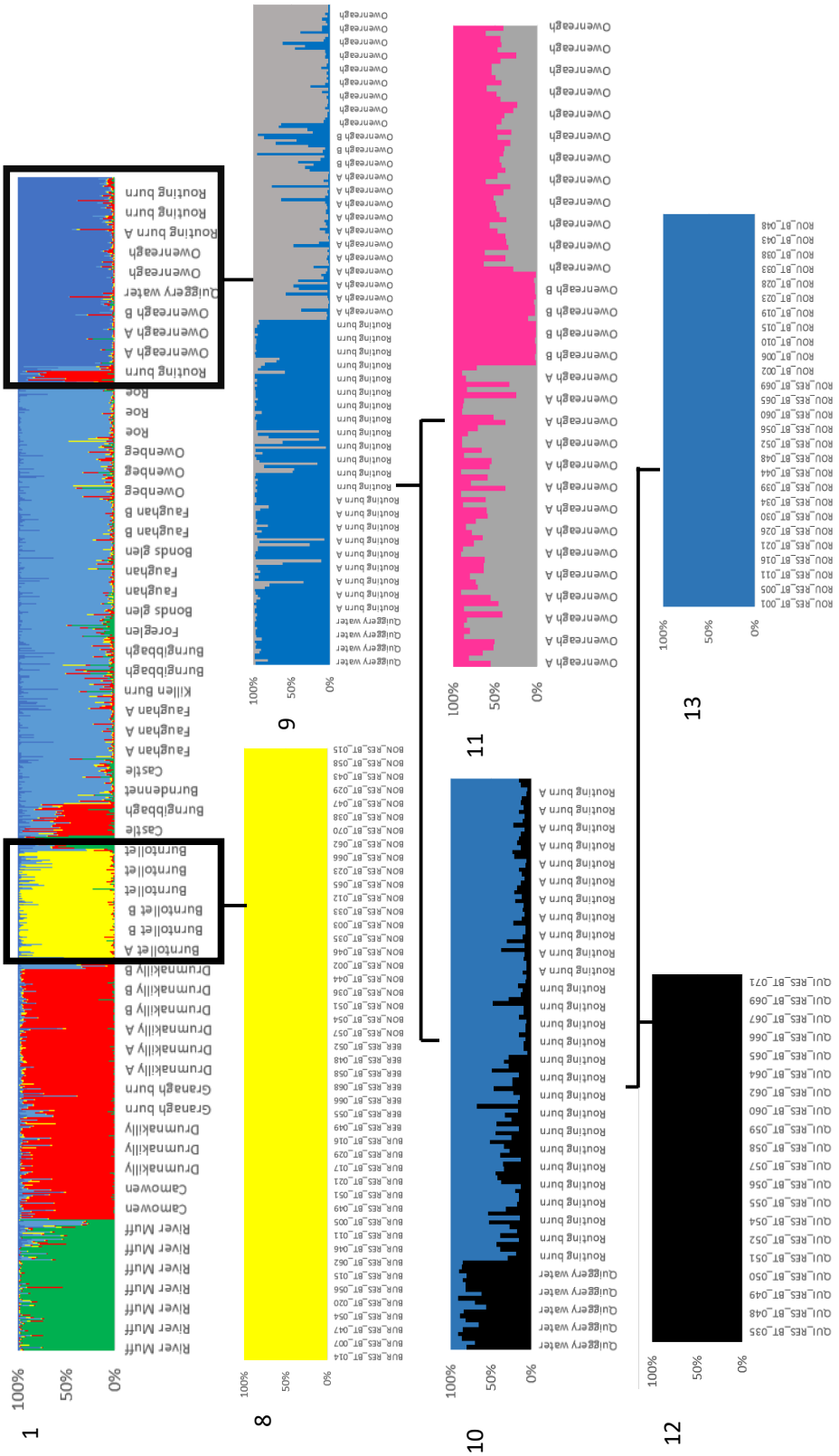
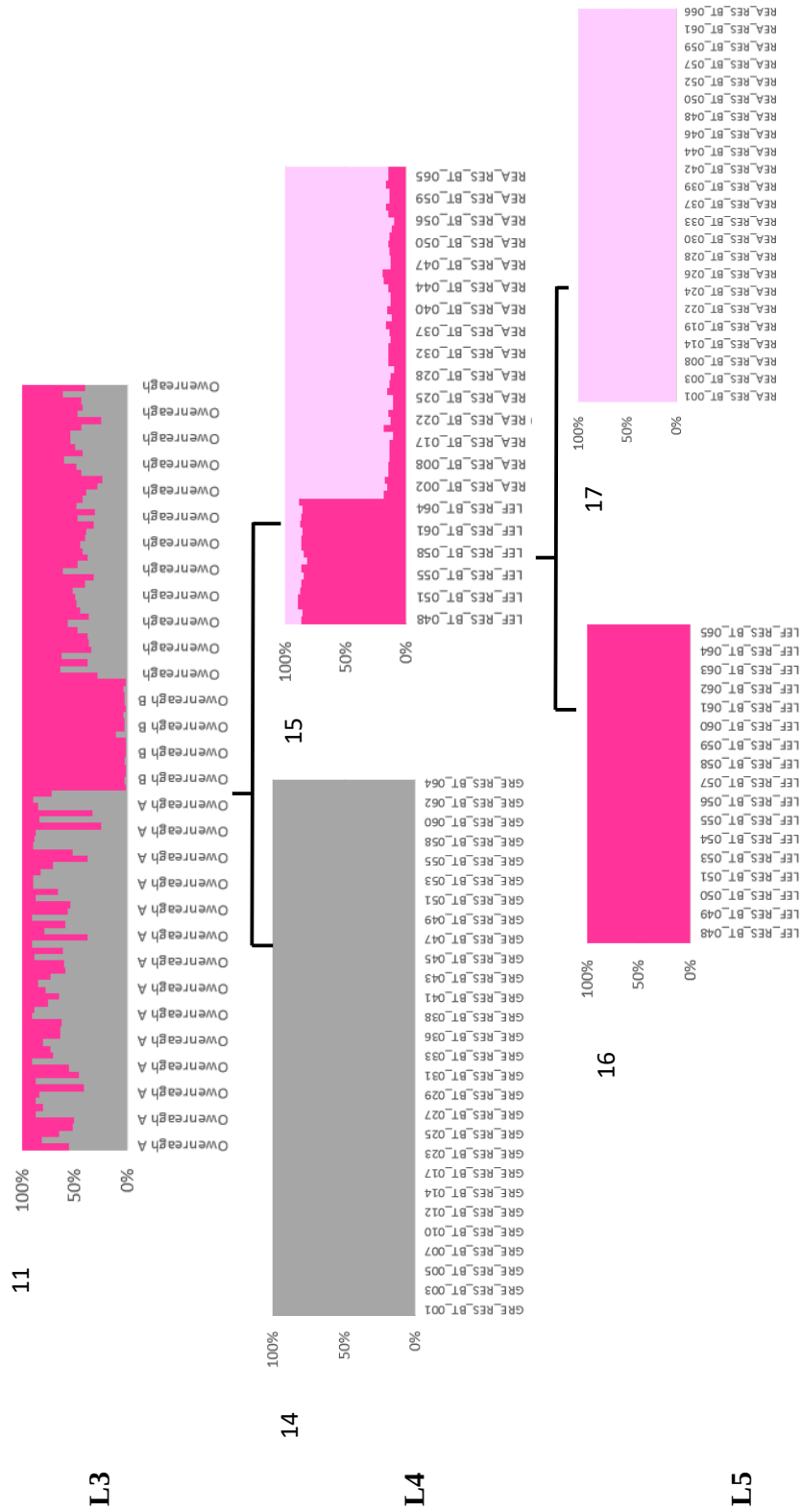


Fig. 2.4 continued



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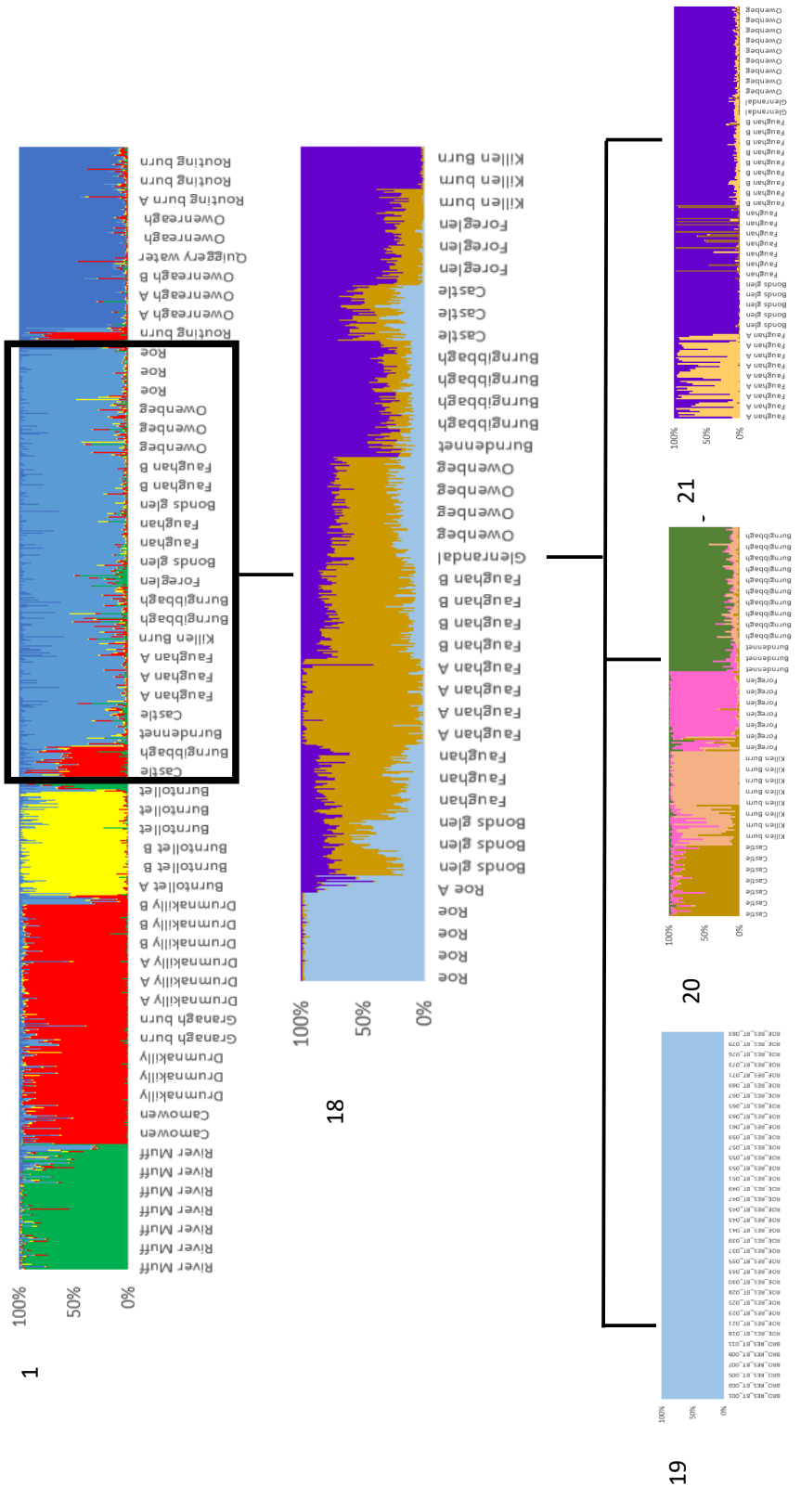

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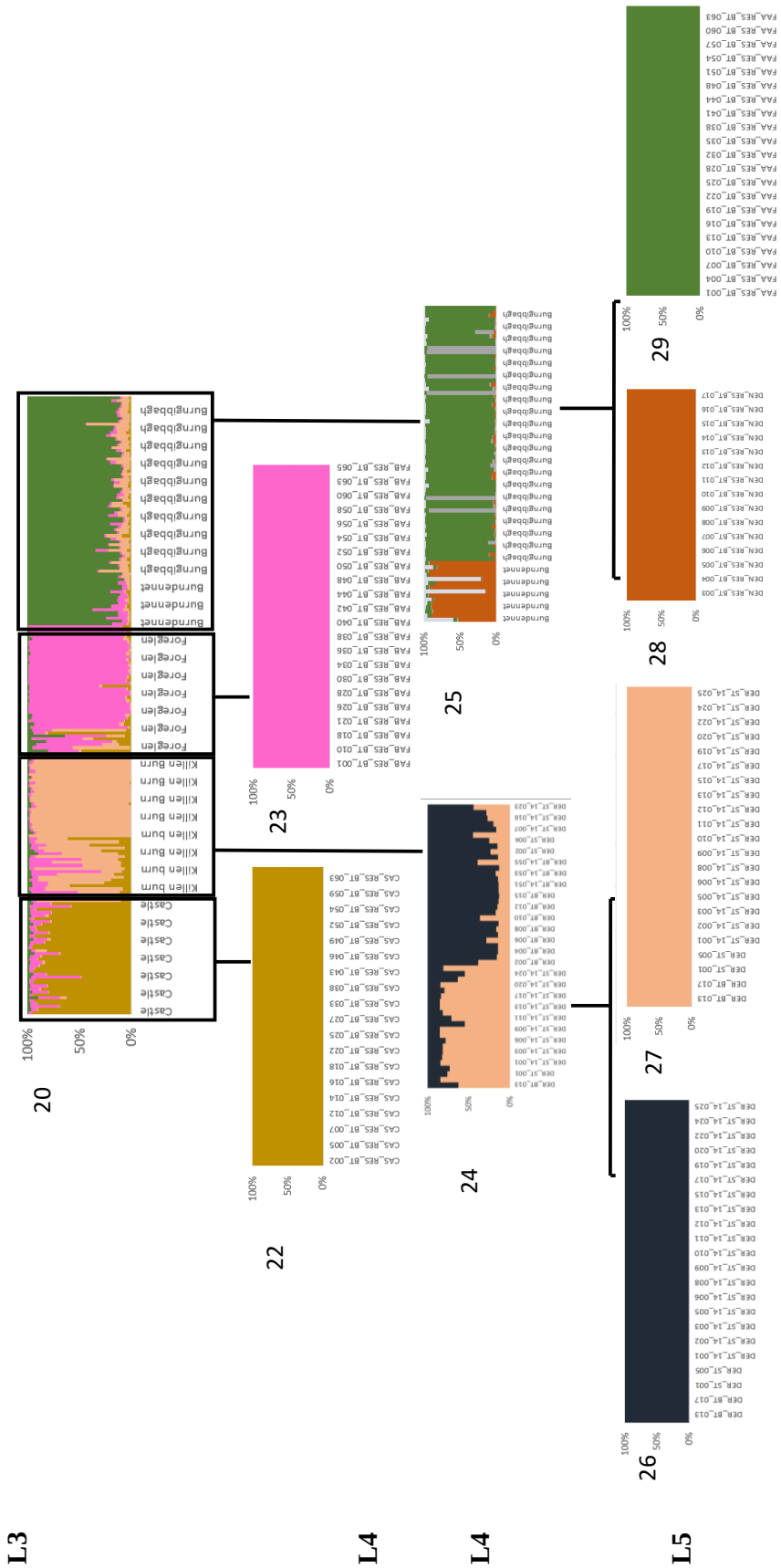

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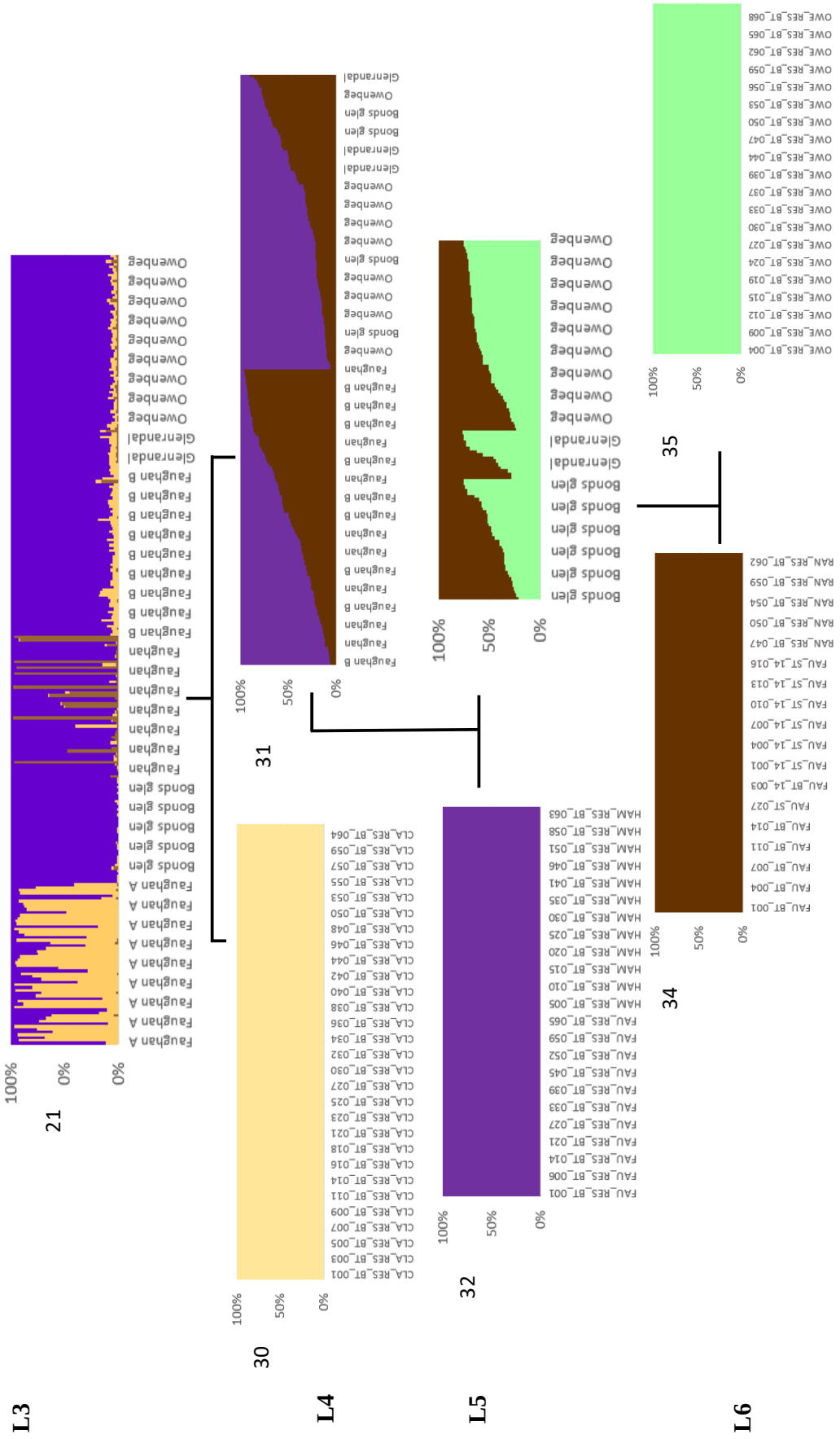

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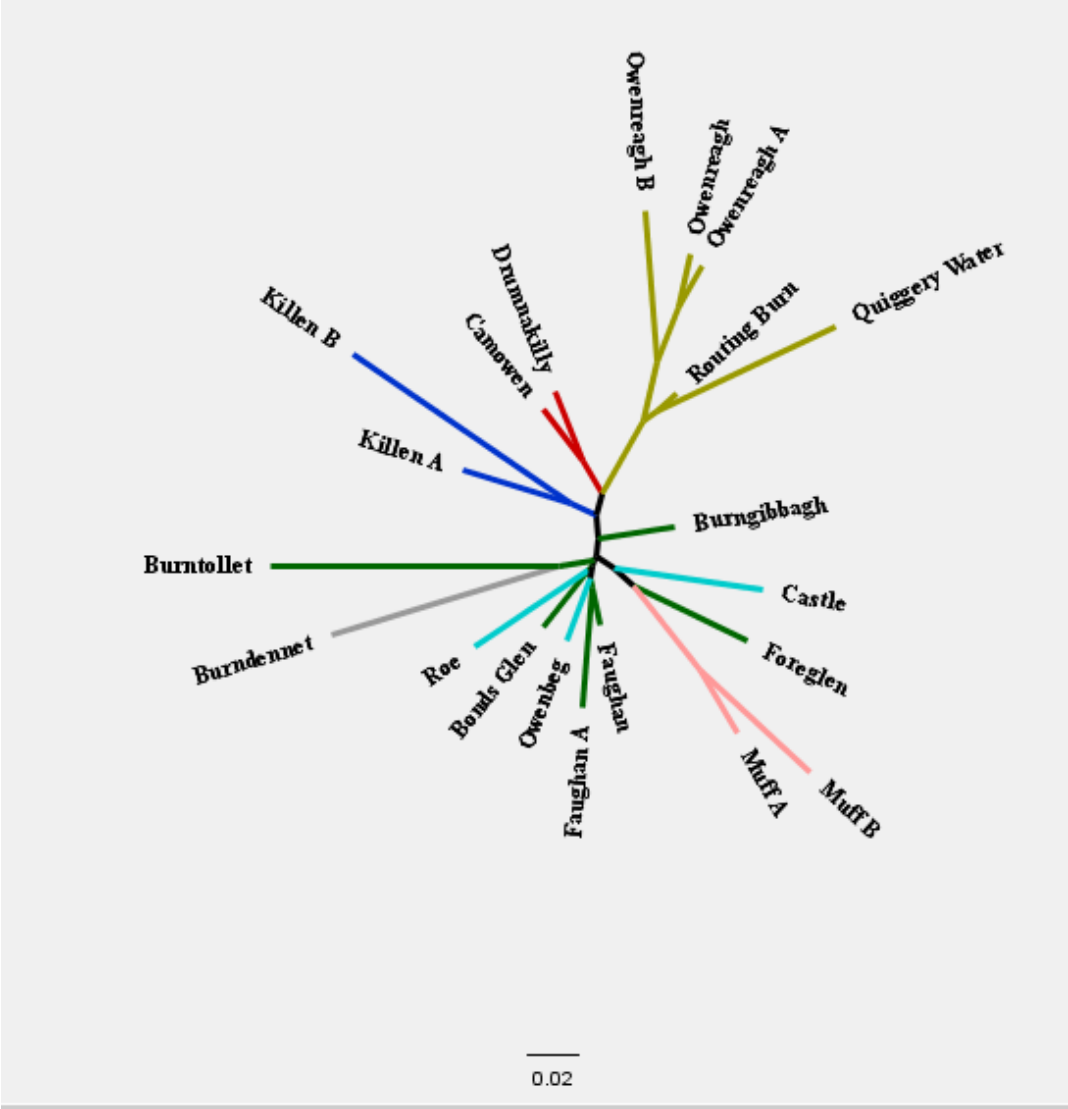

Fig. 2.5: Population tree illustrating genetic distance between all populations identified in STRUCTURE. Tree branches are coloured by sub-catchment; Red-Camowen, Green-Faughan, Yellow- Owenreagh, Grey- Burndennet, Blue- Killen Burn, Pink- River Muff and light blue- River Roe.

	GIB	BUR	BGL	FOR	FAU	FAA	CAS	OWE	ROE	CAM	DRU	REC	REA	REB	ROU	QUI	KILA	KILB	MUFA	MUFB	DEN
GIB		0.108	0.010	0.035	0.012	0.023	0.022	0.012	0.026	0.021	0.029	0.049	0.060	0.058	0.028	0.039	0.017	0.030	0.030	0.064	0.019
BUR	0.210		0.093	0.158	0.105	0.124	0.128	0.099	0.110	0.110	0.122	0.150	0.157	0.158	0.132	0.152	0.128	0.143	0.142	0.184	0.115
BGL	0.019	0.178		0.029	0.008	0.014	0.021	0.010	0.014	0.021	0.027	0.052	0.066	0.063	0.031	0.039	0.020	0.038	0.029	0.059	0.017
FOR	0.069	0.256	0.059		0.026	0.033	0.032	0.024	0.045	0.047	0.043	0.071	0.086	0.084	0.044	0.050	0.021	0.049	0.055	0.033	0.040
FAU	0.032	0.206	**0.011**	0.043		0.012	0.022	0.009	0.021	0.025	0.035	0.042	0.059	0.056	0.030	0.038	0.016	0.036	0.035	0.061	0.022
FAA	0.052	0.232	0.027	0.069	0.026		0.026	0.015	0.029	0.030	0.039	0.059	0.070	0.063	0.036	0.043	0.024	0.046	0.045	0.074	0.039
CAS	0.055	0.228	0.052	0.069	0.051	0.054		0.016	0.027	0.034	0.032	0.063	0.065	0.059	0.031	0.042	0.025	0.052	0.033	0.053	0.043
OWE	0.025	0.177	0.016	0.052	0.019	0.030	0.037		0.020	0.025	0.027	0.048	0.060	0.058	0.029	0.037	0.018	0.034	0.034	0.057	0.022
ROE	0.051	0.197	0.038	0.089	0.049	0.064	0.060	0.045		0.032	0.038	0.053	0.069	0.063	0.042	0.049	0.036	0.049	0.049	0.080	0.044
CAM	0.047	0.217	0.052	0.099	0.061	0.062	0.081	0.060	0.079		0.015	0.044	0.055	0.055	0.020	0.035	0.023	0.048	0.053	0.090	0.042
DRU	0.066	0.251	0.065	0.090	0.087	0.093	0.078	0.071	0.090	0.029		0.049	0.058	0.055	0.022	0.033	0.029	0.053	0.055	0.078	0.047
REC	0.101	0.280	0.094	0.123	0.070	0.125	0.133	0.094	0.090	0.065	0.086		0.018	0.023	0.026	0.038	0.031	0.071	0.085	0.115	0.071
REA	0.130	0.305	0.149	0.193	0.129	0.156	0.143	0.136	0.153	0.124	0.133	**0.026**		0.007	0.037	0.053	0.048	0.078	0.091	0.124	0.084
REB	0.127	0.324	0.146	0.184	0.123	0.127	0.118	0.135	0.137	0.130	0.133	0.044	**0.011**		0.035	0.052	0.048	0.083	0.087	0.120	0.087
ROU	0.067	0.282	0.075	0.089	0.072	0.081	0.072	0.074	0.110	0.046	0.053	0.038	0.063	0.072		0.012	0.022	0.054	0.053	0.079	0.053
QUI	0.079	0.287	0.089	0.098	0.081	0.089	0.088	0.082	0.114	0.073	0.067	0.056	0.093	0.104	0.023		0.029	0.064	0.073	0.092	0.056
KILA	0.043	0.238	0.050	0.051	0.047	0.057	0.060	0.037	0.088	0.050	0.061	0.057	0.093	0.104	0.048	0.063		0.031	0.048	0.068	0.032
KILB	0.056	0.274	0.092	0.088	0.087	0.119	0.115	0.081	0.112	0.120	0.109	0.145	0.162	0.180	0.112	0.107	0.060		0.056	0.083	0.058
MUFA	0.044	0.244	0.048	0.104	0.063	0.090	0.052	0.065	0.094	0.097	0.104	0.162	0.166	0.160	0.102	0.131	0.084	0.086		0.037	0.053
MUFB	0.135	0.311	0.120	0.044	0.129	0.163	0.105	0.117	0.171	0.204	0.166	0.248	0.270	0.265	0.173	0.185	0.140	0.141	0.036		0.075
DEN	0.023	0.192	**0.025**	0.067	0.037	**0.079**	0.068	0.038	0.068	0.100	0.100	0.136	0.190	0.200	0.124	0.120	0.081	0.104	0.080	0.130	

Table 2.3: Heat map indicates the size of the genetic differentiation between populations with large genetic differences highlighted in red and small differences in green. BUR the most genetically differentiated population has not been included in the heatmap to emphasise the similarity between populations within the same sub-catchment. Values above are pairwise F_{ST} distances; values below are pairwise D_{JOST} distances. Note non-significant D_{JOST} values are highlighted in bold. See table 2.1 for population names abbreviations.

2.4.3 POPULATION SUMMARY STATISTICS

The mean number of samples amplified per microsatellite loci ranged from 14.6-152.4 (Table 2.4). The total number of alleles per population ranged from 137-229 and allelic richness ranged from 4.93-7.52. Twenty seven out of 520 tests (comparing 20 loci over 26 sampling locations) were significant for deviations from Hardy-Weinberg Equilibrium after Bonferroni correction ($p<0.0025$). No signs of severe inbreeding were detected based on Wright's inbreeding coefficient (F_{IS}). Linkage disequilibrium tests of the 20 microsatellite markers used showed no pair of loci were consistently linked and there was no evidence of selection for any of the microsatellite markers used.

Pop	River(s)	Sub-catchment	Marker	N	A	%	Ar	HWE	Fis
DEN	DEN	Burndennet	BG935488	14	5	45.45	4.62	0.65	-0.15
			CA048828	12	14	33.33	10.76	**0.00**	0.08
			CA053293	13	7	58.33	5.65	0.17	0.34
			CA054565	15	2	28.57	1.97	1.00	-0.11
			CA060177	15	9	56.25	7.16	**0.00**	0.08
			Cocl-Lav-4	15	5	41.67	4.92	0.08	-0.08
			MHCI	14	7	43.75	5.77	1.00	0.07
			One102-a	15	2	100.00	2.00	1.00	0.00
			One102-b	15	9	42.86	8.49	1.00	-0.14
			One103	15	3	50.00	3.00	0.19	0.25
			One108	15	15	37.50	11.64	**0.00**	0.11
			One9uASC	15	8	66.67	7.19	0.83	-0.17
			ppStr2	15	14	26.92	11.31	0.01	-0.05
			ppStr3	14	3	37.50	2.88	0.58	-0.32
			SaSaTAP2A	15	5	50.00	4.85	0.93	-0.18
			Ssa197	15	3	30.00	2.96	0.72	0.11
			Ssa410UOS	15	17	53.12	12.73	**0.00**	-0.02
			Ssa416	15	3	75.00	2.90	1.00	-0.18
			Ssa85	15	3	37.50	2.95	1.00	-0.11
			SsaD71	15	6	35.29	5.61	0.68	0.07
			Overall	14.6	140	47.49	5.97	**0.00**	-0.01
Pop	**River(s)**	**Sub-catchment**	**Marker**	**N**	**A**	**%**	**Ar**	**HWE**	**Fis**
CAM	CAM, GRA	Camowen	BG935488	112	6	54.55	5.45	0.89	0.05
			CA048828	114	22	52.38	12.91	0.68	0.02
			CA053293	114	9	75.00	7.76	0.36	0.07
			CA054565	114	3	42.86	1.77	1.00	-0.02
			CA060177	108	11	68.75	7.65	0.74	0.00
			Cocl-Lav-4	106	7	58.33	5.77	0.47	0.06
			MHCI	113	11	68.75	7.13	0.39	0.03
			One102-a	114	2	100.00	2.00	1.00	-0.01
			One102-b	113	15	71.43	9.06	0.03	-0.01
			One103	112	5	83.33	3.62	0.63	0.09
			One108	90	26	65.00	12.78	0.20	0.03
			One9uASC	112	8	66.67	5.85	1.00	-0.04
			ppStr2	113	25	48.08	12.40	0.99	0.04
			ppStr3	113	4	50.00	3.11	0.01	-0.09
			SaSaTAP2A	113	9	90.00	6.20	0.99	0.00
			Ssa197	113	7	70.00	6.24	0.53	0.05
			Ssa410UOS	113	24	75.00	13.53	1.00	-0.03
			Ssa416	106	4	100.00	2.73	**0.00**	0.38
			Ssa85	114	4	50.00	3.92	0.92	0.05
			SsaD71	113	9	52.94	6.55	0.84	-0.04
			Overall	111	211	67.15	6.82	0.29	0.02

Pop	River(s)	Sub-catchment	Marker	N	A	%	Ar	HWE	Fis
DRU	DRU, DRA, DRB	Camowen	BG935488	195	8	72.73	5.42	0.87	0.10
			CA048828	195	28	66.67	13.72	0.82	-0.03
			CA053293	194	11	91.67	7.24	0.02	0.25
			CA054565	196	3	42.86	2.29	0.53	-0.09
			CA060177	147	12	75.00	6.46	**0.00**	0.03
			Cocl-Lav-4	189	7	58.33	5.17	0.40	-0.02
			MHCI	191	11	68.75	6.94	0.20	0.07
			One102-a	196	2	100.00	2.00	0.34	-0.08
			One102-b	196	17	80.95	11.06	0.12	0.00
			One103	196	5	83.33	4.33	0.76	-0.07
			One108	144	28	70.00	12.64	0.64	0.02
			One9uASC	195	8	66.67	5.79	0.56	0.00
			ppStr2	194	28	53.85	13.82	0.17	0.02
			ppStr3	194	5	62.50	3.51	0.98	-0.01
			SaSaTAP2A	195	9	90.00	6.23	0.71	0.03
			Ssa197	195	9	90.00	6.56	0.64	-0.10
			Ssa410UOS	195	24	75.00	11.70	**0.00**	-0.01
			Ssa416	189	3	75.00	1.68	1.00	-0.02
			Ssa85	195	5	62.50	4.16	0.18	-0.08
			SsaD71	194	9	52.94	6.76	0.22	0.05
			Overall	189.25	232	71.94	6.87	0.02	0.01
Pop	**River(s)**	**Sub-catchment**	**Marker**	**N**	**A**	**%**	**Ar**	**HWE**	**Fis**
BGL	BGL, GLE	Faughan	BG935488	53	8	72.73	6.47	0.01	0.27
			CA048828	53	23	54.76	13.87	1.00	-0.03
			CA053293	53	10	83.33	8.04	0.65	0.11
			CA054565	53	2	28.57	1.25	N/A	1.00
			CA060177	53	11	68.75	8.37	1.00	-0.01
			Cocl-Lav-4	52	7	58.33	5.52	0.92	0.08
			MHCI	53	13	81.25	8.86	0.24	0.31
			One102-a	53	2	100.00	2.00	0.76	0.07
			One102-b	53	16	76.19	11.06	0.19	-0.01
			One103	52	4	66.67	3.94	0.99	0.01
			One108	50	26	65.00	13.40	0.16	0.06
			One9uASC	50	9	75.00	7.38	1.00	-0.11
			ppStr2	53	28	53.85	15.37	1.00	-0.07
			ppStr3	53	4	50.00	3.73	0.99	0.08
			SaSaTAP2A	53	9	90.00	7.29	0.37	0.01
			Ssa197	53	6	60.00	4.83	0.99	0.06
			Ssa410UOS	53	26	81.25	15.29	0.76	0.00
			Ssa416	50	4	100.00	2.71	0.54	0.12
			Ssa85	52	5	62.50	4.49	0.38	0.06
			SsaD71	53	11	64.71	8.01	1.00	-0.06

			Overall	52.4	224	69.64	7.59	0.96	0.05
Pop	**River(s)**	**Sub-catchment**	**Marker**	**N**	**A**	**%**	**Ar**	**HWE**	**Fis**
GIB	GIB	Faughan	BG935488	59	8	72.73	5.81	0.20	0.12
			CA048828	62	24	57.14	13.90	1.00	0.00
			CA053293	62	9	75.00	7.53	0.21	0.21
			CA054565	62	2	28.57	1.37	1.00	-0.02
			CA060177	62	12	75.00	9.12	0.99	0.06
			Cocl-Lav-4	60	6	50.00	4.96	0.44	-0.09
			MHCI	61	12	75.00	7.68	0.21	0.02
			One102-a	62	2	100.00	2.00	0.21	-0.17
			One102-b	62	15	71.43	10.21	1.00	0.00
			One103	62	5	83.33	4.33	1.00	-0.09
			One108	62	29	72.50	15.37	1.00	0.05
			One9uASC	62	8	66.67	6.06	1.00	0.00
			ppStr2	62	30	57.69	13.99	**0.00**	-0.03
			ppStr3	62	4	50.00	3.72	0.45	-0.10
			SaSaTAP2A	62	8	80.00	7.11	0.77	0.01
			Ssa197	62	7	70.00	5.51	1.00	0.05
			Ssa410UOS	62	25	78.12	14.83	0.90	-0.05
			Ssa416	62	4	100.00	3.24	0.02	0.04
			Ssa85	62	5	62.50	4.57	0.96	-0.10
			SsaD71	62	13	76.47	8.38	0.03	-0.02
			Overall	61.7	228	70.11	7.48	0.21	0.00
Pop	**River(s)**	**Sub-catchment**	**Marker**	**N**	**A**	**%**	**Ar**	**HWE**	**Fis**
BUR	BUR, BUA, BUB	Faughan	BG935488	134	6	54.55	3.60	0.60	0.07
			CA048828	135	23	54.76	8.81	0.25	0.00
			CA053293	135	8	66.67	6.91	**0.00**	0.30
			CA054565	136	2	28.57	1.10	1.00	0.00
			CA060177	85	7	43.75	4.98	0.80	0.00
			Cocl-Lav-4	131	8	66.67	4.79	0.96	-0.02
			MHCI	126	13	81.25	5.40	**0.00**	0.28
			One102-a	136	2	100.00	2.00	0.71	0.03
			One102-b	135	15	71.43	8.23	0.07	0.08
			One103	133	6	100.00	4.24	**0.00**	-0.03
			One108	133	23	57.50	12.80	0.13	0.02
			One9uASC	135	8	66.67	5.34	0.99	0.07
			ppStr2	135	19	36.54	8.10	0.04	0.08
			ppStr3	136	4	50.00	2.90	0.30	0.04
			SaSaTAP2A	136	7	70.00	4.84	0.43	0.10
			Ssa197	135	5	50.00	4.11	0.47	-0.09
			Ssa410UOS	133	25	78.12	11.77	0.88	0.00
			Ssa416	136	3	75.00	2.16	0.27	0.17
			Ssa85	133	5	62.50	3.21	0.04	0.08

			SsaD71	135	10	58.82	5.38	**0.00**	0.08
			Overall	131.65	199	63.64	5.53	**0.00**	0.06
Pop	**River(s)**	**Sub-catchment**	**Marker**	**N**	**A**	**%**	**Ar**	**HWE**	**Fis**
FAU	FAU, FAB	Faughan	BG935488	112	10	90.91	6.14	0.01	0.26
			CA048828	112	30	71.43	15.86	0.82	-0.01
			CA053293	113	8	66.67	7.38	0.11	0.17
			CA054565	113	3	42.86	1.46	1.00	-0.01
			CA060177	108	11	68.75	6.88	0.79	-0.06
			Cocl-Lav-4	111	8	66.67	5.75	0.79	0.07
			MHCI	109	11	68.75	8.48	**0.00**	0.27
			One102-a	113	2	100.00	2.00	0.57	-0.05
			One102-b	112	17	80.95	9.93	0.84	0.01
			One103	113	5	83.33	4.31	0.45	-0.02
			One108	111	33	82.50	15.26	0.20	0.04
			One9uASC	113	9	75.00	7.23	1.00	0.02
			ppStr2	113	33	63.46	16.22	0.12	0.03
			ppStr3	113	4	50.00	3.75	0.85	-0.03
			SaSaTAP2A	113	9	90.00	6.53	0.43	0.00
			Ssa197	112	8	80.00	4.81	0.02	-0.01
			Ssa410UOS	113	29	90.62	15.28	1.00	-0.02
			Ssa416	113	4	100.00	3.11	0.87	0.02
			Ssa85	113	5	62.50	4.59	0.50	0.09
			SsaD71	113	10	58.82	7.48	1.00	-0.01
			Overall	112.15	249	74.66	7.62	0.08	0.04
Pop	**River(s)**	**Sub-catchment**	**Marker**	**N**	**A**	**%**	**Ar**	**HWE**	**Fis**
FAA	FAA	Faughan	BG935488	58	8	72.73	6.36	0.83	0.01
			CA048828	58	23	54.76	14.04	0.94	-0.09
			CA053293	58	8	66.67	7.65	1.00	0.01
			CA054565	58	3	42.86	1.48	1.00	-0.01
			CA060177	53	11	68.75	7.72	0.12	0.01
			Cocl-Lav-4	58	7	58.33	5.83	0.63	0.05
			MHCI	58	11	68.75	7.90	0.88	0.07
			One102-a	58	2	100.00	2.00	0.35	0.14
			One102-b	58	15	71.43	9.74	0.36	0.09
			One103	58	5	83.33	4.14	0.83	-0.03
			One108	57	20	50.00	11.90	1.00	0.01
			One9uASC	58	9	75.00	6.74	0.85	-0.03
			ppStr2	58	21	40.38	13.07	0.20	0.02
			ppStr3	58	4	50.00	3.19	0.21	0.10
			SaSaTAP2A	58	8	80.00	6.23	0.91	-0.03
			Ssa197	58	6	60.00	4.68	0.49	-0.06
			Ssa410UOS	58	20	62.50	13.07	1.00	0.03
			Ssa416	58	3	75.00	2.98	0.94	-0.08
			Ssa85	58	6	75.00	4.64	0.14	-0.07

			SsaD71	58	10	58.82	6.36	0.38	0.10
			Overall	57.7	200	65.72	6.99	0.97	0.01
Pop	**River(s)**	**Sub-catchment**	**Marker**	**N**	**A**	**%**	**Ar**	**HWE**	**Fis**
FOR	FOR	Faughan	BG935488	43	7	63.64	6.17	0.91	0.03
			CA048828	43	20	47.62	12.56	0.07	-0.02
			CA053293	43	7	58.33	6.62	0.55	0.21
			CA054565	43	2	28.57	1.29	1.00	-0.01
			CA060177	39	9	56.25	5.68	0.86	0.01
			Cocl-Lav-4	39	8	66.67	6.14	0.84	0.03
			MHCI	43	9	56.25	7.09	0.39	0.09
			One102-a	43	2	100.00	2.00	0.14	0.26
			One102-b	43	15	71.43	11.16	0.64	-0.01
			One103	43	5	83.33	4.46	0.83	-0.12
			One108	37	21	52.50	11.99	**0.00**	0.03
			One9uASC	43	8	66.67	5.90	0.38	-0.04
			ppStr2	40	24	46.15	13.14	0.00	0.04
			ppStr3	43	4	50.00	3.17	0.11	0.28
			SaSaTAP2A	41	6	60.00	4.23	0.03	-0.03
			Ssa197	43	4	40.00	3.44	0.82	-0.10
			Ssa410UOS	39	22	68.75	13.35	**0.00**	-0.05
			Ssa416	43	3	75.00	2.85	0.11	0.24
			Ssa85	43	5	62.50	4.16	0.72	-0.05
			SsaD71	42	6	35.29	5.21	0.67	0.04
			Overall	41.8	187	59.45	6.53	**0.00**	0.03
Pop	**River(s)**	**Sub-catchment**	**Marker**	**N**	**A**	**%**	**Ar**	**HWE**	**Fis**
KILA	KIL	Killen Burn	BG935488	29	8	72.73	5.17	**0.00**	0.56
			CA048828	29	23	54.76	14.66	**0.00**	-0.04
			CA053293	29	9	75.00	7.78	1.00	0.07
			CA054565	29	2	28.57	1.89	1.00	-0.07
			CA060177	29	10	62.50	7.11	0.30	0.05
			Cocl-Lav-4	29	7	58.33	5.41	0.90	-0.08
			MHCI	29	9	56.25	7.34	0.96	0.07
			One102-a	29	2	100.00	2.00	0.70	0.10
			One102-b	29	14	66.67	10.81	0.99	0.00
			One103	29	5	83.33	4.58	0.99	0.14
			One108	29	25	62.50	15.71	**0.00**	0.05
			One9uASC	29	8	66.67	7.05	0.58	0.02
			ppStr2	29	20	38.46	13.33	0.15	0.03
			ppStr3	29	4	50.00	3.92	0.37	-0.25
			SaSaTAP2A	29	7	70.00	5.00	0.11	0.12
			Ssa197	29	6	60.00	4.84	0.83	-0.12
			Ssa410UOS	29	22	68.75	14.29	**0.00**	0.07
			Ssa416	29	3	75.00	2.94	0.56	0.13
			Ssa85	29	4	50.00	3.99	0.81	0.11
			SsaD71	29	9	52.94	6.97	0.24	-0.12

			Overall	29	197	62.62	7.24	**0.00**	0.04
Pop	**River(s)**	**Sub-catchment**	**Marker**	**N**	**A**	**%**	**Ar**	**HWE**	**Fis**
KILB	KIL	Killen Burn	BG935488	22	5	45.45	4.26	0.60	0.14
			CA048828	22	16	38.10	11.95	**0.00**	-0.05
			CA053293	22	8	66.67	6.54	0.96	-0.08
			CA054565	22	2	28.57	1.50	1.00	-0.02
			CA060177	22	8	50.00	6.29	0.08	0.08
			Cocl-Lav-4	22	5	41.67	4.95	1.00	-0.01
			MHCI	22	7	43.75	6.14	0.40	0.21
			One102-a	22	2	100.00	2.00	0.22	-0.32
			One102-b	22	9	42.86	7.60	0.62	-0.09
			One103	22	4	66.67	3.89	0.93	0.00
			One108	22	17	42.50	12.72	0.05	-0.09
			One9uASC	22	6	50.00	5.67	0.67	0.08
			ppStr2	22	17	32.69	10.58	**0.00**	-0.03
			ppStr3	22	5	62.50	4.77	0.70	-0.15
			SaSaTAP2A	22	5	50.00	4.27	0.22	0.21
			Ssa197	22	5	50.00	4.79	0.35	0.20
			Ssa410UOS	22	12	37.50	9.33	0.94	-0.11
			Ssa416	22	3	75.00	2.99	0.02	0.44
			Ssa85	22	5	62.50	4.18	0.81	0.12
			SsaD71	22	9	52.94	7.79	0.14	0.14
			Overall	22	150	51.97	6.11	0.01	0.03
Pop	**River(s)**	**Sub-catchment**	**Marker**	**N**	**A**	**%**	**Ar**	**HWE**	**Fis**
REA	REA	Owenreagh	BG935488	43	8	72.73	6.27	0.96	0.13
			CA048828	45	20	47.62	13.93	1.00	-0.07
			CA053293	45	9	75.00	6.88	0.01	0.29
			CA054565	45	3	42.86	2.63	1.00	-0.09
			CA060177	40	9	56.25	6.57	1.00	-0.01
			Cocl-Lav-4	43	5	41.67	4.12	0.97	-0.06
			MHCI	45	7	43.75	5.75	0.17	0.17
			One102-a	45	2	100.00	2.00	0.71	0.09
			One102-b	45	11	52.38	8.31	0.96	0.04
			One103	45	4	66.67	3.85	0.02	0.17
			One108	42	18	45.00	9.94	**0.00**	0.01
			One9uASC	45	6	50.00	5.70	0.93	0.05
			ppStr2	45	13	25.00	9.52	0.33	0.14
			ppStr3	45	4	50.00	3.81	0.99	0.07
			SaSaTAP2A	44	6	60.00	5.61	0.94	0.04
			Ssa197	44	7	70.00	4.29	0.26	0.22
			Ssa410UOS	43	17	53.12	11.63	0.78	-0.07
			Ssa416	45	3	75.00	2.11	1.00	-0.06
			Ssa85	45	4	50.00	3.61	0.78	-0.20
			SsaD71	44	8	47.06	7.00	1.00	-0.01

			Overall	44.15	164	56.21	6.18	0.40	0.05
Pop	**River(s)**	**Sub-catchment**	**Marker**	**N**	**A**	**%**	**Ar**	**HWE**	**Fis**
REB	REB	Owenreagh	BG935488	55	8	72.73	5.61	**0.00**	-0.03
			CA048828	55	17	40.48	12.07	0.13	-0.01
			CA053293	55	8	66.67	6.84	0.41	0.15
			CA054565	55	3	42.86	2.74	0.45	0.15
			CA060177	55	10	62.50	7.18	0.32	-0.03
			Cocl-Lav-4	54	5	41.67	4.33	0.80	-0.13
			MHCI	55	9	56.25	6.19	0.04	-0.02
			One102-a	55	2	100.00	2.00	1.00	0.00
			One102-b	54	13	61.90	8.96	0.20	0.09
			One103	54	4	66.67	3.80	**0.00**	0.07
			One108	52	22	55.00	11.71	0.19	0.12
			One9uASC	54	6	50.00	5.47	0.96	-0.03
			ppStr2	55	15	28.85	10.58	0.99	-0.07
			ppStr3	55	4	50.00	3.68	0.28	0.00
			SaSaTAP2A	55	8	80.00	5.33	0.60	0.00
			Ssa197	55	5	50.00	4.26	0.86	-0.09
			Ssa410UOS	55	16	50.00	12.04	1.00	-0.10
			Ssa416	54	2	50.00	1.25	1.00	-0.01
			Ssa85	55	4	50.00	3.42	0.99	0.07
			SsaD71	55	8	47.06	6.84	0.71	-0.04
			Overall	54.6	169	56.13	6.21	0.05	0.00
Pop	**River(s)**	**Sub-catchment**	**Marker**	**N**	**A**	**%**	**Ar**	**HWE**	**Fis**
REC	REC	Owenreagh	BG935488	17	8	72.73	7.08	1.00	-0.05
			CA048828	17	13	30.95	10.87	1.00	-0.11
			CA053293	17	6	50.00	5.65	0.52	0.05
			CA054565	17	2	28.57	1.99	1.00	0.19
			CA060177	17	5	31.25	4.48	0.02	0.00
			Cocl-Lav-4	17	6	50.00	5.17	0.62	0.00
			MHCI	17	8	50.00	7.00	0.10	-0.06
			One102-a	17	2	100.00	2.00	1.00	-0.06
			One102-b	17	10	47.62	7.97	0.28	-0.16
			One103	17	4	66.67	3.98	1.00	-0.04
			One108	17	13	32.50	10.15	**0.00**	0.06
			One9uASC	17	7	58.33	6.34	0.79	-0.05
			ppStr2	17	10	19.23	8.77	1.00	0.09
			ppStr3	17	4	50.00	3.56	0.91	-0.02
			SaSaTAP2A	17	6	60.00	5.34	0.75	-0.22
			Ssa197	17	6	60.00	5.49	0.46	0.04
			Ssa410UOS	17	13	40.62	10.25	**0.00**	-0.14
			Ssa416	17	2	50.00	1.99	1.00	0.19
			Ssa85	17	4	50.00	3.80	0.27	0.23
			SsaD71	17	8	47.06	7.14	0.31	0.01

			Overall	17	137	49.78	5.95	0.09	-0.02
Pop	**River(s)**	**Sub-catchment**	**Marker**	**N**	**A**	**%**	**Ar**	**HWE**	**Fis**
QUI	QUI	Owenreagh	BG935488	20	6	54.55	5.61	0.48	0.16
			CA048828	20	10	23.81	8.16	0.54	-0.02
			CA053293	20	5	41.67	4.97	0.96	-0.04
			CA054565	20	2	28.57	1.92	1.00	-0.08
			CA060177	20	7	43.75	5.77	0.74	0.06
			Cocl-Lav-4	20	6	50.00	4.99	0.07	0.18
			MHCI	20	8	50.00	7.25	1.00	0.11
			One102-a	20	2	100.00	2.00	1.00	0.05
			One102-b	20	6	28.57	5.22	0.67	0.21
			One103	20	4	66.67	3.48	0.43	-0.18
			One108	20	13	32.50	10.30	0.91	-0.08
			One9uASC	20	6	50.00	5.93	1.00	0.02
			ppStr2	20	10	19.23	8.38	0.02	0.02
			ppStr3	20	3	37.50	2.56	1.00	-0.06
			SaSaTAP2A	20	8	80.00	6.26	0.34	0.00
			Ssa197	20	6	60.00	5.30	1.00	-0.02
			Ssa410UOS	20	14	43.75	9.99	0.01	-0.05
			Ssa416	20	1	25.00	1.00	N/A	N/A
			Ssa85	20	4	50.00	3.96	0.02	0.23
			SsaD71	20	7	41.18	5.83	0.01	0.19
			Overall	20	128	46.34	5.44	0.18	0.04
Pop	**River(s)**	**Sub-catchment**	**Marker**	**N**	**A**	**%**	**Ar**	**HWE**	**Fis**
ROU	ROU, RUA	Owenreagh	BG935488	107	8	72.73	7.20	1.00	0.02
			CA048828	108	19	45.24	12.45	0.85	-0.02
			CA053293	108	9	75.00	7.45	0.37	0.12
			CA054565	108	2	28.57	1.87	1.00	0.07
			CA060177	106	11	68.75	7.83	0.06	-0.01
			Cocl-Lav-4	107	6	50.00	5.40	0.60	0.08
			MHCI	107	13	81.25	8.67	0.84	-0.01
			One102-a	108	2	100.00	2.00	0.21	-0.11
			One102-b	108	13	61.90	9.43	0.74	0.00
			One103	108	5	83.33	4.46	0.66	0.04
			One108	107	23	57.50	13.09	1.00	0.08
			One9uASC	106	7	58.33	5.95	0.01	0.04
			ppStr2	108	23	44.23	11.68	**0.00**	-0.02
			ppStr3	107	5	62.50	3.24	0.34	0.17
			SaSaTAP2A	108	8	80.00	5.91	0.34	0.03
			Ssa197	107	6	60.00	5.47	1.00	-0.01
			Ssa410UOS	107	23	71.88	12.76	1.00	-0.01
			Ssa416	106	4	100.00	2.60	1.00	-0.06
			Ssa85	108	5	62.50	4.39	0.50	0.02
			SsaD71	107	10	58.82	7.05	1.00	-0.01

			Overall	107.3	202	66.13	6.95	0.31	0.02
Pop	**River(s)**	**Sub-catchment**	**Marker**	**N**	**A**	**%**	**Ar**	**HWE**	**Fis**
MUFA	MUF	River Muff	BG935488	41	7	63.64	5.52	0.85	0.01
			CA048828	41	14	33.33	9.22	**0.00**	-0.04
			CA053293	41	8	66.67	5.60	0.06	0.04
			CA054565	41	2	28.57	1.33	1.00	-0.01
			CA060177	34	10	62.50	7.57	0.69	0.03
			Cocl-Lav-4	41	5	41.67	3.79	0.33	-0.05
			MHCI	40	9	56.25	6.40	0.06	0.15
			One102-a	41	2	100.00	2.00	0.23	0.21
			One102-b	41	11	52.38	8.47	1.00	-0.17
			One103	41	5	83.33	4.61	1.00	-0.02
			One108	41	15	37.50	10.45	0.96	0.08
			One9uASC	41	6	50.00	4.53	0.07	-0.04
			ppStr2	41	11	21.15	7.88	**0.00**	-0.08
			ppStr3	40	4	50.00	2.59	0.53	-0.06
			SaSaTAP2A	40	7	70.00	6.01	0.97	-0.05
			Ssa197	41	5	50.00	3.99	0.01	-0.04
			Ssa410UOS	40	19	59.38	12.42	0.19	-0.05
			Ssa416	41	2	50.00	1.78	1.00	-0.05
			Ssa85	41	3	37.50	2.99	0.39	0.09
			SsaD71	40	12	70.59	8.62	0.40	0.01
			Overall	40.4	157	54.22	5.79	**0.00**	-0.01
Pop	**River(s)**	**Sub-catchment**	**Marker**	**N**	**A**	**%**	**Ar**	**HWE**	**Fis**
MUFB	MUF	River Muff	BG935488	112	6	54.55	5.38	0.96	0.12
			CA048828	112	17	40.48	10.31	0.97	0.05
			CA053293	114	8	66.67	5.07	**0.00**	0.10
			CA054565	114	1	14.29	1.00	N/A	N/A
			CA060177	104	6	37.50	4.52	1.00	-0.08
			Cocl-Lav-4	106	7	58.33	3.96	0.33	0.00
			MHCI	113	9	56.25	7.59	0.94	0.09
			One102-a	114	2	100.00	2.00	0.70	0.05
			One102-b	114	13	61.90	9.07	0.98	-0.01
			One103	114	5	83.33	4.25	1.00	0.01
			One108	106	17	42.50	10.71	0.87	-0.02
			One9uASC	114	6	50.00	5.20	0.67	0.06
			ppStr2	109	11	21.15	7.53	0.38	0.06
			ppStr3	114	4	50.00	3.25	0.83	0.06
			SaSaTAP2A	113	7	70.00	5.13	0.53	0.05
			Ssa197	114	5	50.00	3.15	0.13	0.01
			Ssa410UOS	113	21	65.62	12.53	**0.00**	0.05
			Ssa416	113	2	50.00	1.35	1.00	-0.01
			Ssa85	114	3	37.50	2.96	0.87	0.02
			SsaD71	113	8	47.06	5.55	0.96	0.00

			Overall	112	158	52.86	5.53	0.52	0.04
Pop	**River(s)**	**Sub-catchment**	**Marker**	**N**	**A**	**%**	**Ar**	**HWE**	**Fis**
CAS	CAS	Roe	BG935488	33	8	72.73	6.03	0.78	0.06
			CA048828	38	21	50.00	14.19	1.00	-0.02
			CA053293	38	8	66.67	7.10	0.82	0.00
			CA054565	38	3	42.86	2.09	1.00	-0.04
			CA060177	35	10	62.50	8.12	1.00	-0.01
			Cocl-Lav-4	30	6	50.00	4.63	0.78	-0.10
			MHCI	36	9	56.25	7.59	0.94	0.20
			One102-a	38	2	100.00	2.00	1.00	0.05
			One102-b	36	13	61.90	9.92	0.86	-0.07
			One103	36	5	83.33	4.24	0.87	0.10
			One108	36	23	57.50	13.73	0.00	-0.02
			One9uASC	35	6	50.00	5.59	1.00	-0.10
			ppStr2	38	17	32.69	11.04	**0.00**	-0.04
			ppStr3	38	5	62.50	3.34	1.00	0.00
			SaSaTAP2A	38	7	70.00	5.08	0.72	-0.16
			Ssa197	38	6	60.00	4.92	0.82	-0.03
			Ssa410UOS	38	21	65.62	13.69	0.15	-0.05
			Ssa416	38	3	75.00	2.34	0.01	0.37
			Ssa85	38	5	62.50	3.64	0.44	0.10
			SsaD71	37	9	52.94	7.00	0.53	0.05
			Overall	36.6	187	61.75	6.81	0.15	0.00
Pop	**River(s)**	**Sub-catchment**	**Marker**	**N**	**A**	**%**	**Ar**	**HWE**	**Fis**
OWE	OWE	Roe	BG935488	58	9	81.82	5.66	0.60	0.13
			CA048828	60	23	54.76	14.03	1.00	0.01
			CA053293	60	9	75.00	7.24	1.00	0.09
			CA054565	60	4	57.14	2.29	0.03	0.21
			CA060177	59	14	87.50	8.40	0.41	0.14
			Cocl-Lav-4	60	7	58.33	5.42	0.91	-0.05
			MHCI	59	12	75.00	8.28	0.74	0.04
			One102-a	59	2	100.00	2.00	0.15	0.17
			One102-b	60	15	71.43	10.96	0.86	-0.04
			One103	58	6	100.00	5.16	0.58	-0.13
			One108	60	25	62.50	14.63	0.63	0.00
			One9uASC	59	8	66.67	6.07	0.91	0.01
			ppStr2	60	29	55.77	14.80	0.05	0.01
			ppStr3	60	5	62.50	2.84	0.11	0.18
			SaSaTAP2A	60	8	80.00	6.99	0.93	0.01
			Ssa197	60	7	70.00	4.79	0.78	-0.07
			Ssa410UOS	60	24	75.00	15.51	1.00	-0.03
			Ssa416	59	4	100.00	2.93	1.00	0.08
			Ssa85	60	5	62.50	4.17	0.42	-0.05
			SsaD71	59	11	64.71	8.01	0.82	0.05

			Overall	59.5	227	73.03	7.51	0.86	0.02
Pop	**River(s)**	**Sub-catchment**	**Marker**	**N**	**A**	**%**	**Ar**	**HWE**	**Fis**
ROE	ROE, ROA	Roe	BG935488	68	7	63.64	5.16	0.02	0.15
			CA048828	71	20	47.62	12.53	1.00	0.01
			CA053293	71	10	83.33	8.59	1.00	0.09
			CA054565	71	1	14.29	1.00	N/A	N/A
			CA060177	70	9	56.25	7.91	1.00	-0.01
			Cocl-Lav-4	71	8	66.67	5.51	0.02	0.06
			MHCI	69	11	68.75	7.77	0.16	0.14
			One102-a	71	2	100.00	1.98	0.16	0.19
			One102-b	71	14	66.67	9.87	0.86	-0.01
			One103	71	5	83.33	4.49	0.05	0.18
			One108	59	21	52.50	11.78	0.94	0.09
			One9uASC	71	8	66.67	7.07	0.95	0.03
			ppStr2	71	21	40.38	12.16	0.98	-0.04
			ppStr3	71	5	62.50	3.42	0.78	0.05
			SaSaTAP2A	71	9	90.00	6.38	0.02	0.03
			Ssa197	71	6	60.00	3.89	0.92	-0.04
			Ssa410UOS	71	23	71.88	12.78	**0.00**	0.05
			Ssa416	71	3	75.00	2.77	0.01	0.31
			Ssa85	71	5	62.50	4.76	1.00	-0.08
			SsaD71	70	12	70.59	8.60	0.09	0.01
			Overall	70.05	200	65.13	6.92	0.00	0.05

Table 2.4: Summary statistics for individuals genotyped for each population (Pop) (see table 2.1 for abbreviations) which includes; N- Number of individuals genotyped for each locus for each population, A- Number of alleles per locus, %- Percentage of total observed alleles per locus, Ar- Allelic richness per locus, HWE- Hardy Weinberg Equilibrium (significant HWE are highlighted in bold) and F_{IS}- Wright's Inbreeding Coefficient.

2.4.4 EFFECTIVE POPULATION SIZE, RELATEDNESS AND SEX RATIO OF POPULATIONS

The effective population size for each population identified from the Foyle catchment was calculated using a linkage disequilibrium method with a lowest allele frequency of 0.001 in LDNe (Waples & Do 2008). N_e of each population ranged from 59.2 (River Faughan A) to 510 (Killen Burn A) (Table 2.5). In comparison N_e of each population ranged from 46 (River Castle) to 224 (River Drumnakilly) when calculated using the linkage disequilibrium method in COLONY (Jones & Wang, 2010).

The relatedness of individuals within populations was established to ensure individuals were samples from multiple families. Using R package 'related' (Pew et al. 2015;

RCoreTeam. 2015) it was found that most individuals were unrelated based on a Wang's coefficient less than 0.1 (Fig. 2.6). The percentage of unrelated individuals per population ranged from 76.3% for CAS to 100% for populations Routing Burn, Quiggery water, Owenreagh, Owenreagh A, Muff A and Killen Burn B (Table 2.6). This was investigated further by establishing the number of full-sibling families within each population using Colony (Jones & Wang 2010). This further demonstrated that most of the individuals were unrelated with the number of full-sibling families (≥ two individuals) ranging from no full-sibling families for populations Routing Burn, Quiggery water, Owenreagh, Owenreagh A, Muff A and Killen Burn B to nine full-sibling families for populations Drumnakilly and Faughan A (Table 2.6). Most full- sibling families were composed of between two and three individuals with only nine families within the entire dataset containing more than three individuals (Hansen et al 1997).

The sex ratio of each population was calculated and a binomial test was used to determine if there was a significant difference between the number of males and females within each population. The overall sex ratio of the Foyle catchment was one female for every 1.2 males (binomial test; $p<0.001$). This sex ratio is driven by certain populations which had a sex ratio which is significantly different from an expected sex ratio of one male for every one female (Table 2.7). The populations which had a sex ratio where there were significantly more males than would be expected were: River Burntollet, River Drumnakilly and Burndennet.

Population	Sub-catchment	N_e (JackKnife) from NeEstimator	N_e (95% confidence interval) from Colony
DEN	Burndennet	76.2(39.6-478.6)	52(26-200)
CAM	Camowen	193.0(154.2-252.9)	105 (48-140)
DRU	Camowen	300.3(224.5-435.4)	224 (180-277)
BGL	Faughan	397.2(249.8-1008.7)	138 (94-228)
GIB	Faughan	225.6(165.8-343.6)	110 (77-159)
BUR	Faughan	106.4 (91.1-126.0)	140 (109-181)
FAU	Faughan	217(182.3-267.6)	129 (98-175)
FAA	Faughan	59.2(519-68.4)	48 (32-76)
FOR	Faughan	101.7(73.8-156.6)	56 (57-142)
KILA	Killen Burn	510.8(210.5-Infinite)	148 (83-404)
KILB	Killen Burn	459.3 (147.2-Infinite)	66 (37-155)
REA	Owenreagh	Infinite(384.6-Infinite)	132 (89-222)
REB	Owenreagh	282.9(168.1-784.4)	94 (65-140)
REC	Owenreagh	463.1(110.1-Infinite)	136 (60-$2.14x10^9$)
QUI	Owenreagh	452.8(119.6-Infinite)	69 (39-168)
ROU	Owenreagh	456.7(319.2-774.8)	178 (136-233)
MUFA	Muff	140.8(94.1-262.1)	76 (50-119)
MUFB	Muff	126.4(101.5-163.3)	109 (80-145)
CAS	Roe	68.4 (57.4-83.8)	46 (29-77)
OWE	Roe	374.8 (257.7-666.8)	120 (86-174)
ROE	Roe	214 (147.2-371.7)	138 (100-193)

Table 2.5: Effective population size of the study populations determined from 20 neutral microsatellite loci. N_e is the effective population size calculated with program NeEstimator using Linkage Disequilibrium method with a lowest allele frequency of 0.001 and Colony using sibship frequency method (Jones & Wang 2010; Wang 2016).

Population	Sub-catchment	Sample size (N)	Number of full-sibling families (≥2 individuals)	Number of independent individuals	Average relatedness of individuals using Wang's coefficient
DEN	Burndennet	15	1	13	0.017
CAM	Camowen	114	4	98	0.040
DRU	Camowen	197	9	165	0.049
BGL	Faughan	53	1	51	0.001
GIB	Faughan	63	3	56	0.022
BUR	Faughan	136	5	126	0.173
FAU	Faughan	113	8	87	0.003
FAA	Faughan	58	9	35	0.033
FOR	Faughan	43	1	451	0.039
KILA	Killen Burn	29	1	27	-0.020
KILB	Killen Burn	22	0	22	0.071
REA	Owenreagh	45	0	45	0.060
REB	Owenreagh	55	3	49	0.076
REC	Owenreagh	17	0	17	0.069
QUI	Owenreagh	20	0	20	0.092
ROU	Owenreagh	108	0	108	0.038
MUFA	Muff	52	0	52	0.138
MUFB	Muff	103	7	83	0.131
CAS	Roe	38	3	29	0.034
OWE	Roe	60	4	51	0.007
ROE	Roe	71	3	67	0.033

Table 2.6: Number of full-sibling families within each population calculated using COLONY and the average of Wang's coefficient for each population calculated using the R package 'related'.

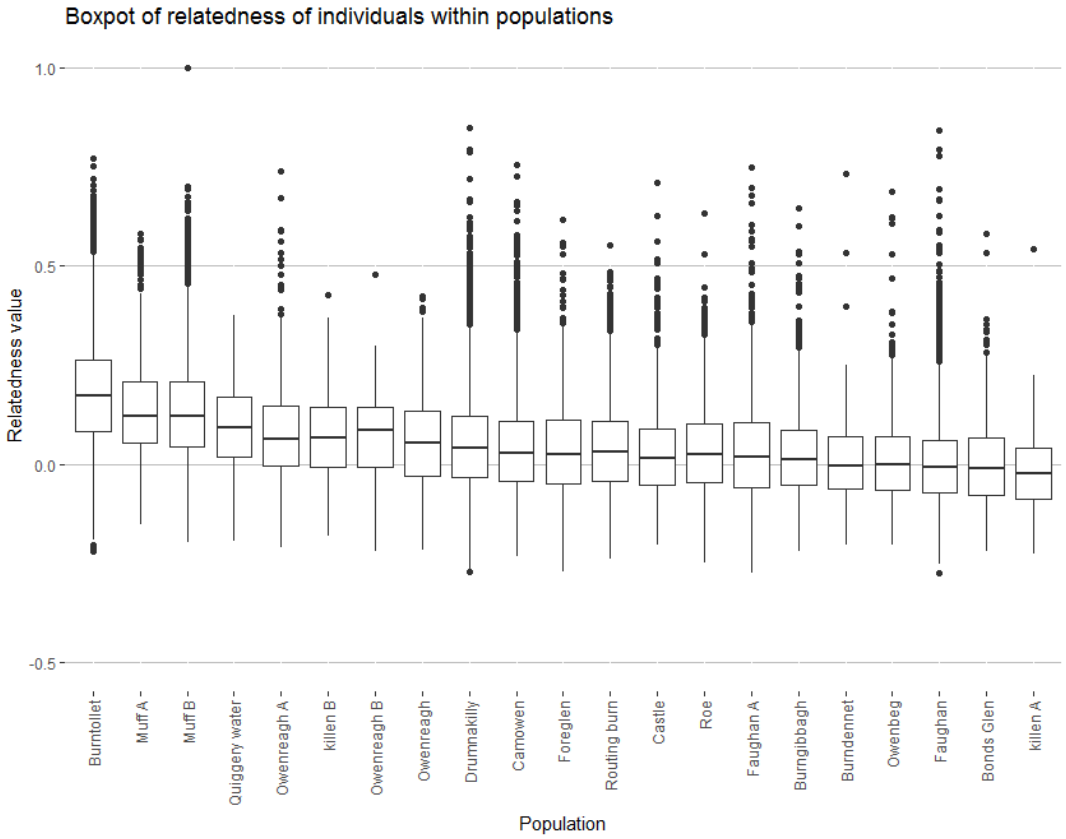

Fig. 2.6: Boxplot of relatedness of individuals calculated using R package 'Related'. Based on simulated data, Wang's coefficient shows pairwise comparisons of individuals within populations with a coefficient <0.1 are unrelated.

Population	Sub-catchment	Sex ratio (female: male)	Binomial test; p-value
DEN	Burndennet	3:13	**0.021**
CAM	Camowen	62:52	0.400
DRU	Camowen	82:115	**0.022**
BGL	Faughan	27:26	1
GIB	Faughan	24:39	0.077
BUR	Faughan	50:86	**0.003**
FAU	Faughan	49:64	0.188
FAA	Faughan	23:35	0.148
FOR	Faughan	17:26	0.222
KILA	Killen Burn	13:16	0.711
KILB	Killen Burn	14:8	0.286
REA	Owenreagh	20:25	0.552
REB	Owenreagh	29:26	0.787
REC	Owenreagh	11:6	0.332
QUI	Owenreagh	7:13	0.263
ROU	Owenreagh	53:55	0.923
MUFA	Muff	19:22	0.755
MUFB	Muff	66:49	0.135
CAS	Roe	24:14	0.143
OWE	Roe	30:30	1.000
ROE	Roe	29:42	0.154

Table 2.7: Sex ratio of 21 populations within the Foyle catchment. Those that are significantly different from an expected sex ratio of 1:1 are highlighted in bold. Overall the Foyle catchment has a sex ratio of 641 females: 773 males (binomial test; $p<0.001$).

2.4.5 CONTEMPORARY GENE FLOW

Contemporary directional gene flow was evident within sub- catchments but not between sub- catchments (Table 2.8). Within the River Faughan sub-catchment all populations except Burntollet showed evidence of directional gene flow towards the mainstem River Faughan. There was also evidence of a directional gene flow both to and from the mainstem River Faughan, Bonds Glen, Burngibbagh and Faughan A. Within the River Camowen sub-catchment contemporary directional gene flow is evident between both populations in both directions. Within the River Roe sub-catchment there is evidence for

directional gene flow between almost all populations in both directions, except for between River Castle and River Roe, where gene flow is only in one direction. Finally, within the River Owenreagh sub-catchment there was only evidence of contemporary directional gene flow from the Quiggery Water to the Routing Burn.

A) Faughan Catchment

From/To	BUR	FOR	BIB	FAA	FAU	BGL
BUR	-	0.116	0.174	0.156	0.175	0.223
FOR	0.110	-	0.485	0.471	**0.626**	0.495
GIB	0.100	0.295	-	0.485	**0.969**	**1**
FAA	0.125	0.312	0.429	-	**0.792**	**0.886**
FAU	0.138	0.400	**0.718**	**0.861**	-	**0.930**
BGL	0.144	0.292	0.743	0.563	**0.996**	-

B) Roe catchment

From/To	ROE	CAS	OWE
ROE	-	0.541	**0.973**
CAS	**0.625**	-	**1.000**
OWE	**0.880**	**0.673**	-

C) Camowen Catchment

From/To	CAM	DRU
CAM	-	**0.864**
DRU	**1.000**	-

D) Owenreagh catchment

From/To	REC	REB	REA	ROU	QUI
REC	-		0.435	0.370	0.173
REB		-			
REA	0.382		-	0.436	0.176
ROU	0.293		0.401	-	0.335
QUI	0.383		0.342	**1.000**	-

Table 2.8: Contemporary gene flow between populations within four sub- catchments based on relative migration networks (Keenan et al., 2013b; R Core Team 2016). A high value (>0.6) indicated strong directional contemporary gene flow (these are highlighted in bold) (Sundqvist el al., 2016) with the direction being from population (table rows) to populations (table columns).

2.4.6 ISOLATION BY DISTANCE AND LANDSCAPE GENETICS

Isolation by distance was tested on geographic distance (km) and genetic distance (D_{JOST}), using mantel tests within IBDWS, which takes into account multiple repeat measures (Jensen et al., 2005). Isolation by distance was tested between the 21 defined

populations (210 pairwise comparisons). There was a significant positive relationship between genetic distance and geographic distance (r^2=0.182; p=0.021) between populations, where genetic distance between populations increased with geographic distance (Fig. 2.7).

Geographic (river) distance, however, only explained around 18% of the genetic variation between population sites. Therefore, to look for other drivers of between site variation, the influence of 31 landscape features on the genetic structuring of brown trout was investigated within the Foyle catchment (Table 2.2). Principle Component Analysis (PCA) was used to reduce the number of highly correlated instream substrate composition variables and to account for correlations between substrate types which arise from the method used to measure them. The first four principle components explained 83.4% of the variance and were retained for further analysis. These principle components derived from a PCA on the seven site specific substrate composition variables (Table 2.2) represented: PC1- driven by high positive loading for cobbles opposing high negative loadings for fines/sands; PC2- which is driven by high positive loadings for mud opposed by high negative loadings for gravel; PC3 which is driven by high positive loadings for boulders opposed to high negative loadings for bedrock; PC4 which is driven by high positive loadings for bedrock opposed to high negative loadings for cobble (Table 2.9).

Correlations between the difference in landscape features between population sampling locations and genetic distance between populations were initially tested using partial mantel tests. These found the difference in ammonia (r^2= 0.379; p=0.008), dissolved oxygen (r^2= 0.361; p=0.016), phosphorus (r^2= -0.186; p=0.031), area of glacial sand gravel in upstream catchment (r^2= -0.209; p=0.032) and wooded area (r^2= 0.324; p=0.021) between sites to be correlated with genetic distance between population sampling locations once the effect of geographic distance was controlled for.

The relationship between landscape features and genetic distance between populations was further investigated using mixed models in the r packages ‘lme4’ and ‘glmulti’ (Table 2.8). A mixed model was used to account for random effects of each population being included in several pairwise combinations in the dataset. Initially, four models were run in glmuti to find the ‘best’ model which explained the variance in genetic distances between populations for each category of environmental variable (instream habitat

characteristics, water quality, geology and landscape factors). The effect of river distance was removed by using the residuals from a Mantel test and Reduced Major Axis (RMA) regressions used to test for Isolation by distance (Table 2.10). Models were selected using AIC_C, which accounts for finite sample sizes and selects the model which best explains the variance in genetic distance between populations. The first model ($Model_{HABITAT}$) examining instream habitat characteristics found principle component one (T=3.113; p=0.002) of substrate composition explained 44.3% of the variance in genetic difference between populations. This was the only statistically significant environmental effect in this model. The second model ($Model_{WATER}$) examining water quality variables found phosphorus (T=3.206; p=0.002) explained 43.0% of the variance in genetic difference between populations. This was the only statistically significant environmental effect in this model. The third model ($Model_{GEOLOGY}$) which examined the geological variables found number of houses per km^2 in the upstream catchment (T=3.889; p<0.001) and distance to nearest farm (T=3.361, p=0.001) explained 37.5% of the variance in genetic difference between populations. These were the only statistically significant environmental effects in this model. The fourth model ($Model_{LANDSCAPE}$) which examined geomorphological variables found area of alluvium (T=2.567; p=0.012) in the upstream catchment to explain 41.7% of the variance in genetic distance between populations. This was the only statistically significant environmental effect in this model.

The final model ($Model_{FINAL}$) combined the predictor variables included in the previous four models representing instream habitat characteristics, water quality, geology and landscape factors. This final model found phosphorus (T=3.209; p=0.002), number of houses per km^2 in the upstream catchment (T=3.751; p<0.001) and distance to the nearest farm (T=3.084; p=0.002) explained 43.2% of the total variation in genetic distance between populations. For each of the variables in this model, as the environmental distance (i.e. the difference in environmental variable between population sampling locations) increases between pairwise sites, so does the genetic distance between populations (Fig. 2.8).

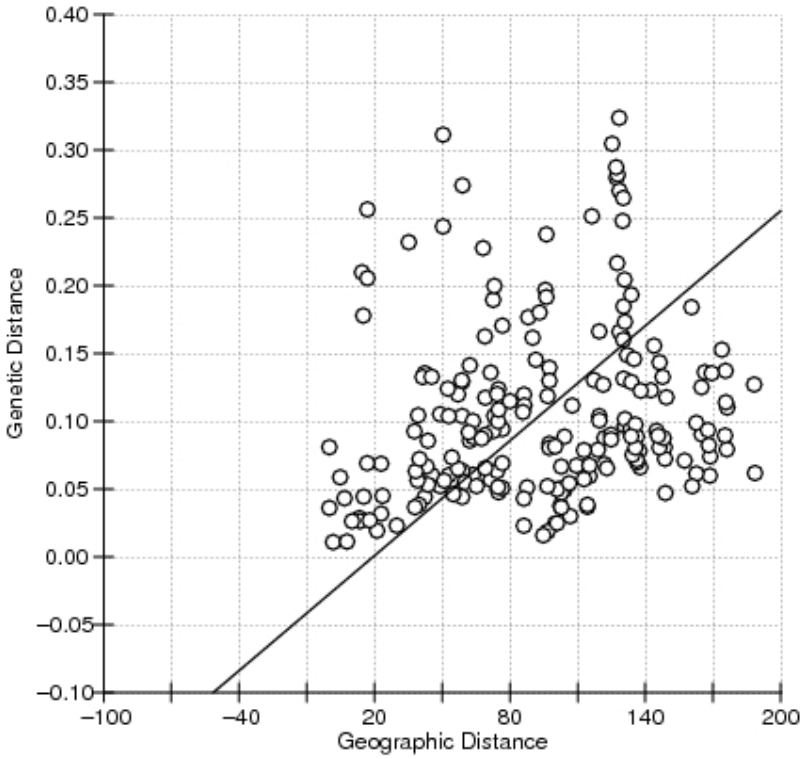

Fig. 2.7: Isolation by distance was evident between 21 populations tested using Isolation by Distance Web Service.

Substrate type	**PC1 loadings**	**PC2 loadings**	**PC3 loadings**	**PC4 loadings**
Percentage of variance explained	31.2%	21.2%	16.4%	13.7%
Bedrock	0.087	0.358	0.372	0.743
Boulder	0.413	0.118	-0.694	0.183
Cobble	0.436	0.127	0.499	-0.528
Gravel	-0.293	-0.594	0.248	0.276
Fines	-0.461	-0.151	-0.253	-0.196
Sand	-0.468	0.421	0.050	-0.131
Mud	-0.338	0.538	-0.048	-0.057

Table 2.9: PC loadings of substrate type in the Foyle catchment and percentage of variance explained by each PC with 82.5% of the total variance explained.

Best Model overall	Fixed Variables	Estimate	Std. Error	df	T value	Pr(>|t|)	IC	R^2_M	R^2_C
Model$_{HABITAT}$	Intercept	0.048	0.009	42.67	5.029	**<0.001**	-519.36	0.081	0.443
	Depth	-0.002	0.001	65.51	-1.447	0.153			
	Substrate PC1	0.011	0.003	128.80	3.113	**0.002**			
Model$_{WATER}$	Intercept	0.034	0.009	37.10	3.856	**<0.001**	-523.37	0.108	0.430
	Phosphorus	0.646	0.201	133.00	3.206	**0.002**			
	pH	0.038	0.024	39.69	1.619	0.113			
Model$_{GEOLOGY}$	Intercept	0.057	0.005	16.52	10.323	**<0.001**	-531.27	0.135	0.375
	Number of houses per km^2 in catchment	0.011	0.003	127.30	3.889	**<0.001**			
	Distance to farm	0.010	0.003	132.30	3.361	**0.001**			
Model$_{LANDSCAPE}$	Intercept	0.050	0.009	24.79	5.919	**<0.001**	-513.74	0.050	0.417
	Alluvium	0.012	0.005	108.30	2.567	**0.012**			
	Diamicton	-0.001	0.001	130.00	-1.579	0.117			
Model$_{FINAL}$	Intercept	0.058	0.005	15.81	10.601	**<0.001**	-515.1	0.198	0.432
	Phosphorus	0.001	0.003	132.00	3.222	**0.002**			
	Number of houses per km^2 in catchment	0.010	0.003	125.10	3.766	**<0.001**			
	Distance to nearest farm	0.009	0.003	131.5	3. 123	**0.002**			

Table 2. 10: Mixed model output for examining the effect of the difference in landscape factors between population sampling locations on genetic distance between populations, accounting for random effects which were the pairwise combination of populations. Model$_{HABITAT}$- habitat variables, Model$_{WATER}$- water quality variables, Model$_{GEOLOGY}$- Geological variables, Model$_{LANDSCAPE}$- geomorphological variables and Model$_{FINAL}$- Final model including important variables from previous four models. R^2M is the variance explained by fixed effects only, whereas, R^2C is the variance explained by both fixed and random effects.

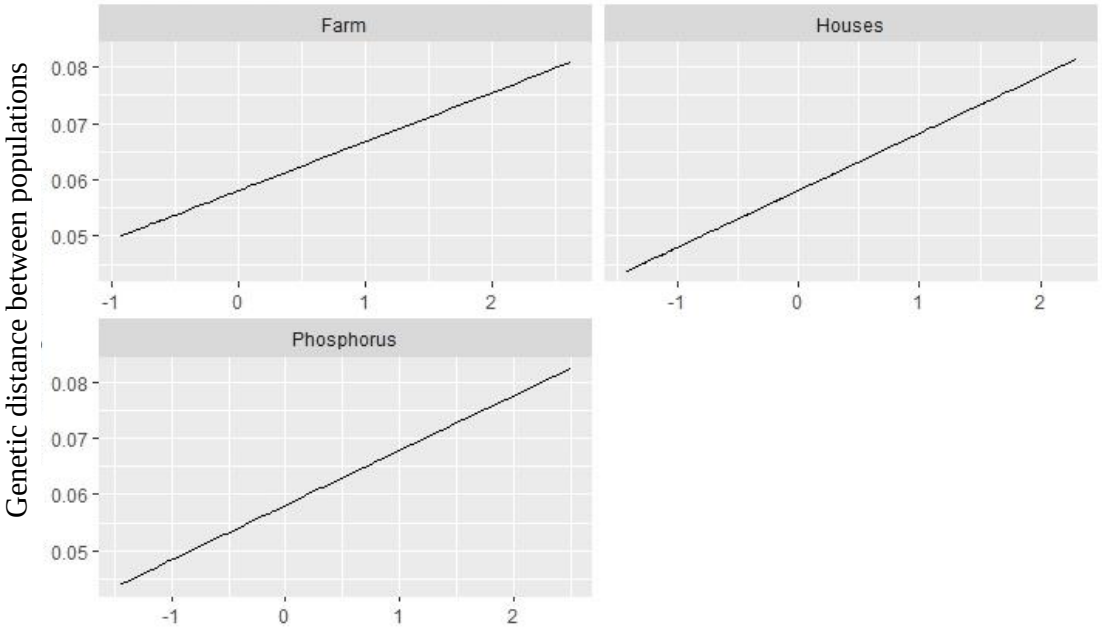

Fig. 2.8: Graphical representation of marginal effects of the predictor variables included in $Model_{FINAL}$ which explains the most variance in genetic distance between populations. Environmental variables which were statistically significant were the pairwise distance between population sites in: distance to nearest farm (km), number of houses per km^2 in the upstream catchment and concentration of phosphorus.

2.5 DISCUSSION

2.5.1 HIERARCHICAL POPULATION STRUCTURING

This study found 21 genetically differentiated populations in the Foyle catchment which were identified at six hierarchical levels (Fig. 2.3.5). Thus, there is clear evidence of intraspecific genetic structuring. Overall, identified brown trout populations in the Foyle catchment had high genetic diversity with a global population differentiation of D_{JOST}= 0.138; F_{ST}= 0.06. The global F_{ST} found here is similar to other studies in similar sized catchments (Crozier & Ferguson, 1986; Lehtonen et al., 2009). The structuring was identified across three spatial scales. Perhaps unsurprisingly, large scale between sub-catchment comparisons had the greatest genetic differentiation with an average genetic distance between populations of 0.09 (D_{JOST}). On a medium scale (i.e. comparisons between tributaries within sub-catchments) there was an average genetic differentiation of 0.05(D_{JOST}). Finally, on a small spatial scale, comparisons between streams in a sub-catchment had the lowest average genetic distance between populations (0.03 D_{JOST}). There

have been studies which have documented population structuring of brown trout across large and medium spatial scales (Ferguson, 1989, 2006; Bernatchez et al., 1992; Massa-Gallucci et al., 2010; Swatdipong et al., 2010; Linløkken et al., 2014). However, the level of population structuring documented in the Foyle catchment, with several populations being identified in sympatry or over geographic (river) distances less than a kilometre apart has not/ or rarely been reported previously.

The extensive population structuring demonstrated by brown trout in the Foyle catchment could have arisen through several evolutionary processes. Post glaciation invasion of new habitats through allopatry and secondary contact, genetic drift, local selection pressures or random chance mutations could explain the population structuring of brown trout on a large and medium spatial scale (Ozerov et al., 2012; McCracken et al., 2013). Under this explanation genetic divergence between populations would have occurred because genetic change in one population could not be easily transmitted to the other as habitat configuration and/or geographic distance are limiting for dispersal/gene flow (Adams et al., 2016). Thus, brown trout occupying each sub-catchment and tributary within sub-catchments evolved in slightly different directions with hybrids between sites being at a selective disadvantage compared with fish from site specific parents (i.e. through divergent natural selection).

The mechanisms through which brown trout could form genetically differentiated populations over small spatial scales, where there are no obvious barriers to gene flow, are less clear. Founder effects, chance mutations or adaptation to small differences in environmental conditions could have driven population structuring over small spatial scales (Stelkens et al., 2012). Riverine systems, particularly within the Foyle catchment are extremely heterogenic over small spatial scales (Niven 2013). This extremely variable environment could result in local selection pressures which drive the population structuring of brown trout found in the Foyle catchment.

The smallest spatial scale over which discrete populations occurred in this study was the identification of populations in sympatry found at two sampling locations. These sympatric populations were composed of anadromous (smolting) brown trout and adult freshwater resident brown trout which are likely to have migrated from their spawning

grounds to co-inhabit in feeding grounds (see chapter 4). Therefore, it seems probable that these sympatric populations have become reproductively isolated due to separate spawning grounds or different timings for spawning (Ferguson 2006). The precise location of these spawning grounds (which as shown by small scale structuring could be less than a few hundred metres apart) must be maintained by a mechanism that prevents effective straying. Precise natal homing has been described in brown trout, whereby they return to their natal grounds to breed (King et al., 2016; Ferguson 2016). Freshwater resident brown trout are likely to migrate downstream to deeper waters with better feeding opportunities (Klemesten et al., 2003). Thus, to maintain the population structuring over small spatial scales, the homing behaviour of brown trout must be exceedingly precise.

This study also identified several distinct populations which were more genetically similar to one another than to populations in their own sub-catchment (i.e. Burndennet and Burngibbagh; Owenbeg and Faughan) (Table 2.3; Fig. 2.5). The genetic similarity of these populations could suggest that either there is effective straying (see chapter five) of anadromous brown trout between rivers (the connection between these sub-catchments is via a sea lough), or that this is the effect of stocking as a management practice, or that the connection between these rivers has subsequently been lost or that genetic similarities have resulted from convergent evolution. As there are no reports of stocking in the Foyle catchment, it seems more likely that effective straying of anadromous brown trout between rivers, or that these rivers were previously connected and these pairs of populations have descended from a common ancestor or that their genetic similarity is due to convergent evolution.

2.5.2 CONTEMPORARY GENE FLOW

In this study, contemporary directional gene flow was calculated in a linear system for which the R package, ‘diveRsity’, has not been validated. However, the results presented do show the direction and relative strength of contemporary gene flow between populations in the Foyle catchment. Contemporary directional gene flow was not evident between populations from different sub-catchments but was apparent within sub-catchments (Table 2.2). In freshwater systems, populations are expected to be arranged into a hierarchical structure, where multiple upstream populations converge into a single downstream population, thus contemporary gene flow would be expected to be from upstream

populations to downstream populations (McCracken et al, 2013). Contemporary directional gene flow was evident mainly in a downstream direction, however, some populations (especially in the sub-catchments Rivers Roe, Camowen and Faughan) also showed evidence of directional gene flow in an upstream direction which could be indicative of small amounts of straying. Therefore, most populations within the Foyle catchment were reproductively isolated.

2.5.3 EFFECTIVE POPULATION SIZE

This study used two methods to calculate the effective population size, N_e, as a recent study by Wang demonstrated sibship frequency as an estimator of N_e is more accurate and robust than the linkage disequilibrium method (Wang 2016; Waples 2016). Using the linkage disequilibrium method of NeEstimator the effective population size of populations ranged from 59.2 (River Faughan A) to 510 (Killen Burn A). Whereas, the effective population size calculated using sibship frequency in COLONY ranged from 46 (River Castle) to 224 (River Drumnakilly). Franklin (1980) stated that populations with an effective population size less than 50 were at risk of extinction. Therefore, this study highlighted Rivers Castle (N_e=46) and Faughan A (N_e=48) could be at risk from extinction due to genetic drift.

N_e also plays a key role for management as it provides information of the evolutionary potential of populations and a populations vulnerability to extinction from demographic, environmental and genetic stochasticity (Palstra & Fraser 2012). Genetic drift is one of the most important stochastic evolutionary forces which can interact with selection and/or mutation (Waples 2010). Genetic drift in populations with a low effective population size would be more pronounced due to small populations having less genetic variation (Waples 2010). Therefore, this study would suggest populations Rivers Castle, Foreglen and Burndennet all have a smaller effective population size relative to other populations and may require management strategies implemented to ensure their survival.

2.5.4 ISOLATION BY DISTANCE, ENVIRONMENT OR A COMBINATION OF BOTH

Local selection pressure, local genetic drift and the chance occurrence of mutations could lead to locally isolated populations, whereby genetic differentiation between populations accumulates with geographic distance (Fraser et al., 2011). This is known as

isolation by distance, a term coined by Wright (1943) (Kimura & Weiss 1964). This study demonstrated isolation by distance had a strong effect on the structuring of brown trout populations in the Foyle catchment. However, many studies on salmonids reveal no effect of isolation by distance (Crozier & Ferguson 1986; Ferguson 1989; Meldgaard et al. 2003; Heggenes & Roed 2006; Stelkens et al., 2012). There are a few studies which have demonstrate isolation by distance (Estoup et al, 1998; Laikre et al., 2002; Linløkken et al., 2014). Many studies may have not detected isolation by distance because the genetic markers used had insufficient resolution or the populations being investigated where separated by small geographic distances (Stelkens et al., 2012). Using Mantel tests in IBDWS (Jensen et al., 2005) a strong positive correlation between geographic (river distance km) and genetic distance (D_{JOST}) was found (r^2=0.182; p=0.021). Evidence of isolation by distance indicates that brown trout populations in the Foyle have been subject to evolutionary pressures resulting in the subdivision of ancestral populations into many genetically differentiated sub-populations.

These evolutionary pressures are likely the result of local environmental drivers, both historical and contemporary, which have resulted in genetic divergence between populations described in this study. Therefore, despite a strong significant effect of isolation by distance a considerable percentage of genetic variation between sites was not explained by geographic (river) distance. The relationship between environmental drivers and population structuring of brown trout was examined in the absence of the effects of geographic (river) distance. Once the effect of isolation by distance was removed from the analysis the difference in distance to nearest farm (km), the number of houses per km^2 in the upstream catchment and the concentration of phosphorus (mg/l) in the river explained an additional 19.6% of the variation in genetic distance between populations. Therefore, population sites which showed no pairwise difference in the environmental variables described here had very little genetic differentiation between them (0-0.05 corrected D_{JOST} removing IBD), whereas population sites which showed a large pairwise difference in environmental variables had large genetic differentiation (0.07-0.08 corrected D_{JOST} removing IBD).

The environmental variables identified as significant in creating genetic structure strongly point to a major effect of the influence of humans on genetic population structuring. Although it is probable that natural variation in environmental factors are also driving

population structuring, environmental variables associated with anthropogenic impacts appear to be the biggest driver (either directly or indirectly). Phosphorus concentration (mg/l) at population sampling locations ranged from 0.029 to 0.094. These concentrations of phosphorus were low and regarded by environmental agencies (NIEA and EPA) as acceptable (<0.03mg/l) to fair (0.04-0.14mg/l). Therefore, it is difficult to separate this into natural or unnatural levels but it is known that phosphorus concentration can be influenced by anthropogenic impacts, such as housing developments and intensive farming practices Daniel et al., 1998). Therefore, the number of houses in the upstream catchment and the distance to the nearest farm also correlated with genetic population structuring.

There have been many studies which have examined the effects of anthropogenic impacts on population structuring of species in freshwater systems (Durrant et al., 2011; Östergren & Nilsson 2012; Stelkens et al., 2012; Thaulow et al., 2013; Hansen et al., 2014). For example, in the River Aare, Switzerland, it was found the number of weirs between populations was a driver of population structuring (Stelkens et al., 2012). Comparing genetic structuring of contemporary and historical populations in Norway, stocking of hatchery bred brown trout and river alterations (construction of barriers and river channelization) drove a complete shift between historic and contemporary population structuring (Thaulow et al., 2013). However, most of these studies have focused on the effects of barriers to migration, such as weirs and dams (Hansen et al., 2014). It has been shown that historical anthropogenic impacts, such as medieval dams and metal pollution from the bronze age, have structured populations of brown trout (Hansen et al., 2014; Paris et al., 2015). Such anthropogenic pressures are important to understand as they drive evolutionary processes shaping population structuring. Habitat fragmentation is one of the biggest threats to biodiversity and is driven by anthropogenic impacts such as, land-use and changes in water chemistry (Stelkens et al., 2012; Hansen et al., 2014). These pressures drive divergence of populations which can have long- and short- term negative impacts through founder effects and genetic drift (Stelkens et al., 2012; Hansen et al., 2014). Therefore, with anthropogenic impacts being inevitable it is important to understand how humans drive the fragmentation of populations and species potential for evolutionary change.

Three scenarios were highlighted as possible mechanisms which could drive population structuring of brown trout. These were: 1- Isolation by distance; 2- Isolation by

environment or 3- a combination of both. This study has shown that both isolation by distance and isolation by environment play an important role in driving population structuring through early evolutionary processes. Isolation by distance was found to have a larger effect over greater geographic (river) distances where there are obvious barriers to gene flow such as weirs or the sea lough. However, isolation by environment shapes population structuring at smaller spatial scales whereby populations whereby the heterogeneity of habitats and natal homing behaviour of brown trout prevents extensive gene flow between neighbouring populations (Fraser et al., 2011). Often these populations are formed where there is no obvious barrier to gene flow and provides some evidence for adaption, which is likely to be driven by changes in environment due to anthropogenic impacts. Future work isolating the adaptive regions of the genome responsible for adaptation to the environmental factors isolated in this study would determine if brown trout have formed locally adapted populations in response to anthropogenic impacts.

CHAPTER THREE: MORPHOLOGICAL STRUCTURING OF BROWN TROUT (*Salmo trutta*) VARIES WITH GENETIC STRUCTURING AND LANDSCAPE FEATURES IN THE FOYLE CATCHMENT, IRELAND.

3.1 ABSTRACT

Morphological structuring of a species can provide useful insights into early evolutionary processes which result in divergence between groups and ultimately, speciation. This study examined the morphological structuring of brown trout in a highly dendritic river catchment, the Foyle, to investigate the degree of morphological differentiation, as well as the role of neutral genetic differentiation, river distance and environmental variables in shaping any structuring. Significant morphological structuring was seen in brown trout from across the Foyle catchment. It was found that genetic distance, river distance and environmental variables, such as substrate composition, influenced the morphological structuring of brown trout. However, environmental variables were more important than neutral genetic differences in driving morphological structuring. It was also demonstrated that the absolute morphology of groups was predicted by differences in human activity. Therefore, it is important to understand morphological structuring of brown trout to determine how it is shaped by anthropogenic impacts.

3.2 INTRODUCTION

It is becoming increasingly apparent that many species in nature show considerable intra-specific structuring in the expression of morphologies, such as body shape (see Pakkasmaa & Piironen 2001a; Drinan et al. 2012 for brown trout (*Salmo trutta*); see Riddell & Leggett 1981; Von Cramon-Taubadel et al., 2005; Páez & Dodson 2017 for Atlantic salmon (*Salmo salar*); see Beacham & Murray 1987 for chum salmon (*Oncorhynchus keta*); see Klingenberg et al., 2003 for cichlid fishes (*Amphilophus citrinellus* species complex); see Guill et al., 2003 for darters; see Cussac et al., 1998 for Percichthys; see Forsman & Shine 1995 for Australian scincid lizard (*Lampropholis delicata)*; see Marchiori et al., 2014 for *Aegla longirostri*). This variation in morphology can reflect ecological, behavioural and genetic differences between populations and provide insights into evolutionary processes, such as natural selection and speciation (Klingenberg et al., 2003; Etheridge et al., 2010). Therefore, where the expression of morphological structuring within a species takes the form of discrete discontinuities, determining the beginning and end of morphologically distinct

groups and their relationship with genetic structuring and environmental factors is of vital importance (Adams et al., 2008; Garduño-Paz et al., 2012; Adams et al., 2016). For example, the European whitefish (*Coregonus lavaretus*) showed extensive morphological and genetic structuring between two lakes in Scotland (Adams et al., 2016). Through common garden experiments, the morphological structuring of these whitefish was shown to be, at least partly, inherited (Adams et al., 2016). Morphological structuring being driven by genetic structuring is further demonstrated by a study on Arctic charr (*Salvelinus alpinus*) in the upper Forth catchment, Scotland. This studied demonstrated morphological differences between Arctic charr from three closely connected lakes with no barriers to movement, as well as neutral genetic differentiation between populations (Adams et al., 2006). This discontinuity between discrete morphologies can also result from environmental factors rather than genetic structuring. For example, two discrete bill morphs of the African estrildid finch (*Pyrenestes ostrinus*) in south-central Cameroon appear to confer different competitive abilities for food resources but this occurs without genetic differences between groups (Smith 1990). Another example of a study demonstrating the influence of environmental factors on morphological structuring is the western rainbow fish (*Melanotaenia australis*) in the Pilbara region, north-western Australia. This study demonstrated significant morphological variation between three geographically distinct sub-catchments providing isolated habitats for morphological differences to develop (Lostrom et al., 2015). Therefore, morphological structuring is likely the result of phenotypic plasticity, whereby differences in environmental factors has driven the expression of different phenotypes. This effect can be thought of as the very earliest stages of intra-specific evolutionary divergence and which can lead to genetic differentiation between phenotypic populations (Adams et al., 2008; Adams et al., 2016).

Morphological variation is particularly prevalent in salmonids, such as Arctic charr (*Salvelinus alpinus*), European whitefish (*Coregonus lavaretus*) and Atlantic salmon (*Salmo salar*) (Riddell & Leggett 1981; Adams et al., 2008; Drinan et al., 2012; Adams et al., 2016). One species that shows considerable discrete within-species morphological structuring is the brown trout (*Salmo trutta*) (Wysujack et al., 2009; Vehanen & Huusko, 2011; Drinan et al., 2012; Stelkens et al., 2012; Westley et al., 2013). This morphological structuring is associated with the exploitation of discrete of niches from freshwater rivers to the marine environment (Chavarie et al., 2015). The most commonly recognised morphologies of brown trout are anadromous (sea) trout, which migrate to salt water, ferox which exist as

piscivorous, long-lived lacustrine trout, and potamodromous trout which migrate between rivers and lakes (Ferguson & Taggart 1991; Meyers et al., 1992; Klemetsen et al., 2003). For example, Lough Melvin supports three morphotypes of brown trout (gillaroo, sonaghen and ferox) which have been found to also show extensive genetic differentiation and reproductive isolation (Ferguson and Mason, 1981; Cawdery and Ferguson 1988; Ferguson & Taggart 1991; Prodöhl et al., 1992; McVeigh et al., 1995; Youngson et al., 2003). However, brown trout also demonstrate morphological structuring between populations (Karakousis et al., 1991; Bernatchez et al., 1992; Pakkasmaa & Piironen, 2001a; Ojanguren & Braña 2003; Stelkens et al., 2012). For example, morphological structuring was found between seven populations in Greece which mainly differed in maximum body depth and distance from anal to caudal fin (Karakousis et al., 1991). A similar pattern of morphological variation between populations was also described in the River Aare, Switzerland, where variation in head and body shape was describe (Stelkens et al., 2012). Such morphological structuring between populations and commonly recognised morphs could be driven by genetic differentiation, or ecological and/or behavioural differences resulting in plasticity effects (Klingenberg et al., 2003; Adams et al., 2016). However, there are very few studies which have examined intra-specific morphological structuring of brown trout and the possible environmental drivers determining structuring (Yevsin 1977; Pakkasmaa & Piironen 2001b; Drinan et al., 2012; Stelkens et al., 2012). An example of one study which has examined how morphological variation is driven by environmental factors was conducted in a Swiss river system which found variation in body shape between brown trout populations could be explained by topographic stream slope and flow regimes (Stelkens et al., 2012). The morphology of brown trout, specifically pectoral fin length, head length and body depth, was also demonstrated to vary with hydraulic forces in the Rivers Barrow and Burrishoole (Drinan et al., 2012). In other species of salmonids flow regime has been identified as an important driver of morphological structuring (Bisson et al., 1988; Obedzinski & Letcher 2004; von Cramon-Taubadel et al., 2005; Pakkasmaa & Piironen 2011b; Drinan et al., 2012). For example, a study in New Brunswick found differences in body morphology and timing of downstream migration in two populations of Atlantic salmon were driven by flow regime and differences in overwintering energetic costs (Riddell & Leggett 1981). This is further demonstrated by comparing morphology of several hatchery and wild river system populations in Ireland. This study found rearing conditions had a significant impact on body shape and growth of Atlantic salmon (von Cramon-Taubadel et al., 2005). The polytypic nature of brown trout, means that they are an ideal model species for the investigation of morphological structuring and the possible environmental drivers of such structuring.

There are three basic proximate mechanisms which may underpin intraspecific morphological structuring: genetic structuring, environmental variables (including landscape variables) or a combination of both. In the first scenario, at its simplest different morphological groups may represent different genetic populations (Pakkasmaa & Piironen 2001a; Adams et al., 2016). If genetic structuring was solely responsible, for intraspecific morphological differences we would expect limited, to no, gene flow between morphological groups. This effect has been shown in the European whitefish (*Coregonus lavaretus*), for example, where there were clear differences in trophic morphology between genetic populations (Adams et al., 2016). Genetic differentiation between three morphotypes (gillaroo, sonaghen and ferox) of brown trout was also found in Lough Melvin (Ferguson & Targett 1991). Therefore, if morphological structuring is explained by genetic structuring, this can be indicative of natural selection or genetic drift and provide insights into the selection processes involved in speciation (Frazer & Russello, 2013).

In the second scenario, morphological structuring is driven by within-generation differences in exposure to environmental variables and, thus, is a plastic response (Pakkasmaa & Piironen 2011). Morphological plasticity can result in the expression of different morphologies in contrasting groups utilising ecologically different niches (Vehanen & Huusko, 2011; Westley et al., 2013; Adams et al., 2016). For example, wild and hatchery-reared brown trout show different morphologies, with wild brown trout having longer heads and shorter anterior trunks compared to hatchery-reared brown trout (Vehanen & Huusko, 2011). When hatchery-reared brown trout were stocked into the rivers inhabited by wild brown trout they developed similar characteristics as the wild form indicating morphological plasticity (Vehanen & Huusko, 2011). Therefore, in this scenario, all individuals comprise the same gene pool but are exposed to different environmental conditions and express different morphologies through plasticity. In the final scenario, both genetic structuring and environmental factors result in the expression of different morphologies (Stelkens et al., 2012). In this case, morphological structuring is the result of divergence through directional selection and environmental modulation through phenotypic plasticity (Ghalambor et al. 2007).

The general aim of the study described here was to evaluate morphological structuring of brown trout across multiple spatial scales in a single, highly dendritic catchment. Specifically, the aims of this study were to:

(i) determine the morphological structuring of brown trout in the Foyle catchment, Ireland

(ii) examine how spatial scale shapes the observed morphological structuring,

(iii) investigate if genetic structuring relates to morphological structure

(iv) determine if environmental factors drive the morphological structuring of brown trout within the Foyle catchment.

3.3 METHODS

3.3.1 STUDY AREA

The River Foyle catchment is a highly dendritic, medium sized catchment of 4500km^2 located both in Northern Ireland and the Republic of Ireland (Niven, 2013). It comprises smaller sub-catchments including the Rivers Camowen, Owenreagh, Derg, Fairywater, Owenkillew, Finn, Faughan, Roe and Burndennet (Fig. 3.1), which drain into the River Foyle and then into a sea lough, Lough Foyle (Niven, 2013). This complex catchment is ideal for studying the morphological structuring of brown trout at different spatial scales.

3.3.2 COLLECTION OF SAMPLES

To establish the morphological structuring of brown trout, 22 sites were electrofished between April and September in 2013/2014 within the Foyle catchment for juvenile brown trout (Fig. 3.1; Table 3.1). The sampling sites chosen were also used in chapter two to establish the population structuring of brown trout. However, a few sampling sites described in chapter two (River Muff, Killen Burn, Bonds Glen and Burndennet) were excluded from this study due to small sample sizes or because many of the brown trout at these sites were anadromous brown trout. These sampling sites were selected based on habitat quality and the abundance of juvenile brown trout. The sites were chosen to represent structuring at three spatial scales (large, medium and small) (Fig. 3.2). The 22 sites chosen represented four major sub- catchments of the Foyle catchment (large scale): The Rivers Faughan, Roe, Owenreagh and Camowen. Three tributaries within each of these sub-catchments were

surveyed to examine structuring on a medium scale. Finally, three sites within one tributary of each sub-catchment were examined for structuring on a small scale (Fig. 3.2). Over 500m of stream length at each chosen site was electrofished; brown trout were collected randomly over this distance to ensure several families were represented at each sampling site (Hansen et al 1997). In total, 1467 brown trout were collected, anesthetised using clove oil, measured for fork length and a scale sample taken. Collected brown trout were also photographed in left lateral view on laminated grey graph paper with a scale.

River ID (abbreviation)	Location ID	Easting	Northing	N	Fork length (mm)
Camowen (CAM)	5	662460.3	870951.2	72	50-269
Drumnakilly (DRU)	7	653773.2	873040.4	71	45-320
Drumnakilly A (DRA)	8	655032.3	874057.7	65	48-145
Drumnakilly B (DRB)	9	654245	873710.5	76	54-189
Granagh Burn (GRA)	15	659846.6	872823.6	69	52-174
Burngibbagh (GIB)	1	644497.4	912857.2	65	14-229
Burntollet (BUR)	2	652919.5	911768.1	66	51-249
Burntollet A (BUA)	3	658370.5	912565.5	69	51-136
Burntollet B (BUB)	4	654962.9	912632	69	44-178
Faughan (FAU)	10	657002.8	905701.6	63	46-273
Faughan A (FAA)	11	660556.6	900607.6	65	44-232
Faughan B (FAB)	12	660476.2	900491.8	65	50-184
Foreglen (FOR)	13	656876.9	908861.8	65	56-208
Glenrandal (GLE)	14	654296.7	904727.1	63	41-205
Owenreagh (REA)	17	632906.1	866020.7	58	56-214
Owenreagh A (REB)	18	632611.8	867336.4	65	50-152
Owenreagh B (REC)	19	638204.2	860452.6	65	56-212
Quiggery water (QUI)	20	644305.9	858990.4	68	53-340
Routing Burn (ROU)	22	646987.2	863690.1	71	63-264
Castle (CAS)	6	671096.8	918932	64	67-191
Owenbeg (OWE)	16	664516.1	905941.5	66	45-187
Roe (ROE)	21	677020.9	903815.1	67	36-194

Table 3.1: Sampling locations with river ID, site number, easting and northing in the coordinate system “Irish Transverse Mercator grid”, the number of brown trout samples collected (N) and fork length range (mm) of sampled brown trout.

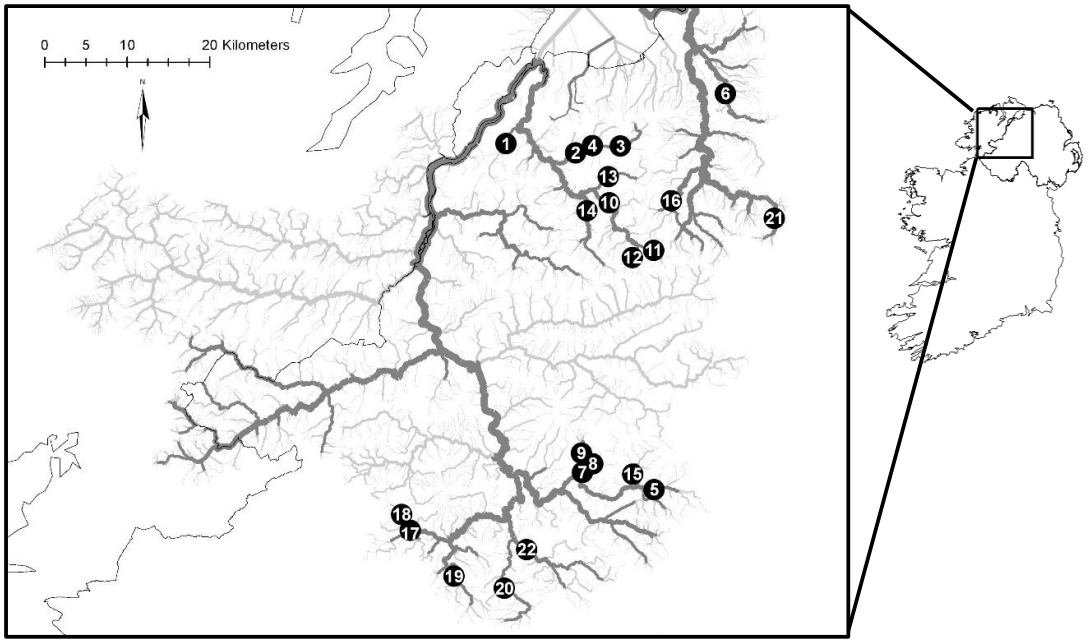

Fig. 3.1: The location of sampling sites surveyed to establish the morphological structure of brown trout within the Foyle catchment. Location ID indicated on map corresponds to information in Table 3.1. Note sampling locations are the same as those described in chapter two with a small number of sampling sites excluded due to presence of anadromous brown trout or small sample size.

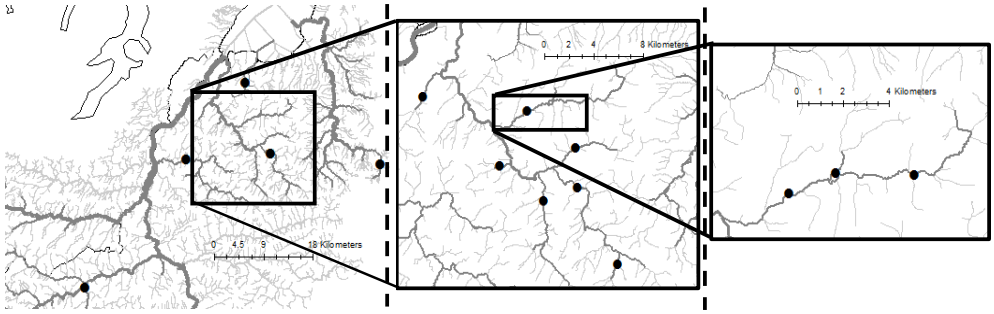

Fig. 3.2: A diagrammatic representation of the three geographic spatial scales used during this study to investigate the relationship between geographic distance and population structure. Large scale geographic distances were between different sub-catchments and ranged from 52km- 176km. Medium scale geographic distances were between tributaries within sub-catchments and ranged from 7km-65km. Finally, small scale geographic distances were between streams within tributaries and ranged from 0.3km-10km.

3.3.3 STATISTICAL ANALYSIS

Digital images of individual brown trout were used to determine morphological structuring of body shape. Seventeen consistently identifiable landmarks, chosen based on previous work (Stelkens et al., 2012; Vehanen & Huusko 2011; Adams & Huntingford 2004; Garduño-Paz et al., 2012), were digitised in two dimensions on each digitised photograph and a scale was added, to allow for size correction, using tpsDig2 and tpsUtil (Fig. 3.3) (Rohlf 2006a; Rohlf 2006b). Geometric morphometric analysis was then performed using MorphoJ v1.06b (Klingenberg, 2011). Procrustes superimposition, which scales, translates and rotates individual image landmarks to a mean shape derived from all specimens by minimising the sum of squared distances between corresponding landmarks (Stelkens et al., 2012), was used prior to geometric morphometric analysis. Residuals from a pooled within-group regression of Procrustes coordinates on log centroid size was used to provide a shape measure free from allometric scaling (Klingenberg, 2011).

Canonical Variate Analysis (CVA) assumes that all samples may be assigned to pre-defined groups (in this case sampling site) and determines whether the multivariate data (in this case the position of the landmarks) supports group partitioning (Webster and Sheets 2010). The resulting CV axes are scaled by patterns of within-group variation and those which are significant can be used to distinguish between groups (Webster and Sheets 2010). CVA was used to analyse the residuals from the pooled within-group regression as a measure of shape independent of allometric scaling, to determine the morphological structure of brown trout using 10 000 permutations (Klingenberg & Monteiro 2005). CVA analysis was conducted on all sampling locations, as well as within each spatial scale (large, medium and small). The magnitude of pairwise ‘morphological distances’ between each possible pair of sampling sites was determined using the mahalanobis distance.

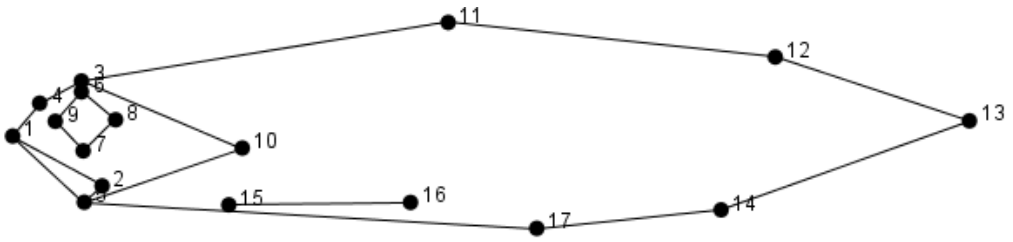

Fig. 3.3: Seventeen Landmarks were used to estimate the shape of brown trout. Landmark 1: the tip of the snout; 2: the posterior part of maxilla; 3: edge of cranium directly above centre of eye; 4: edge of cranium, central to 1 and 3 giving curvature of head; 5: edge of the buccal cavity directly below centre of eye; 6-9: upper, lower, posterior and anterior parts of eye, respectively; 10: posterior edge of gill operculum; 11: anterior edge of dorsal fin; 12: anterior edge of adipose fin; 13: point where lateral line meets caudal fin; 14: anterior base of anal fin; 15: anterior base of pectoral fin; 16: tip of pectoral fin with position corrected by placing landmark collinear to landmark 15, representing length of pectoral fin; 17: anterior base of pelvic fin.

Correlations between morphological distance between groups and genetic distance (D_{JOST}) or geographic (river) distance (km) were each tested using Mantel and Partial Mantel tests in the Isolation by Distance web service (Jensen et al., 2005). River distance (km) was calculated using ArcGISV10.2 (ESRI 2011) and was measured as the distance between two sampling locations following the watercourse, as opposed to a straight-line distance. Genetic structuring of brown trout within the Foyle catchment had previously been established (see Chapter two) and was used to determine whether morphological structuring could be explained by genetic structuring. Using the genotypic information from Chapter two, the R package 'diveRsity' was used to calculate the genetic distance (D_{JOST}) between morphological groups (Keenan et al., 2013b; R CoreTeam 2016).

Thirty-one environmental factors were used to examine the relationship between environmental variables and morphological structuring of brown trout. These variables represent four major categories of environmental variable types: site specific habitat characteristics; site specific water quality; geology; and landscape features of the catchment upstream of each sampling site (Table 3.2). Environmental variables were collated from data collected by the Loughs Agency. Locations sampled by the Loughs Agency to determine these landscape variables did not always exactly match locations sampled for determining morphological structuring. Therefore, where possible, the nearest location with information on environmental variables to the sampling sites was used. If this was not possible, information on geology and landscape features were calculated using the methods described

in Table 3.2. However, landscape features for instream habitat features and water quality, for which there were no data, were estimated by taking an average of all sampling sites for each missing variable.

Instream substrate composition, collected by the Loughs Agency, for site specific habitat characteristics was composed of seven variables: percentages of bedrock, boulder, cobble, gravel, fines, sands and muds. These variables were highly correlated. Therefore, to reduce the number of co-correlated variables, Principle Component Analysis (PCA) was conducted.

Mixed models were run using the R packages 'lme4' and 'glmulti' (Bates et al., 2015; Calcagno & de Mazancourt 2010) to investigate the influence of environmental variables on the 'morphological distance' between populations. The 'distance' between environmental variables was calculated as Euclidian distance using the 'distance function' in the R package 'ecodist' (Goslee & Urban, 2007). Mixed models were run separately on each of the four categories of environmental variables (site specific habitat, water quality, geology and landscape features) to investigate their relationship with morphological structuring. The model which best fitted the data was selected using AICc for each category of environmental variables and the environmental variables included in the best model for each category were then collated into a final model to determine which landscape variables influenced morphological structuring. The final model also included genetic distance as one of the variables to determine if genetic distance or landscape variables played a bigger role in shaping morphological groups. Mixed models were run using the genetic algorithm of 'glmulti' with no interactions included in the models due to the high number of possible combinations of variables (often more than a billion possible models).

The links between landscape variables and group specific morphology, such as eye size, were established using linear models in the R package 'glmulti' with no interactions (again due to the number of possible models). Individual Canonical Variables (CV) scores were averaged for each morphological group to represent site specific morphology. A linear model was conducted separately on each category of landscape variables (site specific habitat, water quality, geology and landscape features) to investigate which variables in each category act as a driver of group specific morphology. The landscape variables included in

the best model for each category were then collated into a final model to conclude which landscape variables influenced group specific morphology.

Variable	Sampling methodology	Year(s) data collected
Category: Water Quality		
Biological Oxygen Demand (mg/l)		2009-2014
Ammonia (mg/l)		2009-2014
Phosphorus (mg/l)		2009-2014
Suspended Solids (mg/l)		2009-2014
Dissolved oxygen (mg/l)		2009-2014
Conductivity		2009-2014
pH		2009-2014
Category: Site specific habitat characteristics		
Depth (m)	Average depth at sampling site	1998-2006
Width (m)	Average width at sampling site	1998-2006
Cover (%)	The cover provided by trees was estimated for both the right and left river bank and then averaged at each sampling site.	1998-2006
Overhang (%)	The overhang of vegetation on both the right and left river bank was estimated and then averaged at each sampling site.	1998-2006
Bedrock (%)	Percentage of sampling area containing bedrock (exposed solid rock)	1998-2006
Boulder (%)	Percentage of sampling area containing boulder (large rocks >256mm)	1998-2006
Cobble (%)	Percentage of sampling area containing cobble (loose rock 64-256mm)	1998-2006

Gravel (%)	Percentage of sampling area containing gravel (loose material 16-64mm)	1998-2006
Fines (%)	Percentage of sampling area containing fines (loose material 2-16mm)	1998-2006
Sand (%)	Percentage of sampling area containing sand (loose material <2mm)	1998-2006
Mud (%)	Percentage of sampling area containing mud	1998-2006
Category: Geology		
Stream order	Stream order was calculated using methodology explained by Horton (1945)	2002
Catchment area (km^2)	Catchment area above each sampling site was determined using the river network boundary.	2002
Elevation (m)	Calculated from height contours on either side of sampling site	2002
Stream gradient	Horizontal distance between the two nearest contour lines and dividing by the change in elevation.	2002
Number of houses per km^2 upstream of site- representative of Urban area	Number of houses upstream of sampling site divided by the catchment area above the sampling site	2002
Distance to nearest farm (km)- representative of proximity to farmland	Straight line distance from sampling site to nearest farm house	2002
Category: Landscape features		
Area of peat upstream (km^2)	Area of peat upstream of sampling site was measured using 'Drift and Quaternary editions of Geological Survey of N Ireland' maps.	2002

Area of glacial alluvium upstream (km^2)	Area of glacial alluvium upstream of sampling site was measured using 'Drift and Quaternary editions of Geological Survey of N Ireland' maps.	2002
Area of glacial sand and gravel upstream (km^2)	Area of glacial sand and gravel upstream of sampling site was measured using 'Drift and Quaternary editions of Geological Survey of N Ireland' maps.	2002
Area of Diamicton upstream (km^2)	Area of glacial boulder and clay upstream of sampling site was measured using 'Drift and Quaternary editions of Geological Survey of N Ireland' maps.	2002
Area of urban upstream (km^2)	Defined from 1:50,000 'OSNI Discoverer Series' maps. Urban area above sampling sites was calculated.	2002
Area of woodland upstream (km^2)	Defined from 1:50,000 'OSNI Discoverer Series' maps. Woodland area above sampling sites was calculated.	2002
Area of grassland upstream (km^2)	Defined from 1:50,000 'OSNI Discoverer Series' maps. Grassland area above sampling sites was calculated.	2002

Table 3.2: Environmental variables and their units from each of the four major categories used to test if landscape and environmental features influence population structure with the year(s) the data was collected and the methodology used to collect the data. These data were collated from data collected by the Loughs Agency

3.4 RESULTS

Some digital photographs were excluded from shape analysis based on poor quality or lighting; the sampling location Owenreagh A was removed from the analysis due to a small sample size. Thus, 968 out of 1467 digitalised photographs of brown trout were

included in the analysis of morphological structuring, with all sampling locations having more than 30 individual brown trout within them.

3.4.1 MORPHOLOGICAL STRUCTURING

Morphological structuring across 21 sampling locations within the Foyle catchment was examined using CVA in MorphoJ v1.06b (Klingenberg, 2011). This analysis showed all 21 sampling locations formed morphologically separate groups. These 21 morphological groups were significantly different from one another based on pairwise tests (all $p<0.05$) and pairwise mahalanobis distances ranged from 1.625 to 5.045 (Table 3.3). On the largest spatial scale, pairwise comparison of sampling locations from different sub-catchments, the mean mahalanobis distance was 3.17. On a medium spatial scale, pairwise comparison between tributary sampling locations within each sub-catchment, the mean mahalanobis distance was 3.11. On a small spatial scale, pairwise comparison of sampling sites in streams within tributaries, the mean mahalanobis distance was 2.64 (Table 3.4).

The morphologies of brown trout were examined using CV's 1-4, which together represented 57.7% of the variation in shape (Fig. 3.4). Each canonical variate explained variation in shape change across an axis. CV1 captured variation ranging between a streamlined morphology and robust deep bodied morphology (Fig. 3.4A). Fish with a low CV1 score had a streamlined morphology with a shallow body, long head, long pectoral fin and large eye. Those with a high CV1 score had a robust deep bodied morphology with a short pectoral fin, small eye and shorter snout. CV2 depicted variation in eye structuring (Fig. 3.4B). Those with a low CV2 score had a shorter snout, short pectoral fin, smaller eye placed more dorsally on the head and a smaller mouth. CV3 summarised variation in head shape (Fig. 3.4C). Fish with a low CV3 score had a short head length, a short deeper curved snout and a short maxillary bone. Finally, CV4 captured variation in snout shape and eye size (Fig. 3.4D). Fish with a low CV4 score had a long shallow curved snout, small eye, long maxilla and long pectoral fin placed lower on the body.

	CAM	DRU	DRA	DUB	GRA	GIB	BUR	BUA	BUB	FAU	FAA	FAB	FOR	GLE	REA	REC	QUI	ROU	CAS	OWE	ROE
CAM		0.033	0.049	0.041	0.022	0.061	0.210	0.236	0.247	0.076	0.076	0.076	0.109	0.078	0.129	0.079	0.074	0.050	0.081	0.076	0.086
DRU	2.701		0.011	0.001	0.020	0.064	0.225	0.243	0.275	0.083	0.078	0.080	0.091	0.073	0.130	0.068	0.062	0.045	0.080	0.067	0.095
DRA	2.984	2.590		0.012	0.047	0.079	0.221	0.232	0.255	0.108	0.107	0.100	0.099	0.081	0.131	0.101	0.068	0.059	0.082	0.078	0.093
DUB	3.062	2.635	2.446		0.038	0.065	0.232	0.238	0.275	0.093	0.098	0.087	0.086	0.083	0.135	0.094	0.074	0.060	0.075	0.071	0.088
GRA	3.110	2.692	3.181	2.508		0.047	0.184	0.215	0.208	0.071	0.063	0.064	0.105	0.066	0.127	0.072	0.081	0.055	0.089	0.058	0.083
GIB	3.041	3.325	3.525	3.374	3.570		0.195	0.224	0.210	0.032	0.052	0.035	0.069	0.014	0.130	0.101	0.079	0.067	0.055	0.025	0.051
BUR	3.673	3.215	3.835	3.569	2.653	3.956		0.009	0.012	0.195	0.207	0.191	0.250	0.153	0.284	0.254	0.262	0.255	0.225	0.176	0.177
BUA	3.054	3.315	3.665	3.224	2.704	3.409	2.196		0.019	0.205	0.243	0.224	0.257	0.177	0.318	0.287	0.300	0.273	0.224	0.190	0.203
BUB	4.354	4.258	4.596	3.918	3.716	5.045	3.490	3.498		0.204	0.228	0.213	0.260	0.166	0.308	0.288	0.296	0.290	0.228	0.177	0.206
FAU	2.728	2.265	2.555	2.095	2.607	2.957	2.919	2.677	3.287		0.044	0.023	0.073	0.005	0.142	0.083	0.099	0.072	0.050	0.029	0.055
FAA	3.431	3.653	3.818	3.453	2.690	3.673	3.042	2.611	3.573	2.816		0.025	0.069	0.020	0.156	0.125	0.089	0.081	0.054	0.030	0.064
FAB	3.515	3.770	4.004	3.546	2.779	3.753	3.384	3.179	3.599	2.737	1.625		0.032	0.013	0.124	0.068	0.074	0.079	0.061	0.023	0.049
FOR	3.653	3.262	3.231	2.835	3.683	2.861	4.213	3.474	4.510	2.646	3.958	4.120		0.048	0.193	0.123	0.098	0.089	0.069	0.052	0.089
GLE	2.592	2.487	2.708	2.453	2.586	3.232	3.245	2.962	3.965	2.002	2.265	2.556	3.303		0.149	0.107	0.076	0.067	0.042	0.012	0.036
REA	2.130	2.880	2.628	3.117	3.055	3.504	3.188	3.071	4.518	2.763	3.013	3.472	4.076	2.145		0.026	0.093	0.063	0.143	0.136	0.153
REC	2.903	2.681	2.633	2.418	1.906	3.701	3.015	3.151	4.072	2.473	2.768	3.156	3.613	2.260	2.352		0.056	0.038	0.133	0.094	0.090
QUI	2.732	2.448	3.117	2.562	2.473	3.616	2.868	2.918	3.573	2.379	2.894	3.461	3.346	2.304	2.815	2.140		0.023	0.088	0.082	0.114
ROU	2.837	2.757	2.455	2.053	2.809	3.443	3.603	3.226	3.636	2.080	3.407	3.479	3.297	2.742	2.750	2.536	2.749		0.072	0.074	0.110
CAS	2.930	3.128	3.246	3.491	3.110	3.099	3.666	3.304	4.723	2.991	3.786	3.344	3.834	3.332	3.464	3.408	3.918	3.653		0.037	0.060
OWE	2.645	2.938	3.343	2.983	2.747	3.222	3.058	3.148	3.892	2.101	2.657	2.494	3.863	1.947	2.184	2.697	2.884	2.922	3.338		0.045
ROE	3.536	3.288	4.101	3.754	3.040	3.990	3.193	3.099	3.941	2.775	2.660	2.592	3.809	2.926	3.347	3.347	3.406	3.620	3.414	2.730	

Table 3.3: Heat map indicates the size of morphological and genetic distance between populations with large genetic differences highlighted in red and small differences in green. Values above are pairwise DJOST (genetic) distances between sampling sites; values below are pairwise mahalanobis (morphological) distances between sampling sites. Note all pairwise mahalanobis distances were significant. See table 3.1 for sampling site abbreviations.

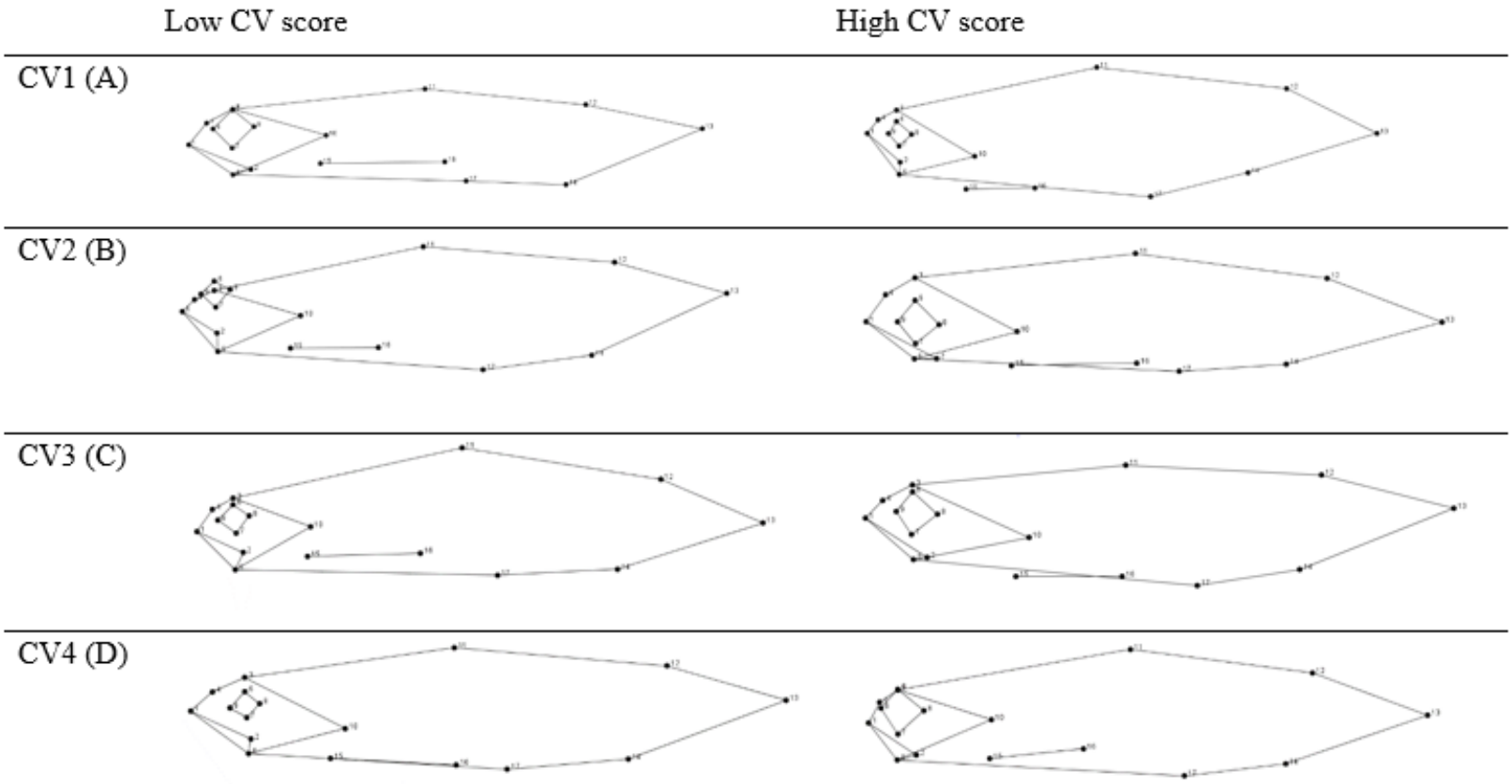

Fig. 3.4: Diagrammatic representation of morphology of brown trout explained by each CV shape extremes emphasised by a factor of two. A=CV1, B=CV2, C=CV3, D=CV4.

Spatial scale	Number of pairwise comparisons	Mean mahalanobis distance	Range of mahalanobis distances
Large (comparison between sub-catchments)	151	3.17	1.91-4.72
Medium (comparison between tributaries within sub-catchment)	49	3.11	1.95-5.04
Small (comparison of streams within tributaries)	10	2.64	1.62-3.50

Table 3.4: Mean and range of mahalanobis distance at three spatial scales investigated: large, medium and small.

3.4.2 ENVIRONMENTAL VARIABLES, GENETIC AND GEOGRAPHIC DISTANCE SHAPING MORPHOLOGICAL DIFFERENCES BETWEEN SITES

The effect of genetic population structuring and river distance on morphological structuring was tested using Mantel and Partial Mantel tests (Fig. 3.5). Mahalanobis distance between all morphological groups was correlated with genetic distance ($r=0.427$; $p=0.005$) and with river distance ($r=0.191$; $p=0.011$). When the effect of river distance was controlled for in a Partial Mantel test, mahalanobis distance (representing morphological distance between pairwise groups) was still correlated with genetic distance between morphological groups ($r=0.407$, $p=0.01$).

The influence of 31 landscape features on the morphological structuring of brown trout was investigated in the Foyle catchment (Table 3.2). Principle Component Analysis was used prior to any further analysis on instream substrate composition to account for correlations between substrate types which arise from the method used to calculate them. The first four principle components explained 80.7% of the variance and were retained for further analysis. These principle components derived from a PCA on the seven site specific substrate composition variables (Table 3.2) comprised substrate PC1- which is driven by high positive loading for cobble opposing high negative loadings for fines/sands; substrate PC2- which is driven by high positive loadings for mud opposed by high negative loadings

for gravel; substrate PC3 which is driven by high positive loadings for boulders opposed to high negative loadings for bedrock; substrate PC4 which is driven by high positive loadings for gravel opposed to high negative loadings for bedrock (Table 3.5).

The relationship between the difference in environmental variables between any two collection sites and the 'morphological distance' between groups was investigated using mixed models (Table 3.6, Fig. 3.6). Four models were run to find the best model which explained the variance in morphological distances between populations for four categories of environmental variables: instream habitat characteristic, water quality, geology and landscape features (Table 3.2). As genetic distance was established to correlate with 'morphological distance', one final model was run to determine whether genetic distance and/or between site differences in environmental variables were more important in driving morphological structuring as measured by the 'morphological distance' between groups. Therefore, the final model combined variables from the best models for each of the four categories of environmental variables and genetic distance as explanatory variables (Table 3.6; Fig. 3.6).

The model examining instream habitat characteristics ($Model_{Habitat}$) found that as the difference in substrate PC1 (T=5.40; p=<0.001) and substrate PC4 (T=2.82; p=0.008) increased, so did the morphological difference between populations. Substrate PC1 and PC4 together explained 6.5% of the fixed effect variance in morphological difference between populations. The model examining water quality variables ($Model_{Water}$) found no variables explained the fixed effect variance in morphological population structuring. The model which examined geological variables ($Model_{Geology}$) found that as the difference in river slope decreased (T=-2.92, p=0.004), the morphological difference between populations increased, whereas as the number of houses per km^2 in the upstream catchment (T=7.30, p=<0.001) increased, so did the morphological difference between populations. River slope and the number of houses per km^2 in the upstream catchment together explained 27.5% of the fixed effect variance in morphological difference between populations. The model which examined the landscape variables ($Model_{Landscape}$) found as the difference in area of peat (T=2.45, p=0.016) and of urban habitat (T=2.50, p=0.014) in the upstream catchment increased, the morphological difference between populations also increased. Peat and urban

habitat area in the upstream catchment together explained 7% of the fixed effect variance in morphological difference between populations (Table 3.6; Fig. 3.6).

The variables included in the best fitting models for each landscape category (instream habitat characteristics, water quality, geology and landscape features) were combined into a final model, along with genetic distance between morphological groups. The environmental factors which explained 44.7% of the variance in the morphological population structure of brown trout were differences in substrate PC1 (T=5.20, p<0.001) and substrate PC4 (T=3.65, p=0.001), stream order (T=2.14, p=0.035), elevation (T=2.47, p=0.018), slope (T=-4.39, p=<0.001) and number of houses per km^2 in the upstream catchment (T=6.01, p=<0.001). Although genetic distance between pairwise sites was included in the model and had previously been shown as an important factor driving morphological structuring, once the effects of these environmental variables were included genetic effects no longer contributed significantly to explain the variance in morphology between populations. Therefore, environmental factors were better predictors of variation in morphology between sites than were genetic differences (Table 3.6; Fig. 3.6).

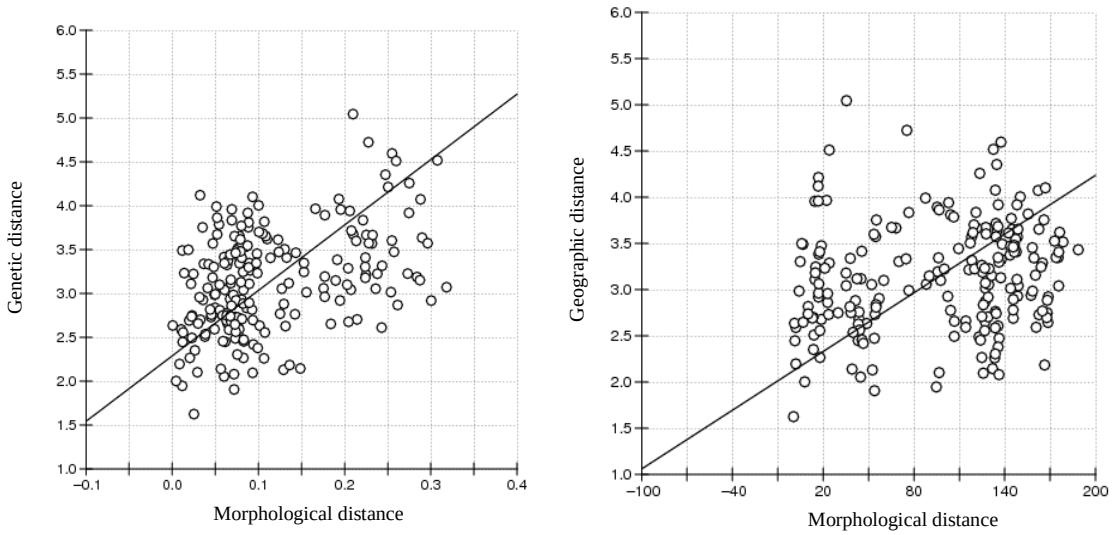

Fig 3.5: Graphic representation of mantel tests between shape distance (mahalanobis distance) and (left) genetic distance (D_{JOST}) and (right) geographic distance (km) for morphological groups.

Substrate type	PC1 loadings	PC2 loadings	PC3 loadings	PC4 loadings
Percentage of variance explained	31.43	21.94	16.31	11.04
Bedrock	0.109	0.341	-0.487	-0.692
Boulder	0.416	0.128	0.659	0.178
Cobble	0.453	0.129	-0.389	-0.096
Gravel	-0.260	-0.607	-0.289	0.441
Fines	-0.464	-0.144	0.292	-0.689
Sand	-0.464	0.418	-0.071	0.140
Mud	-0.334	0.535	0.052	0.399

Table 3.5: PC loadings of substrate type in the Foyle catchment and percentage of variance explained by each PC with 80.72% of total variance explained by PC1-4.

Best model	Fixed variables	Estimate	Std.Error	Df	T value	P value	R^2_M	R^2_C
$Model_{Habitat}$	Intercept	2.617	0.101	36.72	25.874	**<0.001**	0.196	0.496
	PC1 of substrate composition	0.200	0.037	145.68	5.401	**<0.001**		
	PC4 of substrate composition	0.099	0.035	31.62	2.821	**0.008**		
$Model_{Geology}$	Intercept	3.025	0.092	23.24	33.00	**<0.001**	0.275	0.664
	Stream order	0.061	0.035	144.73	1.766	**0.08**		
	Elevation	0.094	0.053	118.08	1.793	**0.08**		
	Slope	-0.161	0.055	95.80	-2.916	**0.004**		
	Number of houses per km^2 in upstream catchment	0.273	0.037	146.62	7.296	**<0.001**		
$Model_{Landscape}$	Intercept	2.753	0.118	44.38	23.27	**<0.001**	0.068	0.522
	Peat area	0.030	0.012	145.44	2.446	**0.016**		
	Urban area	0.853	0.341	121.62	2.504	**0.014**		
$Model_{Final}$	Intercept	2.674	0.083	30.01	32.306	**<0.001**	0.447	0.626
	PC1 of substrate composition	0.169	0.032	127.21	5.201	**<0.001**		
	PC4 of substrate composition	0.102	0.028	24.16	3.605	**0.001**		
	Stream order	0.067	0.031	116.83	2.136	**0.035**		
	Elevation	0.105	0.042	41.16	2.467	**0.018**		
	Slope	-0.200	0.046	56.90	-4.389	**<0.001**		
	Number of houses per km^2	0.205	0.034	93.03	6.012	**<0.001**		

Table 3.6: Mixed effect models output. $Model_{Habitat}$- included all pairwise differences in instream habitat variables and geographic distance; $Model_{Geology}$-included all pairwise differences in geology variables; $Model_{Landscape}$- included all pairwise differences in landscape features; $Model_{Final}$- final models included all variables deemed important from previous models and genetic distance between morphological groups.

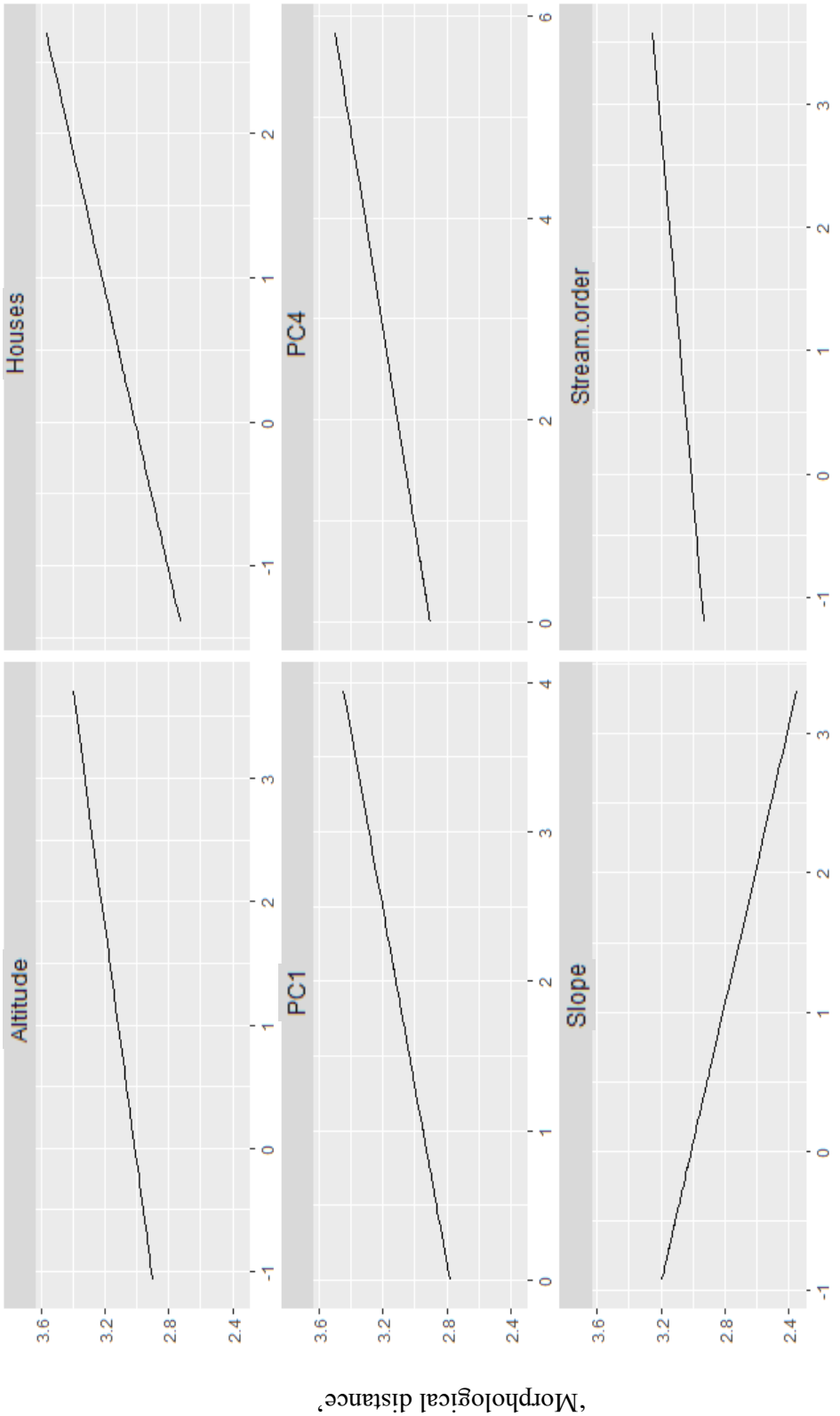

Fig. 3.6: Marginal effects of variables included in best model describing the effect of landscape variables on morphological structuring of brown trout ($Model_{Final}$). The predictor variables included in the final model were (from top to bottom): Elevation, number of house per km^2 in the upstream catchment area, substrate PC1, substrate PC4, slope and Stream order.

3.4.3 LANDSCAPE FEATURES SHAPING ABSOLUTE VARIATION IN SHAPE OF BROWN TROUT

The above analysis strongly points to landscape and environmental variables predicting morphological expression. Linear models were used to test for landscape and environmental effects on absolute variation in morphology. The morphology of brown trout from across the Foyle catchment was represented by CV 1-4 scores derived from a CVA (Fig. 3.4). For each CV, a linear model was run firstly for each category of landscape variables (using absolute values for instream habitat, water quality, geology and landscape features). Following this, a final linear model which contained all variables which were included in the best model for each category of landscape variable was analysed.

CV1 represented the change in morphology from a streamlined body shape to a robust-deep bodied morphology (Fig. 3.4A). It was found that elevation (T=-2.38, p=0.031) and the number of houses per km^2 (T=2.82, p= 0.013) in the upstream catchment best described the variance of CV1, explaining 52.7% of the shape variance ($F_{(2,15)}$=10.46; p=0.001) (Table 3.7). It was shown as elevation increased the morphology of brown trout tended towards a stream-lined body shape and as the density of houses increased, the morphology of brown trout tended towards a robust, deep bodied shape (Fig. 3.7). CV2, which represented shape variation in eye shape (Fig. 3.4B) was predicted by substrate PC1 (T=2.23, p=0.046), stream order (T=2.36, p=0.054), elevation (T=-3.52, p=0.004), the number of houses per km^2 (T=-2.94, p= 0.012) in the upstream catchment and upstream catchment area (T=-3.29, p=0.006) with 60.1% of shape variance explained ($F_{(5,12)}$=6.128; p=0.004) (Table 3.8). It was found that brown trout in higher order stream had a larger eye morphology. It was also shown that the brown trout had larger eyes in streams with a greater percentage of cobble substrate. Whereas, as elevation, the number of houses per km^2 and upstream catchment area increased, brown trout morphology tended towards having a smaller eyed morphology (Fig. 3.8). CV3, which represented variance in shape from a small headed morphology to a large headed morphology (Fig. 3.4C), was significantly driven by substrate PC1 (T=3.067, p=0.007) and substrate PC2 (T= -3.46, p=0.004), explaining 44.8% of shape variance ($F_{(4,13)}$=6.43; p=0.004). (Table 3.9). It was found that substrates rich in cobble and mud where associated with a larger headed morphology, in comparison to a smaller headed morphology which inhabited areas of gravel, fines and sands (Fig. 3.9). Finally, CV4, which represents shape variation in snout size (Fig. 3.4D), was significantly shaped by pH (T=-3.13, p=0.006) explaining 34.1% of shape variance ($F_{(3,14)}$= 6.95;

p=0.004) (Table 3.10). As pH decreased a longer snout and smaller eye was expressed (Fig. 3.10).

Best model	Variables	Estimate	Std. Error	T value	P value	R^2
Model$_{HABITAT}$	Intercept	-0.432	0.324	-1.332	0.204	0.434
	Bankside overhang	0.031	0.015	2.137	0.051	
	PC1 of substrate composition	-0.498	0.144	-3.459	**0.004**	
	PC4 of substrate composition	0.283	0.142	1.993	0.066	
Model$_{WATER}$	Intercept	25.207	15.976	1.578	0.139	0.210
	Biological Oxygen Demand	0.831	0.461	1.803	0.095	
	Phosphorus	21.490	13.074	1.644	0.124	
	Suspended solids	-0.363	0.145	-2.502	**0.027**	
	pH	-3.465	2.259	-1.534	0.149	
Model$_{GEOLOGY}$	Intercept	0.439	0.678	0.648	0.527	0.527
	Elevation	-0.010	0.004	-2.382	**0.031**	
	Number of houses per km^2	0.125	0.044	2.824	**0.013**	
Model$_{LANDSCAPE}$	Intercept	-0.045	0.440	-0.102	0.920	0.270
	Peat area	-0.100	0.053	-1.900	0.078	
	Urban area	3.281	1.611	2.037	0.061	
	Grass area	0.024	0.015	1.590	0.134	
Model$_{FINAL}$	Intercept	0.439	0.678	0.648	0.527	0.527
	Elevation	-0.010	0.004	-2.382	**0.031**	
	Number of houses per km^2	0.125	0.044	2.824	**0.013**	

Table 3.7: The absolute effect of environmental variables on group specific morphology (absolute variation in shape) explained by CV1 (streamlined morphology vs robust deep bodies morphology). Model$_{HABITAT}$- included all instream habiat variables; Model$_{WATER}$- included all water quality variables, Model$_{GEOLOGY}$- included all geological variables; Model$_{LANDSCAPE}$- included all landscape factors; Model$_{FINAL}$- final model included all variables from best models selected by AICc from previous four models.

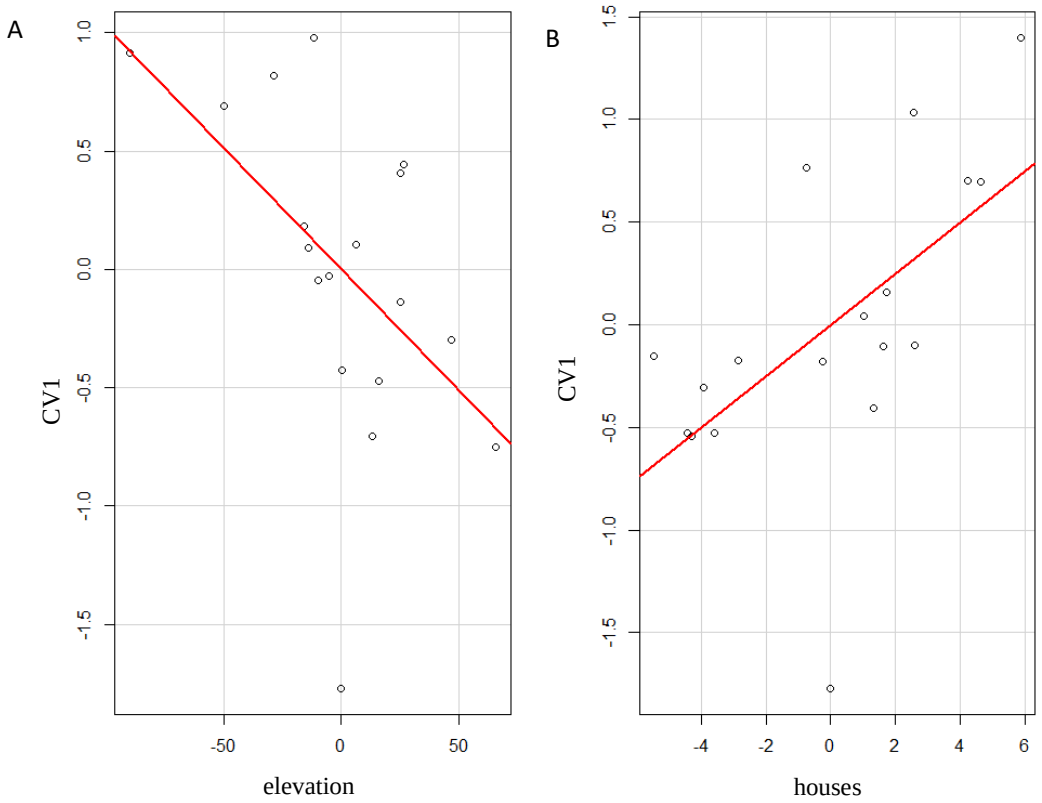

Fig. 3.7: Added variable plot, showing the relationship between the response variable and the predictors in a regression model, after controlling for the presence of the other predictors. In this case, the above added variable plots show the relationship between predictor variables (elevation (A) and Number of houses per km^2 in the upstream catchment (B)) which significantly explain the variation in absolute morphology explained by CV1, where a low CV score indicates a streamlined morphology.

Best model	Variables	Estimate	Std. Error	T value	P value	Adjusted R^2
Model$_{HABITAT}$	Intercept	0.912	0.438	2.083	0.055	0.299
	Depth	-0.061	0.033	-1.845	0.085	
	PC1	0.201	0.146	1.376	0.189	
Model$_{GEOLOGY}$	Intercept	2.259	0.708	3.189	**0.007**	0.480
	Stream order	0.513	0.243	2.112	0.055	
	Elevation	-0.013	0.005	-2.738	**0.017**	
	Houses	-0.163	0.042	-3.928	**0.002**	
	Area	-0.029	0.010	-2.846	**0.014**	
Model$_{LANDSCAPE}$	Intercept	0.605	0.354	1.707	0.107	0.063
	Grass	-0.018	0.013	-1.462	0.163	
Model$_{FINAL}$	Intercept	2.311	0.621	3.724	**0.003**	0.601
	PC1	0.280	0.126	2.227	**0.046**	
	Stream order	0.457	0.214	2.36	**0.054**	
	Elevation	-0.015	0.004	-3.519	**0.004**	
	Houses	-0121	0.041	-2.943	**0.012**	
	Area	-0.029	0.009	-3.291	**0.006**	

Table 3.8: The absolute effect of environmental variables on group specific morphology (absolute variation in shape) explained by CV2 (small eyed morphology vs large eyed morphology). Model$_{HABITAT}$- included all instream habiat variables; Model$_{WATER}$- included all water quality variables, Model$_{GEOLOGY}$- included all geological variables; Model$_{LANDSCAPE}$- included all landscape factors; Model$_{FINAL}$- final model included all variales from best models selected by AICc from previous four models. Note stream order was included in Model$_{FINAL}$ as significant as the p-value was only slightly greater than 0.05.

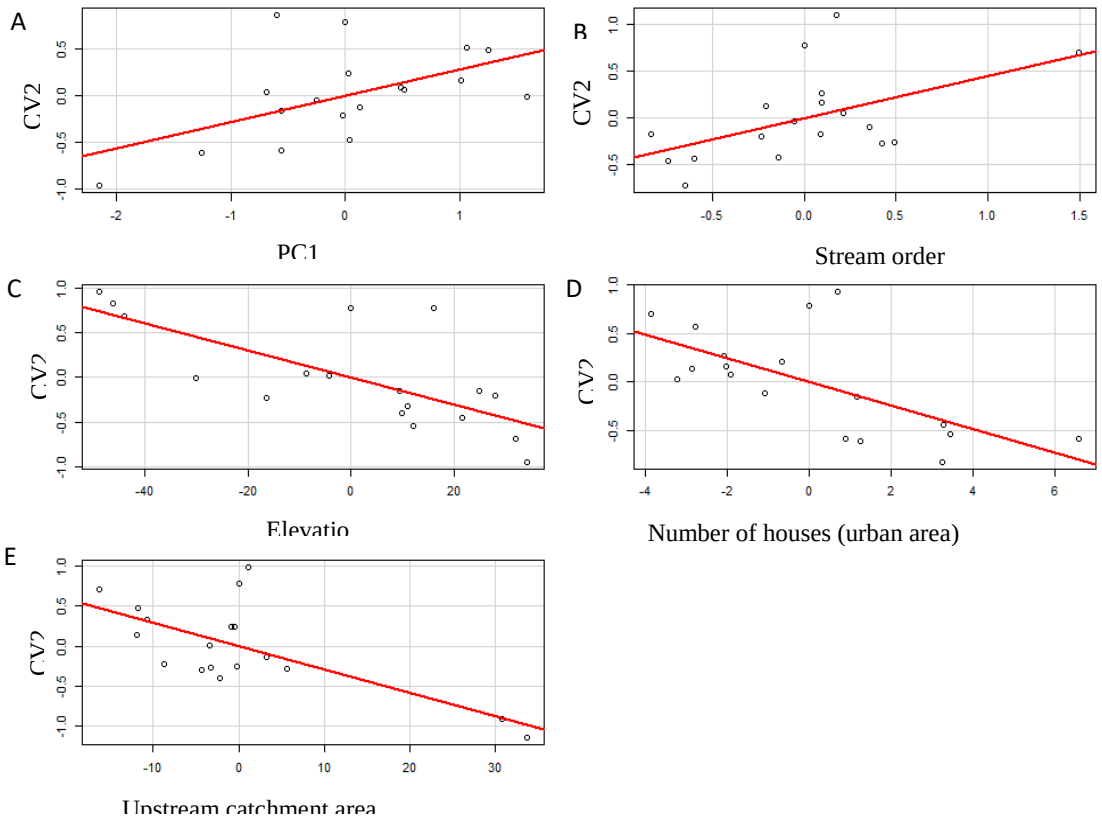

Fig. 3.8: Added variable plots of the relationship between environmental factors which significantly explain the variation in absolute morphology explained by CV2, where a low CV score indicates a morphology with a shorter snout, short pectoral fin, smaller eye placed more dorsally on the head and a smaller mouth. The predictor variables included in the final model were: A-PC1, B- Stream order, C- Elevation, D- Number of houses per km^2 in the upstream catchment, E- upstream catchment area.

Best model	Best model	Variables	Std.Error	T value	P value	R^2
Model$_{HABITAT}$	Intercept	0.055	0.151	0.365	0.721	0.500
	PC1	0.455	0.135	3.364	**0.005**	
	PC2	-0.843	0.219	-3.845	**0.002**	
	PC4	0.242	0.152	1.591	0.134	
Model$_{WATER}$	Intercept	-13.396	8.348	-1.605	0.128	0.085
	pH	1.864	1.160	1.607	0.128	
Model$_{GEOLOGY}$	Intercept	-2.042	0.610	-3.350	**0.004**	0.003
	Stream order	0.818	0.234	3.496	**0.003**	
Model$_{FINAL}$	Intercept	0.118	0.153	0.774	0.451	0.448
	PC1	0.433	0.141	3.067	**0.007**	
	PC2	-0.630	0.182	-3.456	**0.004**	

Table 3.9: The absolute effect of landscape variables on group specific morphology (absolute variation in shape) explained by CV3 (small headed morphology vs large headed morphology). Model$_{HABITAT}$- included all instream habiat variables; Model$_{WATER}$- included all water quality variables, Model$_{GEOLOGY}$- included all geological variables; Model$_{LANDSCAPE}$- included all geomorphological variables; Model$_{FINAL}$- final model included all variables from best models selected by AICc from previous four models.

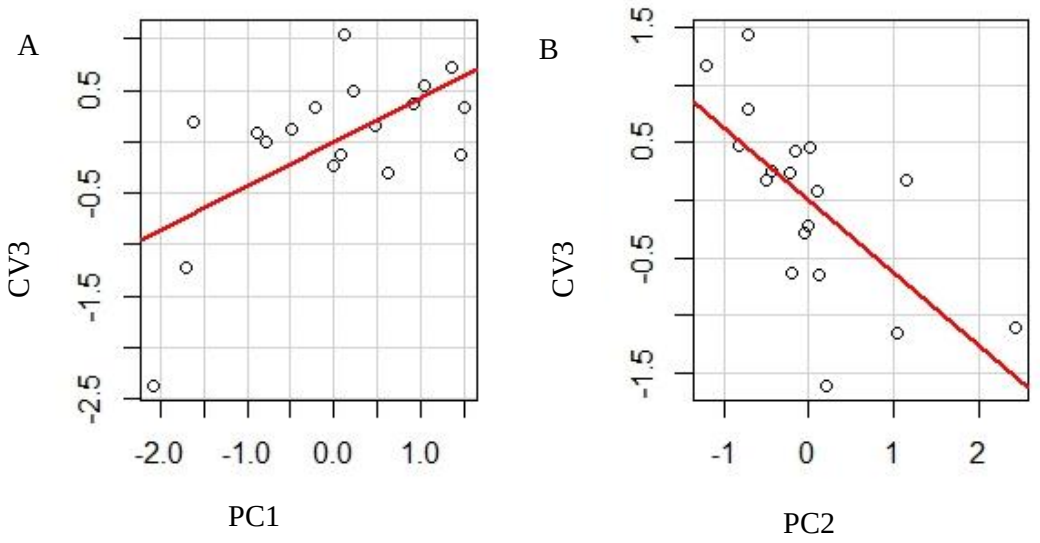

Fig. 3.9: Added variable plots of the relationship between environmental factors which significantly explain the variation in absolute morphology explained by CV3. Fish with a low CV score had a short head length, a short deeper curved snout and a short maxillary bone. The predictor variables included in the final model were: A- PC1, B-PC2.

Best model	Variables	Estimate	Std.Error	T value	P value	R^2
Model$_{HABITAT}$	Intercept	0.179	0.104	1.719	0.105	0.282
	PC3	0.223	0.081	2.772	**0.014**	
Model$_{WATER}$	Intercept	13.631	4.302	3.168	**0.006**	0.341
	pH	-1.872	0.598	-3.132	**0.006**	
Model$_{LANDSCAPE}$	Intercept	0.327	0.257	1.272	0.224	0.195
	Peat	-0.055	0.034	-1.651	0.121	
	Alluvium	-0.360	0.148	-2.432	**0.029**	
	Grass	0.026	0.011	2.289	**0.038**	
Model$_{FINAL}$	Intercept	13.631	4.302	3.168	**0.005**	0.341
	pH	-1.872	0.598	-3.132	**0.006**	

Table 3.10: The absolute effect of landscape variables on group specific morphology (absolute variation in shape) explained by CV4 (short nose large eye morphology vs long nose large eyed morphology). Model$_{HABITAT}$- included all instream habiat variables and geographic distance; Model$_{GENETIC}$- morphological distance versus genetic distance, Model$_{GEOLOGY}$- included all geological variables; Model$_{LANDSCAPE}$- included all geomorphological variables; Model$_{FINAL}$- final model included all variables from best models selected by AICc from previous four models.

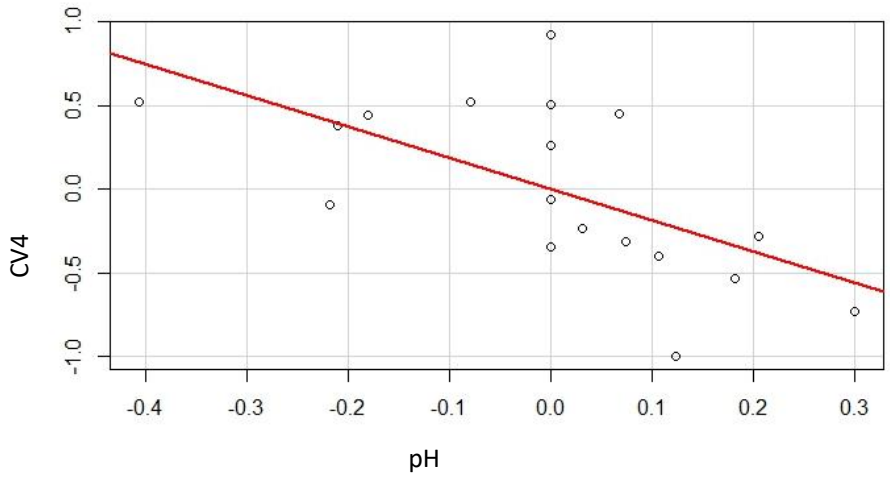

Fig. 3.10: Added variable plots of the relationship between environmental factors which significantly explain the variation in absolute morphology explained by CV4. Fish with a low CV4 score had a long shallow curved snout, small eye, long maxilla and long pectoral fin placed lower on the body. The predictor variable included in the final model was pH.

3.5 DISCUSSION

3.5.1 MORPHOLOGICAL STRUCTURING AND ENVIRONMENTAL DRIVERS OF ABSOLUTE SHAPE VARIATION

This study investigated the morphological structuring of brown trout in the Foyle catchment across 21 sampling sites. CVA revealed the 21 sampling sites formed 21 morphologically distinct groups which were significantly different from one another (Table 3.3). These morphologically distinct populations were differentiated by a continuum of morphological variation between populations represented across four CV axes from CVA (Fig. 3.4). CV1 represented variation between a streamlined morph and a robust deep bodied morph. CV2 captured variation in eye shape with a low CV2 score representing fish with a smaller eye placed more dorsally on the head. CV3 summarised variation in head shape and CV4 captured variation in snout shape and eye size with a low CV score representing fish with a short nose large eye morphology (Fig. 3.4). The relationship between environmental variables driving shape variation between morphological groups was then investigated using linear models.

Several environmental factors were found to drive morphological variation of brown trout in the Foyle catchment. Elevation and number of houses per km^2 in the upstream catchment (representative of urban area) was found to drive morphological variation described by CV1. At higher elevations and in areas with lower densities of houses (less urbanised) a more streamlined morphology of brown trout was expressed. Shallow, faster flowing waters are expected at higher elevations were a stream-lined morphology would be advantageous (Pakkasmaa & Piironen 2001b). A streamlined morphology in this study also had long pectoral fins which would be used to maintain the fish's position in fast-flowing waters (Pakkasmaa & Piironen 2001). In comparison at lower elevations where there was a higher density of houses waters would be slower flowing and deeper as they near the lower reaches of the river (Taylor & McPhail, 1985; Swain & Holtby, 1989; Zhen-Ghan 2017). Therefore, a deep bodied robust morphology may be more advantageous. A similar effect has been demonstrated in several other studies which have examined the relationship between flow regimes and body morphology in brown trout and other species of salmonids (Riddell & Leggett 1981; Taylor & Foote 1991; Pakkasmaa & Piironen 2001a; Langerhans 2008; Stelkens et al., 2012; Drinan et al., 2012). For example, body morphology and time of downstream migrations differed significantly between two populations of Atlantic salmon

in the Miramichi River, New Brunswick and was driven by differences in flow regime (Riddell & Leggett 1981). Another study found morphological differences in body morphology of brown trout, specifically body height and fin sizes, where driven by differences in water velocity and are likely to be of functional importance (Pakkasmaa & Piironen 2001b).

The landscape variables, cobble and fines/sand substrate composition, elevation, stream order, number of houses per km^2 upstream catchment area, were responsible for driving a divergence in eye shape (CV2). Higher order streams with a substrate composition dominated by cobble would be found at lower elevations where there is a higher density of houses (Zhen-Ghan 2017). Therefore, the morphology of populations at lower elevations had larger eyes. Although there is limited literature examining the relationship between eye size and the environmental characteristics identified in river systems, there has been work, particularly in the Arctic charr (*Salvelinus alpinus*), in lakes which have examined morphology variation and prey type (Lavin & McPhail 1986; Walker et al., 1988; Snorrason et al., 1994; Adams et al., 1998; Alekseyev et al., 2002). For example, a study on three discrete morphs of Arctic charr in Loch Rannoch, Scotland were differentiated by head length, depth and eye diameter (Adams et al., 1998). This study demonstrated the morph of Arctic charr with the largest eyes had a different diet, primarily feeding on larger benthic macro-invertebrate species and other fish, whereas Arctic charr with smaller eyes fed on zooplankton (Adams et al., 1998). Therefore, a difference in diet could explain the difference in eye size between morphological populations in this study.

The landscape variable which drives the morphological variation explained by CV3, representing head shape variation, was substrate composition, specifically differences in the proportion of cobble, fines/sand, gravel and mud. In areas where substrate was composed of cobble and mud a larger headed morphology was present in comparison to areas where substrate composition was mostly fines, sands and gravel were a smaller headed morphology was adopted. Whereas, pH drove the variation in snout size represented by CV4. In areas with a more alkaline pH a large eyed, short snouted morphology was adopted. There has been little literature which has examined the relationship between head morphology and substrate composition or pH in river systems. However, it is likely that different substrate compositions support different macro-invertebrate communities thus, head morphology is

driven by differences in prey items (Culp et al., 1983; Erman & Erman 1984; Brown & Brussock 1990; Quinn & Hickey 1990; Bourassa & Morin 1995; Fraser et al., 1998; Siwertsson et al., 2013). Moreover, a higher pH is often found in chalk streams which are shallow and clear, where a shorter snout would be advantageous as prey sizes are smaller (Ormerod et al, 1985; Ormerod et al., 1987). There have been many studies which have examined head morphology of fish and prey items in lakes (Skúlason et al., 1989; Snorrason et al., 1994; Kahilainen & Østbye 2006). For example, head morphology of Arctic charr in lake Thingvallavatan, Iceland was related to feeding habitats. Common garden experiments also revealed these morphological differences also had a genetic basis with significant maternal effects (Skúlason et al., 1989). In another common garden experiment two species of *Geophagus* (Pisces: Chiclidae) showed variation in snout morphology when fed on a diet of ferine shrimp nauplii or chironomid larvae (Wimberger 1992). Therefore, it is possible that the differing substrate composition and pH supports different communities of macro-invertebrates driving head morphology of brown trout.

Therefore, this suggests morphological variation of brown trout in the Foyle catchment varies between built up areas in the lower reaches of rivers which are often deeper but also have higher levels of anthropogenic impacts in comparison to areas higher in river catchments which are smaller, faster flowing streams less likely to be impacted by humans. The variation in morphology between groups enable brown trout to exist in a wide range of habitats and as has been demonstrated the differing morphologies of brown trout enables them to exist in different flow regimes through their swimming ability and their head shape varied to feed on different prey items.

3.5.2 MORPHOLOGICAL STRUCTURING AND ISOLATION BY DISTANCE

This study has been shown absolute shape variation is driven by environmental variables, therefore, the next aim of this study was to disentangle whether geographic distance, genetic and/or environmental factors where driving morphological structuring. The 21 morphological populations found in this study represented three spatial scales (large, medium and small (Fig. 3.2)). The mean mahalanobis distance between pairwise morphological groups varied between spatial scales, with the mean mahalanobis distance being 3.17 at the largest spatial scale, 3.11 on a medium spatial scale and 2.64 at the smallest spatial scale. Therefore, morphological groups which are in different sub-catchments show

the greatest morphological differences, whereas, morphological groups located in different streams (small spatial scale) within the same tributary show the smallest morphological differences. This would indicate that isolation by distance was structuring morphological populations of brown trout in the Foyle catchment and was confirmed using Mantel tests (r=0.191; p=0.011) (Fig. 3.5).

There have been many studies which have examined morphological variability between brown trout populations (Wysujack et al., 2009; Vehanen & Huusko, 2011; Drinan et al., 2012; Stelkens et al., 2012; Westley et al., 2013). However, there have been relatively few studies which have examined the morphological structuring of brown trout populations within river systems and across varying spatial scales (Stelkens et al., 2012). One example of a study which has examined this relationship on a micro-geographic scale was in the River Aare, Switzerland. This study found pairwise geographic distance between sampling locations positively correlated with morphological variation (Stelkens et al., 2012). The relationship between morphological structuring and geographic distance has been investigated in other species of fish (Adams et al., 1998; Valentin et al., 2014; Adams et al., 2016). For example, morphological variation was investigated between ten populations of Coho salmon (*Oncorhynchus kisutch*) in British Columbia where it was found juvenile populations in coastal tributaries displayed a different morphology to juvenile populations further inland (Taylor & McPhail 1985). Therefore, this relationship between geographic distance and morphological structuring would suggest there is either environmental and/ or genetic variables which are resulting in isolation between morphological populations.

3.5.3 MORPHOLOGICAL STRUCTURING AND GENETIC DIFFERENTIATION

Partial mantel tests were used to investigate whether genetic structuring drives morphological structuring of brown trout in the Foyle catchment. In chapter two it was demonstrated that isolation by distance was a significant driver of genetic population structuring of brown trout. Therefore, partial mantel tests were used to determine if genetic distance was correlated with morphological structuring when geographic distance was controlled for. These showed that genetic distance indeed was a driver of morphological structuring of brown trout in the Foyle catchment. There are few studies on brown trout which have investigated the relationship between morphological structuring and genetic population structuring (Stelkens et al., 2012). However, studies which have examined this

relationship in brown trout have found different results. For example, in Lough Melvin it was found three morphotypes (ferox, sonaghen and gillaroo) were genetically differentiated as well as being morphologically differentiated (Ferguson & Taggart 1991). In contrast, within the River Aare, Switzerland, no relationship was found between genetic structuring of brown trout and morphological structuring (Stelkens et al., 2012). There have been slightly more studies examining the relationship between morphological differentiation and genetic population structuring in other species of fish, especially salmonids, but these studies tend to be between morphological groups within lakes (Ferguson & Taggart 1991; Varian & Nichols 2010; Valentin et al., 2014; Adams et al., 2016). For example, European whitefish (*Coregonus lavaretus*) in Loch Eck and Loch Lomond, Scotland showed both morphological and genetic differentiation (Adams et al., 2016). This relationship between neutral genetic differentiation and morphological structuring can provide insights into early stages of divergence (Adams et al., 2016). Processes such as genetic drift, founder effects, chance mutations or natural selection could have led to populations which are genetically, and as a result, morphologically differentiated (Ortego et al., 2015). Therefore, scenario one (morphological structuring is shaped by genetic population structuring) is evident, which may suggest to some extent the morphological traits observed are the result of divergent natural selection (Ortego et al. 2015).

3.5.4 MORPHOLOGICAL STRUCTURING AND ENVIRONMENTAL VARIABLES

Genetic differentiation between populations was more pronounced at larger spatial scales, therefore, it is likely that environmental variation was also a driver of morphological structuring. This was investigated using mixed models. This demonstrated that differences in substrate composition between groups, specifically fines, sands and cobbles, were significantly positively correlated with 'morphological distance' between groups. As the difference in the number of houses per km^2, area of peat and urban area in the upstream catchment increased so did the 'morphological distance' between groups. However, as the difference in river slope between groups increased the 'morphological distance' decreased.

There have been few studies which have examined the relationship between environmental variables and morphological structuring in brown trout (Riddell & Leggett 1981; Taylor & Foote 1991; Pakkasmaa & Piironen 2001; Langerhans 2008; Stelkens et al., 2012; Drinan et al., 2012). The River Aare study found total length of subterranean

canalization correlated with morphological distance between populations (Stelkens et al., 2012). Another study examined morphological differentiation of brown trout with flow regime in the Burrow and Burrishoole Rivers and found brown trout in faster flowing waters had a more streamlined morphology compared to those in slower flowing waters (Drinan et al., 2012). However, there have been more studies which have examined this relationship in other species of salmonids (Lavin & McPhail 1986; Hindar & Jonsson 1993; Obedzinski & Letcher 2004; Garduño-Paz et al., 2012; Adams et al., 2016). These results would suggest phenotypic plasticity is a driver of morphological differentiation between populations of brown trout in the Foyle catchment. The environmental characteristics highlighted in this study as drivers of morphological differentiation between populations were substrate composition and anthropogenic drivers associated with habitat fragmentation (Wang et al., 2001). There have been studies which have investigated the influence of anthropogenic impacts on fish morphology (Haas et al., 2010; Franssen 2011). These studies have shown that habitat alteration by human impact could be a factor which is driving trait divergence in fish (Franssen 2011). Therefore, scenario two (morphological structuring is shaped by environmental factors) is evident, which may suggest to some extent the morphological traits observed are the result of phenotypic plasticity (Ortego et al. 2015).

3.5.5 IS NEUTRAL GENETIC DIFFERENTIATION OR ENVIRONMENTAL VARIABLES MORE IMPORTANT IN DRIVING MORPHOLGICAL STRUTCURING IN BROWN TROUT?

Finally, as this study has highlighted, both neutral genetic differentiation and environmental factors can drive morphological variability between populations of brown trout. However, to establish if genetic distance and environmental variables both explain the morphological structuring of brown trout a final mixed model was run with included genetic distance between morphological populations and the environmental variables included in the best model for each category of environmental variables (Table 3.6; Fig. 3.6). Genetic distance between morphological groups was not included as a significant variable in the final model, demonstrating that although genetic distance did correlate with morphological distance, landscape variables (specifically substrate PC1, substrate PC4, stream order, slope, elevation and number of houses per km^2) were more important in explaining morphological structuring. This is particularly evident on a small spatial scale where morphological groups were formed within the same genetic population. For example, in chapter two it was shown the River Drumnakilly contained one genetic population (DRU) but this chapter has shown there are three morphological groups (DRU, DRA, DRB). Therefore, the effect of genetic

population structuring or environmental variables may be partially affected by spatial scale. Morphological structuring of groups separated by large geographic distances is driven more by genetic distance than structuring of morphological groups separated by a short geographic distance which is driven more by landscape variables.

Therefore, the evidence of this study is that scenario three, a combination of both genetic and environmental factors, drives morphological divergence between groups but is scale dependent with genetic differentiation and environmental factors driving morphological structuring across medium and large spatial scales. In contrast, environmental factors are responsible for driving morphological structuring on a small spatial scale. This further suggests local adaptation may play a role in the morphological structuring of brown trout at larger spatial scales (Taylor 1991). In comparison, at smaller spatial scales this study would suggest phenotypic plasticity is likely to drive morphological differentiation between populations (Stelkens et al., 2012). Determining morphological and genetic structuring of a species can give insights into the evolutionary processes which drive very early stages of divergence. As anthropogenic impacts, such as climate change, alter freshwater habitats, it is increasingly important to understand both phenotypic plasticity and local adaptation of a species to determine how populations will respond to these anthropogenic impacts (Drinan et al. 2012).

CHAPTER FOUR: GENETIC STRUCTURING ACROSS ALTERNATIVE LIFE HISTORY STRATEGIES AND SMALL SPATIAL SCALES IN BROWN TROUT (*SALMO TRUTTA*).

4.1 ABSTRACT

Partial migration occurs when some individuals in a population migrate but others do not. The brown trout (*Salmo trutta*) is a species that exhibits partial migration where in some populations a variable proportion of the population migrates to sea to feed, whilst the other individuals complete their life cycle in fresh water. This study attempts to separate two apparently alternative hypotheses for the population structuring that underpins the expression of partial migration in this species: a) that migrants and residents comprise two distinct gene pools; b) that individual genetic variation or individual variation in gene-environment interactions is responsible for the expression of different life history strategies. This study identified five genetic populations from three sampling locations with no evidence of population structuring being attributed to life history strategy but rather differences were based on geographical site. Two sympatric populations were identified in each of the River Muff and Killen Burn. Although both populations in each river were composed of freshwater resident and anadromous brown trout, the frequency of each life history strategy significantly differed between rivers. Therefore, this study supports the concept that partial migration in brown trout is most likely driven by a quantitative threshold trait, where the threshold value varies both between populations and between individuals within populations.

4.2 INTRODUCTION

Partial migration, one of the most common migratory patterns observed in nature, is defined as when some individuals in a population migrate whilst others do not (Dingle & Drake, 2007; Chapman et al., 2011). Depending upon the form of partial migration individuals from resident or migratory life history strategies are most likely to segregate spatially either for foraging or breeding (Chapman et al., 2011). A wide range of species across multiple taxa exhibit some form of partial migration, see for example birds: Lapwings (*Vanellus vanellus*) (Lundberg, 1988), mammals: moose (*Alces alces*) (Ball et al., 2001) and reptiles: green turtles (*Chelonia mydas*) (Mortimer & Carr, 1987). However, even in the best studied systems there is only an incomplete understanding of the mechanisms driving

individuals towards one of the alternative life history strategies (Chapman et al., 2011; Dodson et al., 2013). The brown trout (*Salmo trutta*) is an example of a species which exhibits non-breeding, partial migration (migrants and non-migrants breed in sympatry but forage in different habitats for much of their life cycle) (Hendry et al., 2003; Klemetsen et al., 2003; Charles et al., 2004; Wysujack et al., 2009). Brown trout express a number of different life history strategies which involve migrating across varying geographic distances and differing habitats (Jonsson & Jonsson, 1993). The two most extreme life history strategies are freshwater river residency (where individuals remain in the river system throughout their entire lifecycle) and anadromy (where individuals migrate to sea to feed before returning to breed in their natal rivers) (Samuilovienė & Kontautas, 2012). The decision that initiates the adoption of a particular life history strategy is most likely controlled by a quantitative threshold trait in brown trout (Ferguson 2006; Ferguson et al 2016). It is presumed that this operates as a genetically predefined threshold value, controlled by one or more loci, which is sensitive to a continuously varying environmental cue, such as food availability and/or temperature, and which will ultimately influence which life history strategy is adopted (Ferguson 2006). A low threshold value would trigger physiological and behavioural processes resulting in anadromy and a high threshold value would result in freshwater residency (Roff et al., 1996; Dodson et al., 2013; Ferguson et al., 2016).

As a consequence of homing behaviour to natal breeding sites, brown trout have been shown to form fragmented genetic populations across relatively short geographic distances (Crozier & Ferguson, 1986; Ferguson, 1989, 2006; Bernatchez et al., 1992; Carlsson et al., 1999; Massa-Gallucci et al., 2010; Swatdipong et al., 2010). In some places this leads to more than one brown trout population co-existing in sympatry (Bernatchez et al., 1992; Carlsson et al., 1999; Stelkens et al., 2012). The composition of sympatric (here defined as co-habiting during the breeding season) freshwater resident and anadromous brown trout might result from either of two scenarios. In the first scenario, freshwater resident and anadromous brown trout comprise two separate genetic groups. Under this hypothesis, a population difference in threshold value for anadromy drives the expression of alternative life history strategies. One population would have a lower average genetically predefined threshold trait value, promoting a high incidence of anadromy, and the second population would have a higher average genetically predefined threshold trait value promoting a high incidence of freshwater residency. At its extreme, this would result in one population

expressing only anadromous brown trout and the other only freshwater resident brown trout (Chapman et al., 2011; Pulido 2011; Dodson et al., 2013).

An alternative scenario is that sympatric anadromous and freshwater resident brown trout comprise a single gene pool. In this scenario, the two life history strategies are maintained by individual variation in a single population (Pulido 2011; Chapman et al., 2011; Dodson et al., 2013). Under this model, it is individual variation regarding the interaction between the gene(s) responsible for migration and the environment that results in partial migration. Individual fish drawn from a common gene pool differ in the genetically predefined threshold value for the expression of migration or in their exposure to environmental variables that result in the threshold being reached, or a combination of both (Chapman et al., 2011).

There have been several studies which have investigated whether sympatric, co-habiting freshwater resident and anadromous brown trout originate from the same or separate gene pools. Most studies have shown that sympatric anadromous and freshwater resident brown trout are from one gene pool (Fleming et al., 1983; Hindar et al., 1991; Cross et al., 1992; Petersson et al., 2001). However, there have been a few studies which have demonstrated sympatric anadromous and freshwater resident originate from separate gene pools (Krieg & Guyomard, 1985; Skaala & Naevdal, 1989). Discriminating between these two possibilities is a challenge. It is also often difficult to properly differentiate between individuals adopting alternative life history strategies, which is often only possible once anadromous brown trout have begun the metamorphosis (smolting) prior to sea migration. Taking samples from juvenile brown trout before external indicators of transformation have begun to show can lead to misidentification of life history strategy (Ferguson 2006). This has led to difficulties in discriminating between alterative explanations for partial migration in brown trout.

The aim of the work described here is to discriminate between the two alternative explanations for partial migration. Specifically, this study examines the effect of within-river genetic population structuring and geographic location on the adoption of alternative life history strategies by brown trout within the Foyle catchment, Ireland.

4.3 METHODS

4.3.1 COLLECTION OF SAMPLES

The River Foyle is a large dendritic river catchment of around 4500km^2 (Fig. 4.1) (Dauphin et al., 2010). Four sites, from across the Foyle catchment, were selected for detailed examination of structuring of sympatric anadromous and freshwater brown trout (Fig. 4.1). At each sampling location, samples from anadromous and freshwater resident brown trout were collected by electrofishing the same area of river (~1km in length) in both 2013 and 2014 (Table 4.1). Anadromous brown trout were sampled by electrofishing in April/May and freshwater resident brown trout were sampled in June-August. Samples were only collected from brown trout which met the following criteria as either anadromous or freshwater resident: anadromous brown trout were defined by a silvering on the epidermis and an elongated body, while freshwater residents were defined as brown trout which lacked silvering on their epidermis retaining their juvenile colouration and had a fork length (mm) greater than the longest anadromous brown trout caught at each individual site each year (Fig. 4.2) (Le Gentil et al., 2013). In total 226 fish were classified (82 as freshwater resident and 144 as anadromous) and anaesthetised using clove oil. A non-destructive (fin-clip) tissue sample was collected and stored in 95% ethanol. A reference photograph was taken of each brown trout, fork length (mm) and weight (g) were measured and scale samples were collected.

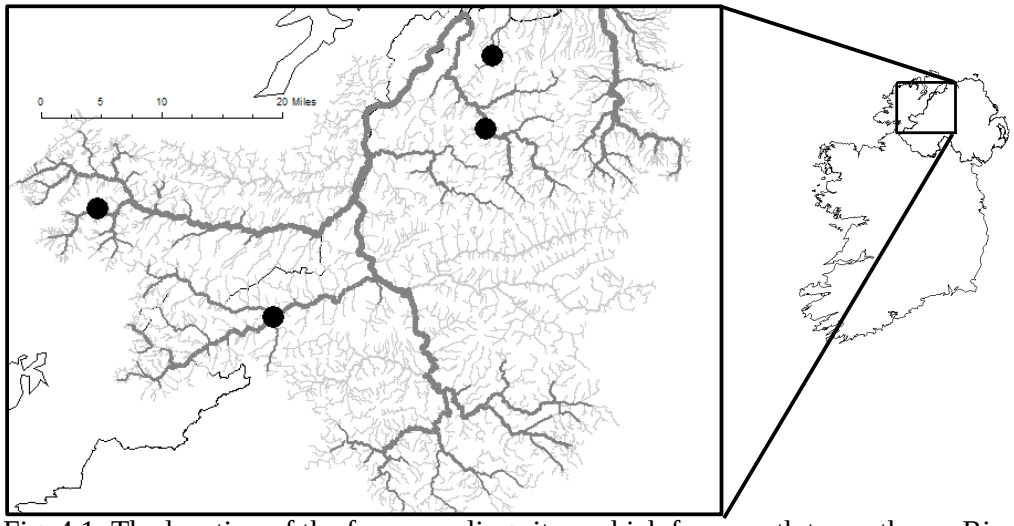

Fig. 4.1: The location of the four sampling sites, which from north to south are: River Muff, Bonds Glen, River Reelan and Killen Burn.

River	Year sampled	Life History	Sample size (N)	Fork length range (mm)
Bonds Glen	2013	Freshwater resident	15	186-226
Bonds Glen	2013	Anadromous	3	154-185
Bonds Glen	2014	Freshwater resident	5	196-236
Bonds Glen	2014	Anadromous	17	133-196
River Muff	2013	Freshwater resident	18	152-186
River Muff	2013	Anadromous	36	110-151
River Muff	2014	Freshwater resident	19	172-245
River Muff	2014	Anadromous	51	111-170
Killen Burn	2013	Freshwater resident	17	205-250
Killen Burn	2013	Anadromous	5	165-205
Killen Burn	2014	Freshwater resident	7	228-298
Killen Burn	2014	Anadromous	24	141-227
River Reelan	2013	Freshwater resident	1	250
River Reelan	2013	Anadromous	8	154-215

Table 4.1: The number and fork length range for freshwater resident and anadromous brown trout samples collected from each site.

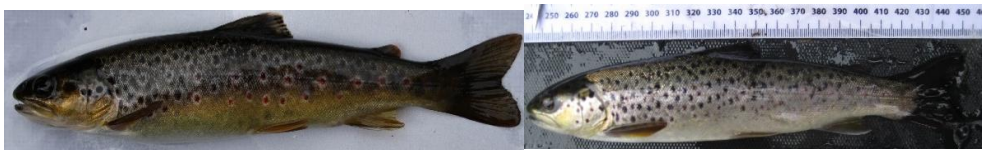

Fig. 4.2: Examples of life history classification. Photograph on left is an example of a freshwater resident brown trout phenotype and the photograph on right is example of an anadromous brown trout phenotype.

4.2.2 MICROSATELLITE AMPLIFICATION

Genomic DNA was extracted following methods described in Chapter two and genotyped using the same suite of microsatellite markers (see appendix). Twenty-one microsatellite markers were initially screened, however, SsaD48 was removed from the analysis due to inconsistences in banding patterns making it unreliable.

4.2.3 STATISTICAL ANALYSIS

The genetic population structure of anadromous and freshwater resident brown trout was established using a Bayesian statistical framework implemented in STRUCTURE (Pritchard et al., 2000). STRUCTURE was run hierarchically to reveal cryptic structuring based on sampling site and life history type, using 100 000 Markov Chain Monte Carlo steps after a burn-in period of 100 000. Runs were performed for 1 to 10 clusters (K) with 20 iterations for each individual sample. STRUCTURE Harvester (Earl & vonHoldt 2012) was then used to determine ΔK using the *ad hoc* method used by Evanno et al. (2005) and the most likely value of K was calculated. CLUMPP (Jakobsson & Rosenberg 2007) was used with the "Greedy" method to consolidate the probability of each individual belonging to each cluster from the 20 iterations used in STRUCTURE. The resulting clusters were visualised using ArcGISv10.2 (ESRI 2011). The genetic distance between populations was then calculated using the R package 'diveRsity' and was calculated using D_{JOST} as opposed to F_{ST} (Keenan et al., 2013b; R Core Team 2016), although F_{ST} is presented for comparative purposes.

Summary statistics were calculated using the R package 'diveRsity' (Keenan et al., 2013b; R Core Team 2016) for each life history type, at for each population. This analysis established the number of individual samples per population, the number of alleles across loci, the percentage of total observed alleles, allelic richness, Hardy-Weinberg equilibrium and Wright's inbreeding coefficient. Populations were also tested for deviations from Hardy-Weinberg equilibrium and linkage disequilibrium was tested with Markov Chain parameters comprising 1000 dememorizations, 100 batches and 1000 iterations per batch using 'Genepop on the web' (Raymond & Rousset 1995; Rousset 2008). Bonferroni correction was used to reduce the probability of Type 1 errors. LOSITAN workbench was used to ensure that the microsatellite markers used in this study were not subject to selection (Antao et al., 2008). It was run using a stepwise mutation model with 50000 simulations, a confidence interval of 0.99 and a false discovery rate of 0.1.

The co-ancestry function within the R package 'related' (Pew et al., 2015; R Core Team 2016) was used to estimate the relatedness of individuals within each sampling location using Wang's coefficient (Wang 2002) with 500 bootstraps (Wang 2011; Pew et al., 2015). COLONY (Jones & Wang, 2010) was used to further quantify the relatedness of individuals by identifying the number of full-sibling families within each sampling location.

4.4 RESULTS

194 of the 226 tissue samples yielded good quality DNA that amplified for more than 70% of the microsatellite markers used. The River Reelan was excluded from further analysis due to its low sample size and lack of samples of freshwater resident brown trout.

4.4.1 POPULATION STRUCTURE

The first level of analysis in STRUCTURE identified two genetic clusters, River Muff and Bonds Glen & Killen Burn (Fig. 4.3.1). This initial level of analysis did not discriminate between life history strategies but rather separated fish on geographic location. The second level of analysis analysed the River Muff cluster and Bonds Glen/Killen Burn cluster separately. This identified two genetic clusters with the River Muff (Fig. 4.3.3) and two genetic clusters in the Bonds Glen & Killen Burn cluster, Bonds Glen and Killen Burn (Fig. 4.3.2). The third level of analysis identified no further structuring in the River Muff clusters (Fig. 4.3.5, 4.3.6). No further structuring was found in the Bonds Glen genetic cluster (Fig. 4.3.4). Two genetic clusters were identified within the Killen Burn at the third level (Fig. 4.3.7), which after further analysis showed no further structuring (Fig. 4.3.8, 4.3.9). Thus, in total five genetic populations were identified from the three sampling locations, with two sympatric genetic clusters in each of the River Muff and the Killen Burn (Fig. 4.3.2; Table 4.2). The genetic clustering identified was based on geographical site and showed no evidence of being based on life history strategy or year of sample collection. Overall genetic differentiation ranged from 0.037 to 0.165, based on D_{JOST}, (0.030 to 0.091 based of F_{ST}) with a global differentiation of 0.049 D_{JOST}; 0.052 F_{ST} (Table 4.3).

Two sympatric populations were identified in the Killen Burn and River Muff. The proportion of freshwater resident and anadromous brown trout significantly differed between

each sympatric population (Table 4.4). Population A in the Killen Burn had a significantly higher proportion of freshwater resident brown trout than population B and vice versa for anadromous brown trout ($\chi^2=15.92_{(1)}$; $p<0.001$). Whereas, population C in the River Muff had a significantly higher proportion of freshwater resident brown trout than population D and vice versa for anadromous brown trout ($\chi^2=13.37_{(1)}$; $p<0.001$). Therefore, the probability of anadromous brown trout belonging to population B in the Killen Burn and population D in the River Muff is greater than for population A in the Killen Burn and population C in the River Muff.

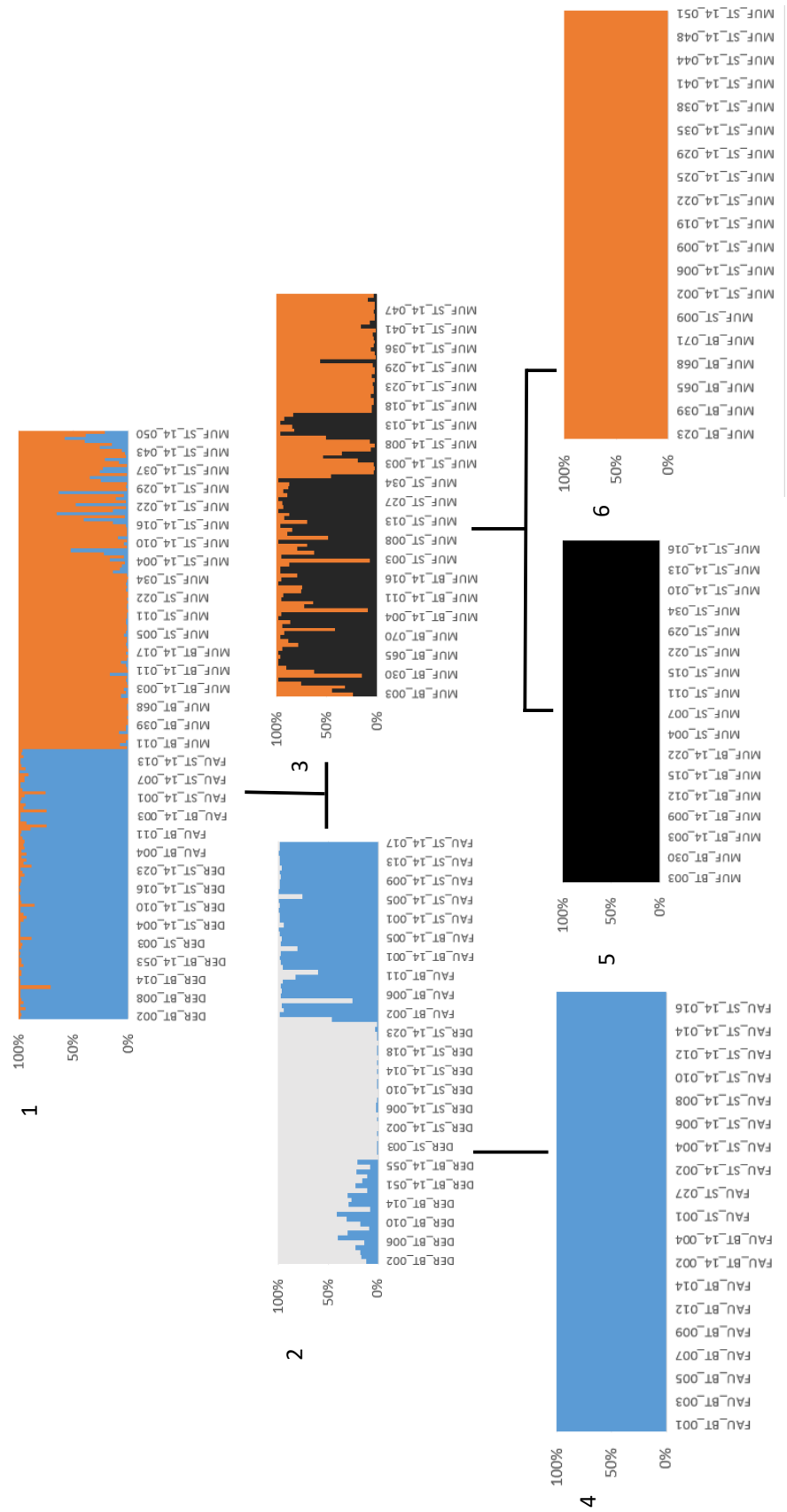

Fig 4.3: Barplots of the hierarchical population structure of three sampling locations in the Foyle catchment. Populations are: 4- Bonds Glen, 8- Killen Burn A, 9- Killen Burn B, 5- River Muff C and 6- River Muff D. Final populations are represented by solid coloured barplots.

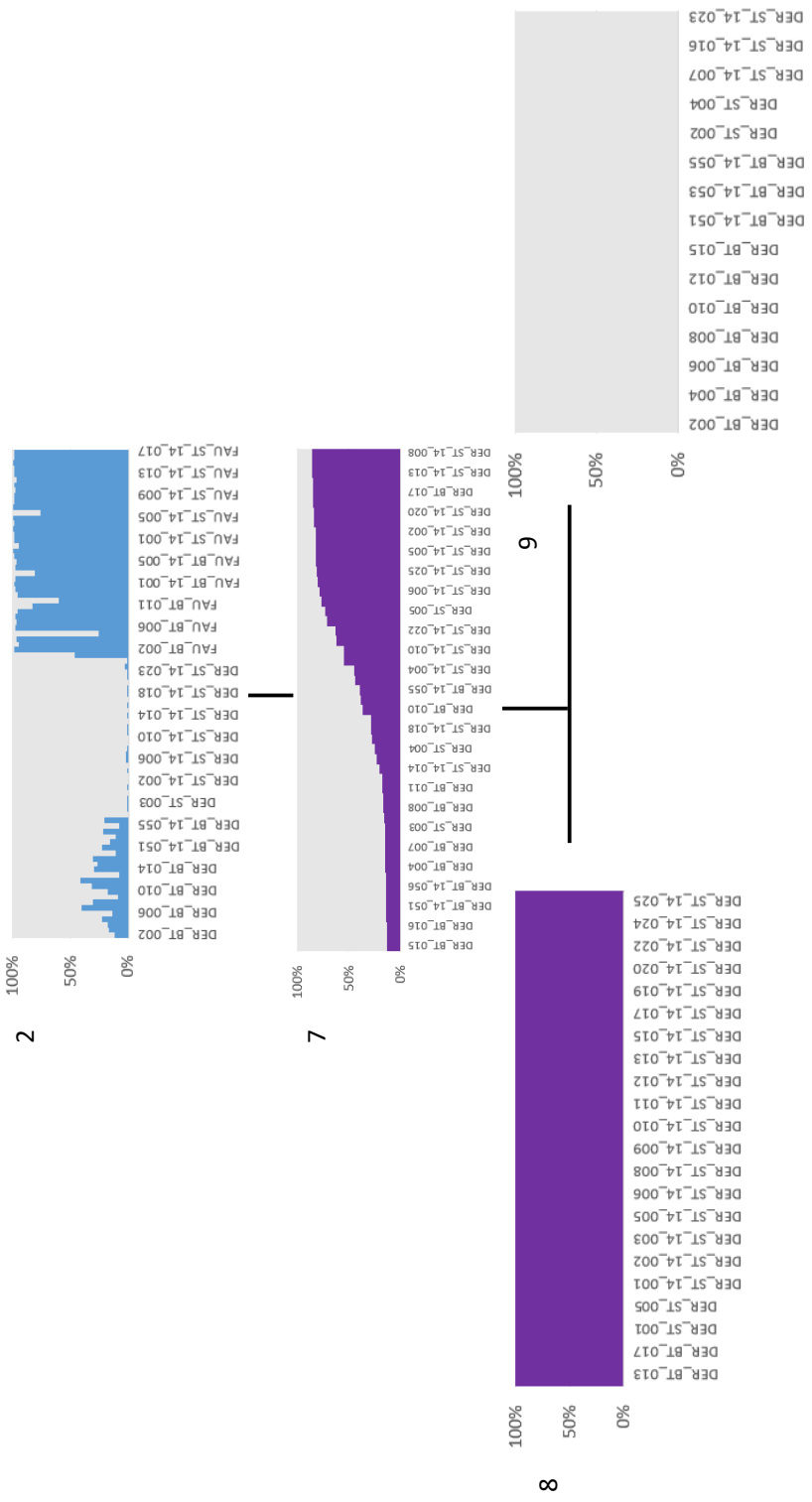

Fig 4.3: Continued.

Genetic Cluster	Number of freshwater resident brown trout	Year(s) freshwater resident samples collected	Number of anadromous brown trout	Year(s) anadromous samples collected
River Muff C	28	2013/2014	30	2013/2014
River Muff D	6	2013/2014	41	2013/2014
Bonds Glen	18	2013/2014	20	2013/2014
Killen Burn A	20	2013/2014	9	2013/2014
Killen Burn B	2	2013	20	2013/2014

Table 4.2: The number of freshwater and anadromous brown trout in each genetic cluster identified and the year(s) the samples were collected.

	Killen Burn A	**Killen Burn B**	**River Muff C**	**River Muff D**
Killen Burn B	0.060			
River Muff A	0.072	0.089		
River Muff B	0.165	0.152	0.037	
Bonds Glen	0.057	0.103	0.044	0.141

Table 4.3: pairwise genetic distances between rivers based on D_{JOST}. All pairwise distances showed a significant difference between population pairs based on the upper and lower 95% Confidence Interval.

	Killen Burn A	**Killen Burn B**	**River Muff C**	**River Muff D**
Probability of anadromous brown trout belonging to cluster	0.31	0.69	0.42	0.58
Probability of freshwater resident brown trout belonging to cluster	0.91	0.09	0.82	0.176
χ^2 (df), p-value	15.92(1); p<0.001		13.37(1); p<0.001	

Table 4.4: Probability of anadromous and freshwater resident brown trout belonging to each sympatric population within the River Muff and Killen Burn with significance tested for using a chi-squared test with Yates' correction.

4.3.2 SUMMARY STATISTICS

The mean number of individual samples (comprising an individual brown trout) which amplified for each microsatellite marker ranged from 22-58. The total number of alleles per site ranged from 138 to 207. The percentage of the total number of alleles across all populations ranged from 58.6%-77.0% and the mean allelic richness of each population ranged from 5.61 to 8.06. Three of 100 tests (comprising 20 loci over five populations) were significant for deviations from Hardy Weinberg Equilibrium (HWE) ($p<0.0025$). The maximum detected Wrights Inbreeding coefficient (F_{IS}) was 0.053, indicating no signs of inbreeding within any population (Table 4.5). Linkage disequilibrium tests of the 20 microsatellite markers used showed that no pair of loci were consistently linked. Using the LOSITAN workbench (Antao et al., 2008) none of the microsatellite markers were found to be under selection.

The R package 'related' was used to test the relatedness of individuals in each population. Wang's coefficient revealed that very few individuals were from the same sibling family (Table 4.3). Confirmed using Colony, each population was composed of mostly unrelated individuals (Table 4.6). The River Muff D contained the greatest number of full-sibling families relative to sample size, with all three of the families in the sample containing individuals from both life history strategies.

River	Catchment	N	A	%	Ar	HWE	F_{IS}
Killen Burn A	River Derg	29	197	81.1	8.06	0.00	0.044
Killen Burn B	River Derg	22	150	68.2	6.62	0.01	0.029
River Muff C	River Muff	47	168	71.5	6.57	0.001	0.015
River Muff D	River Muff	58	138	59.7	5.61	0.868	0.053
Bonds Glen	Faughan	38	202	83.8	8.05	0.014	0.033

Table 4.5: Summary statistics for populations which includes; N- The number of individual samples in each population, A- Number of alleles per population, %- Percentage of total observed alleles, Ar- Allelic richness, HWE- Overall Hardy Weinberg Equilibrium per population, F_{IS}- Overall Wright's Inbreeding Coefficient for each population with values close to 0 indicating no inbreeding.

Population	Average relatedness of individuals based on Wangs coefficient	Number of full-sibling families	Number of independent individuals
Bonds Glen	-0.032	1	51
Killen Burn A	-0.064	1	27
Killen Burn B	0.030	0	22
River Muff C	0.116	0	47
River Muff D	0.083	3	50

Table 4.6: Relatedness of anadromous and freshwater resident brown trout, sample size and number of full sibling families for each population.

4.4.3 SEX RATIO

The overall sex ratio of both freshwater resident and anadromous brown trout deviated significantly from an expected ratio of 1:1. Amongst freshwater resident fish, across all sampling sites, there was one female for every 2.9 males (binomial test, $p<0.001$). In contrast, females dominated in the anadromous brown trout, with two females for every male (binomial test, $p<0.001$). However, the sex ratio for both freshwater resident and

anadromous brown trout was population specific (Table 4.7). The sex ratio of freshwater resident brown trout in the River Muff C was highly skewed, with one female for every 8.3 males (binomial test, p<0.001) and River Muff D with no females detected (binomial test, p<0.001); these were the only populations where the sex ratio of freshwater resident brown trout significantly deviated from an expected sex ratio of 1:1. For anadromous brown trout, both the river Muff C (2.3 females for every one male (binomial test, p=<0.001)) and Bonds Glen (four females for every one male (binomial test, p=0.012)) had a significantly higher proportion of females than expected (Table 4.7).

River	Life history	N	Sex ratio (female:male)	p-value
River Muff C	Resident	28	3:25	**<0.001**
River Muff C	Anadromous	30	21:9	**0.042**
River Muff D	Resident	6	0:6	**0.031**
River Muff D	Anadromous	41	25:16	0.211
Bonds Glen	Resident	18	6:12	0.238
Bonds Glen	Anadromous	20	16:4	**0.012**
Killen Burn A	Resident	20	11:9	0.824
Killen Burn A	Anadromous	9	5:4	1
Killen Burn B	Resident	2	1:1	1
Killen Burn B	Anadromous	20	13:7	0.263

Table 4.7: Sex ratio of freshwater resident and anadromous brown trout for populations.

4.5 DISCUSSION

4.5.1 POPULATION STRUCTURING

Brown trout often form genetically differentiated populations separated by short geographic distances (Carlsson et al., 1999; Stelkens et al., 2012). Five populations were identified at the three sites sampled in this study: Bonds Glen, Killen Burn A, Killen Burn B, River Muff C and River Muff D. Two sympatric populations were identified in each of the Killen Burn and River Muff. All five populations, however, were composed of brown trout that adopted both freshwater resident and anadromous life history strategies. This study found no evidence of population level genetic differentiation between anadromous and freshwater resident brown trout. Most genetic differentiation discovered was between populations separated geographically and not between life-history strategies. Thus, this

study does not support scenario one, that partial migration is simply the result of two genetically distinct groups expressing alterative life history strategies. This result is in line with the finding of other studies, such as in the River Jörlanda, Sweden where no genetic differentiation was detected between anadromous and resident brown trout using mitochondrial haplotypes and microsatellite markers (Petersson et al., 2001). Petersson and colleagues demonstrated that there was a greater genetic difference between populations above and below a migration barrier than there was between the coexisting freshwater resident and anadromous brown trout. Similarly, in the Voss River, Western Norway, there were greater genetic differences between brown trout at different localities than between co-existing life history strategies (Hindar et al., 1991).

4.4.2 SEX RATIO

In this study, the sex ratio of brown trout exhibiting each of the alternative life history strategies of partial migration deviated significantly from an expected ratio of 1:1. Such deviations have been demonstrated elsewhere, for example in Vangsvatnet Lake, Norway, and the Kirk Burn, Scotland (Campbell, 1977; Jonsson, 1985). The costs and benefits of a non-breeding partial migration, which is an energetically and metabolically demanding process, have been shown to be sex specific (Jonsson & Jonsson, 1993; Sahashi & Morita, 2013). For females, the costs of migration, which include a higher chance of mortality and a higher energy expenditure, are more likely to be outweighed by the fitness benefits accruing from the ability to reach a larger body size due to an increased food availability. In females, a larger body size enables them to produce larger eggs and in greater numbers, gain better territories and have a higher success defending their nests (Jonsson & Jonsson 1993; Dodson et al., 2013). On the other hand, for males the benefits of a large body size accruing from anadromy are not as clear, as the energetic cost of gamete production is relatively small. Smaller males can use sneaky tactics for mating and become principle spawners in the absence of larger males (Jonsson & Jonsson, 1993).

This study demonstrated the deviation in sex ratio was not only specific to life history strategy but was also site specific, with anadromous brown trout in the River Muff and Bonds Glen and freshwater resident brown trout in the River Muff having a sex ratio which significantly deviated from an expected 1:1. The Killen Burn, having the longest migration distance to the sea, was the only sampling location with no deviation from an expected 1:1

sex ratio. It has been demonstrated the costs of adopting an anadromous life history strategy increase with migration distance (Sahashi & Morita, 2013). Therefore, it is plausible that the higher chance of mortality and increased energy demand associated with the longer migration from the Killen burn may mitigate any additional advantages for females to adopt an anadromous life history strategy.

4.4.3 ADOPTION OF ALTERNATIVE LIFE HISTORY STRATEGIES

Although this study demonstrated that freshwater resident and anadromous brown trout were present in all populations, the frequency of each life history strategy varied significantly between sympatric populations in the River Muff and Killen Burn. The environmental variables that trigger the physiological and behavioural processes leading to migration are, at least partly, understood for salmonids (Dodson et al., 2013). For example, it has been shown in salmonid males that life-history divergence is dependent on current growth rate, body size and condition (Sahashi & Morita, 2013). Males which have exceeded the threshold value for these characteristics tend to mature as freshwater residents, whereas, males which have not reached the threshold size tend to adopt an anadromous life history strategy (Sahashi & Morita 2013; Dodson et al., 2013; Ferguson et al., 2016). If males mature quickly they have more opportunities to spawn compared to males which become anadromous, as the latter group must delay maturation until they return from the marine environment (Sahashi & Morita 2013). Sympatric populations in the River Muff and Killen Burn showed different rates of expression of each of the two life history strategies, despite being subject to the same broad environmental variables. There are two possible explanations for this. Firstly, as anadromous brown trout were sampled during the smolt run it is possible that resident freshwater brown trout were not sampled due to the population originating upstream. The second possibility is that this result of different average thresholds between different populations at each site is the result of a threshold difference which is inherited (Dodson et al., 2013; Piché et al., 2008). To address whether a difference in threshold trait is responsible for the difference in frequency of anadromous and freshwater brown trout in sympatric populations, the genomics of the quantitative threshold trait would need to be further investigated. However, despite evidence of between population variation in the expression of the alternative life history traits of partial migration, the clear conclusion of this study is that the biggest driver in differences in the expression of alternative life history strategies of partial migration is within population, between individual variation in the probability of migration (scenario 2). There are three mechanisms through which this

could occur. This could be the result of within-family inherited differences in the quantitative threshold value of the traits that triggers migration (Dodson et al., 2013; Ferguson 2006). This explanation is in part supported by the finding of differences in the expression of migratory life history strategy of the populations in sympatry (Killen Burn and River Muff) in this study. Alternatively, individual variation in the expression of the migratory life history strategy may result from between-individual differences in exposure to the environmental conditions that trigger migration (Metcalfe et al., 1989). We cannot discount this mechanism with the data from this study. Most likely, individual variation in expression of life history strategy is resulting from a combination of both.

One consequence of the presence of anadromous and freshwater resident brown trout in a single gene pool is that it is likely to act as a mechanism to prevent directional selection and will maintain partial migration as an adaptive trait (Dodson et al., 2013). The expression of partial migration as an adaptive trait would have significant fitness advantages for species that live in highly variable environments, such as brown trout. Thus, partial migration will persist in a population when the relative fitness advantage of migration differs between individuals in the population and the relative proportion of the population expressing migration may fluctuate in response to prevailing environmental conditions, such as food availability (Ferguson et al., 2016). There are several environmental conditions which could promote an anadromous life history strategy, which include food availability, temperature and flow rate (Chapman et al., 2011; Ferguson et al., 2016). An individual's condition in terms of size, growth rate, lipid levels and metabolic rate determine whether an individual adopts a freshwater residency or anadromous life history strategy (Ferguson et al., 2016). Therefore, food availability has been suggested as one of the environmental drivers of partial migration (Hendry et al., 2003). It has been demonstrated through laboratory experiments that when brown trout are kept on a high food availability diet, a higher proportion adopt a freshwater residency life history in comparison to those kept on a low food availability diet (Wysujack et al., 2009). This could be due to low food availability being associated with low growth rate which is known to trigger migratory behaviour in brown trout (Wysujack et al., 2009). In another common garden experiment, offspring from anadromous parents and freshwater resident parents where kept on low, medium and high food availability diets (Van Leeuwen et al., 2015). Offspring from anadromous parents kept on an intermediate food availability diet dominated similar sized offspring from freshwater resident parents when competing for feeding territories. This suggests that parental effects interact with

environmental conditions to influence the probability of migration (Van Leeuwen et al., 2015). Therefore, populations with a lower threshold trait value have a higher proportion of individuals to begin the physiological and behavioural processes resulting in anadromy (Dodson et al., 2013; Roff et al., 1996; Ferguson et al 2017).

CHAPTER FIVE: EVIDENCE OF STRAYING IN A HOMING SALMONID? THE USE OF GENETIC STOCK IDENTIFICATION IN A COMPLEX RIVER CATCHMENT TO DETERMINE THE POPULATION OF ORIGIN FOR A MIXED STOCK OF ANADROMOUS BROWN TROUT.

5.1 ABSTRACT

Effective management of mixed stock fisheries is of vital importance to prevent the exploitation of vulnerable stocks. Genetic Stock Identification (GSI) is a powerful tool for this and can provide real time assessments of stock exploitation. This study investigated the stock composition of anadromous brown trout caught using a Rotary Screw Trap (RST) in the lower reaches of the River Faughan during downstream migration in spring. It was determined that two populations (mainstem River Faughan and Bonds Glen) disproportionately contributed to the production of anadromous brown trout. There was evidence of straying, with 9% of anadromous brown trout caught in the River Faughan, assigned with a probability greater than 0.7 to a population which was relatively distant (River Owenbeg). This has important consequences for management to prevent the exploitation of vulnerable populations within mixed stocks of anadromous brown.

5.2 INTRODUCTION

Identifying intraspecific population structuring is an important issue for the effective conservation and management of a species (Ferguson 2016). Specifically, identifying where an effective population (groups with no, or limited, gene flow between them) begins and ends is of critical importance (Vähä et al., 2016). Thus, with the advancement of molecular markers there have been many studies which have examined intraspecific structuring within a species (Ferguson & Taggart 1991; Lu et al., 2001; Bowen et al., 2005; Charruau et al., 2011). For example, there is little to no population structuring of loggerhead turtles (*Caretta caretta*) in south-eastern United States based on nuclear DNA, however, when examining maternally inherited mtDNA there is genetic structuring of multiple populations (Bowen et al., 2005). This has important implications for effective conservation of a species and identifying management units. Management units are used to conserve evolutionary important genetic divergence between populations and are defined by the intraspecific structuring of a species (Palsbøll et al., 2007). For species which are exploited, or for species which require conservation intervention, what constitutes an exploited gene pool or which genetic group requires most immediate management is vital information. This effect may

become even more acute where a species under management is migratory and where exploitation of mixed stocks occurs (Östergren et al., 2016). For example, there have been losses of anadromous Pacific salmonids (*Oncorhynchus* spp.) populations and habitat since the 1850s due to anthropogenic activities such as fishing and mining. To conserve and manage remaining populations adequately it is important to maintain neutral genetic diversity by identifying intraspecific population structuring (Nehlsen et al., 1991). However, the level of exploitation on any effective population may be difficult to measure and even more difficult to manage.

Molecular techniques can be used to study the relative proportions of exploited migratory species in a mixed stock (Anderson et al., 2008). Genetic Stock Identification (GSI) techniques have been developed to allow the analysis of mixed stock fisheries using mixed stock analysis (MSA), which identifies the proportion of individuals which assign to different geographic regions (Östergren et al., 2016). For example, using MSA for Atlantic salmon (*Salmo salar*) in the Greenland mixed stock fishery it was found that the contribution of nine geographic regions varied substantially, with contribution of groups ranging from <1% of the captured Atlantic salmon originating from Maine to 40% of them originating from Southern Québec (Gauthier-Ouellet et al., 2009). This work highlighted not only the importance of identifying exploited regions but also that contributions from regions are temporally variable and should be considered when managing a species (Gauthier-Ouellet et al., 2009). Therefore, such techniques are extremely useful for management purposes as they identify specific geographic regions which are vulnerable and threatened from overharvest, as well as providing the knowledge required to establish targeted stock management (Östergren et al., 2016; Vähä et al., 2016). GSI can also be used for real time analysis of mixed stocks to determine the proportion of different populations in a catch (Anderson et al., 2008). For example, Beachman et al. (2004) used GSI for the rapid assessment of sockeye salmon (*Oncorhynchus nerka*) from the Fraser River, Canada, allowing near real time management decisions since stock compositions of the catches were analysed within 9-30 hours of samples being received. More recently, individual assignment (IA) has been implemented as a technique, whereby a mixture of individuals can be assigned to predefined populations. There have been some studies using GSI analysis in salmonids (see Bradbury et al. 2015; Vähä et al. 2016 for examples of Atlantic salmon (*Salmo salar*), Winans et al. 2004 for example of Steelhead salmon (*Oncorhynchus mykiss*) and Mäkinen et al. 2015; Swatdipong et al. 2013 for examples of lake run trout (*Salmo trutta*). However, there have

been very few studies, with only a few exceptions (Kolijonen & Koskiniemi 2014; King et al., 2016) which have used GSI analysis on mixed populations of anadromous brown trout (*Salmo trutta*).

Brown trout often form highly structured populations over short geographic ranges (see Chapter 2) with a high degree of genetic differentiation between populations (Crozier & Ferguson, 1986; Ferguson, 1989, 2007; Bernatchez et al., 1992; Carlsson et al., 1999; Massa-Gallucci et al., 2010; Swatdipong et al., 2010; Stelkens et al., 2012). This makes them an ideal study species for individual assignment from mixed stocks using GSI. Brown trout also exhibit a continuum of different life history strategies, the two commonest being freshwater resident (river dwelling) and anadromous (colloquially known as sea) trout. There have been a few studies which have examined the stock composition of lake dwelling brown trout but there are very few studies which have examined the stock composition of anadromous brown trout caught in salt water (see Knutsen et al., 2001; King et al., 2016; Ostergren et al., 2016). Anadromous brown trout reside in freshwater rivers until they reach a genetically predefined threshold (see Chapter 4), which triggers a migratory life history strategy whereby they migrate to estuarine and coastal marine environments to feed until they become sexual mature. At this point, they migrate back towards their natal river to spawn (Klemetsen et al., 2003). Anadromous brown trout numbers in Britain and Ireland have decreased substantially in recent years (Youngson et al., 2003). This could be due to overharvest by commercial or recreational fisheries as anadromous brown trout are caught in estuaries and lower reaches of river systems (Limburg & Waldman, 2009). But habitat degradation, barriers to migration, stocking practices and the effects of aquaculture might also have an effect (Limburg & Waldman, 2009). For example, stocking hatchery- raised brown trout could lead to a decrease in genetic potential for anadromy in wild brown trout populations, as hatchery-raised anadromous brown trout have lower survival rates at sea (Ferguson, 2006; Ferguson 2016). Sea lice infestations from salmon aquaculture could also contribute to the decline in anadromous brown trout numbers. A study in the River Burrishoole demonstrated salmon farming can have both direct and indirect effects on the genetics of cohabiting anadromous brown trout by reducing variance at major histocompatibility class I genes (Coughlan et al., 2006).

The River Foyle catchment, Co. Londonderry is a medium sized catchment, of around 4500km^2, located both in Northern Ireland and the Republic of Ireland (Niven, 2013). It is a complex, highly dendritic river catchment which comprises of many smaller sub-catchments (Figure 5.1). These small sub-catchments drain either into the River Foyle which in turn drains into a sea lough, Lough Foyle. However, some of the sub-catchments drain directly into Lough Foyle (Niven, 2013). As with most regions, anadromous brown trout numbers in the River Foyle catchment have declined in recent decades (Niven 2013), therefore, it is important for management to understand which populations are contributing to anadromous brown trout numbers in the Foyle system and where management should be focused to ensure anadromous brown trout production.

This study used the River Faughan sub-catchment to determine which populations are contributing to the production of anadromous brown trout in that River. The River Faughan sub-catchment was used as a case study as it is one of the most productive anadromous brown trout rivers in the Foyle system (Niven 2013). This study specifically aimed to:

(i) test the quality of the genetic baseline for individual assignment from a mixed stock;

(ii) determine which populations or tributaries within the River Faughan contribute to the production of anadromous brown trout;

(iii) determine if individuals from the mixed stock of anadromous brown trout assign to populations outside the River Faughan sub-catchment and if this is evidence of straying.

5.3 METHODS

5.3.1 COLLECTION OF SAMPLES

A rotary screw trap (RST) was placed at the same location in the lower reaches of the River Faughan, almost at the confluence with Lough Foyle and lower than any likely juvenile brown trout habitat, during the spring smolt run in April and May between 2005-2008 and in 2014. A total of 701 anadromous brown trout were collected (Fig. 5.1; Table 5.1) primarily from smolting anadromous brown trout, with an average fork length of 206±42mm. Samples collected between 2005 and 2008 were archived biological material (scale samples) which

were collected by the Loughs Agency (the managing body for the Foyle catchment). Samples collected in 2014 were non-destructive tissue samples (fin clips). Anadromous brown trout collected from the RST in 2014 were anesthetised using clove oil, a non-destructive genetic sample (adipose fin clip) was then collected and stored in 95% ethanol. A record of fork length (mm) and weight (g) was made and a scale sample collected.

5.3.2 GENETIC BASELINE

Previous work (presented in chapter two) establishing the hierarchical population structure of brown trout in the Foyle catchment was used as a genetic baseline for Genetic Stock Identification (GSI) of the anadromous brown trout samples collected. In summary, 1426 genetic samples from 28 sampling locations were used to establish a hierarchical population structuring (Figure 5.1; Table 5.1). The population structure was established using a hierarchical Bayesian approach in STRUCTURE (Pritchard et al., 2000) with all major tributaries in the River Faughan being surveyed. The study established 21 genetically distinct populations. Six genetically distinct populations were identified from 10 sampling sites within the River Faughan. Overall, genetic differentiation ranged from 0.011 to 0.324 based on D_{JOST} (0.008-0.124 F_{ST}) with a global differentiation of 0.138 D_{JOST}; 0.057 F_{ST} (see chapter 2). The genetic baseline for this study was formed from the genotypes of all 21 populations to investigate which populations in the River Faughan produce anadromous brown trout as well as identifying any possible straying.

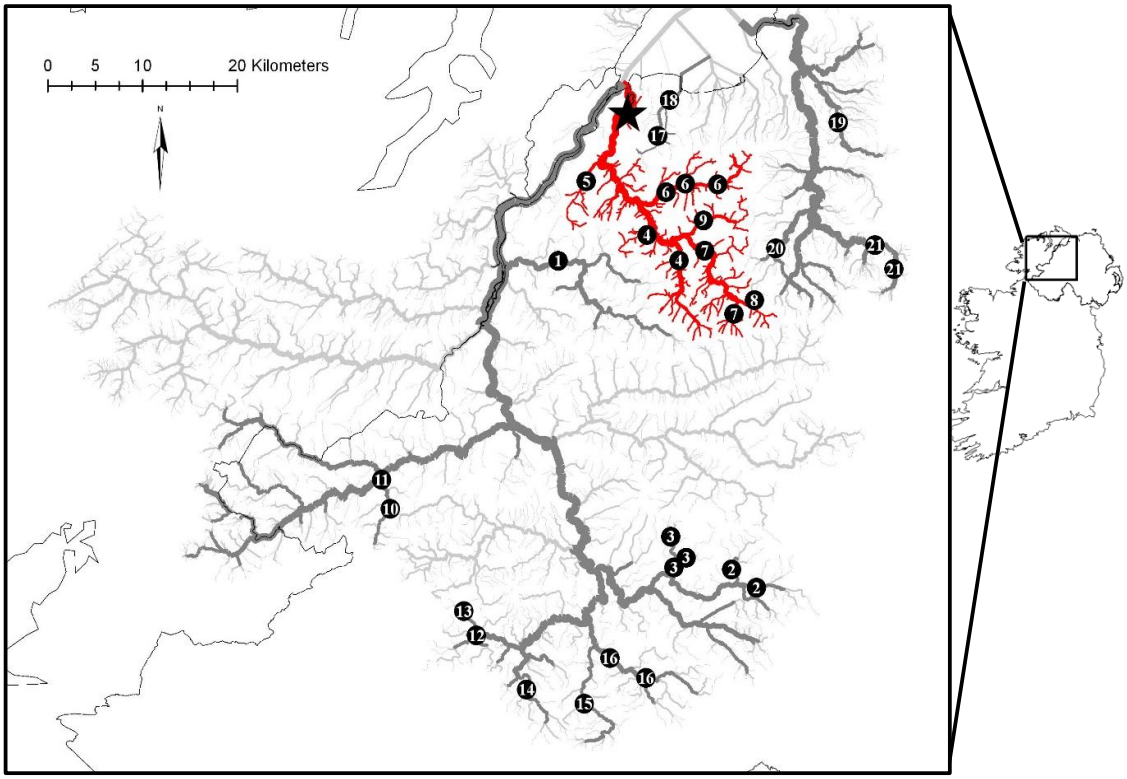

Fig 5.1: The location of the RST used to collect genetic samples from migrating anadromous brown trout (marked *) and the location of populations used for the genetic baseline. Population sampling location IDs can be found in Table 5.1. The River Faughan sub-catchment has been highlighted in red.

Population (abbreviation)	**Sub-catchment**	**Location ID**	**Easting**	**Northing**	**N**
Burndennet (DEN)	Burndennet	11	641530.6	904685.1	16
Camowen (CAM)	Camowen	2	662460.3	870951.2	114
Drumnakilly (DRU)	Camowen	3	653773.2	873040.4	197
Bonds Glen (BGL)	**Faughan**	16	650703.4	907420.5	53
Burngibbagh (GIB)	**Faughan**	12	644497.4	912857.2	63
Burntollet (BUR)	**Faughan**	1	652919.5	911768.1	136

Faughan (FAU)	**Faughan**	14	657002.8	905701.6	113
Faughan A (FAA)	**Faughan**	6	660556.6	900607.6	58
Foreglen (FOR)	**Faughan**	15	656876.9	908861.8	43
Rotary screw trap (RST)	**Faughan**	star	592899.9	899121.4	607
Killen Burn A (KILA)	Derg	17	622887.4	882083.9	29
Killen Burn B (KILB)	Derg	18	622887.4	882083.9	22
Owenreagh (REA)	Owenreagh	19	632906.1	866020.7	45
Owenreagh A (REB)	Owenreagh	7	632611.8	867336.4	55
Owenreagh B (REC)	Owenreagh	20	638204.2	860452.6	17
Quiggery water (QUI)	Owenreagh	8	644305.9	858990.4	20
Routing Burn (ROU)	Owenreagh	9	646987.2	863690.1	108
River Muff A (MUFA)	Muff	4	652304.2	918250.8	52
River Muff B (MUFB)	Muff	5	652304.2	918250.8	103
Castle (CAS)	Roe	13	671096.8	918932	38
Owenbeg (OWE)	Roe	21	664516.1	905941.5	60
Roe (ROE)	Roe	10	677020.9	903815.1	71

Table 5.1: Genetic populations (from Chapter 2) with population ID number, sub-catchment population, Easting and Northing in "Irish Transverse Mercator grid" coordinate system and the number of brown trout samples obtained from each population (N). Collection sites from the River Faughan sub-catchment are highlighted in bold.

5.3.3 MICROSATELLITE AMPLIFICATION

Genomic DNA was extracted following the methods by Kennan et al., 2013 used in Chapter two and genotyped using the same suite of 21 microsatellite markers (see appendix; chapter 2). Twenty-one microsatellite markers were initially screened, however, SsaD48 was removed from the analysis due to inconsistences in banding patterns making it unreliable.

5.3.4 STATISTICAL ANALYSIS

The quality of the genetic baseline used in this study was established using the 'leave one out' test in ONCOR (Kalinowski et al., 2007). The proportion of individuals correctly assigned to each populations and sub-catchment is tested in this protocol by removing one fish at a time from each baseline population and then estimating their origin (Kalinowski et al., 2007).

A GSI framework was then used to assign individual anadromous brown trout of unknown origin to populations from which they most likely emanate from using ONCOR (Kalinowski et al., 2007). Genetic samples collected from smolts (1st year marine migrants) and adults (≥2nd year marine migrants) were analysed separately as smolts should all assign to the River Faughan, whereas adults could have strayed. Anadromous brown trout were classified as smolts if they had a fork length less than 229mm and as adults if they had a fork length greater than 230mm. This classification was based on length data used by the Loughs Agency to differentiate between smolts and adults (Niven & McCauley 2017). ONCOR estimates the probability of individuals belonging to a baseline population by using Rannala and Mountain's (1997) method (Kalinowski et al., 2007).

4.4 RESULTS

A total of 701 genetic samples from anadromous brown trout were collected from the rotary screw trap between 2005-2008 and 2014. From the 701 samples, 607 yielded good quality DNA with a high molecular weight and amplified for more than 70% of the 20 microsatellite markers used, and were thus analysed further.

The quality of the genetic baseline was determined using 'leave-one-out' tests in ONCOR (Kalinowski et al., 2007). These test the probability of self-assigning individuals of known origin to the correct population/ reporting group (sub-catchment). Reporting groups are collections of populations which are genetically similar, in this case populations were grouped by sub-catchment. On average, more than 60% of individuals where re-assigned to the correct sub-catchment, except for the Burndennet sub-catchment where an average of 30% of individuals were correctly reassigned (Figure 5.2). On average, 60% of brown trout of known origin were correctly assigned to the correct populations, except for Castle (59%), Faughan (55%), Burngibbagh (54%), Owenbeg (53%), Quiggery water (50%), Owenreagh B (47%), Killen Burn A (41%), Bonds Glen (39%) and Burndennet (30%) (Figure 5.3). However, individuals from populations with a lower proportion of individuals correctly re-assigned tended to be re-assigned to neighbouring populations from the same sub-catchment.

The mixed stock of anadromous brown trout from the RST were assigned to populations within the Foyle catchment using assignment tests in ONCOR. A total of 75.8% of individual assignments (smolts and adults) were assigned to populations with a probability greater than 0.7 (Table 5.2; Figure 5.4). This showed that 90.6% of the remaining 336 smolts and 69.7% of the remaining 89 adults were assigned to populations within the River Faughan sub-catchment. From the anadromous brown trout smolts assigned to the River Faughan sub-catchment, 66.1% were assigned to a population collected from the main stem of the River Faughan which was located towards the top of the sub-catchment. The second population, with 18.8% of anadromous brown trout smolts assigned to it, was the Bonds Glen population (Table 5.2; Figure 5.4). For a few anadromous brown trout smolts (9.4%), there were high levels of allocation to populations relatively distant to the River Faughan sub-catchment, which could be indicative of non-random straying. The highest assignment was to the River Owenbeg population (in the River Roe sub-catchment), with 8.1% of anadromous brown trout smolts being assigned to this population. In comparison, 44.9% of anadromous brown trout adults were assigned to the River Faughan mainstem population and 11.2% assigned to the Bonds Glen populations (Table 5.2; Figure 5.4). However, 30.3% of anadromous brown trout adults were assigned to populations not in the River Faughan sub-catchment. The highest assignment was to the River Owenbeg population with 13.5% of adult anadromous brown trout assigned.

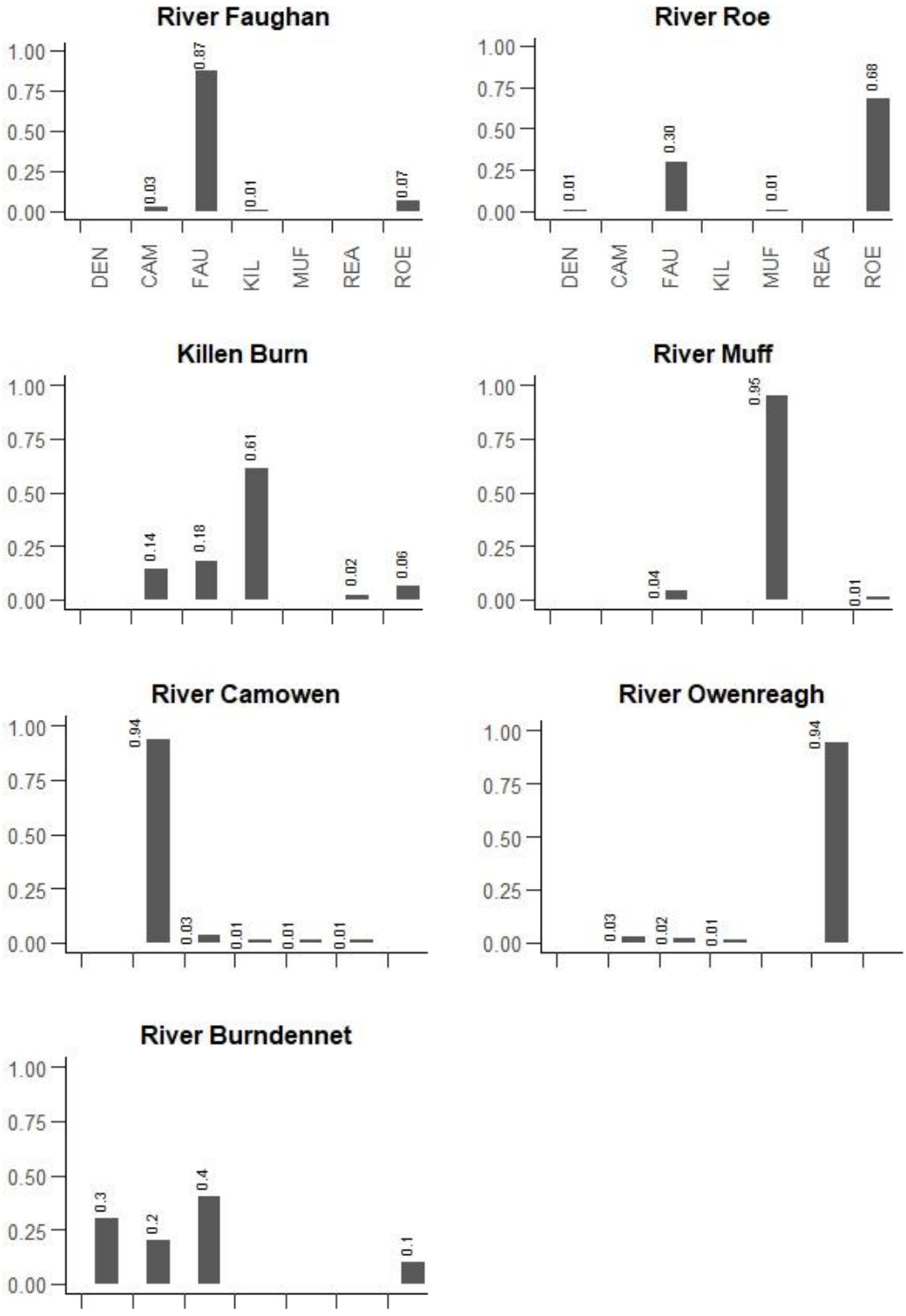

Figure 5.2: Average percentage of correct self-assignment to sub-catchment (reporting groups) and percentage of individuals mis-assigned to other regions. Note sub-catchments are in the same order in all graphs (DEN, CAM, FAU, KIL, MUF, REA, ROE). The sub-catchments are marked in the top two graphs and all graphs below follow the same order.

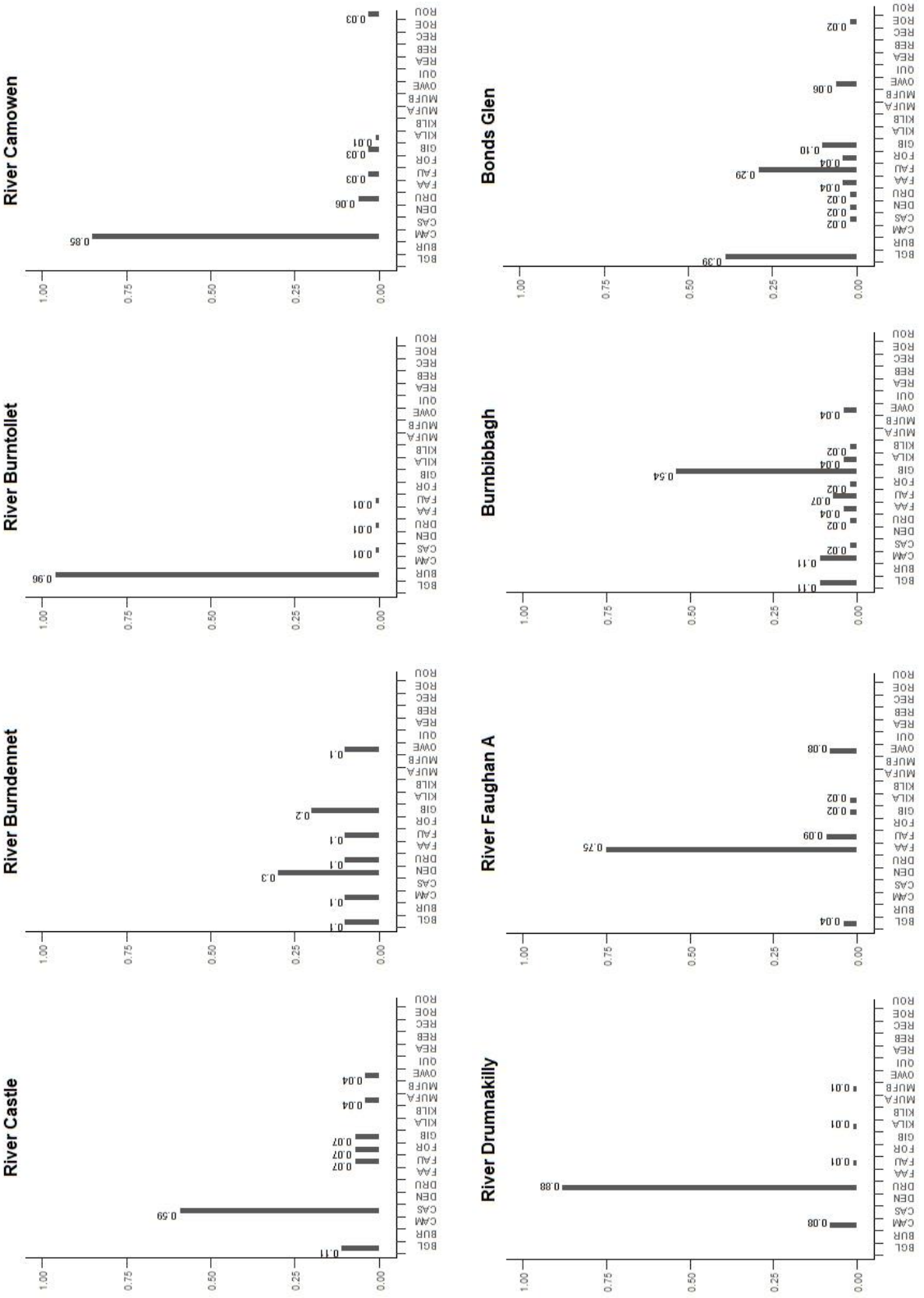

Figure 5.3: Average percentage of correct self-assignment to genetic population and percentage of individuals mis-assigned to other regions.

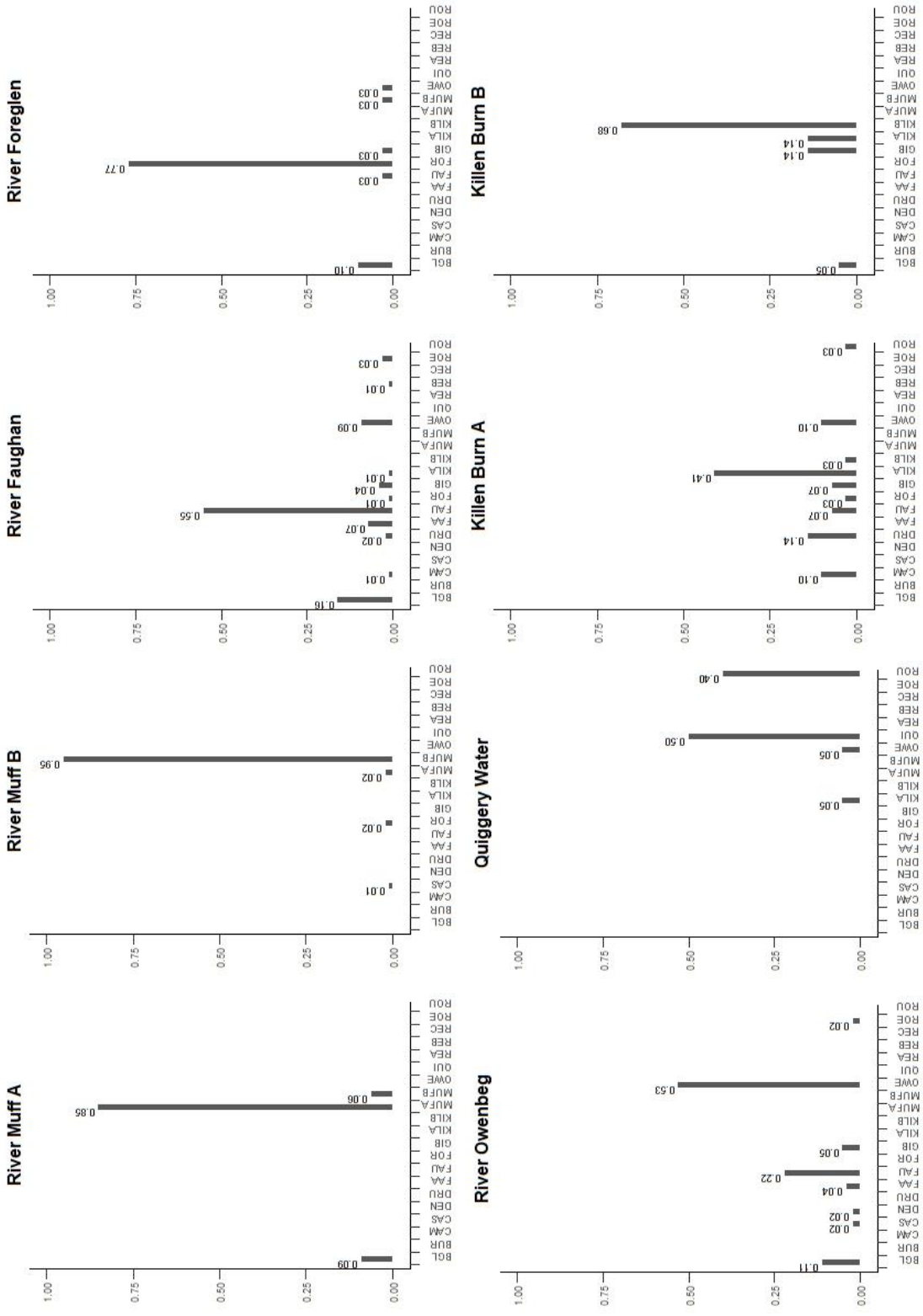

Figure 5.3: Continuation- Average percentage of correct self-assignment to genetic population and percentage of individuals mis-assigned to other regions.

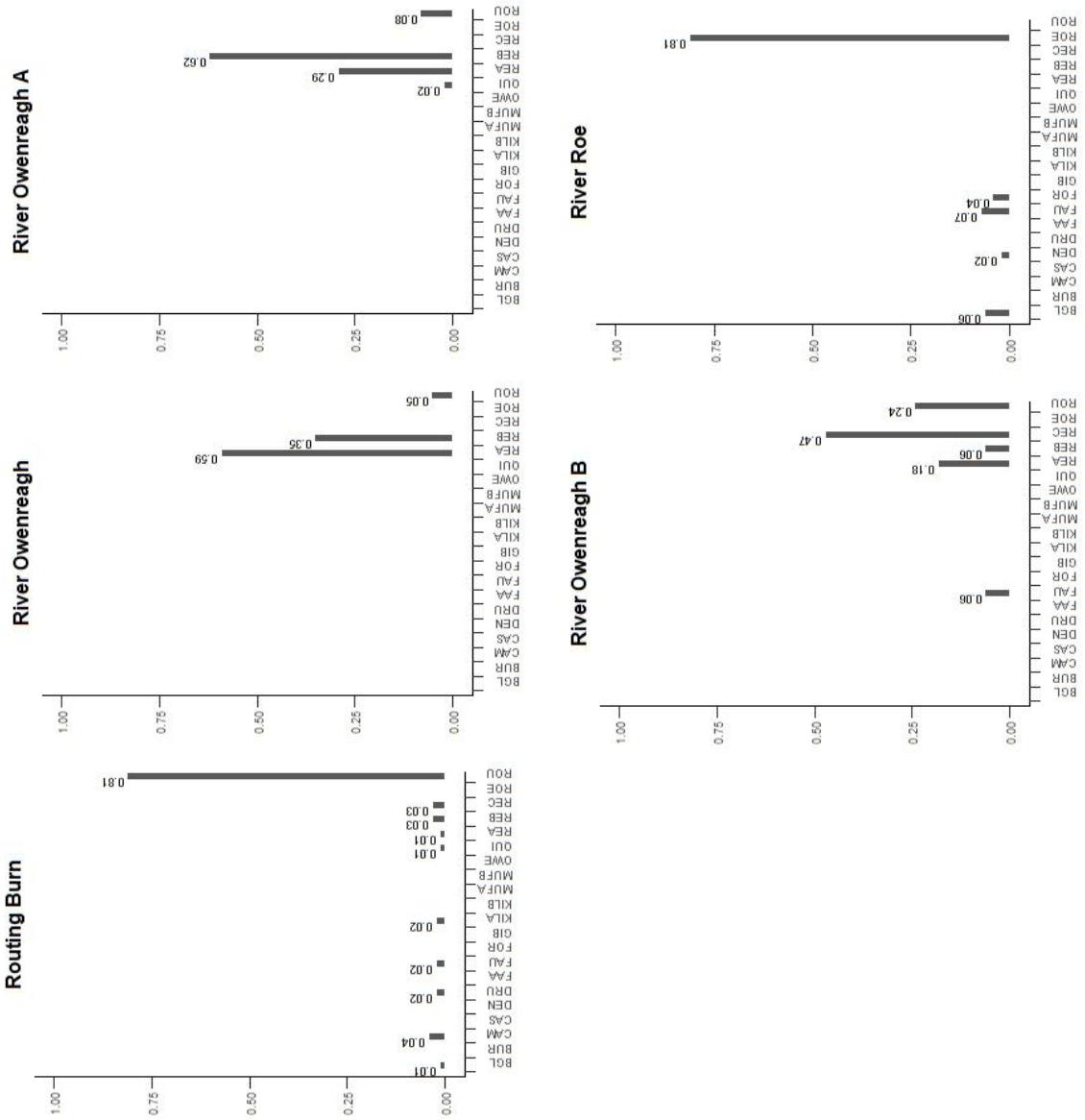

Figure 5.3: Continuation- Average percentage of correct self-assignment to genetic population and percentage of individuals mis-assigned to other regions.

Population	Sub-catchment	Number of smolts assigned	Average probability	Number of adults assigned	Average probability
Drumnakilly	Camowen	1	0.868	0	0
Killen Burn A	Derg	3	0.813	9	0.942
Faughan	**Faughan**	**222**	**0.920**	**40**	**0.912**
Bonds Glen	**Faughan**	**63**	**0.916**	**10**	**0.913**
Burngibbagh	**Faughan**	**38**	**0.908**	**8**	**0.916**
Faughan A	**Faughan**	**13**	**0.905**	**4**	**0.825**
River Muff A	Muff	1	0.788	0	0
Routing Burn	Owenreagh	0	0	1	0.963
Owenreagh	Owenreagh	0	0	1	1
Owenbeg	Roe	30	0.853	12	0.903
Roe	Roe	0	0	4	0.959

Table 5.2: Assignment of mixed origin anadromous brown trout to genetic populations, but only considering fish with an assignment value (P) equal to or greater than 0.7 using assignment tests in ONCOR and Geneclass2. Assignments to populations within the River Faughan sub-catchment are highlighted in bold.

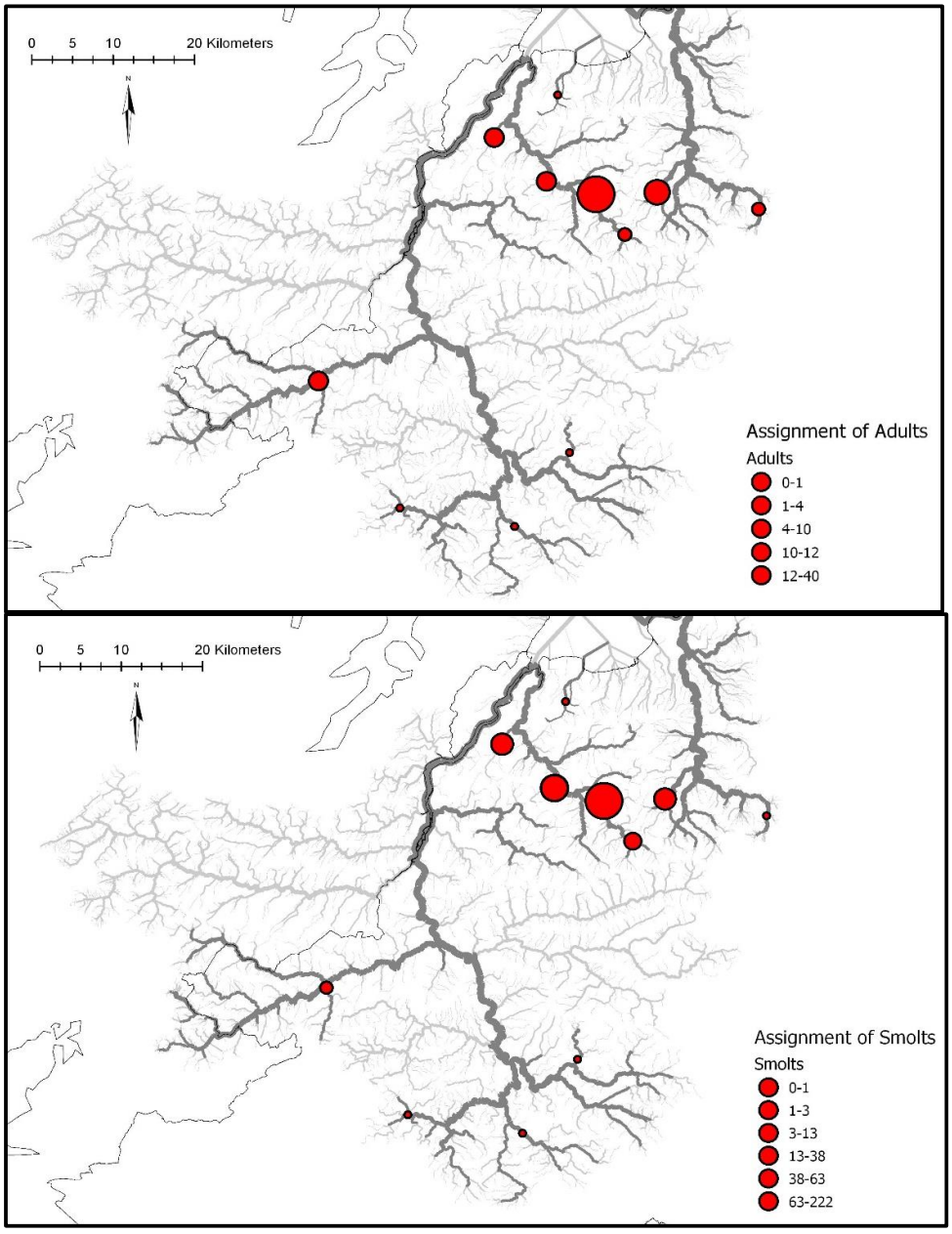

Figure 5.4: Anadromous brown trout assignment of adults and smolts to genetic populations, only considering fish with an assignment value (P) equal to or greater than 0.7. Pie size is indicative of the number of anadromous brown trout assigned**.**

5.5 DISCUSSION

5.5.1 QUALITY OF GENETIC BASELINE

The genetic baseline used in this study (see Chapter 2) was composed of 21 populations which were hierarchically structured, six of which were in the River Faughan (Fig. 5.1). The quality of this genetic baseline used for individual assignment was estimated by determining the proportion of individuals, of known origin, which correctly assign back to each population and sub-catchment (Kalinowski et al., 2007). This demonstrated the genetic baseline used for this study was of generally good quality as a high proportion of individuals (>60%) were correctly re-assigned to their sub-catchment and population of origin.

The accuracy of individual re-assignment to the genetic baseline is dependent on several factors. Firstly, it is important that populations forming the genetic baseline are representative of any populations which are likely to contribute to the production of anadromous brown trout (Pella & Masuda 2006; King et al., 2016). This study included populations in the genetic baseline from each of the major tributaries within the River Faughan sub-catchment. However, only one population was sampled within each tributary and if more populations had been sampled this would have improved the accuracy of assignment. The distribution of sampling was not even across the Foyle catchment with between two and five populations representing each sub-catchment other than the River Faughan. This affects the detection of strays and reliability of assigning them to a population of origin (Ikediashi et al., 2012). However, assignment of strays to sub-catchment of origin will be more accurate as all sub-catchments had a high proportion of individuals correctly re-assigned to them. Another factor which is important to have is a high level of divergence between populations a high accuracy rate of individual assignment, ideally with a global F_{ST}>0.03 (King et al., 2016). The genetic baseline used in this study had a global F_{ST} of 0.057 indicating a high level of genetic differentiation between populations which improves the accuracy of individual assignment. Inter-river or population F_{ST} values are recommended to be more than 0.05 for 97% assignment accuracy (Latch et al., 2006). Pairwise F_{ST} values between populations were less than 0.05 for 58.1% of 210 pairwise comparisons. However, the majority (71.4%) of pairwise comparisons had an F_{ST} value greater than 0.03. Therefore, the genetic baseline used in this study would have a high assignment accuracy. Finally, small sample sizes (<30 individuals) within each population can lead to mis-assignment during

individual assignment. Baseline populations in this study mostly had sample sizes greater than 30 individuals for each population (Prodöhl et al., 2017). However, populations Burndennet, Killen Burn A, Killen Burn B and Owenreagh B had sample sizes less than 30 and additional sampling at these populations locations may improve assignment. The population in Bonds Glen had a lower proportion of individuals which were correctly re-assigned due to the population being composed of two sampling locations (Bonds Glen and River Glenrandal) which each had small sample sizes. Further sampling at these locations may resolve the population structuring of brown trout at this site. Therefore, the quality of the genetic baseline used in this study was generally of high quality.

5.5.2 ASSIGNMENT OF INDIVIDUALS FROM MIXED STOCK

A mixed stock of anadromous brown trout with unknown origin were collected during the downstream migration of smolting anadromous and 2nd year marine migrant brown trout in April in the lower reaches of the River Faughan. The mixed stock of anadromous brown trout was composed of 487 smolts (1st year marine migrants) and 120 adults (≥2nd year marine migrants) which were differentiated based on fork length (mm) (Niven & McCauley 2017). Therefore, smolts and adults (2nd year migrants) were analysed separately as it is expected anadromous brown trout smolts would assign to populations within the River Faughan sub-catchment, whereas adult anadromous brown trout could assign to any catchment as the result of straying. Individual assignment tests (24.2%) with correct assignment probabilities less than 0.7 were excluded from further analysis. The individuals which assigned with low probabilities indicates that these individuals belong to populations which were not sampled in the genetic baseline. Therefore, further sampling effort for populations with low sample sizes would be required to increase the accuracy of the genetic baseline and sampling additional sites for a more complete coverage of the Foyle catchment. Of the remaining individual anadromous brown trout with assignment probability >0.7 a high proportion of smolts and adults did assign to populations in the River Faughan sub-catchment (86.6%). However, a higher proportion of anadromous brown trout smolts assigned to the River Faughan sub-catchment (90.6%) than adults (69.7%). The two populations which most anadromous brown trout smolts assigned to, were a population in the upper reaches of the mainstem River Faughan (66.1%) and the Bonds Glen (18.8%) population. In contrast, the two populations which most anadromous brown trout adults assigned to where the River Faughan (44.9%) and the River Owenbeg (13.5%), which is a relatively distant population to the River Faughan sub-catchment.

This showed that the main stem River Faughan population and Bonds Glen disproportionately contribute to the production of anadromous brown trout smolts and adults in the River Faughan sub-catchment. Disproportionate contributions of certain parts of a catchment to anadromous life histories has been demonstrated in other studies, for example the sub-catchments Rivers Tamar and Tavy contributed significantly to mixed stocks of anadromous brown trout, in the South-West of England, whereas the River Lynher did not (King et al. 2016). In the Inari Basin, northern Finland, 12 populations out of 30 sampled contributed to the production of lake-run brown trout (Swatdipong et al., 2013). Situations where only a few populations contribute to the production of anadromous brown trout have important consequences for the effective management of a migratory life history. Management strategies need to not only ensure the continued production of anadromous brown trout within these tributaries but also to target rivers which contribute small numbers of anadromous brown trout, to potentially improve their levels of production. For example, it was shown in this study that the River Burngibbagh and River Faughan A populations produced a smaller number of anadromous brown trout smolts. This production of anadromous brown trout smolts may be natural but if it is the result of habitat degradation it may require targeted management to ensure future production of anadromous brown trout.

5.5.3 EVIDENCE OF STRAYING?

A large proportion of anadromous brown trout smolts (9.4%) and adults (30.3%) were assigned to populations in sub-catchments which were not the River Faughan. The River Owenbeg, which is relatively distant to the River Faughan sub-catchment, had the largest proportion of anadromous brown trout assigned to it. There are alternative explanations as to why anadromous brown trout smolts and adults would assign to populations not in the River Faughan sub-catchment. It would be expected that all anadromous brown trout smolts would assign to populations in the River Faughan, however, 8.1% of anadromous brown trout smolts were assigned to the River Owenbeg. By examining the genetic baseline with the 'leave-one-out' test in ONCOR a high proportion of individuals from the River Owenbeg (42%) were assigned incorrectly to populations in the River Faughan sub-catchment. This is despite a high probability (>68%) of correct re-assignment to sub-catchment of origin for River Faughan and River Roe sub-catchments. This indicated that the River Owenbeg population is genetically more like the River Faughan sub-catchment populations. This is supported by the hierarchical genetic structuring of brown trout (established in Chapter two), where the River Owenbeg population was shown to be more

like the Bonds Glen population than to any other population in the River Roe sub-catchment (see Figs. 2.3/ 2.4 from Chapter two). Given the River Owenbeg and River Faughan populations are around 97km (river distance) apart but the mouth of the River Faughan and Roe are relatively close together, the combination of mis-assignment and genetic similarity between the River Owenbeg and populations in the River Faughan points toward possible straying of adults and effective introgression between these two populations.

Possible straying of adults from other sub-catchments to the River Faughan is supported by the fact that 30.1% of anadromous brown trout adults were assigned to populations not in the River Faughan. The two populations with the highest proportion of adult anadromous brown trout assigned, with an assignment probability greater than 0.7, were the River Owenbeg population (13.5%) and the Killen Burn A population (10.1%). The River Roe sub-catchment where the River Owenbeg population is located and the River Derg sub-catchment where the Killen Burn A population is located, are both relatively productive for anadromous brown trout (P. Boylan, pers. comm). Therefore, this could be indicative of straying by adult anadromous brown trout. Straying is an evolutionarily important feature of salmonids, especially for colonisation, recolonization and range expansion (King et al., 2016). Tagging studies have demonstrated 1-10% of salmonid straying occurs between rivers in close proximity to one another (Palstra et al., 2007). Higher levels of straying have been demonstrated in anadromous brown trout compared with Atlantic salmon populations (Thorstad et al., 2016). However, strong population structuring and genetic differentiation of brown trout populations demonstrates limited effective straying between populations (Thorstad et al., 2016).

The anadromous brown trout adults which showed evidence of straying may have been overwintering in the River Faughan sub-catchment. It has been shown elsewhere that anadromous brown trout overwinter in non-natal rivers if overwintering conditions are more favourable than in their river of origin (King et al., 2016; Thorstad et al., 2016). The data from the study presented here would indicate that some of the anadromous brown trout which were caught in the lower reaches of the River Faughan sub-catchment were fish that had been over wintering in the River Faughan where presumably conditions are more favourable than the River Roe sub-catchment or Lough Foyle and were migrating to summer feeding grounds when caught. However, this does not explain the finding that River Faughan and

River Owenbeg trout are genetically more similar than the River Owenbeg population is to populations within its own sub-catchment (River Roe). This finding indicates potential interbreeding between the two populations. Known effective straying rates (individuals which successfully breed and contribute to succeeding generations) for anadromous brown trout range from 1% to 3% (Thorstad et al., 2016). If rates of effective straying were higher, genetic differences between populations would not be detectable (Thorstad et al., 2016). Therefore, the anadromous brown trout adults caught in the River Faughan sub-catchment which originated from the River Owenbeg and Killen Burn A populations could have strayed for overwintering. The anadromous brown trout adults which originated from the River Owenbeg could also be successfully interbreading with the Faughan population.

This study has important implications for management. Firstly, it has highlighted that the Rivers Faughan (main stem population) and Bonds Glen produce the largest number of anadromous brown trout smolts leaving the River Faughan sub-catchment. Therefore, this work provides key knowledge on the populations which produce anadromous brown trout and management decisions can be based on this to maintain and ensure the survival of the highlight anadromous brown trout populations. Secondly, it has been highlighted that a high proportion of anadromous brown trout adults assign to populations not in the River Faughan sub-catchment which could be indicative of straying/ overwintering. Future work could be to create a more comprehensive baseline in the Foyle catchment which would improve the accuracy of assignment of anadromous brown trout smolts and adults, giving more of an insight into which populations are contributing to the production of anadromous brown tout which could be used for the basis of management decisions.

CHAPTER 6: GENERAL DISCUSSION

6.1 INTRASPECIFIC GENETIC, MORPHOLOGICAL AND LIFE HISTORY STRUCTURING OF BROWN TROUT

Intraspecific genetic, morphological and life history structuring can provide insights into macro-evolutionary processes, such as genetic drift, mutation and natural selection, and early stages of divergence (Trontelj and Fiser 2008; Adams et al., 2016). Therefore, there have been many studies examining patterns of genetic, morphological and life history variation in species of freshwater fish (see Ward et al., 1994; Wong et al., 2004; Primmer et al., 2006; Stewart et al., 2006; Sanches et al., 2007; for genetic structuring, Eigenmann & Eigenmann 1982; Skúlason et al., 1999; Olafsdóttir et al. 2007; Garduño-Paz et al., 2012; Gowell et al. 2012; Gagnaire et al., 2013; Siwertsson et al., 2013; Chavarie et al., 2014; Faulks et al. 2015 for morphological structuring and Leggett & Carscadden 1978; Thorpe et al., 1998; Charles et al., 2004; Ferguson 2006, 2016; Blanck & Lamourous 2007; Wysujack et al., 2009 for life history structuring). However, there have been relatively few studies examining intraspecific genetic, morphological and life history structuring of brown trout across varying spatial scales in the same riverine system.

Intraspecific structuring of brown trout has been examined across large spatial scales, i.e. between catchments, and at medium spatial scales, i.e. between tributaries within a catchment (Crozier & Ferguson, 1986; Ferguson, 1989, 2006; Bernatchez et al., 1992; Carlsson et al., 1999; Massa-Gallucci et al., 2010; Swatdipong et al., 2010; Ensing et al., 2011). There have also been a few studies which have examined intraspecific structuring of brown trout on a small scale (McRae 2006). For example, in the River Aare, Switzerland, large genetic differentiation was found between populations within a 40km stretch of river (Stelkens et al., 2012). However, there have been no studies examining how structuring varies between several spatial scales and identifying potential environmental drivers of such structuring. Examining environmental drivers of population structuring can provide insights into potential barriers to gene flow, which result in such structuring (Ozerov et al., 2012; McCracken et al., 2013). Environmental factors driving structuring may be anthropogenic in origin and a knowledge of how these operate could highlight impacts which should be mitigated against to protect the evolutionary potential of a species (Dionne et al., 2008).

Morphological intraspecific structuring of a species can also provide important insights into evolutionary processes, such as natural selection and phenotypic plasticity, and the early stages of divergence. There have been many studies which have investigated morphological structuring of salmonids (Riddell & Leggett 1981; Beacham & Murray 1987; Pakkasmaa & Piironen 2001a; Paakasmaa & Pirronen 2001b; Von Cramon-Taubadel et al., 2005; Adams et al., 2008; Garduño-Paz et al., 2012; Drinan et al. 2012; Adams et al., 2016; Páez & Dodson 2017). For example, three morphotypes of brown trout (gillaroo, sonaghen and ferox) found in Lough Melvin showed extensive morphological variation, genetic differentiation and reproductive isolation (Ferguson and Mason, 1981; Cawdery and Ferguson 1988; Ferguson & Taggart 1991; Prodöhl et al., 1992; McVeigh et al., 1995; Youngson et al., 2003). There have been studies which have also investigated the morphological structuring of brown trout (Karakousis et al., 1991; Bernatchez et al., 1992; Pakkasmaa & Piironen, 2001a; Ojanguren & Braña 2003; Stelkens et al., 2012). However, few studies have examined morphological structuring across several spatial scales and there has been almost no attention given to potential environmental drivers of such variation in morphology between populations. There have been studies which have investigated environmental drivers of morphology in other species of salmonids (Bisson et al., 1988; Obedzinski & Letcher 2004; von Cramon-Taubadel et al., 2005; Pakkasmaa & Piironen 2011b; Drinan et al., 2012). For example, a study in New Brunswick found differences in body morphology and timing of downstream migration in two populations of Atlantic salmon were driven by flow regime and differences in overwintering energetic costs (Riddell & Leggett 1981). This study suggests that environmental drivers might also shape variation in morphology between populations of other species.

Although less often defined as such, intraspecific structuring of life history strategies is another form of intraspecific variation but in this case variation is expressed as discrete expression of alternative life histories. One of the most common forms of differential life history strategy expression is partial migration, where a proportion of a population undertakes a migration whereas other individuals do not (Dingle & Drake 2007; Chapman et al., 2011). Partial migration can be seen across a wide range of taxa, such as, for example birds: lapwings (*Vanellus vanellus*) (Lundberg 1988), mammals: moose (*Alces alces*) (Ball et al., 2001) and reptiles: green turtles (*Chelonia mydas*) (Mortimer & Carr 1987). Brown trout show extensive variation in life history strategies, with the most common life history strategy being anadromous (known colloquially as sea) trout and freshwater resident brown

trout (Ferguson 2006). There has been much controversy surrounding whether sympatric anadromous and freshwater resident brown trout originate from a single gene pool (Fleming et al., 1983; Hindar et al., 1991; Cross et al., 1992; Petersson et al., 2001) or whether each life history strategy forms a separate gene pool (Krieg & Guyomard, 1985; Skaala & Naevdal, 1989). Resolving this and defining whether the frequency of anadromous and freshwater life history strategies varies between populations can provide insights into the threshold trait value which can trigger a migratory life history.

Therefore, in the studies presented for this thesis I examined all three forms of possible intraspecific structuring: genetic, morphological and life history, in the brown trout (*Salmo trutta*), across a single but relatively complex, dendritic catchment, the River Foyle. The first step in this work was initially to attempt to define any pattern of intraspecific variation in each of the three possible types of structuring; the second was to attempt to look for possible drivers of this structuring.

6.2 BROWN TROUT STATUS

Due to the intraspecific diversity of brown trout, particularly in terms of their morphology, since 1750 more than 57 species of brown trout have been described (Ferguson 1989, 2004). However, brown trout are now generally considered one polymorphic species (Klemetsen et al., 2003). Describing brown trout as a single species has led to many debates about whether to describe distinctive morphs, such as ferox, gillaroo, sonaghen and fine spotted trout, as a separate sub-species (Ferguson 2004). For example, for many years it has been debated whether Ferox trout should be classed as a sub-species of brown trout (Ferguson 2004). Ferox are a long lived, piscivourous brown trout which have extensive morphological and genetic differentiation (based on LDH-5 ‘100’) from other populations of brown trout in Ireland (Ferguson 2004). LDH-5 ‘100’ has been described as an ancestral allele and is found in some ferox populations in Ireland and Scotland which has been replaced LDH-5 ‘90’ in modern brown trout (Ferguson & Mason 1981). Therefore, Ferguson (2004) suggested that ferox should be described as a sub-species to recognise and manage its genetic and morphological differences (Ferguson 2004). Therefore, brown trout demonstrate extensive and often complex patterns of genetic and morphological variation between populations. This is important to understand to define management units for effective management of the species.

Another commonly recognised morphology and life history strategy of brown trout is anadromy, these fish being known colloquially as sea trout. Anadromous brown trout are economically important for recreational fisheries. However, in Britain and Ireland in recent years there has been a large decline in anadromous brown trout numbers. For example, in the Foyle catchment using rod catch information there has been a decrease in anadromous brown trout catches from around 8000 in the 1970s to 280 anadromous brown trout caught in 2015 (Niven 2015). Therefore, it is important to understand potential drivers of genetic, morphological and life history structuring to manage anadromous brown trout numbers and prevent a complete collapse in their production

6.3 THREATS TO BROWN TROUT POPULATIONS

Brown trout populations face several threats from anthropogenic activities which could decrease their genetic diversity or frequency of the anadromous life history strategy (Jonsson & Jonsson 2011). These threats include: stocking, climate change, habitat fragmentation, pollution events and overharvesting (Ferguson 2016). Stocking of hatchery-reared brown trout could lead to a decrease in the genetic potential for anadromy in wild brown trout populations, as hatchery-reared anadromous brown trout have lower survival rates at sea (Ferguson, 2006; Ferguson 2016). Stocking of hatchery- reared brown trout could also reduce the genetic diversity and homing capabilities of wild brown trout populations which would lead to a breakdown in the extensive population structuring of brown trout (Ferguson 2016). Sea lice infestations from salmon aquaculture could also contribute to the decline in anadromous brown trout numbers. A study in the River Burrishoole demonstrated that salmon farming escapees can have both direct and indirect effects on the genetics of cohabiting anadromous brown trout by reducing variance at major histocompatibility class I genes (Coughlan et al., 2006). Climate change could also have a big impact on the frequency of the anadromous life history strategy. It is predicted that anadromous brown trout numbers will decline with warmer climates. This can be seen from the current climatic gradient with greater proportions of anadromous brown trout being found at higher latitudes because the productivity of rivers decreases relative to the productivity of the marine environment. Therefore, as temperatures increase so will productivity in rivers, thus, a larger proportion of brown trout will be more likely to adopt a freshwater residency life history strategy (Jonsson & Jonsson 2011). Finally, habitat fragmentation is considered one of the biggest

threats to biodiversity, as reduced connectivity between habitats leads to inbreeding, genetic drift, erosion of genetic variation and loss of rare alleles (Stelkens et al., 2012; Hansen et al., 2014). Therefore, it is imperative to understand how these impacts are driving intraspecific structuring, not only for conservation and management purposes but also to determine how humans are driving evolutionary processes within a species and the rate at which these processes are occurring (Moritz 2002).

6.4 IMPORTANCE OF INTRASPECIFIC STRUCTURING AND MANAGEMENT

Understanding both intraspecific structuring (genetic, morphological or life history) has the potential to provide insights into the evolutionary forces that may be driving the earliest stages of evolutionary divergence that may ultimately lead to speciation (Adams et al., 2016). Intraspecific genetic and morphological structuring could be assumed to be the result of an adaptive response reflecting differential selection pressures (Hendry & Stearns 2004) but it could also be the result of non-directional genome change such as, random genetic drift or population bottlenecking (Frazer & Rusello 2013). In the expression of morphological structuring it is also possible that phenotypic plasticity and differential exposure to local environmental conditions may also play an important role (Garant et al., 2007; Bolnick et al., 2011).

Determining the patterns and drivers of intraspecific structuring can inform our understanding of important evolutionary processes. However, an understanding of intraspecific structuring has important implications for management. The conservation and management of intraspecific variation is an important component of management of natural ecosystems. To achieve this requires managers to be able to identify units for conservation that will allow for actions which will sensibly maintain both the genetic, morphological and life history variation within a species (Ryder 1986). Management units are traditionally defined as populations whose population dynamics depend on birth and death rates (Palsbøll et al., 2007). However, determining intraspecific genetic structuring of a species has become a more common approach for inferring management units, with management units being defined as populations with significant genetic differentiation between them (Palsbøll et al., 2007). It is also important to include morphological variation between populations as a factor when designating management units. For example, Lough Melvin morphotypes, gillaroo, sonaghen and ferox show extensive genetic and morphological variation (Ferguson 2004),

thus, each morphotype would be classified as a management unit. Therefore, it is important to define genetic and morphological differentiation between populations and designate management units based on these findings.

6.5 FURTHERING OUR UNDERSTANDING AND APPLIED CONSEQUENCES OF THIS STUDY

The novel results presented in this thesis make a significant contribution to our understanding of intraspecific structuring of brown trout. The studies in this thesis all further our understanding of intraspecific structuring and provides insights into drivers of such genetic and morphological differentiation between populations of brown trout. This thesis also demonstrated how life history structuring can provide useful insights into how the frequency of anadromous and freshwater brown trout changes between populations which has important implications for management. Finally, using the information gained on the genetic population structuring of brown trout it was possible to identify tributaries within a sub-catchment which should be targeted for effective management of anadromous brown trout.

6.5.1 INTRASPECIFIC GENETIC STRUCTURING

In Chapter two, I investigated intraspecific structuring of brown trout at three different spatial scales (large, medium and small) and determined environmental drivers of such structuring. This study found 21 genetically differentiated populations in the Foyle catchment which were identified at six hierarchical levels (Fig. 2.3.6). Several other studies have investigated the genetic population structuring of brown trout but few have demonstrated structuring over such a range of spatial scales or between co-existing populations in riverine systems (Griffiths et al., 2009). The level of population structuring identified in this thesis has been demonstrated by studies examining population structuring of brown trout between tributaries of a catchment (Crozier & Ferguson, 1986; Ferguson, 1989, 2006; Bernatchez et al., 1992; Carlsson et al., 1999; Massa-Gallucci et al., 2010; Swatdipong et al., 2010; Ensing et al., 2011), between morphotypes within a lake (Ferguson & Taggart 1991; Ferguson 2004) and between populations in differing stream (McRae 2006; Stelkens et al., 2012). This has important evolutionary consequences with genetic divergence between populations possibly being driven by post glaciation invasion of new habitats through allopatry and secondary contact, genetic drift, local selection pressures or random

chance mutations (Ozerov et al., 2012; McCracken et al., 2013). However, this also has vital implications for effective management of a species. As has been previously described management units are often identified as having significant genetic differentiation between them (Palsbøll et al., 2007). Therefore, since this study identified significant differentiation between all populations, except for five pairwise comparisons (out of a total of 210), it would suggest that each population identified in this study should be managed as a separate management unit.

This study also investigated potential drivers, geographic distance and environmental factors, of intraspecific genetic structuring of brown trout across the Foyle catchment. This showed geographic distance was a significant driver of genetic population structuring in the Foyle catchment. There are a few studies which have also detected isolation by distance as a potential driver of genetic population structuring in brown trout (Estoup et al, 1998; Laikre et al., 2002; Linløkken et al., 2014). However, many other studies reveal no effect of isolation by distance (Crozier & Ferguson 1986; Ferguson 1989; Meldgaard et al. 2003; Heggenes & Røed 2006; Stelkens et al., 2012). Evidence of isolation by distance shaping populations structuring of brown trout indicates brown trout populations have been subject to evolutionary pressures resulting in the subdivision of ancestral populations into many genetically differentiated sub-populations. This further supports assigning management units based on significant genetic differentiation between populations.

Finally, analysis of landscape and environmental features that might be driving this variation showed that the population structuring of brown trout was shaped by river phosphorus concentration, urbanised area (represented by number of houses per km^2 in the upstream catchment) and proximity to farmland (represented by the distance to the nearest farm (km)). This has important implications for management as these variables are strongly altered by anthropogenic pressures. Anthropogenic impacts have been shown to drive population structuring of brown trout in several other studies (Durrant et al., 2011; Östergren & Nilsson 2012; Stelkens et al., 2012; Thaulow et al., 2013; Hansen et al., 2014). For example, medieval dams and metal pollution from the bronze age have been shown to have driven population strutcuring of modern brown trout populations (Hansen et al., 2014; Paris et al., 2015). The number of weirs between populations have also been show to act as barriers to gene flow between populations of brown trout (Stelkens et al., 2012). Therefore, despite

anthropogenic impacts being inevitable it is important to identify possible anthropogenic drivers of population structuring and mitigate against such pressures for effective management of a species.

6.5.2 INTRASPECIFIC MORPHOLOGICAL STRUCTURING

In chapter three, I describe the morphological variation between populations and identify possible genetic, geographic and/or environmental drivers. As was described in chapter two, there was extensive genetic structuring of brown trout populations, therefore, it could be presumed that the level of morphologically structuring would be similar. This study identified extensive morphological structuring of brown trout across three spatial scales with morphological variation between 21 populations. There have been other studies which have examined morphological variation between populations of brown trout (Karakousis et al., 1991; Bernatchez et al., 1992; Pakkasmaa & Piironen, 2001a; Ojanguren & Braña 2003; Stelkens et al., 2012). However, there have been no studies which have examined morphological structuring between many populations and across three different spatial scales. Therefore, this study has highlighted the variability of brown trout morphologies between populations. This is important to consider when allocating management units as morphological populations may or may not also be genetically differentiated. This also has important evolutionary consequences as the variation in morphology could be driven by neutral genetic variation or phenotypic plasticity (Pakkasmaa & Piironen 2011b; Stelkens et al., 2012).

The morphological structuring of brown trout was driven by several environmental factors, however, unlike in the analyses presented in Chapter two only one of these variables was found to be linked to anthropogenic impacts (urbanised area- represented by number of houses per km^2 in the upstream catchment area). Environmental variables which were found to drive morphological structuring were: stream order, substrate PC1 representing differences between cobbles and fines/sand, elevation and upstream catchment area. The differences in these environmental variables have been linked to flow rate (Zhen-Ghan 2017) which has been demonstrated in other studies on salmonids to drive variation in morphology (Riddell & Leggett 1981; Taylor & Foote 1991; Pakkasmaa & Piironen 2001b; Langerhans 2008; Stelkens et al., 2012; Drinan et al., 2012). It was also demonstrated that genetic differentiation between morphological populations could be a potential driver of

morphological structuring but when analysed in a model including the mentioned environmental variables, it was found the environmental variables described more of the variation in morphology between populations and were, thus, more important drivers. This leads to the conclusion that phenotypic plasticity is probably the driving force of morphological variation between populations. As anthropogenic impacts, such as climate change, alter freshwater habitats, it is increasingly important to understand both phenotypic plasticity and adaptation of species to its local environment to determine how brown trout populations will respond to these impacts (Drinan et al. 2012).

These findings lead to several conclusions with application to conservation and fisheries management. Firstly, that genetic structuring (measured by an examination of markers that are neutral) is underpinned by phenotypic differences, thus, suggesting that the expressed variation between groups has some functional significance (see Adams and Huntingford 2004) and is not simply the result of random genetic drift. This plus the fact that variation in morphology was correlated with variation in broad scale environmental and landscape features, further emphasises the need for discrete populations to be managed as separate entities. One conclusion of this is that any mixing of morphological or genetic groups across the catchment is likely to result in a mismatch between the local environmental conditions and the morphological/genotypic group and would, thus, be counterproductive as a conservation measure (see comparisons with McGinnity et al. 2009).

6.5.3 INTRASPECIFIC LIFE HISTORY STRUCTURING

Chapter four, examined a specific case of intraspecific morphological structuring by examined the adoption of alternative life history strategies at three sampling locations within the Foyle catchment. Specifically, I aimed to determine which of two possible alternative mechanisms were driving partial migration within populations (a difference in the threshold trait value between populations or a difference between individuals within populations). This study demonstrated genetic differentiation between brown trout populations from different streams but no evidence of genetic differences between migration strategies. A lack of genetic differentiation between anadromous and freshwater resident brown trout co-existing in sympatry has been described by several other studies (Fleming et al., 1983; Hindar et al., 1991; Cross et al., 1992; Petersson et al., 2001). However, little/no studies have identified sympatric (here defined as co-existing) populations with differing frequencies of

anadromous and freshwater resident brown trout. This therefore, suggests that the mechanism driving partial migration varies both between populations and between individuals within a population. It is likely partial migration, in most species, is driven by quantitative trait loci which are highly heritable (Chapman et al., 2011). In brown trout it has been suggested that these loci are threshold traits which are triggered by individual condition, food availability, sex and parental effects (Ferguson 2016). Therefore, there will be a difference in environmental variables between rivers, such as food availability or distance to sea, which is driving a difference in the frequency of anadromous and freshwater resident brown trout. However, between sympatric populations with differing frequencies of anadromous and freshwater resident brown trout it is possible that either the nursery areas for both populations differed in environmental characteristics or that the threshold trait value for each population differs. Thus, this study provides some insights into the mechanisms which drive partial migration as the threshold trait value is likely to vary between populations due to inheritance and within populations due to environmental factors (such as resource availability).

Similarly to those presented in Chapters two and three, these findings point towards several important conservation and fisheries management conclusions. Firstly, they show that the valuable anadromous brown trout resource is not evenly distributed amongst the brown trout populations of the Foyle. Thus, for effective management of anadromous brown trout, activity needs to focus more on some populations than others. These data also show that for populations that do produce individuals with an anadromous life history, the management of all individuals in that population is important (as all have the potential to produce anadromous fish in the next generation).

6.5.4 USING INTRASPECIFIC GENETIC STRUCTURING FOR GENETIC ASSIGNMENT OF ANADROMOUS BROWN TROUT OF UNKNOWN ORIGIN

Intraspecific structuring of populations can further be used to influence management decisions by using Genetic Stock Identification (GSI) analytical framework to determine which tributaries produce anadromous brown trout. Therefore, Chapter five used the information gained from the genetic population structuring of brown trout presented in Chapter two, in an applied manner to inform management where management resources would be most effective in managing anadromous brown trout. GSI has been used in several

studies either for mixed stock analysis or individual assignments (Winans et al. 2004; Swatdipong et al. 2013; Bradbury et al. 2015; Mäkinen et al. 2015; Vähä et al. 2016). However, there have been relatively few studies which have examined GSI in anadromous brown trout (Koljonen et al., 2014; King et al., 2016) and no studies which have used GSI within a riverine system on a relatively small spatial scale. This study demonstrated that two populations (mainstem river Faughan and Bonds Glen) disproportionately contributed to the production of anadromous brown trout in the River Faughan sub-catchment. This was true for both smolting anadromous brown trout and adults. However, the finding with the biggest implication for management was the evidence of effective straying between sub-catchments. A large percentage of adult anadromous brown trout assigned to the river Owenbeg, a river relatively distant to the river Faughan sub-catchment. However, the river mouths of the rivers Roe (sub-catchment of river Owenbeg) and Faughan are relatively close to one another in the sea lough. Therefore, this would suggest that there is possible overwintering or effective straying of anadromous brown trout adults between sub-catchments. Staying was found between sub-catchments in the Rivers Tamar, Tavy and Lynher and suggested this could be due to fish overwintering in the lower reaches of freshwater rivers (King et al., 2016). The applied implications of these findings are that it is possible to very precisely define, down to a very small stream reach, the nursery sites that produce anadromous trout if there is enough genetic differentiation between populations to allow for a high probability of accurate assignment. This enables effective management of anadromous brown trout to be highly focussed. It also highlights that there are (until now) unknown habitat requirements, particularly with regards to overwintering needs for non-breeding anadromous trout.

A general conclusion that relates specifically to management of brown trout in the Foyle system (and potentially other dendritic catchments) is that the brown trout population is highly genetically and morphologically structured even at very small spatial scales. These studies also show that this variation is most likely driven by macroevolutionary processes, such as genetic drift, natural selection and phenotypic plasticity. However, specifically in the case of intraspecific genetic structuring, anthropogenic impacts, both past and present, are likely to impact population structuring. A consequence of this is that populations need to be managed at a small spatial scale (stream level) and that management policies that do not take account of the localised adaptations and variations are very likely to be at best ineffective and worst damaging for the populations.

6.6 LIMITATIONS OF THIS STUDY

There were some limitations to this study. The sampling sites used in this study encompassed both the north and south of the Foyle catchment but the middle west and east of the Foyle catchment were not sampled. Sampling of these regions would give greater insight into the genetic and morphological structuring of brown trout in the Foyle catchment. Using the north and south of the Foyle catchment, as a case study, would suggest that if the rest of the Foyle catchment was to be sampled, it is likely that many additional morphological and genetic populations would be identified. Sampling more sites within the Foyle catchment would also enable the environmental variables driving genetic and morphological structuring to be examined in more detail. There were a few sampling locations which had sample sizes less than 30 due to poor amplification of microsatellite markers or poor quality digital photographs for morphometric analysis. As digital photographs were taken in the field, despite great efforts it was difficult to standardise each photograph for camera height, lighting and the levelness of the board the fish were placed on for each photograph. Therefore, resampling at these sites would give more power to the overall analysis. The samples used in this study were collected over two years (2013 and 2014) but only in three locations were samples collected in both years for genetic analysis. Therefore, temporal stability of the genetic population structuring identified cannot be confirmed. Collecting samples over multiple years (>five years) would confirm the temporal stability of the findings presented (Tessier & Bernatchez 1999).

Examining intraspecific life history structuring of brown trout provided insights into the variation in frequency of anadromous and freshwater resident brown trout between populations. This study, however, was only conducted over three sampling locations. By investigating more sites, greater insights would be gained about the differences in frequency of life history traits between populations within the same tributary and between rivers. Finally, with a greater genetic baseline created for the whole of the Foyle catchment, it would be possible to assign individual anadromous brown trout of unknown origin caught in a rotary screw trap in the lower reaches of the rive Faughan to a population of origin with a higher accuracy. This would also allow for straying of anadromous brown trout to examined in more detail. By reading scale samples to define whether each anadromous brown trout

caught in the rotary screw trap were 1st year migrants or 2nd year migrants it would be possible to investigate straying further.

Despite these limitations, this thesis did result in some important key findings with applied conservation and fisheries application. Chapter two demonstrated that brown trout in a dendritic catchment can exhibit highly genetically structured, discrete populations over multiple spatial scales. This genetic structuring is driven by both isolation by distance and isolation by environment, with environmental variables being broadly anthropogenic in nature. This has important consequences for the identification of management units and effective management of the species. Chapter three demonstrated that genetically differentiated populations can also show morphological differences, as well as morphologically differences being identified between sampling sites which represented one genetic population. Despite morphological differences being identified between genetic populations, environmental variables were found to be an important driver of morphological structuring. This demonstrated that phenotypic plasticity was a driving force of morphological variation between populations. Chapter four demonstrated anadromous and freshwater resident brown trout are derived from the same gene pool. However, the frequency of each life history strategy varies both between populations and between individuals. Finally, Chapter five demonstrated the possibilities of using molecular techniques to influence management decisions. This chapter used information from Chapter two to form a genetic baseline to with anadromous brown trout of unknown origin could be assigned. Therefore, it was possible to identify the population of origin of the anadromous component of the trout population to a relatively small geographic region of the catchment. This study showed that a relatively small number of streams in a broader catchment were responsible for the production of anadromous trout.

6.7 FUTURE DIRECTIONS

Building on the work conducted in this thesis, future studies might develop on the study presented in Chapter two to establish the population structuring in both the west and east of the Foyle catchment. This would determine if population structuring was found across the same three spatial scale and determine if environmental variables associated with anthropogenic impacts also shape the population structuring in these locations. This would provide important information for management on the extent that anthropogenic impacts shape the population structuring of brown trout in the Foyle catchment. This would also provide insight into the evolutionary processes driving the population structuring of brown

trout in the Foyle catchment. It is often assumed genetic structuring of populations is the result of adaptation and this seems very likely in this study. Therefore, genomic studies could be used to establish if the population structuring identified was the result of local adaption or random genetic drift. Building of chapter three, morphological structuring would also be examined in the west and east of the Foyle catchment to determine how environmental variable drive morphological structuring. It was likely the morphological structuring of brown trout was the result of phenotypic plasticity or local adaptation. It was not possible in this current study to separate the origins of morphological variation and determine whether phenotypic plasticity or adaptation drives morphological variation. Therefore, this could be further investigated through common garden experiments to quantify the extent of phenotypic plasticity demonstrated by brown trout in the Foyle catchment. As well as, isolating the regions of the genome responsible for such adaptations. Another interesting direction for future work would to be further investigate life history structuring of brown trout. By investigating if a similar pattern of life history structuring is found within other areas of the Foyle catchment and other river systems, it would be possible to identify if the pattern described in this thesis of differences in frequencies of life history traits between populations is also found in other geographic areas. Finally, individual assignment of individual anadromous brown trout from a mixed stock was conducted in the River Faughan, as a case study. Hence, repeating this study in other sub-catchments of the Foyle would provide key information for management. With a more comprehensive genetic baseline of the Foyle catchment, particularly in sub-catchments anadromous brown trout are known to originate, it would be possible to establish the population of origin for a mixed stock of anadromous brown trout caught at the mouths of the Rivers Foyle, Roe and Faughan. It would also be interesting to repeat this study in other river catchments to investigate if other rivers have populations which disproportionately contribute to anadromous brown trout production and if there is evidence of straying.

APPENDIX

This table is a list of the 22 primers used in this study (Keenan et al., 2013a)

Locus	Forward primer	Reverse primer
Panel- 1		
Ssa85	NED-AGGTGGGTCCTCCAAGCTAC	gtttACCCGCTCCTCACTTAATC
Oneμ9ASC	NED-CTCTCTTTGGCTCGGGGAATGTT	gtttGCATGTTCTGACAGCCTACAGCT
Ssa416UoS	FAM-TGACCAACAACAAACGCACAT	gtttCCCACCCATTAACACAACTAT
CA054565a	VIC-TCTGTGGTTCCCGATCTTTC	gtttCAACATTTGCCTAGCCCAGA
One102	NED-GGGATTATTCTTACTTTGGCTGTT	gtttCCTGGTTGGGAATCACTGC
CA048828	VIC-GAGGGCTTCCCATACAACAA	gtttGTTTAAGCGGTGAGTTGACGAGAG
salmoY	Unpublished primer- refer to Prof. Paulo Prodohl	Unpublished primer- refer to Prof. Paulo Prodohl
One103	FAM-TGCTAAATGACTGAAATGTTGAGA	GAGAATGAATGGCTGAATGGA (no pig tail)
ppStr2	PET-CTGGGGTCCACAGCCTATAA	gtttGAGCTACAACCTGATCCACCA
CA053293	PET-TCTCATGGTGAGCAACAAACA	gtttACTCTGGGGCATTCATTCAG
One108	VIC-GTCATACTACTCATTCCACATTA	gtttACACAGTCACCTCAGTCTATTC
SsaD48	FAM-GAGCCTGTTCAGAGAAATGAG	gtttCAGAGGTGTTGAGTCAGAGAAG
Cocl-lav-4	VIC-TGGTGTAATGGCTTTTCCTG	gtttGGGAGCAACATTGGACTCTC
Ssa406UoS	NED-ACCAACCTGCACATGTCTTCTATG	gtttGCTGCCGCCTGTTGTCTCTTT
Panel- 2		
BG935488	gttTGACCCCACCAAGTTTTTCT	NED-AAACACAGTAAGCCCATCTATTG
Ssa197	VIC-GGGTTGAGTAGGGAGGCTTG	gttTGGCAGGGATTTGACATAAC
MHC-I	PET-AGGAAGGTGCTGAAGAGGAAC	gtttCAATTACCACAAGCCCGCTC
SsaD71	NED-AACGTGAAACATAAATCGATGG	gtTTAAGAATGGGTTGCCTATGAG
ppStr3	FAM-CTGACCGCTGCACACTAA	gtttGGCTCTAATCGACTGGCAGA
Sasa-TAP2	gtttGTCCTGATGTTGGCTCCCAGG	NED-GCGGGACACCGTCAGGGCAGT
CA060177	VIC-CGCTTCCTGGACAAAAATTA	gtttGAGCACACCCATTCTCA
Ssa410UoS	gtttGGAAAATAATCAATGCTGCTGGTT	PET-CTACAATCTGGACTATCTTCTTCA

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