The Consequences Of Phenotypic Plasticity On Postglacial Fishes

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ABSTRACT

Phenotypic differences within a species significantly contribute to the variation we see among plants and animals. Plasticity as a concept helps us to understand some of this variation. Phenotypic plasticity plays a significant role in multiple ecological and evolutionary processes. Because plasticity can be driven by the environment it is more likely to produce beneficial alternative phenotypes than rare and often deleterious genetic mutations. Furthermore, differences in phenotypes that arise in response to the environment can affect multiple individuals from the same population (or entire populations) simultaneously and are therefore of greater evolutionary significance. This allows similar, beneficial alternative phenotypes to increase quickly within a single generation and allow new environments to produce and select for new phenotypes instantly. The direction of the present thesis is to increase our understanding of how phenotypic plasticity, coupled with contrasting environmental conditions, can produce alternative phenotypes within a population. Plasticity provides a source of variation for natural selection to act upon, and may lead to genetic isolation as a by-product. For example, there are multiple cases of polymorphic populations of fish, where groups belonging to multiple isolated gene pools, have arisen in sympatry. Here it is shown that although plasticity is important in sympatric speciation events, plasticity alone is not responsible for the frequency in which sympatric polymorphic populations occur. The most frequently observed differences among sympatric polymorphic populations are morphological differences associated with parts of the anatomy used in the detection, handling and capture of prey. Moreover, it is shown here that there are physiological effects associated with foraging on alternative prey that may significantly contribute towards ecological speciation. It is also shown in this study that anthropogenic abiotic factors can disrupt developmental processes during early ontogeny, significantly influencing morphology, and therefore having ecological consequences. Phenotypic structuring in postglacial fish is most frequently based around a divergence towards either pelagic or littoral benthic foraging specialisms. Divergences that deviate from this pattern are of greater scientific interest as they increase our understanding of how evolutionary processes and selection pressures work. Here we describe a rare divergence not based around the typical pelagic/littoral benthic foraging specialisms. Finally, in this study, the effectiveness of local level conservation policy shows that species of fish which are highly variable in their life history strategies are harder to effectively manage and often poorly represented at a local level.

"Thus, from the war of nature, from famine and death, the most exalted object which we are capable of conceiving, namely, the production of the higher animals, directly follows. There is grandeur in this view of life, with its several powers, having been originally breathed into a few forms or into one; and that, whilst this planet has gone cycling on according to the fixed law of gravity, from so simple a beginning……

……endless forms most beautiful and most wonderful have been, and are being, evolved."

– CHARLES DARWIN, THE ORIGIN OF SPECIES

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BMR	Basal Metabolic Rate
CBBT	College Burn Brown Trout
cm	Centimetre
CVA	Canonical Variate Analysis
D	day
Dd	degree days
DFA	Discriminant Function Analysis
G	gram
GLS	Generalised Least Squares
GLMM	Generalised Linead Mixed Model
GPA	Generalised Procrustes Analysis
Hr	hour
ILAC	Iceland Arctic charr
Km	Kilometre
L	Litres
LBAPs	Local Biodiversity Action Plans
LLEW	Loch Lomond European Whitefish

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m	Metres
mg	Milligram
mm	Millimetres
PC	Principal Component
PCA	Principal Component Analysis
rGrowth	Residual Instantaneous Growth
s	Seconds
SCENE	Scottish Centre for Ecology and the Natural Environment
rSMR	Residual Standard Metabolic Rate
SDA	Specific Dynamic Action
SE	Standard Error
SMR	Standard Metabolic Rate
WABT	Whiteadder Brown Trout
WRAC	Wester Ross Arctic charr

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Declaration

I hereby declare that the material presented in this thesis is the result of original research, conducted between June 2012 and March 2016, under the supervision of Professor Colin E. Adams. This thesis has not been submitted, in whole or in part towards the fulfilment of any other degree. It is entirely my own composition and that the research described herein was carried out by me unless otherwise stated or acknowledged

Signature _____

Oliver Hooker
September 2016

CHAPTER 1.

INTRODUCTION

1.1. PHENOTYPIC VARIATION

A phenotype is most often referred to as the physical appearance of an organism but it can also include non-physical characteristics. A phenotype can be used to describe the whole organism, but an individual organism is ultimately comprised of multiple phenotypes made up of different observable characteristics across a range of traits (West-Eberhard, 1989). These characteristics and traits can be passed from parents to offspring as inherited information contained within the genome, as a result of interactions between an individual and the environment (West-Eberhard, 2005), or be a combination of both (Adams and Huntingford, 2002a).

In nature, the phenotype of individuals varies within a species. Differences in phenotype between individuals can manifest through a wide range of trait types. These include life history traits such as longevity and maturation; traits such as behaviour i.e. spawning, foraging and habitat use (and the products of these different behaviours) as well as differences in physiology (Dawkins, 1978; Reznik and Ghalambor, 2001). Differences in bird nest structure within a species have been suggested as alternative phenotypes based on behaviour (Dawkins, 1972). Variation in phenotype can also be induced as seen in zombie ants (*Camponotus leonardi*) when infected by *Ophiocordyceps* fungi. Alternative phenotypes across a species, or within a single population, are however most frequently observed as differences in morphology and colouration which are often less cryptic than physiological or behavioural differences (Van-Leeuwen *et al.,* 2011).

Variation among individuals most frequently occurs along a continuum and is common for most traits (West-Eberhard, 1989). One example of this is body shape. Some traits however are discontinuous such as eye colour (White and Rabago-Smith, 2011). Discontinuous traits categorise individuals to groups, but continuous traits (or their ranges) can themselves be discontinuous to some extent, this results in the ranges in trait variation between individuals becoming fragmented. This can lead to multimodal trait frequencies also referred to as phenotypic structuring (Doebeli, 1996). Size is a prime example of a continuous trait that frequently shows structuring in fish (Adams *et al.,* 2003; Silwertsson *et al.,* 2013).

Phenotypic structuring occurs where multimodal trait frequencies exist and can be seen throughout nature. The products of these are frequently referred to as morphs or ecomoprhs as the phenotype is often a functional response to the organisms' 'ecology'. When multiple forms (morphs) coexist within a species at the same stage of development they are called polymorphisms. This term is most frequently used to describe multiple morphological forms or differences in physiology, but is can also be applied to different behaviours (polyethism) (Komdeur, 2006). Ford (1966) insists that a polymorphism must have some genetic predetermination which results in contrasting differences that do not overlap and thus should not include continuous variation within a uni-modal distribution, which instead is just phenotypic variation rather than structuring. Multiple forms should appear fractured at opposite ends of a range creating a multimodal distribution rather than a continuum or alternative morphs. Ford (1966) also states that polymorphism should not be the result of different environmental interactions (plasticity). However, the occurrence of non-continuous phenotypic variation (phenotypic structuring) is possible without any genetic predetermination (Ghalambor *et al.*, 2007), this can be seen in the butterfly *Junonia octavia* in which a plastic response in colour results in colour morphs that are either blue or orange depending on the season (McLeod, 1969).

The developmental mechanism that can trigger the expression of alternative phenotypes can be both genetic (allelic-switch) and environmental (Wakano and Whiteman, 2008). A genome input, like a mutation, leads to the production of a small phenotypic change in a single individual. If this change in phenotype results in an increase in fitness associated with the mutant allele, the mutant gene and the associated phenotype increase in the population over subsequent generations (West-Eberhard, 2008). Thus, the frequency of the trait will increase slowly (West-Eberhard, 2005). Phenotypic changes can also result from an environmental input. When individuals in a population encounter different environmental interactions during development different individuals may simultaneously express different phenotypes, referred to as polyphenisms, in response to different environmental interactions. Environmental drivers can present themselves in many ways and unlike a genome input (or chance mutation) an environmental input allows the frequency of a beneficial trait to increase rapidly within a single generation.

1.2. Environmental Drivers Of Phenotypic Variation

Environmental drivers of phenotypic change can present themselves in many ways and nearly all environmental factors can influence an organism's ecology either directly or indirectly. Aquatic systems show high levels of heterogeneity and thus provide different environmental parameters with which to interact and cause a phenotypic response.

Highly heterogeneous environments, in general, might be expected to promote high levels of phenotypic variation. Where the environment is structured into two or more discrete habitat types this may induce discrete alternative phenotypes to be expressed. Freshwater lake habitats in particular present such multiple habitat types, e.g. limnetic, littoral and profundal zones, with discrete alternative environments

The kinds of environmental drivers that have been shown to influence phenotype are many and varied. Chemical factors such as the amount of dissolved calcium concentration can influence the number of bony plates formed in three-spined sticklebacks (Marchinko and Schluter, 2007). Oxygen levels have been shown to directly influence gill size (Langerhans *et al.,* 2007; Crispo and Chapman, 2010) and gill morphology among fish (Sollid *et al.,* 2003).

Flow rate is a physical property of water that can affect many parts of a fish's anatomy. It has directly influenced body shape, caudal fin shape and gill size in the African cyprinid, *Barbus meumayeri* (Langerhans *et al.,* 2007). Atlantic salmon, *Salmo salar,* and brown trout, *Salmo trutta,* show phenotypic variation due to differing flow rates across their environments. In both species differences in flow rate positively correlated with body shape, fish from the faster flowing rivers developed a more fusiform body and larger paired fins (Riddell and Leggett, 1981).

Light is a physical parameter of water that is often overlooked but can be responsible for observed differences in eye size in Arctic charr, *Salvelinus alpius* (Gardner *et al.,* 1988). Light can result in the loss of phenotypic variation within populations through the loss of sexual dimorphic characteristics which have reduced benefits when light levels are low. This has been reported in the wild in Arctic charr (Adams and Huntingford, 2002a) and through controlled laboratory experiments using sticklebacks (Spoljaric and Reimchen, 2008).

Temperature is frequently documented as having an effect on morphology in fish (Hubbs, 1922; Beacham, 1990; Camphino *et al.,* 2004). Ramler *et al.,* (2014) claimed that this effect was non-linear with different temperatures affecting the potential range of phenotypic variation that can potentially be expressed. McPhee (2012) speculated that temperature may cause the expression of different phenotypes due to heterochrony of various anatomical features such as vertebrae number and fin ray counts. The variation in phenotpype is likely to be casued by changes in the rate and timing of crondogenesis (development of cartilidge) and ossification (development of bone) (Koumoundouros *et al.*, 2001; Camphinho *et al.*, 2004; Ramler *et al.*, 2014).

Predator prey interactions can be an important driver influencing morphological adaptation. Predation is a biological factor apparent in all ecosystems and thus examples can be seen across different taxa (Miner *et al.,* 2005). It can either be the cause or the effect of differences in phenotype, thus facilitating, as opposed to hindering, sympatric divergence (Vamosi and Schluter, 2004). This has been shown in three-spined sticklebacks from Iceland and British Columbia. In Lake Thingvallavatn, Iceland, individuals that live in the lava habitat, which they can use as a protective refuge, have shorter spines than those that live in the mud habitat, which provides no protective refuge resulting in a higher predation pressure (Kristjansson *et al.,* 2002). Across different lakes in British Columbia the same pattern was seen in stickleback populations with limnetic individuals having increased investment in armour plating compared to benthic individuals in response to increased predation risk from foraging in open water (Vamosi and Schluter, 2004). Predation pressures can also influence escape responses, as seen in crucian carp, *Carassisius carrassisius*. Where crucian carp coexist with pike, *Esox lucius*, individuals develop a deeper body to act as a deterrent, but are also more streamlined with superior locomotor performance to help evade predation (Domenici *et al.,* 2008).

The most fundamental driver behind alternative phenotypes and ecological speciation and the most commonly reported are differences in foraging strategies rooted in different prey types. This driver can give rise to different morphological features of functional significance to the predator in response to the detection, handling and consumption of prey (Skúlason and Smith, 1995; Robinson, 2000; Adams and Huntingford, 2002a). As diet has a significant role in driving phenotypic responses (Parsons and Robinson, 2007), multiple taxa have been tested under laboratory conditions

to examine their phenotypic response to varying prey items (Lavin and McPhail, 1986; Relyea, 2004; Ruehl and DeWitt, 2007; Mougi and Kishida, 2009).

Understanding the environmental drivers behind phenotypic structuring is important. Phenotypic structuring reflects differential selection pressures of the environment that shape adaptation and are thus localised evolutionary responses (Garant *et al.,* 2007). For example, phenotypic structuring can be indicative of early stages of divergence, it can also highlight possible selection pressures at work (Schluter, 2001; Rundle and Nosil, 2005). The observable patterns in phenotypic structuring can uncover both past and current diversifying selection pressures that individuals are exposed to, providing insight into the drivers behind phenotypic structuring (Urban *et al.,* 2008). Changes in the phenotypes that are expressed in a population can also uncover spatial and temporal ecosystem changes which are important to conservation.

Phenotypic structuring is particularly prevalent in many fish species that inhabit post-glacial lakes making them an excellent model for studying ecological speciation (Skúlason *et al.,* 1999). In the British Isles, as well as other Northern (Arctic) areas such as Finland (Kahilainen *et al.,* 2011), Norway (Jonsson and Hindar, 1982), Sweden (Svanbäck *et al.,* 2008), Russia (Alekseyev *et al.,* 2002) and Canada (McPhail, 1984) the existence of sympatric polymorphic populations of species of freshwater fish that differ in functional traits associated with foraging are now known to be relatively common (Schluter and McPhail, 1992; Wimberger, 1994; Skúlason and Smith, 1995; Skúlason *et al.,* 1999). This is in part because postglacial lakes provide geographically disjunct habitats with often discrete, and to some extent replicated environments, that vary in available resources and species communities. Such lakes have provided multiple examples where phenotypic structuring has arisen within and between different populations of the same species. Where structuring within a catchment is most acute, groups are phenotypically discrete and have well-defined and differing ecology; frequently comprise of separate gene pools with no, or at least very limited, gene flow. Three species that exhibit significant amounts of within species phenotypic differences include Arctic charr (*Salvelinus alpinus*) (Snorrason *et al.,* 1994; Adams *et al.,* 1998), brown trout (*Salmo trutta*) (Cawdrey and Ferguson, 1988) and European whitefish (*Coregonas laveratus*) (Kahilainen and Østbye, 2006; Siwertsson *et al.,* 2013).

1.3. SPECIATION

Speciation defines the process that gives rise to multiple reproductively isolated populations which are (usually) absent of gene flow. Speciation may result as a product of divergent natural selection with alternative species emerging from different selection pressures.

Speciation most frequently occurs in allopatry due to the physical isolation between individuals of the same species. This can be in the form of a geographical or physiological barrier that restricts movement, and thus gene flow between populations (Schliewen *et al.*, 1994; Coyne and Price, 2000). Phenotypic variation therefore presents itself more readily when in allopatry because individuals inhabiting different habitats are exposed to different environmental conditions. and populations occupy separate gene pools. Phenotypic structuring can therefore be an indicator of incipient speciation (Adams *et al.*, 2016).

1.4. ECOLOGICAL SPECIATION

Ecological interactions are repeatedly implicated as being fundamental to speciation events. Ecological speciation is the process that leads to the development of barriers to gene flow between populations as a consequence of divergent selection pressures of differing environments. This process is most acute when the selection pressures are highly contrasting and favouring extreme phenotypes within a single population. Thus, ecological speciation is underpinned by natural selection on morphological, physiological or behavioural traits (Schluter, 2001) in which reproductive isolation occurs as a by-product of divergent selection on certain traits caused by different environments (Schluter and McPhail, 1992; Schluter, 2001).

There are three main modes of divergent selection associated with ecological speciation; 1) differences in environment are the most common and include resources, habitat structure, species community, especially coexisting predators, inter and intra specific competition (Schluter, 2001); 2) Sexual selection, which selects for traits linked with mate recognition (Boughman, 2001); 3) Frequency dependant ecological interactions that generate disruptive selection (Rundle and Nosil, 2005).

However, it is with resource (food) acquisition, and interactions associated with this such as behaviour and habitat use, that ecological speciation is most strongly associated

(Rundle and Nosil, 2005). Early stages of incipient ecological speciation involve differences in behaviour such as foraging strategies; this is often followed by subsequent modifications in morphology and behaviour, through plasticity, to increase efficiency in foraging for alternative resources before gene pool segregation (Skúlason *et al.*, 1999).

1.5. Sympatric Speciation

Sympatric speciation, where reproductively isolated populations occur with no geographical or physiological barrier is also possible (Coyne, 2007). This process is much subtler and complex and was for a long time contested (Mayr, 1963). Sympatric speciation is ecological speciation in its truest form.

The process of sympatric speciation is a constant battle between natural selection and recombination. Selection is divergent, trying to separate groups of individuals from within a population. It is constantly trying to be counteracted by interbreeding; this breaks up any evolving gene complexes that may contribute to reproductive isolation. Thus sympatric speciation is more likely to occur if the possibility of recombination is reduced. This can happen through differential habitat use, assortative mating, and/or temporal changes in reproductive habits. Reproductive isolation and the ability to coexist must coevolve for enough ecological differentiation to be present; otherwise reproductive barriers may not fully develop (Coyne and Orr, 2004).

It is worth highlighting that where sympatric populations of the same species occur these populations may not have arisen in sympatry. Sympatric polymorphic populations can also be the product of multiple invasions of the same species but originating from different lineages that diverged previously in allopatry (i.e. secondary contact) (Bernatchez and Dodson, 1990; Pigeon *et al.*, 1997; Skúlason *et al.*, 1999; Robinson *et al.*, 2000; Alekseyev *et al.*, 2002).

1.6. Phenotypic Plasticity

Phenotypic plasticity can be easily seen in nature and it is most frequently expressed as ecologically relevant morphological, behavioural and physiological traits (Miner *et al.*, 2005). Phenotypic plasticity is the ability of a single genotype to express multiple alternative phenotypes in response to contemporary environmental conditions, both intrinsic and extrinsic (Pigliucci, 2004). It can be instantaneous, anticipatory or

delayed, permanent or reversible, adaptive or non-adaptive, beneficial or harmful, passive, discrete, continuous and generational (Whitman and Agrawal, 2009).

Plasticity allows adaptation during the process of ontogeny to an environment. It increases the chance of survival in organisms where selection for such a phenotype is under pressure (Via *et al,* 1995). Phenotypic plasticity has multiple ecological effects which can facilitate evolutionary processes and speciation by increasing fitness and generating phenotypic novelty which can then be exposed to natural selection (Whitman and Agrawal, 2009).

Plasticity can drive rapid (in evolutionary timescales) phenotypic responses in organisms (West-Eberhard, 1989) thus the ecological consequences of plasticity can manifest over a single generation. These consequences can range from simple environmental susceptibilities such as changes in movement patterns, to mediating intraspecific interactions, such as assortative mating and can lead, through indirect effects, to the structuring of ecological communities (Whitman and Agrawal, 2009). Indirect effects can include changes in habitat use associated with exploiting different prey which can cause phenotypic differences not directly associated with the acquisition of resources. These localised environmental effects that result in individuals more suited to a suite of biophysical conditions, contribute to and help maintain phenotypic structuring by further fragmenting populations in sympatry and allows adaptation as a direct response to the environment during ontogeny. Thus, plasticity is a fundamental component of ecological speciation (West-Eberhard, 1989).

The degree of divergence between alternative phenotypes that are expressed under plasticity alone is partly related to how plastic an organism is. Because the degree of plastic response not only differs between species but also characteristics and behaviours expressed within a species, and there is genetic variation associated with the capacity for plasticity responses, plasticity can itself be considered as a trait (Jong, 2009). Thus different genotypes may show different widths in their reaction norms i.e. species or traits may be more or less plastic than another (Whitman and Agrawal, 2009; Appendix 1). Teasing apart how much phenotypic variation is attributable to plasticity or is already predetermined by the genome is difficult. The European eel provides an ideal model, as it is a broadcast spawner and there is no detectable genetic variation between populations (Als *et al.,* 2011). Any phenotypic structuring has to be wholly modulated through the

environment. The occurrence of dramatic and fragmented phenotypic structuring in the European eel is expressed by 'broad headed' and 'narrow headed' individuals (Barry *et al.*, 2016). They are almost exclusively found living in sympatry. Broad headed individuals feed exclusively on fish and larger macro invertebrates. Narrow headed individuals specialise in feeding on small benthic invertebrates and larger zoo plankton (Barry, 2015). These differences in trophic ecology can affect different aspects of the European eel's ecology such as movement patterns including home range size, rate of movement and differences in diel patterns as well as their susceptibility to infection (Barry, 2015).

The adaptive values of plasticity are reliant on timing, speed of response and specificity. To understand plasticity, we must understand these adaptive values, understand the environment that drives them and understand the fitness outcomes. Organisms with high levels of phenotypic plasticity may have a significant fitness advantage in heterogeneous environments, when invading new habitats or when exploiting new resources (West-Eberhard, 1989; Scheiner, 1993; Via *et al.*, 1995; Schlichting, 2004).

For species that inhabit fluctuating environments, phenotypic plasticity has fitness benefits closely linked with the ability to adapt to contemporary environmental conditions. The result are adjustments to various aspects of an individual's phenotype following novel input during development which are completely non-genetic; ultimately it is an organism's ability to functionally respond to offset any loss of fitness associated with an environmental change. It is shown in Appendix 1 that different populations of brown trout of migratory and residential origin had different levels of phenotypic flexibility. Residential trout showed a greater phenotypic response in morphology to changes in diet, probably to assist in continually adapting to a changing and competitive freshwater environment, compared with brown trout from migratory (anadromous) origins, which would likely encounter a more homeostatic marine environment in the wild.

Plasticity operates by producing a non-genetic adjustment to the phenotype during development in response to some form of intrinsic or extrinsic input. Plasticity is most likely governed by gene regulation that responds to changes in the environment, the product is a phenotype that it is better suited to that environment. Each phenotype (or trait) has a specific set of genes whose expression is regulated by an environmental or allelic (or both) cue (West-Eberhard, 1989). A simple change in environmental conditions such as an increase in temperature can cause different genes to be expressed resulting in the

expression of a different phenotype. This is a very simplified example, the process is much more complex with multiple genes acting simultaneously via transcription, translation, enzyme, hormone, and morphogen regulation, morphogenesis, apoptosis or a combination of some or all of these (Miura, 2005; Amdam, 2007; Emlen *et al.*, 2007; Wolschin and Amdam, 2007; Zhou *et al.*, 2007). Between receiving an environmental input cue and a phenotypic response there may be dozens of steps, influenced by hundreds of genes and untold environmental/physiological factors before the production of the ultimate phenotype. Because regulatory genes can also be selected for by the environment, and respond to changes in the environment, genes responsible for plasticity can also evolve (Via and Lande, 1985). Thus the capacity to be plastic, not the end product of the plasticity is selected for.

Novel characteristics that arise through phenotypic plasticity can become genetically fixed by altering how the genome responds to environmental input (Pigliucci, 2004). This leads to an individual's alternative phenotype having a specific set of distinctive or distinctively expressed modifier genes whose expression is regulated by a simple cue (West-Eberhard, 1989). Selection can drive alternative phenotypes towards different evolutionary outcomes by acting semi-independently upon one or more discrete alternative phenotypes (West-Eberhard, 2003). Smtih (1990) showed how differences in seed size simultaneously selected for contrasting beak sizes in thre African finch, *Pyrenestes ostrinus*. This phenomenon is particularly true for alternative phenotypes with a strong functional significance living in sympatry (Schluter and McPhail, 1992; Adams and Huntingford, 2002b; West-Eberhard, 2005). Thus, investigating alternative phenotypes of ecological importance that have arisen in sympatry can help increase our understanding of the selective pressures and evolutionary processes that shape change.

1.7. Reproductive Isolation In Sympatry

Reproductive isolation in sympatry occurs in the absence of geographical boundaries. If the pressures driving natural selection are strong it can cause a population to divide into subpopulations, each specialising on a different resource (Whitman and Agrawal, 2009). This process is more likely to occur following the invasion of new habitats or a decrease in interspecific competition. Where there are underutilised resources that require a unique trophic character to aid in its exploitation (Smith and Skúlason 1996), or in ecosystems with a highly variable habitat (Garduno-Paz *et al.*, 2010a).

Because of the close association between sympatric speciation and the exploitation of new niches we often see adaptive radiations in new and distinct habitats, such as those influenced by volcanoes which can cause rapid and extreme changes to the environment (Smith and Skúlason, 1996). In the northern hemisphere which is dominated by recently glaciated lakes similar patterns are seen. Here there are frequent reports of discrete morphs of fish that occupy either a benthic or limnetic habitat (Skulason *et al.*, 1999; Kristjansson et al., 2002; Siwertsson *et al.*, 2010).

Sympatric speciation events often begin with the successful invasion or exploitation of a newly available niche, new and novel phenotypes that arise through plasticity can rapidly increase in frequency. Plasticity allows the trait distribution to become extended, divergent selection pressures then favour individuals at the tail end of a trait distribution (disruptive selection) selecting for phenotypes that are suited to contrasting environments. The result is environmentally driven phenotypes of ecological significance on which natural selection can act. The alternative polyphesnisms that assist adaptation to contrasting and discrete resources drive the evolution of polymoprhisms. Sympatric (Ecological) speciation is strongly associated with resource acquisition, interactions associated with this such as behaviour and habitat use are also important because they have the potential to contribute to non-random mating (Rundle and Nosil, 2005). Individuals either mate exclusively in the habitat of the resource they use (habitat isolation), or choose as mates only individuals using the same resources (sexual isolation) or mates that look phenotypically similar (positive assortative mating) (Coyne, 2007). Non-random mating may also arise due to a mismatch in the timing of reproduction as a response to different prey ecology. It may also arise in response to different environmental conditions on spawning sites. As a result of non-random mating, adaptive modifications that resulted from plasticity can now potentially become fixed within an individual's genome making them stable. This leads to the development of genetically isloated subpopulations (or groups) of individuals with differing phenotypes forming from a single population.

This model of sympatric speciation through niche expansion is thought to be responsible for much of the intraspecific variation in phenotype that we see among populations of fishes inhabiting postglacial lakes in the northern hemisphere.

1.8. POSTGLACIAL FISH AS MODEL SPECIES

Fish living in freahwaters recently glaciated include a range of species that have recolonized freshwaters in the northern hemisphere at the end of the last glacial epoch approximately 10,000-15,000 years ago (Schluter & McPhail, 1992; Skúlason & Smith, 1995; Skúlason et al., 1999). During this period numerous lakes and rivers, with only vacant niches for fish, were formed in Arctic areas as the ice cap retreated (Schluter and McPhail, 1992; Wimberger, 1994; Skúlason and Smith, 1995; Skúlason *et al.,* 1999). In the northern reaches of the British Isles, specifically Scotland, melt water left behind by the retreating glaciers created many large unconnected lakes. At the time the British Isles were surrounded by water, for these species to colonise their ancestors needed to be anadromous however many have now adapted to a permanently freshwater life (Table 1.1.).

Table 1.1. Native fish species of Scotland that colonised freshwaters after the last postglacial epoch.

Species name	Family	Common name
Alosa alosa#	Clupeidae	Allis shad
Alosa fallax#	Clupeidae	Twait shad
*Anguilla anguilla^*	Anguillidae	European eel
*Coregonus lavaretus**	Salmonidae	Powan
*Coregonus albula**	Salmonidae	Vendace
*Lampetra fluviatalis**	Petromyzontidae	River lamprey
Osmerus eperlanus#	Osmeridae	Smelt
Petromyzon marinus#	Petromyzontidae	Sea lamprey
Salmo salar#	Salmonidae	Atlantic salmon
Salmo trutta#*	Salmonidae	Brown trout
*Salvelinus alpinus**	Salmonidae	Arctic charr

*** Fully freshwater; # spawn in freshwater and feeds at sea (anadromous); ^ spawn in the sea and feeds in freshwater (catadromous)**

1.8.1. ARCTIC CHARR

Arctic charr, *Salvelinus alpinus* (Linneaus 1758), are the most northerly distributed freshwater fish in the Holarctic (Skúlason *et al.,* 1999; Wilson *et al.,* 2004). Glaciation during the Pleistocene period is believed to have heavily influenced their zoogeography and genetic structuring (Wilson *et al.,* 1996; 2004). Throughout the British Isles, Arctic charr are believed to have originated (colonised) from a single Atlantic lineage (Brunner *et al.,* 2001). Arctic charr are extremely plastic, their phenotype can respond quickly and dramatically to their prevailing environment (Snorrason *et al.,* 1994; Skúlason and Smith, 1995; Smith and Skúlason, 1996; Adams *et al.,* 1998; Alexander and Adams, 2000;

Klemetsen *et al.*, 2002; Alekseyev *et al.*, 2002; Adams *et al.*, 2003; Andersson *et al.*, 2005; Power *et al.*, 2005). Arctic charr can frequently be seen forming subgroups within a single location in which they coexist, often exploiting quite specific prey (high niche specificity). The resulting phenotypic structuring observed (as morphological differences) are most frequently seen as differences in; head and body size and shape; feeding apparatus, most notably jaw length; fin shape and size; eye diameter; gill raker number as well as differences in their feeding behaviour (polyethisms) and habitat choice (Nordeng, 1983; Hindar and Jonsson, 1993; Adams *et al.*, 1998; 2003; Chapter 5). By having high niche specificity and phenotypes that reflect these, Arctic charr may be able to grow better and retain higher densities than intermediate species or forms that adopt a generalist foraging tactic (Jonsson and Jonsson, 2001). Arctic charr are the most documented species in the British Isles where sympatric polymorphic populations exist (Elliott and Baroudy, 1995; Adams and Huntingford 1998; Fraser *et al.*, 1998; Adams *et al.*, 2003; Adams *et al.*, 2008; Garduno-Paz *et al.*, 2010b; Chapter 5).

1.8.2. BROWN TROUT

Brown trout, *Salmo trutta* (Linneaus 1758), exhibit three major alternative polymorphisms, the 'common' or 'freshwater-resident' brown trout, the 'sea' trout and the 'ferox' trout. Common brown trout spend all their lives in freshwater in either rivers or lakes. Sea trout adopt an anadromous life history, migrating to sea to grow and mature before returning to freshwater to spawn. Ferox trout are characterised as being piscivorous, growing very large with an oversized head and increased longevity. However, there is much speculation regarding their authenticity as examples of true sympatric polymorphism (Campbell, 1979; Ferguson and Mason, 1981; Ferguson and Taggart, 1991; Duguid *et al.*, 2006) and this will not be addressed in this thesis. Sympatric polymorphic populations that are solely based around foraging specialisations are seen in brown trout, but much less frequently than in Arctic charr, only one documented case in Scotland in Loch Laidon (Eric Verspoor pers. comm.), and one in Ireland, Lough Melvin (Cawdrey and Ferguson, 1988). We know brown trout to also be highly plastic and variable in their phenotype so it is unclear as to why phenotypic structuring within brown trout populations is much less frequent. Where trout and charr coexist, trout dominate the littoral zone forcing Arctic charr to adopt an alternative foraging strategy which is often either a pelagic (limnetic) or benthic (Hammar, 2014). One possibility why less structuring is seen within brown trout may be as a result of their exploitation of the littoral zone. The littoral zone is highly productive and heterogeneous in resources available to consumers (Stoffel *et al.*, 2005);

therefore, this area may be better suited to generalist foragers compared with specialist foragers. Given the broad and connected range of available niches within this zone it may make the appearance of discrete alternative phenotypes more cryptic.

1.8.3. ATLANTIC SALMON

Atlantic salmon, *Salmo salar* (Linneaus 1758), are often overlooked as a polymorphic species. Most focus tends to be based around foraging strategies as a good indicator of polymorphisms. However subtle differences in life history strategies are common in salmon (Klemetsen *et al.,* 2003). Polymorphisms in salmon can be seen in the different numbers of years spent in freshwater and seawater as either parr (juveniles) or maturing adults who then return at different stages of their life and in many areas interbreed (Klemetsen *et al.,* 2003) in other locations there is strong genetic structuring between some catchments (McConnell *et al.,* 1997; Garant *et al.,* 2000). A less frequently reported life history polymorphism can be seen in populations that adopt a non-anadromous life history (Berg, 1985). However, sympatric polymorphism as a result of alternative foraging techniques has yet to be discovered in Atlantic salmon.

1.8.4. WHITEFISH

Within the British Isles there are no clear polymorphic populations of European whitefish, *Coregonus lavaretus* (Linneaus 1758), vendace, *Coregonus albula* (Linneaus 1758) or pollan, *Coregonus autumnalis* (Pallas 1776). In Loch Lomond there is some evidence of the beginning of a divergence of the whitefish population there with small differences in foraging strategies between groups that show subtle genetic differences (Adams *et al.,* 2016). However, in the British Isles there are very few whitefish populations, and even fewer that are natural and not the result of translocations to secure endangered populations (Maitland and Lyle, 1991). Many examples of phenotypic structuring within whitefish populations can be found in Finland (Kahilainen and Østbye, 2006), Norway (Østbye *et al.,* 2005a), Sweden (Lindsay, 1981) and Russia (Sendek, 2004). Most frequently seen are differences in ecology, habitat use, morphology, size and most noticeably in gill raker number (Lindsay, 1981; Turgeon and Bernatchez, 2003; Kahilainen and Østbye, 2006). A fascinating example of this in lake whitefish, *Coregonus clupeaformis* (Mitchill 1818) exists outside of the British Isles, across six lakes that belong to the St John river basin (eastern Canada and Northern Maine) where a number of parallelisms can be seen. These six lakes are home to both dwarf (small body-size) and normal (large body size) ecotypes that express different traits associated with trophic

specialisation. However, the similarity in the traits suggests that selection forces driving differences in morphology are similar across lakes. (Lu and Bernatchez, 1999).

1.9. Biodiversity And Conservation

Biodiversity is a measure that tries to encapsulate all the variety of life on the Earth. It can be used to describe diversity at a fine scale, such as within species diversity (phenotypic or genetic), or across larger scales such as the number of species within a habitat, or even the number of habitats within an ecosystem. Polymorphic populations are an important component of biodiversity that are often overlooked. Recent studies have shown that phenotypic plasticity not only contributes directly to biodiversity but it can influence local biodiversity indirectly through animal behaviours (Schmitz, 2003; Duffy, 2008; Mouritsen and Poulin, 2005). Thus, the effects of plasticity on biodiversity may have beneficial ecological consequences as biodiversity contributes towards ecosystem functioning (Hooper *et al.,* 2005).

Biodiversity is generally thought to increase nearer to the equator, northern and southern extremities are classed as being depauperate by comparison (Dirzo and Raven, 2003). It is not until you look closer at the within species variation at a genetic and phenotypic level you start to see the wealth of biodiversity contained in postglacial fish, in particular salmonid species. However, conservation of species poor systems may also be important because they often represent unsusal or rare species communities.

It is believed that a combination of restricted gene flow between populations coupled with micro-evolutionary processes has resulted in much of the phenotypic diversity that we see (Bush and Adams, 2007). Arctic charr probably show the most extreme within-species variation (Behnke, 1989). Some of this variation we know to be the result of plasticity (Adams *et al.,* 2003; Adams and Huntingford, 2004), and some has a genetic basis (Skúlason *et al.,* 1993; Gíslason *et al.,* 1999; Adams and Huntingford, 2002a; Alexander and Adams, 2004). Brown trout are also highly variable between-populations; this is most easily observed as differences in colour pattern, but a wide range of other traits vary across populations (Behnke, 1986; Ferguson, 1989). Distinct genetic differences between populations of brown trout as a result of postglacial isolation in different fresh waters are thought to underpin many of the observed phenotypic differences (Ferguson, 1989; Prodohl *et al.,* 1994; Hynes *et al.,* 1996).

When novel characters are expressed within a population the range of expressed characters is extended and diversity increases. Thus, phenotypic variation and structuring contributes significantly to biodiversity. There are a number of consequences associated with species that are highly variable, not only because they increase biodiversity but because they are often rare and of scientific value often meaning that there is a strong case for the conservation of populations with extreme phenotypes. In addition, some populations within a species may be more divergent than others potentially raising their conservation status (Bush and Adams, 2007). However, defining suitable and effective conservation policies that provide protection for species that are extremely variable in their expressed phenotypes can be highly problematic (Meffe, 1987; Ferguson, 1989). This is because it may not be appropriate to develop conservation policy aimed at the species level because the needs of the species will most likely vary across populations. Policy makers may end up being faced with a large number of populations potentially requiring different management strategies (Bush and Adams, 2007). An alternative method would be to adopt an ecosystem approach by conserving all habitat and species important to that ecosystem.

Biodiversity loss is a global issue (Hooper *et al.,* 2012) and halting its loss has received a lot of attention in recent years. Conserving biodiversity is a component of the Rio Convention on biological diversity 1992, thus countries are legally obligated to conserve it. Biodiversity loss can be attributed to climate change, invasive species, habitat loss, degradation and fragmentation and growing energy demands. All of these strain the environment and its ability to support diverse ecosystems (Hooper *et al.,* 2012). Ecosystem services are defined as the beneifts mankind can obtain from healthy functioning ecosystems. Increased awareness of the importance of biodiversity and how it can contribute to ecosystem services has resulted in conservation policy and programme actions aimed at protecting biodiversity from all of its many threats (Cullen *et al.,* 2001). The existence of phenotypic (and genetic) structuring is likely to have significant implications. It not only complicates the identification of useful management units (Rader *et al.,* 2005) but also the application of conservation strategies for species that are highly variable in their ecology such as alternative life history strategies (Chapter 6).

To provide effective conservation strategies for highly variable, and often more valuable species (economically, culturally, scientifically) means we need to understand all aspects of a species ecology and how populations may differ from one location to the next. Environmental changes in an ecosystem, either natural or anthropogenic may affect

different individuals to different degrees. For example, where sympatric populations persist, ecosystem changes may affect the ratio between phenotypes (Ide *et al.*, 2011). This potentially two-way relationship between phenotype and ecology suggests that efforts towards conservation may require the assessment of morphological variation within the population and an understanding of its proximate causes if the full diversity within a species (often called cryptic, genetic and phenotypic diversity) is to be adequately maintained, managed and protected.

1.10. Quantifying Phenotypes In Fish

Phenotypic differences can have effects across the whole organism and thus there are many ways of identifying and explain patterns in structuring. In this thesis, using resource use as a fundamental driver of structuring, I use differences in body and head morphology, Standard Metabolic Rate (SMR), carbon and nitrogen stable isotope ratios, parasite fauna and spatial data to identify and compare differences in phenotype.

1.10.1. GEOMETRIC MORPHOMETRICS

Measuring morphological differences to quantify levels of phenotypic structuring is a core part of this thesis and relevant to helping to describe morphological differences among alternative phenotypes within wild populations and fish raised in the laboratory. Arctic charr were the primary species selected for this study because Arctic charr exhibit high levels of phenotypic variation and a wealth of data exist on the ecology and evolutionary biology. I mainly concentrate on morphological characteristics as a method to describe and measure plasticity.

Quantifying differences in shape has increased in sophistication in recent years with the development of geometric morphometrics which adopt a multivariate approach to comparing shapes (unlike its predecessor of morphometrics which compared linear measurements of features) (Rohlf, 1990). Geometric morphometrics allows the multivariate analysis of shape variation (or change) and its covariation with other variables (Bookstein, 1991) in either two or three dimensions.

Throughout this thesis all geometric morphometrics analysis was conducted in two dimensions using the software MorphoJ (Kilngenberg, 2011) using the following protocol.

Coordinates for shape analysis were generated using homologous, consistently definable landmarks, such as those in areas of the body close to skeletal structure and areas around the skull and the base of leading fin spines. Landmarks were carefully chosen to highlight morphological and anatomical features that would identify changes in shape that were of functional significance relevant to foraging. In addition, information on individual size was added using a scale (in mm).

Prior to all geometric morphometric analysis, any variation in shape that resulted from position, scale and orientation was mathematically removed. This is done using a Procrustes fit (or superimposition). The process was given the name after Procrustes, who according to Greek mythology was a bandit from Attica who attacked people by stretching them or cutting off their legs in order to fit them to his iron bed (Fernández-Cano *et al.*, 2012). The Procrustes fit removes non-shape variation by superimposing all landmarks coordinates from all samples so that the squared sum of the variation between corresponding landmarks is minimised by translating, scaling and rotating. This process is also referred to as the Generalised Procrustes Analysis (GPA) or Generalised Least Squares (GLS) (due to the way the variation is measured).

The centroid for each landmark configuration is calculated as the averaged coordinate for all the landmarks (the most central position relative to all coordinates). All centroids for each sample are then translated to the origin; this removes variation caused by position (translation). The configuration is then scaled so that all centroid sizes have common unit of one (Bookstein, 1991). The centroid size is calculated as the square root of the sum of squares for all the Euclidean distances from each landmark in the sample to its centroid, this removes variation between landmarks caused by size. Finally, in order to minimise the squared differences between corresponding landmarks due to orientation, configurations are rotated (optimal rotation) (Rohlf and Slice, 1990).

The purpose of using geometric morphometrics in this thesis was to look at differences in shape that arise through plasticity in response to different environmental drivers and not those that may occur from examining fish of different sizes. In many species, ontogenetic processes may cause size to have an allometric effect on shape. To remove variation in shape caused by allometry all configurations were corrected for size using a pooled within-group regression of the Procrustes coordinates on the centroid size, or log centroid size. Information on the true size of the centroid is still retained after the

Procrustes fit (which scales the centroid to one). The regression then identifies any correlation in the configuration, or movement of landmark coordinates, associated with an increase in size. The residuals from this regression which represent a measure of landmark configuration free from the influence of ontogeny are then used for all further analysis.

Following size correcting, all data are explored for unwanted non-biological distortion. This is done by performing a Principal Component Analysis (PCA) and then the shape changes associated with each Principal Component (PC) examined for normality. Geometric morphometric studies on fish can often harbour lunate distortions (Figure 1.1). This type of artefact manifests during image collection process and is caused by *rigor mortis* of the body muscles (Siwertsson *et al.,* 2013). This unwanted variation in shape can again be removed; this time by regressing Procrustes coordinates against the PC that contains the unwanted distortion. The new set of Procrustes coordinates (the residuals from this regression) are independent of the PC that contained distortion and are thus free of any shape variation associated with the lunate bending effect. This process can also be used to remove other types of shape variation that may want to be excluded, such as sexual dimorphic characteristics. Care needs to be taken so that additional variation from other parts of the anatomy is not lost. This can be done by visually assessing the amount of variation or movement in other landmarks associated with the PC in question.

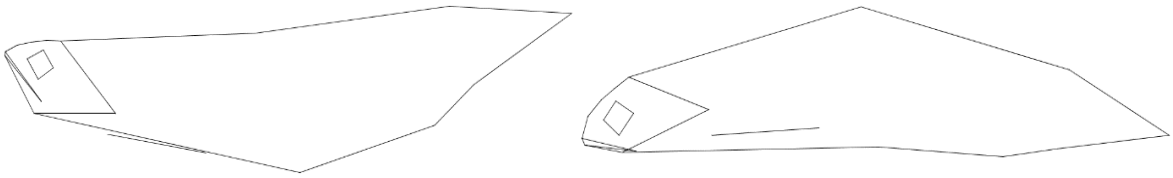

Figure 1.1. Lunate shape change associated with a PC axis frequently seen in geometric morphometric analysis involving fish. The wireframe to the left has a negative PC score and the wireframe to the right has a positive PC score.

1.10.2. STANDARD METABOLIC RATE

Standard Metabolic Rate (SMR) in ectotherms is the equivalent of basal metabolic rate (BMR) in endotherms. It is defined as the minimal maintenance metabolic rate of an ectotherm in a post-absorptive and inactive state (Finstad *et al.,* 2007). Metabolic rate is an important trait in evolutionary processes, in part because it constitutes the fundamental energy budget of an individual and because it has a strong influence on behavioural aspects (such as dominance) and physical aspects (such as growth) of an individual (Metcalfe *et*

al., 1995; Forseth *et al.,* 1999). Selection on SMR can be modulated by environmental factors, such as resource acquisition (Steyermark *et al.*, 2005; Alvarez and Nicieza, 2005) which can affect physiology and life history trajectories (Finstad *et al.,* 2007). SMR has been found to be repeatable and possibly inherited (Metcalfe *et al.,* 2016) with up to a three-fold difference between sibling individuals, making SMR an ideal factor for understanding the role of physiology in the occurrence of resource specialisation. Comparative studies have shown that SMR is of ecological and evolutionary importance (Glazier, 2005; Steyermark *et al.*, 2005; Careau, 2008; Artacho and Nespolo, 2009; Burton *et al.*, 2011). However, differences in metabolic rate in direct response to different foraging strategies are poorly documented.

1.10.3. PARASITE FAUNA

Differences in the frequency of specific internal parasites or the composition of the parasitic faunal community can result from different foraging techniques. When investigating foraging strategies, parasite fauna can provide a valuable insight in to long term foraging preferences. For example, the *Diphyllobothrium* parasite (a parasitic cestode) has an intermediate host and can only be transmitted through ingestion of a planktonic copepod (Knudsen *et al.,* 1996), thus a high abundance of *Diphyllobothrium* cysts, which are easily observed attached to the swim bladder and gut, are typically found in planktivorous-feeding fish. These parasites remain attached to the internal organs for a considerable time after the prey that infected the host has been digested. Thus the presence (or absence) of some parasites can help elucidate the prey choices of individuals with empty stomachs and add support to the stomach contents and stable isotope data. Some parasites that have novel or very specific life cycles can provide more detailed information regarding foraging, such as niche width (Knudsen *et al.*, 1996). Parasitism has even been shown as a factor helping to maintain trophic segregation, contributing towards genetic segregation (Karvonen *et al.*, 2013).

1.10.4. STABLE ISOTOPE RATIOS

Stable isotope ratios are often used to examine long term foraging patterns. Most commonly used is that of white muscle tissue which has a relatively low isotopic turnover, however, other tissues are now being used to gather different information (Tieszen *et al.*, 1983; Hobson and Clark, 1992). Organs that have high lipid content, such as the liver (Tieszen *et al.,* 1983) can be used to provide recent and short term data as they have a relatively high isotopic turnover. Hairs, feathers and scales can provide temporal

information through chronologies. This can help in explaining ontogenetic niche shifts or changes in foraging patterns in responses to environmental changes by assigning changes in isotope signatures to a specific time period (Darimont and Reimchen, 2002; Weber *et al.,* 2002; Podlesak *et al.,* 2005).

1.11. Overall Aims Of This Thesis

The focus of this thesis was to expand our understanding of the consequences of phenotypic plasticity on phenotypic structuring. Phenotypic structuring, we know to have some genetic and environmental reliance but we focus purely on the ecological or environmental effect/potential to modify phenotypes. Using a series of controlled laboratory based experiments and observations made in the field, I test how the combination of the environment and the plastic ability of a species can contribute to the modification of its phenotype with an emphasis on its importance to early divergence. I address factors that may help to maintain or fuel these processes and factors that may change the direction of divergences. I also address how phenotypic structuring contributes to biodiversity and relate this to conservation.

1.11.1. CHAPTER 2. HOW PLASTIC ARE POSTGLACIAL FISH?

- **Question** - Does the level of plasticity within a species correlate with the frequency in which we see sympatric polymorphic populations?
- **Approach** - The number of sympatric polymorphic populations that species exhibit in the wild as a result of a benthic – pelagic divergence varies considerably. For example, there are many sympatric polymorphic populations of Arctic charr and whitefish. Sympatric polymorphic populations are however rarely seen in brown trout and never in Atlantic salmon. We measured a plastic response across these four species to test if the level of plasticity within a species is related to the frequency in which we see sympatric polymorphic populations in the wild.

1.11.2. CHAPTER 3. THE PHYSIOLOGICAL COSTS OF PREY SWITCHING REINFORCE FORAGING SPECIALISATION.

- **Question** - Does the effect of early prey specialisation have a physiological impact on an individual's ability to switch its foraging strategy?
- **Approach** - For resource driven phenotypic structuring to occur (when mediated through plasticity alone) foraging specialisations need to be consistent over space and time. Physiological adaptation to aid digestion of a specific resource permits an

increase in efficiency to a specific food item whilst increasing the cost of deviating. Changes in prey abundance due to natural variation in the environment may not always warrant a switch in foraging tactic to a more abundant food source if there is a cost involved. In this study I raised fish on specific diets and then switched them. During these diet trials I measured standard metabolic rate (SMR) and growth to see if there was a physiological response to diet type. Adaptations to a specific prey were measured as a change in SMR and growth when prey were switched.

1.11.3. CHAPTER 4. TEMPERATURE MODULATES THE EXPRESSION OF PHENOTYPE IN A FRESHWATER FISH.

- **Question** - Will abrupt climate changes affect the expression of functionally significant phenotypes?
- **Approach** - Climate change is the greatest anthropogenic modification of the environment and is thus likely to impact on the expression of phenotypes through plasticity. In this laboratory based experiment I tested how an increase in temperature can modify an individual's phenotype and how this may affect phenotypic structuring. In addition, I considered the ecological consequences by comparing laboratory raised individuals to wild sympatric polymorphic populations of Arctic charr.

1.11.4. CHAPTER 5. MORPHOLOGICAL, ECOLOGICAL AND BEHAVIOURAL DIFFERENTIATION OF SYMPATRIC PROFUNDAL AND PELAGIC ARCTIC CHARR (*SALVELINUS ALPINUS*) IN LOCH DUGHAILL, SCOTLAND.

- **Question** - What are the consequences of differential resource use on two sympatric populations of Arctic charr when adopted in sympatry and how do these facilitate ecological speciation?
- **Approach** - Using a previously undescribed sympatric polymorphic population of Arctic charr, I identify and correlate differing ecological responses caused by alternative foraging specialisms. We summarise how the consequences of these differences contribute towards driving and maintain the process of ecological speciation.

1.11.5. CHAPTER 6. EFFECTIVENESS OF LOCAL BIODIVERSITY ACTION PLANS TO IDENTIFY LOCALLY RARE AND ENDANGERED FRESHWATER FISH IN SCOTLAND.

- **Question** - Do fish with high levels of phenotypic and life history variation receive adequate conservation cover at a local level?
- **Method** – Local Biodiversity Action Plans (LBAPs) were implemented as a tool to provide conservation for locally and internationally endangered species at a local level. In this chapter I calculated the amount of effective representation each of the 12 species of freshwater fish, listed on the LBAP list, receive from all LBAPs producing authorites in Scotland and then discuss the level of representation of species that are highly variable.

CHAPTER 2.
HOW PLASTIC ARE POSTGLACIAL FISH?

2.1. ABSTRACT

Phenotypic plasticity is fundamental to ecological speciation in sympatry as it allows a single genotype to express multiple alternative phenotypes in response to different environmental conditions. One common environmental driver that can cause the expression of alternative phenotypes is foraging. Foraging adaptations in fish that reside in postglacial lakes are often seen as a divergence to either zooplankton (pelagic) or macro-invertebrate benthos (benthic) specialists. Using this pattern in foraging as a divergent selection pressure, we drove a phenotypic plastic response in European whitefish, Arctic charr, brown trout and Atlantic salmon to see which is most plastic. A literature search on populations of these species from Europe, Iceland and Russia revealed 26 European whitefish, Arctic charr 61, 1 brown trout and 0 Atlantic salmon populations to exhibit evidence of polymorphism based around a pelagic / benthic divergence. Based on this we anticipated Arctic charr to be the most plastic, followed by European whitefish, brown trout and then Atlantic salmon. Brown trout were found to be the most plastic, but as expected Atlantic salmon were the least plastic. This suggests that plasticity alone is not responsible for the frequency with which sympatric polymorphic populations occur. Freshwater fish were more plastic than anadromous, possibly due to inhabiting a more heterogeneousenvironment. Wild fish were more plastic than hatchery fish, probably due to the plastic potential for fish being selected against due to living in homogenous and sterile environment such as those that would be encountered in hatchery conditions

2.2. INTRODUCTION

Ecological speciation is central to the formation of new species in sympatry (sympatric speciation) (Rundle & Nosil, 2005). Sympatric speciation involves reproductive isolation between populations independent of geographical or physical barriers. Factors that contribute towards sympatric speciation include sexual selection such as assortative mating, ecological interactions, contrasting environmental conditions and foraging strategies (Skúlason and Smith, 1995; Schluter, 2001). Disparity in foraging behaviours are most frequently reported to be a fundamental driver behind the occurence of discrete alternative phenotypes in sympatry and thus early stages of incipient ecological speciation. Differences in foraging i.e. prey types, are often followed by subsequent modifications in morphology through plasticity to increase efficiency in foraging on alternative prey (Skúlason and Smith, 1995; Skúlason *et al.,* 1999).

Phenotypic plasticity allows a single genotype to express multiple alternative phenotypes in response to different interactions with the environment (Pigliucci, 2004). Phenotypic plasticity is the phenomenon that permits environmentally induced, novel, alternative phenotypes to be expressed by individuals in sympatry. Because of this phenotypic plasticity is frequently implicated in the expression of discrete alternative phenotypes within a single population (Adams *et al.,* 2016) and is fundamental to ecological speciation (West-Eberhard, 1989).

Different phenotypes that are the result of plasticity are partly reliant on how contrasting the selection pressures are and how plastic an organism is. Depending on the reaction norm of the characteristics being expressed, some species (or individuals) are more or less plastic than others (Via, 1993) which can in turn differ between characteristics and behaviours. For species with high levels of plasticity, alternative phenotypes are able to increase in frequency more rapidly, potentially arising within a single generation (West-Eberhard, 2005b). Thus it could be speculated that sympatric speciation events may be more frequently seen in animals that are more plastic because plasticity can give rise to novelty and contribute to the processes of speciation and macroevolution (West-Eberhard, 1989).

The aquatic environment is three dimensional and contains many chemical, physical and biological variables making it one of the most heterogeneous environments on earth, capable of influencing many components on an organism's phenotype. For example,

calcium levels have been shown to positively correlate with the number of bony plates in three-spined sticklebacks, *Gasterosteus aculeatus* (Bergstrom, 2002); flow rate can directly influence body shape in the African cyprinid, *Barbus meumayeri* (Langerhans *et al.,* 2007); and predation pressures can improve escape responses in Crucian carp, *Carassius carrassius* (Domenici *et al.,* 2008). Phenotypic variation within populations is frequently seen in habitats that provide new or novel opportunities to forage on alternative resources.

Postglacial lakes have proven to be an excellent model for examining phenotypic structuring in sympatry as they provide geographically disjunct habitats (Adams *et al.,* 2016) with often discrete, and to some extent replicated environments that vary in available resources and specie communities. Multiple examples of phenotypic structuring have arisen across species (Olsson and Eklöv, 2005; Aguirre *et al.,* 2008; Præbel *et al.,* 2013). Where similar patterns in structuring occur it is possible that similar environmental drivers are at work. Resource acquisition is strongly linked to phenotypic structuring in sympatry in postglacial lakes, thus incipient ecological speciation events often start with a disparity in expressed behaviours closely linked to foraging (Skúlason and Smith, 1995; Skúlason *et al.,* 1999; Rundle and Nosil, 2005). These behaviours often follow or precede plastic morphological modifications to forage on alternative resources. Differences in morphology and behaviours related to different resource use can lead to the development of sub-populations and non-random mating, thus sympatric speciation has strong links with acquiring resources (Skúlason *et al.,* 1999). The sympatric speciation model through niche expansion (Smith and Skúlason, 1996) is thought to be the main driver behind a lot of the intraspecific phenotypic structuring we see in postglacial fish.

The most frequently seen foraging specialisms in fish that reside in postglacial lakes are a divergence to either zooplankton (pelagic) or macro-invertebrate benthos (benthic) specialists. Similarities in morphological traits that are expressed can be seen as adaptive modifications to the anatomy important to foraging. Parallelisms can be seen across different species and locations, clearly indicating that these morphological modifications have a functional role; examples of which are repeatedly seen in European whitefish (Bernatchez *et al.,* 1996; Pigeon *et al.,* 1997; Amundsen, 2004; Østbye *et al.,* 2005a) (see Table 2.1. for more examples), Arctic charr (Skúlason *et al.,* 1989; Adams *et al.,* 1998; Knudsen *et al.,* 2006; Chapter 5) see Table 2.2. for more examples), and to a lesser extent brown trout (Cawdrey and Ferguson, 1988) (see Table 2.3.). A literature search on populations of European whitefish (*Coregonus laveratus*), Arctic charr

(*Salvelinus alpinus*), brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*) from Europe, Iceland and Russia revealed 25, 61, 1 and 0 populations respectively to exhibit evidence of polymorphism based around a pelagic/benthic divergence and to have arisen in sympatry from a single colonising event (Tables 2.1. – 2.3.).

Table 2.1. Recorded sympatric polymorphic populations of European whitefish in Europe and Russia.

European whitefish	Country	Lake	No. of morphs	Source
Coregonus lavaretus	Norway	Vaggatem	3	Siwertsson *et al.,* 2010
Coregonus lavaretus	Norway	Skrukkebukt	3	Siwertsson *et al.,* 2010
Coregonus lavaretus	Norway	Vuoddasjavri	2	Østbye *et al.,* 2006
Coregonus lavaretus	Norway	Iddjajavri	2	Østbye *et al.,* 2006
Coregonus lavaretus	Norway	Stuorajavri	2	Østbye *et al.,* 2006
Coregonus lavaretus	Norway	Njallajavri	2	Østbye *et al.,* 2006
Coregonus lavaretus	Norway	Vuolgamasjavri	2	Østbye *et al.,* 2006
Coregonus lavaretus	Norway	Ladnetjavri	2	Østbye *et al.,* 2006
Coregonus lavaretus	Norway	Bajasjavri	2	Siwertsson *et al.,* 2010
Coregonus lavaretus	Norway	Virdnejavri	2	Siwertsson *et al.,* 2010
Coregonus lavaretus	Norway	Dåvajavri	2	Siwertsson *et al.,* 2010
Coregonus lavaretus	Norway	Lahpojavri	2	Siwertsson *et al.,* 2010
Coregonus lavaretus	Norway	Suopatjavri	2	Siwertsson *et al.,* 2010
Coregonus lavaretus	Norway	Bjørnevatn	3	Siwertsson *et al.,* 2010
Coregonus lavaretus	Norway	Skrukkebukta	3	Siwertsson *et al.,* 2010
Coregonus lavaretus	Norway	Vaggatem	3	Siwertsson *et al.,* 2010
Coregonus lavaretus	Norway	Langfjordvatn	3	Siwertsson *et al.,* 2010
Coregonus lavaretus	Norway	Femund	4	Østbye *et al.,* 2005b
Coregonus lavaretus	Finland	Muddusjärvi	3	Kahilainen and Østbye 2006
Coregonus lavaretus	Finland	Vastusjärvi	2	Siwertsson *et al.,* 2010
Coregonus lavaretus	Finland	Paadar	4	Kahilainen *et al.,* 2014
Coregonus lavaretus	Finland	Inarijärvi	4	Thomas *et al.,* 2016
Coregonus lavaretus	Finland	Pulmankijärvi	2	Østbye *et al.,* 2005a
Coregonus lavaretus	Finland	Baltic Sea	2	Ozerov *et al.,* 2015
Coregonus lavaretus	Russia	Kuetsjavri	2	Siwertsson *et al.,* 2010

To test which of these species is more plastic we artificially drove a plastic response in phenotype based around a planktonic/benthic divergence. This was achieved by feeding either an exclusively pelagic (*Daphnia pulex*) or exclusively benthic (*Chironomid sp.*) diet and to then compare the morphological response to these diets. These two preys are commonly found in the diet of sympatric polymorphic lake-dwelling Arctic charr (Adams & Huntingford, 2002b; Knudsen *et al.*, 2006). In whitefish populations, pelagic prey also consists of zooplankton species, however benthic prey are typically molluscs (Østbye *et al.*, 2006). Based on these records of sympatric polymorphic populations we expected Arctic charr to show the greatest phenotypic response to the two diets, followed by European whitefish, we expected brown trout to show some phenotypic response but very small and Atlantic salmon to exhibit no response.

Table 2.2. Recorded sympatric polymorphic populations of Arctic charr in Europe, Iceland and Russia.

Arctic charr	Country	Lake	No. of morphs	Source
Salvelinus alpinus	Austria	Attersee	3	Brenner, 1980
Salvelinus alpinus	England	Windermere	4	Elliott and Baroudy, 1995
Salvelinus alpinus	Germany	Constance	2	Dorfel, 1974
Salvelinus alpinus	Iceland	Galtaból	2	Gíslason *et al.,* 2011
Salvelinus alpinus	Iceland	Skerjalon	2	Gíslason *et al.,* 2011
Salvelinus alpinus	Iceland	Stóra Viðarvatn	2	Gíslason *et al.,* 2011
Salvelinus alpinus	Iceland	Svínavatn	3	Gíslason *et al.,* 2011
Salvelinus alpinus	Iceland	Thingvallavatn	4	Skúlason *et al.*, 1989
Salvelinus alpinus	Iceland	Vatnshlíðarvatn	2	Gíslason *et al.,* 2011
Salvelinus alpinus	Norway	Båtsvatn	2	Klemetsen and Grotnes, 1980
Salvelinus alpinus	Norway	Bear Island	2	Klemetsen *et al.,* 1985
Salvelinus alpinus	Norway	Eikesdalsvatnet	2	Hesthagen *et al.,* 2009
Salvelinus alpinus	Norway	Fjellfrosvatn	2	Knudsen *et al.,* 2006
Salvelinus alpinus	Norway	Granvinvatn	2	Hindar *et al.,* 1986
Salvelinus alpinus	Norway	Salangen	2	Nordeng, 1983
Salvelinus alpinus	Norway	Selura	2	Hindar *et al.,* 1986
Salvelinus alpinus	Norway	Sirdalsvatn	2	Hesthagen *et al.,* 1995
Salvelinus alpinus	Norway	Skogsfjordvatn	3	Skoglund *et al.,* 2015
Salvelinus alpinus	Norway	Store Rennen	2	Bjøru and Sandlund, 1995
Salvelinus alpinus	Norway	Tinnsjøen	2	Soreide *et al.,* 2006
Salvelinus alpinus	Norway	Tunnsjøen	3	Sandlund et al., 2015
Salvelinus alpinus	Norway	Vangsvatn (et)	2	Hindar and Jonsson, 1982
Salvelinus alpinus	Norway	Vouma	2	Pers.comm. Marianne Simonsen
Salvelinus alpinus	Russia	Bol'shoe Leprindo	2	Alekseyev *et al.,* 2002
Salvelinus alpinus	Russia	Bol'shoe Namarakit	2	Alekseyev *et al.,* 2002
Salvelinus alpinus	Russia	Davatchan	2	Alekseyev and Pichugin, 1998
Salvelinus alpinus	Russia	Gol'tsovoe	2	Alekseyev *et al.,* 2002
Salvelinus alpinus	Russia	Kalarskii Davatchan	3	Alekseyev *et al.,* 2009
Salvelinus alpinus	Russia	Kamakanda	2	Alekseyev *et al.,* 2002
Salvelinus alpinus	Russia	Kiryalta – 3	2	Alekseyev *et al.,* 2002
Salvelinus alpinus	Russia	Kiryalta – 4	2	Alekseyev *et al.,* 2002
Salvelinus alpinus	Russia	Krestaki – 1	2	Alekseyev *et al.,* 2002
Salvelinus alpinus	Russia	Kudushkit	2	Alekseyev *et al.,* 2002
Salvelinus alpinus	Russia	Leprindokan	2	Alekseyev *et al.,* 2002
Salvelinus alpinus	Russia	Maloe Leprindo	2	Chapter 5 of this thesis
Salvelinus alpinus	Russia	Severonichatskoe	2	Alekseyev *et al.,* 2002
Salvelinus alpinus	Russia	Svetlinskoe	2	Samusenok *et al.,* 2006
Salvelinus alpinus	Russia	Tokko	2	Alekseyev *et al.,* 2002
Salvelinus alpinus	Scotland	Dughaill	2	Chapter 5 of this thesis
Salvelinus alpinus	Scotland	Ericht	2	Fraser *et al.,* 1998
Salvelinus alpinus	Scotland	Maree	2	Adams *et al.,* 2008
Salvelinus alpinus	Scotland	Rannoch	3	Adams *et al.,* 1998
Salvelinus alpinus	Scotland	Stack	2	Adams *et al.,* 2008
Salvelinus alpinus	Scotland	Tay	2	Adams *et al.,* 2003
Salvelinus alpinus	Sweden	Ajaure	2	Hill *et al.,* 1990
Salvelinus alpinus	Sweden	Ankarvattnet	2	Amundsen and Klemetsen, 1988
Salvelinus alpinus	Sweden	Bjellojaure	2	Nyman *et al.,* 1981
Salvelinus alpinus	Sweden	Fättjaure	2	Henricson and Nyman, 1976
Salvelinus alpinus	Sweden	Faxälven	2	Määr, 1950
Salvelinus alpinus	Sweden	Kvarnbergsvattnet	3	Hammar, 1981
Salvelinus alpinus	Sweden	Lake Blåsjön	2	Hammar *et al.,* 1993
Salvelinus alpinus	Sweden	Övre Bjökvattn	2	Nilsson and Filipsson, 1971
Salvelinus alpinus	Sweden	Sitasjaure	3	Conejeros *et al.,* 2008
Salvelinus alpinus	Sweden	Suorva	3	Hanson and Lindstroem 1979
Salvelinus alpinus	Sweden	Stora Rösjön	2	Svedäng, 1990
Salvelinus alpinus	Sweden	Visjön	2	Näslund, 1990
Salvelinus alpinus	Sweden	Fulufjäll	2	Lindström and Andersson, 1980
Salvelinus alpinus	Switzerland	Neuchatel	2	Quartier, 1951

Table 2.3. Recorded sympatric polymorphic populations of brown trout in Europe, Iceland and Russia.

Brown trout	Country	Lake	No. of morphs	Source
Salmo trutta	Scotland	Leidon	3	Pers.comm. (Eric Verspoor)

2.3. MATERIALS AND METHODS

2.3.1. LIVESTOCK PREPARATION AND ARRIVAL

2.3.1.1. EUROPEAN WHITEFISH

European whitefish were collected from a morphologically uni-modal, plankton feeding population that inhabit Loch Lomond, Scotland (56°10.335'N, -4°58.609'W) (referred to as LLEW herewith). Adult fish were caught using 33, 36, and 38 mm knot to knot pelagic gill nets measuring 30m x 1.5m. Nets were set over night at the water surface from 16:00 – 09:00 on the 7th and 10th January 2014. Ripe fish were anaesthetized, blotted dry, and their eggs or sperm extruded by abdominal massage. Eggs from six females were fertilized with sperm from six haphazardly chosen males to create six full-sib families. Water hardened eggs were immediately transferred to incubation bottles (keeping families separate) at the Scottish Centre for Ecology and the Natural Environment (SCENE), Loch Lomond, Glasgow (where sampling took place). The powan eggs were incubated using 1 litre hatching bottle systems adapted from the design of Rottmann & Shireman (1988) which had been used previously for this purpose (Lyle *et al.*, 2010).

2.3.1.2. ARCTIC CHARR

Arctic charr were collected from a morphologically uni-modal, plankton feeding population that inhabit Loch Glair, Wester Ross, Scotland (57°32.648'N, 5°19.125'W) (referred to as WRAC herewith). Fish were caught using fyke nets, from the 4th – 9th November 2012 from the River Coulin. Adults were kept in square holding tanks 1.8m x 1.8m x 0.5m (1620 L) supplied with water from the River Coulin for 2-4 days until ripe. Ripe fish were anaesthetized, blotted dry, and their eggs or sperm extruded by abdominal massage. Eggs from nine females were fertilized with sperm from nine haphazardly chosen males to create nine full-sib families. Fertilised eggs were water hardened and immediately transferred to incubation facilities at SCENE. A second Arctic charr population consisted of six full-sib families derived from an anadromous cultured stock of Arctic charr from Iceland (referred to as ILAC herewith) and arrived at SCENE on 11th of March 2013 as eyed eggs and immediately transferred to incubation facilities.

2.3.1.3. BROWN TROUT

Brown trout from a fully freshwater population were collected on the 11th October 2013 above an impassable dam on the Whiteadder River, a tributary that flows in to the River Tweed, Scotland (55°88=N, 2°57=W) (referred to as WABT herewith). Brown trout derived from a fully anadromous population were collected on the 23rd October 2013 from College Burn, River Tweed, Scotland (55°77=N, 2°18=W) (referred to as CBBT herewith). All fish were caught by electrofishing. Additional confirmation of life history was applied based on size and coloration (Eek and Bohlin, 1997). Freshwater fish were smaller and dark brown in colour with red spots, while anadromous fish were larger and silvery-grey in colour with black spots. Adult fish were transported to the Belhaven Trout Company, Scotland, where they were held in two round 1530 L aluminium tanks keeping freshwater and anadromous fish separate. Ripe fish were anaesthetized, blotted dry, and their eggs or sperm extruded by abdominal massage. Eggs from twelve female freshwater brown trout were fertilised with sperm from twelve haphazardly chosen freshwater males. Eggs from seven female anadromous brown trout were fertilised with sperm from seven haphazardly chosen anadromous males. Freshwater and anadromous fish were spawned from the 3rd of November to the 29th of November and from the 17th of November to the 4th of December 2013, respectively. Fertilised eggs were water hardened and then held in meshed rearing baskets suspended in a holding tank keeping families separate. When eggs hatched, alevins were removed from rearing baskets and placed in the stock aquaria still keeping families separate. Alevins were then transferred to SCENE on 31st January 2014. A third population of brown trout was obtained on the 5th March 2014 as newly hatched alevins from Houietoun Fishery, Scotland (referred to as HTBT herewith). Original brown trout used to stock the fishery were of freshwater ancestry. Fish were selected haphazardly from a holding tank containing a mixture of alevins from 14 full-sib families and immediately transferred to fish holding facilities at SCENE.

2.3.1.4 ATLANTIC SALMON

Atlantic salmon from an anadromous river running population were sampled from mature sea-run Atlantic salmon undertaking their spawning migration and were captured at the Loch na Croic fish trap on the river Blackwater, northern Scotland (57° 60'N, 4°63'W) (referred to as BWAS herewith). Males and females were held separately at the trap site in circular tanks measuring 4m x 1.5m (18,850 L) supplied directly with water from the River Blackwater until ripe. Fertilised eggs were water hardened and then transferred to the nearby SSE hatchery at Contin, Scotland, where they were reared as separate family

groups until the eyed stage. On March 11th 2014, 50-100 eyed eggs from nine different full-sib families were immediately transferred to incubation facilities at SCENE.

2.3.2. LIVESTOCK CARE AND FEEDING POST ARRIVAL

Upon arrival LLEW and WRAC water hardened eggs, ILAC and RCAS eyed eggs, and CBBT, WABT, HTBT alevins (Table 2.4.) were acclimatised over a two-hour period to the new water supply. All eggs were placed in meshed rearing baskets suspended in holding aquaria keeping families separate. Upon hatching individuals were removed from rearing baskets and placed in to stock aquaria keeping families separate. Fish that arrived as alevins were directly transferred to stock aquaria keeping families separate. All aquaria were supplied with water directly from Loch Lomond on a flow through system and kept at ambient loch temperature which ranged from 4ºC – 16ºC throughout the duration of the experiment.

Table 2.4. Species, their origin, differences in life history and time of sampling.

Abbre-viation	Common Name	Origin	Wild / Hatchery	Life History	Pairings	Arrived at SCENE as	Date of Arrival
LLEW	Whitefish	Loch Lomond	Wild	Freshwater	6	Eggs	09/01/2013
WRAC	Arctic charr	Wester Ross	Wild	Freshwater	9	Eggs	10/11/2012
ILAC	Arctic charr	Iceland	Hatchery	Anadromous	6	Eyed eggs	11/03/2013
CBBT	Brown trout	White adder	Wild	Freshwater	12	Alevins	31/01/ 2014
WABT	Brown trout	College burn	Wild	Anadromous	7	Alevins	31/01/ 2014
HTBT	Brown trout	Houietoun	Hatchery	Freshwater	14	Alevins	05/03/2014
RCAS	Atlantic salmon	River Connan	Wild	Anadromous	9	Eyed eggs	11/03/2014

To control for differences in development due to different temperatures and sampling times, the number of degree-days (dd) were used as a measure of developmental rate. Degree-days is the cumulative count of the water temperature for a known period of time in days. Count commenced when all fish (Species and location specific) had hatched. When the egg sacs of alevins were close to exhaustion and approaching the 'first feed' stage chopped liver was introduced *ad libitum* as an exogenous food source. WRAC and ILAC reached the 'first feed' stage, when the yolk sac was almost exhausted and fish started actively seeking food at 102dd, HTBT at 115dd, WABT and CBBT at 123dd and RCAS at 187dd. Powan first feed stage commenced at 1dd as they immediately feed after hatching (Table 2.5.). After 14 days' fry were fed a ground fishery pellet until they were large enough to be put onto their treatment diets.

Table 2.5. Sample periods for each group with information on dietary exposure times in degree days (dd).

Group	Age at first feed	Age when benthic or pelagic diet started	Age when sampled	Total time exposure to benthic or pelagic diet
LLEW	1	690	1207	517
WRAC	102	328	835	507
ILAC	102	325	866	541
HTBT	115	355	854	499
WABT	123	346	902	556
CBBT	123	346	902	556
RCAS	187	406	902	496

2.3.3. DIETARY TREATMENTS

For each of the seven experimental groups, juveniles from all pairings were mixed evenly. Once all the fry from each group were feeding on an exogenous source an even number of each group was distributed across a total of 140 aquaria (N= 48 fish per aquarium) all measuring 38x23x18cm thus providing 10 replicates of each group to control for potential tank effects. Five of these tank replicates were then exclusively fed *Daphnia pulex* (zooplankton) and the other five exclusively bloodworm (*Chironomus sp.*). *Daphnia pulex* represented a pelagic prey item, whereas bloodworm represented a benthic prey item. Pelagic and benthic diets started when Arctic charr were between 325-328dd and brown trout were between 346-355dd. RCAS and LLEW started later at 406dd and 690dd respectively (Table 2.5.) due to slower development and therefore not being large enough to consume the prey (Figure 2.0.)

2.3.4. DATA COLLECTION

Samples were collected after being exposed to a novel diet for approximately 526±30dd (Table 2.5.). Five specimens were sampled haphazardly from each of the five aquaria, providing 25 replicates from each dietary treatment (n = 350). Fish were killed by Benzocaine overdose following regulatory ethical procedures. All specimens were weighed and lateral view photographs taken of their left side using a Cannon EOS 350D digital camera for geometric morphometric analysis. For each photograph, a scale reference was added to allow for size correction (removal of size associated shape change). Twenty one consistently identifiable landmarks on the head and body (Figure 2.1.) were digitised in two dimensions on each fish image using tpsDig2 (Rohlf, 2006a) and tpsUtil (Rohlf, 2006b).

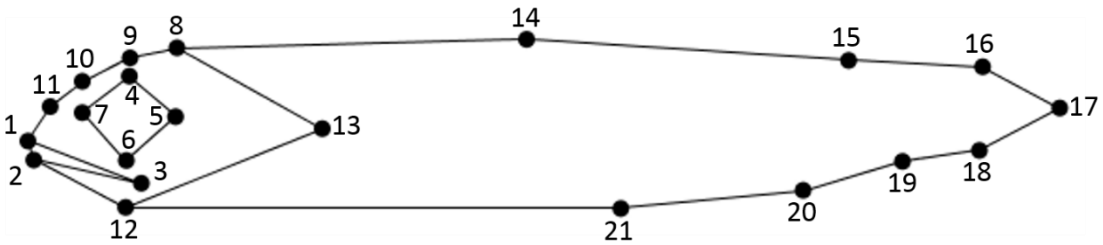

Figure 2.1. Landmark 1, the tip of the nose; 2, the anterior tip of the lower jaw; 3, the most posterior part of the upper jaw; 4-7, most upper, posterior, lower and anterior parts of eye respectively; 8, edge of the cranium directly above the most posterior edge of the eye; 9, edge of the cranium directly above the centre on the eye; 10, edge of the cranium directly above the most anterior edge of the eye; 11, edge of the cranium and central to 1 and 10; 12, the join between gill operculum's that fuse the bucchal cavity; 13, most posterior edge of the gill operculum; 14 base of front dorsal fin spine; 15, most anterior edge of the adipose fin where it joins the body; 16, base of top caudal fin spine; 17, where the lateral line meets the caudal fin; 18, base of bottom caudal fin spine; 19, base of last anal fin spine; 20, base of first anal fin spine; 21, base of first pectoral fin spine.

2.3.5. DATA ANALYSIS

Geometric morphometric analysis was performed using the software MorphoJ (Klingenberg, 2011). Prior to geometric morphometric analysis, landmark data were subject to a Procrustes superimposition to remove least squares variation in the data created by size, position and orientation (Rohlf and Slice, 1990; Mitteroecker and Gunz, 2009). The mean shape configuration was then computed and the variation around this mean calculated (Dryden and Mardia, 1998). Before comparing any groups a pooled within-group regression of Procrustes co-ordinates on log centroid size was used to derive residuals. This provided a measure of shape free from allometric scaling associated with early ontogeny (Klingenberg, 1998).

A single Discriminant Function Analysis (DFA) (1000 permutations) was used to compare and quantify (Viscosi and Cardnin, 2011) any morphological responses to the different diets across groups and a further seven individual DFA (1000 permutations) of each group to test if there was a plastic morphological response within groups exposed to different diets. Mahalanobis distance was used as pairwise measures of the magnitude of shape difference.

A single Canonical Variate Analysis (CVA) (1000 permutations) was used to test if the plastic morphological response within groups exposed to different diets was the same

or if it differed across species. Mahalanobis distance was used as pairwise measures of the magnitude of shape difference.

2.4. Results

The single Disciminant Function Analysis (DFA) found diet to have a significant effect on morphology (Mahalanobis distance 1.7928, p = <.0001) (Figure 2.2.A.). Fish raised on a benthic diet developed a deeper body and a shorter more robust head. Fish that were raised on a pelagic diet had a shallower body and a more pointed head. Individual DFA for each group found morphological responses to be similar. All showed a significant morphological response to the experimental diets (Figure 2.2.B. LLEW, Mahalanobis distance 6.34, p = 0.0002; Figure 2.3.A. ILAC, Mahalanobis distance 5.62, p = 0.0086; Figure 2.3.B. WRAC, Mahalanobis distance 5.56, p = 0.0097; Figure 2.3.C. COAS, Mahalanobis distance 4.19, p = 0.0108; Figure 2.4.A. CBBT, Mahalanobis distance 7.64, p = 0.0003, Figure 2.4.B. HTBT, Mahalanobis distance 6.98, p = 0.0008; Figure 2.4.C. WABT, Mahalanobis distance 7.18, p = 0.0006.

2.5. Discussion

We found varying degrees of morphological divergence across species. Our predictions that Arctic charr would be the most plastic, followed by European whitefish, brown trout, and finally Atlantic salmon were not completely accurate. Wild freshwater brown trout were in fact the most plastic, wild Arctic charr were the second most plastic and wild European whitefish third, followed by hatchery freshwater brown trout, wild anadromous brown trout, hatchery anadromous Arctic charr and finally anadromous Atlantic salmon.

Theories of sympatric speciation through the exploitation of new resources state that plasticity is an important component of this process for many species. Plasticity permits new and often novel phenotypes to simultaneously manifest in across a population because the environment can affect multiple individuals at the same time thus the range of potential traits and the frequency in which they occur increases Under certain conditions divergent selection pressures can favour individuals at either tail end of a trait distribution (disruptive selection). Thus, alternative phenotpyes that are a product of the environment and thus of ecological significance provide new sources of variation upon which natural selection can act. Alternative polyphesnisms within a population as a result of plastic

responses to the environment can assist adaptation to discrete and contrasting resources which is known to underpin evolution of sympatric polymorphic populations. Subpopulations can then form from a single population and then through processes that help to drive and maintain non-random mating can permit genetic isolation among the subpopulations. This model of sympatric speciation is believed to underpin many of the sympatric polymorphic populations of fishes recorded in postglacial lake.

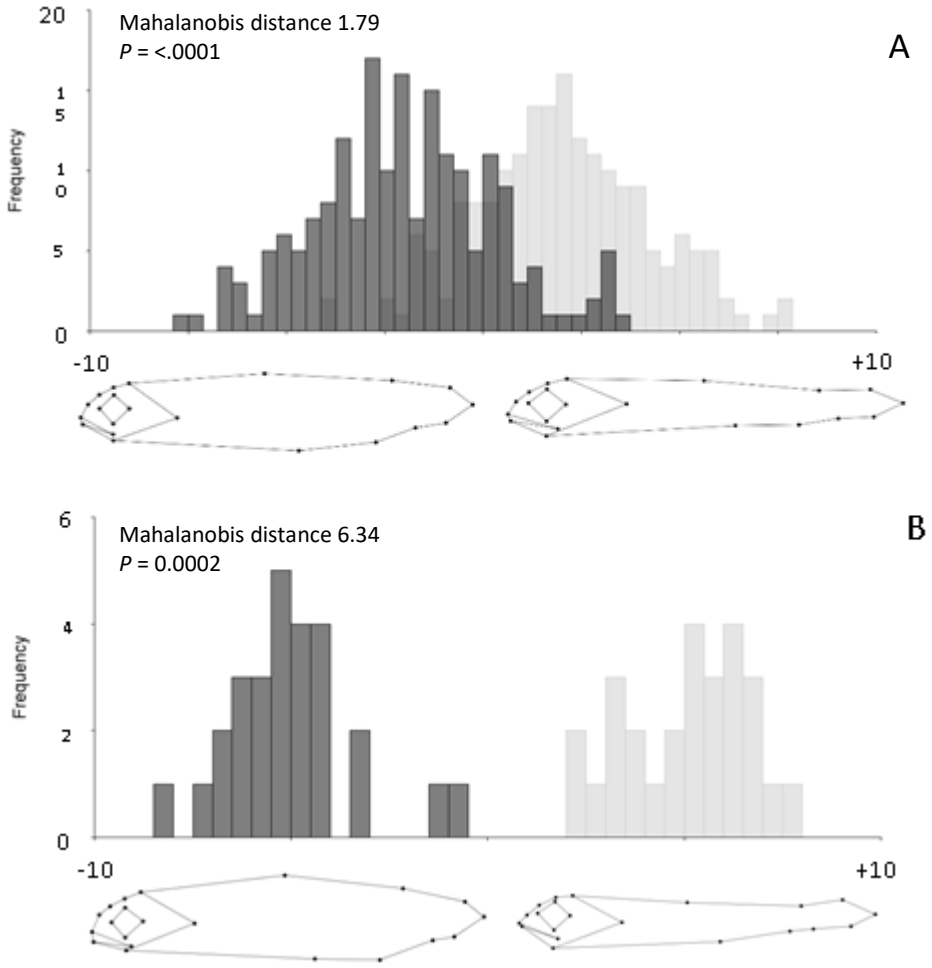

Figure 2.2. Discriminant Function Analysis for A) all groups and B) Loch Lomond European Whitefish (LLEW). Wire frames depict body shape at outer most point of the X axis and scaled to a factor of two to aid visual representation.

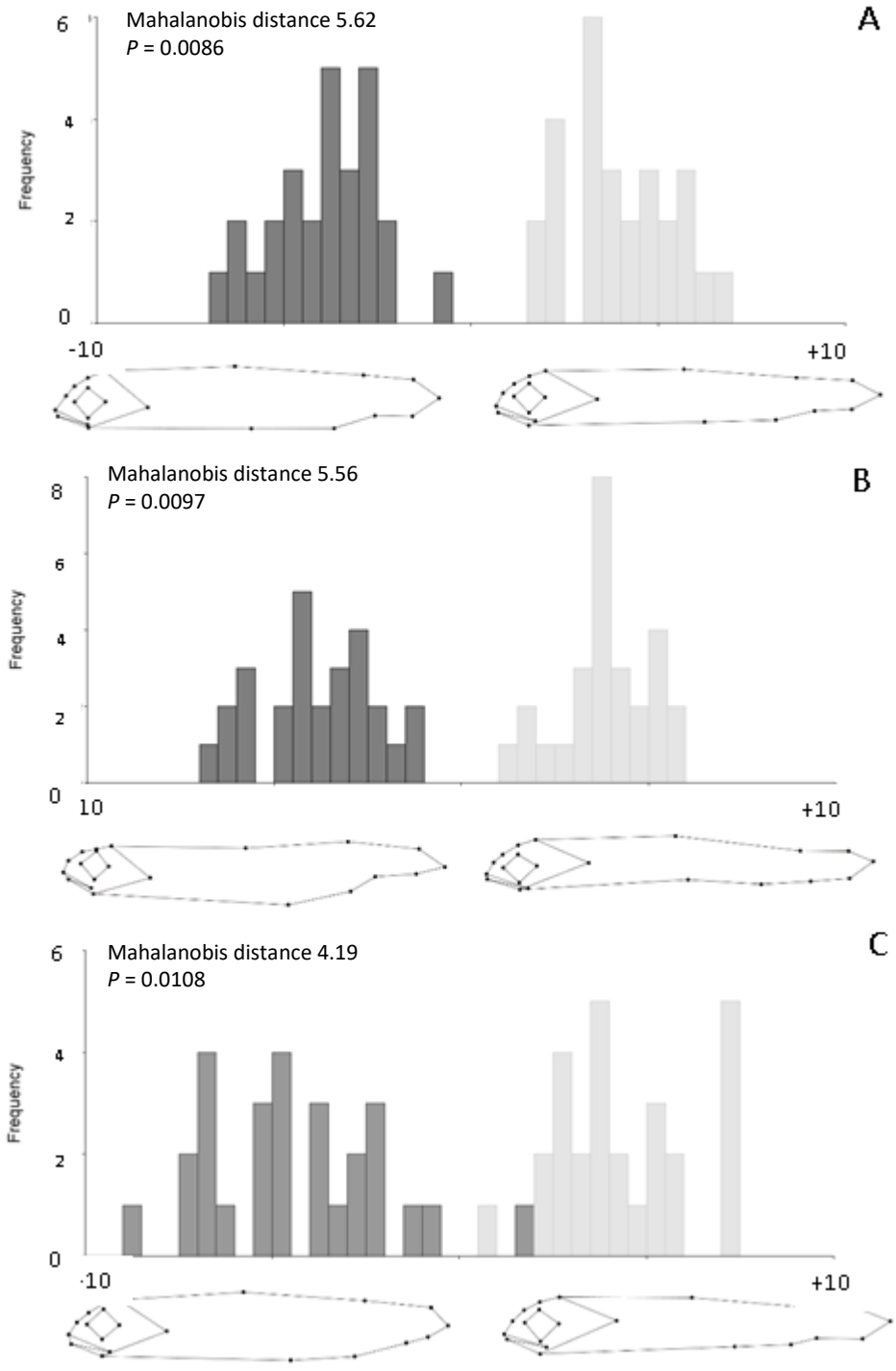

Figure 2.3. Discriminant Function Analysis for A) Holar Arctic Charr (ILAC), B) Wester Ross Arcitc Charr (WRAC) and C) Conan Atlantic Salmon (COAS). Wire frames depict body shape at outer most point of the X axis and scaled to a factor of two to aid visual representation.

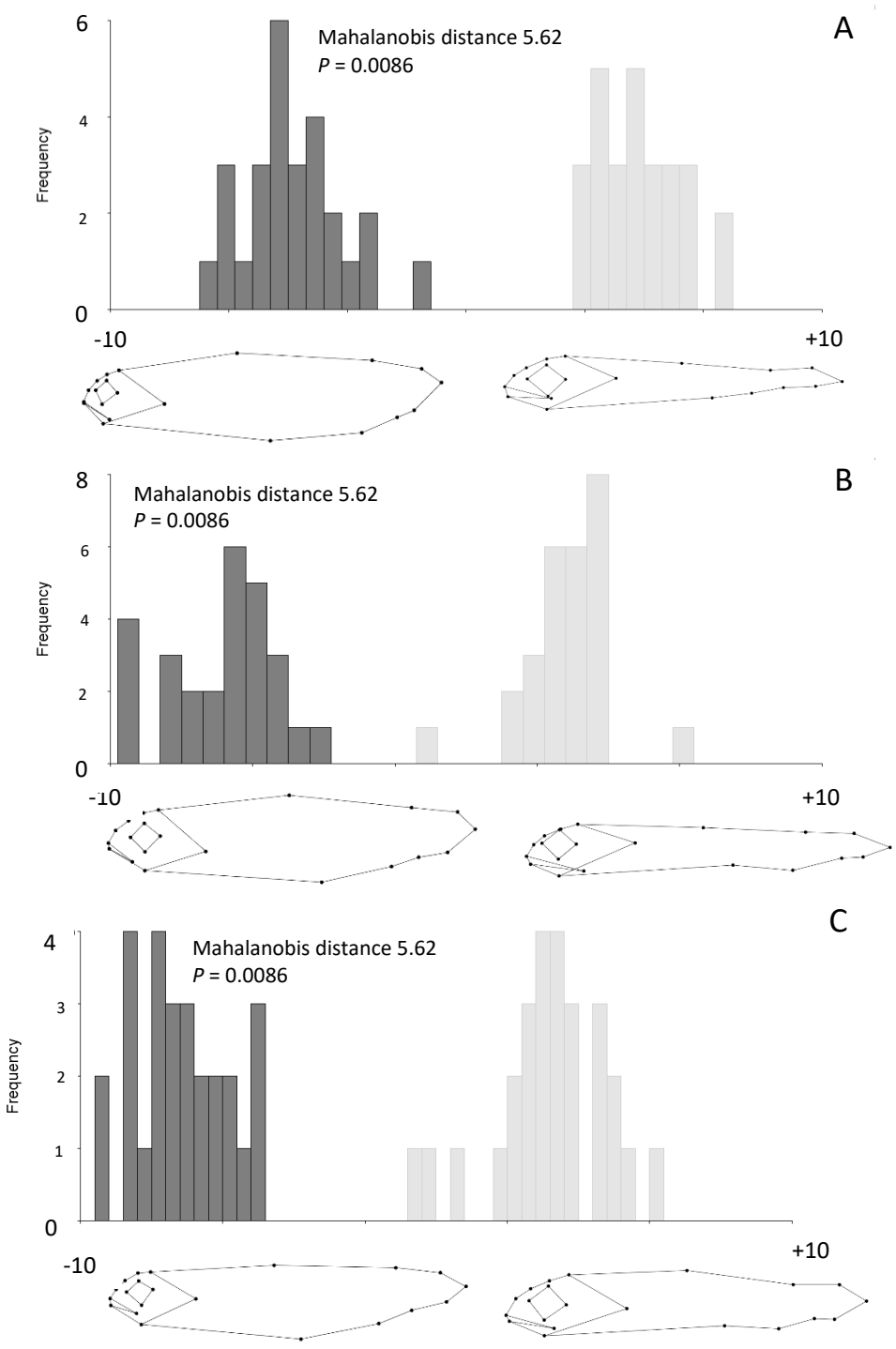

Figure 2.4. Discriminant Function Analysis for A) College Burn Brown Trout (CBBT), B) Houtie Toun Brown Trout (HTBT) and C) White Addar Brown Trout (WABT). Wire frames depict body shape at outer most point of the X axis and scaled to a factor of two to aid visual representation.

Canonical Variate Analysis (CVA) supported the results from the individual DFA showing morphology to be significantly different between siblings raised on different diets for all groups (ILAC, Mahalanobis distance 2.51, p = <.0001; WRAC, Mahalanobis distance 3.48, p = <.0001; CBBT, Mahalanobis distance 2.53, p = 0.0001; HTBT, Mahalanobis distance 3.14, p = <.0001; WABT, Mahalanobis distance 4.03, p = <.0001; COAS, Mahalanobis distance 2.04, p = 0.0022; LLEW, Mahalanobis distance 3.21, p = <.0001) (Figure 2.5. and 2.6.). There was no relative species order in the level of plasticity expressed. However, it was interesting to note that fish from freshwater populations were more plastic than those from anadromous populations and wild fish were more plastic than hatchery fish.

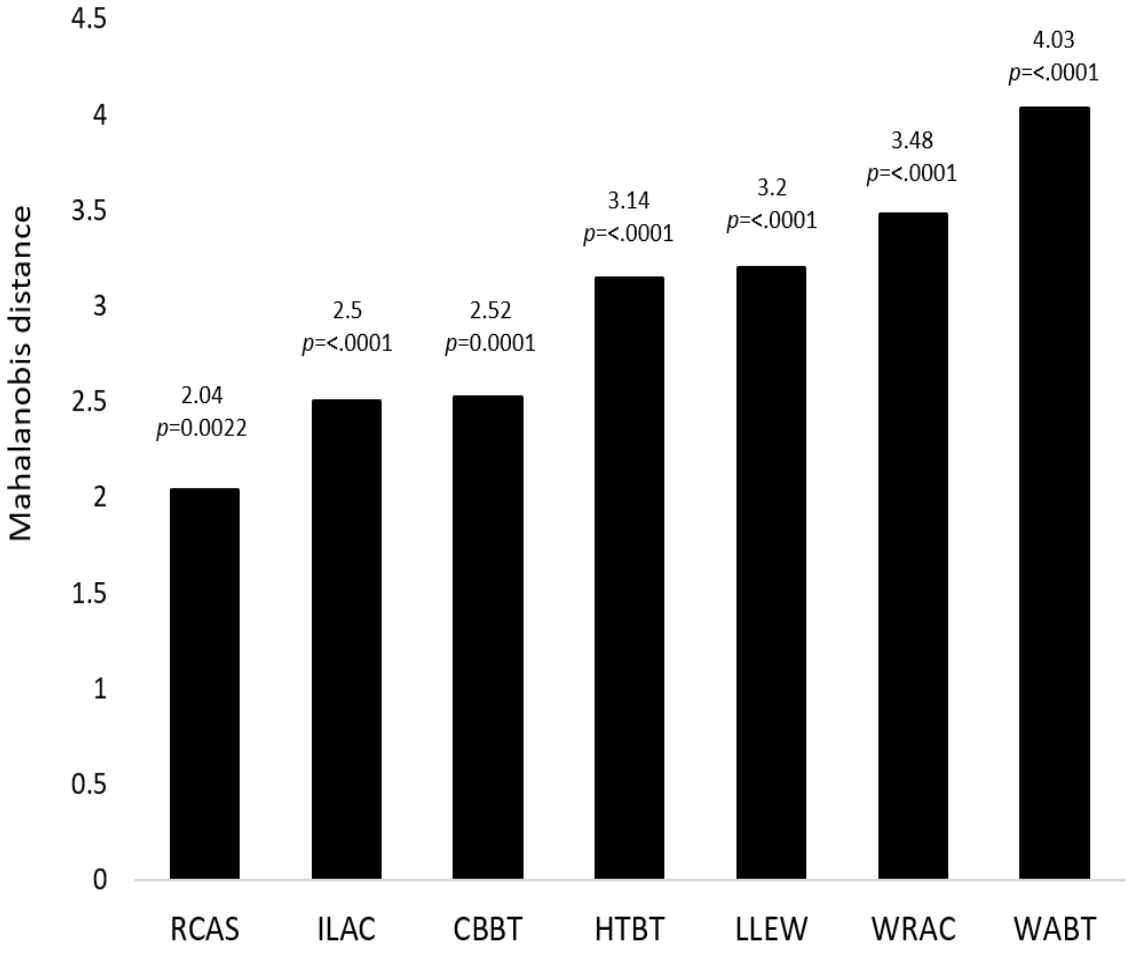

Figure 2.5. Mahalanobis distances for each group, *P* value for significance is given above the corresponding bar.

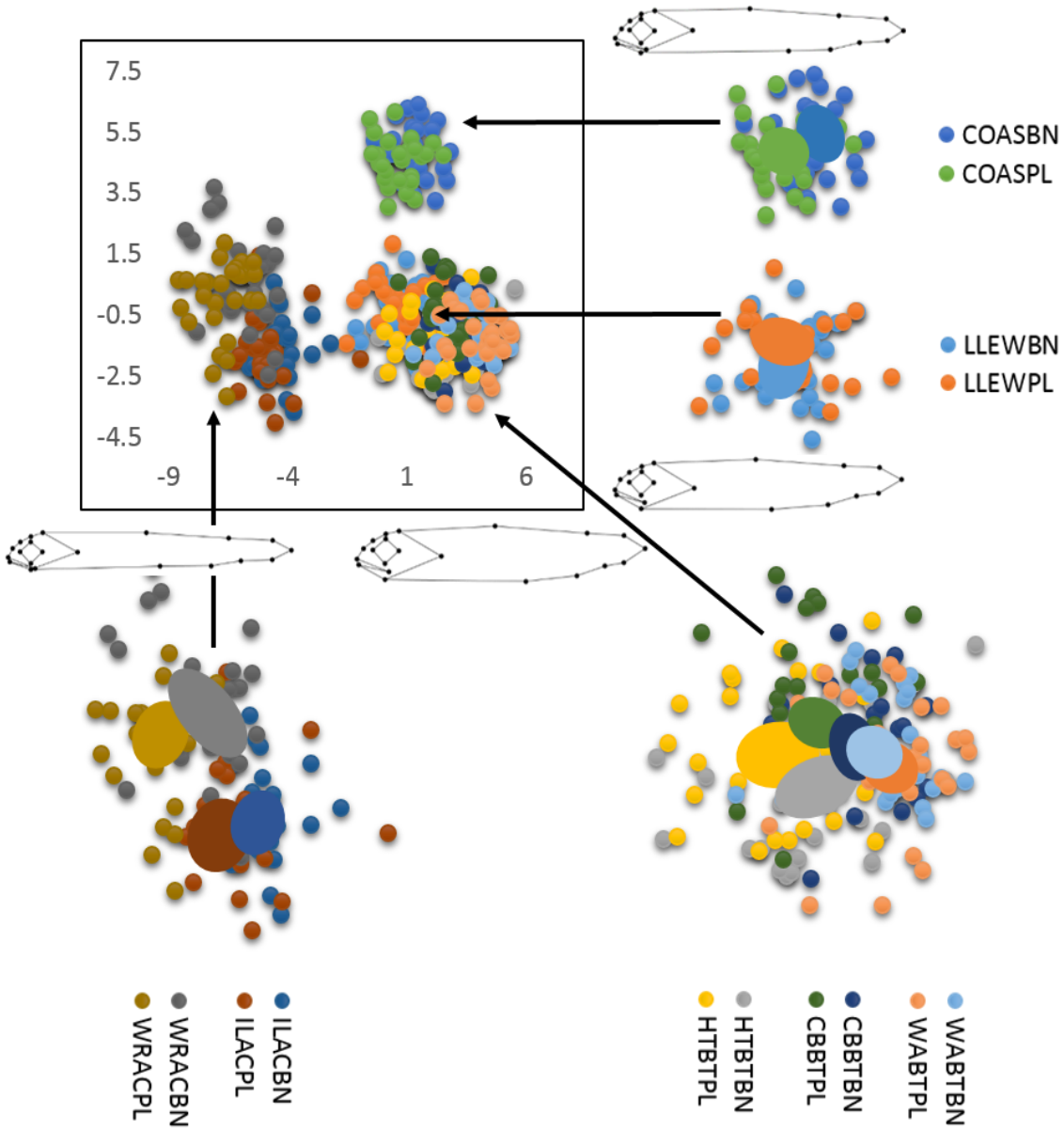

Figure 2.6. Canonical Variate Analysis for all pairwise comparisons of fish raised on either a benthic (BN) or pelagic (PL) for Wester Ross (WR) and Iceland (IL) Arctic charr, Houie toun (HT), college burn (CB) and white adder (WA) brown trout, Loch Lomond (LL) white fish and River Conan (CO) Atlantic salmon. CV1 (shown on the X axis) accounts for 57% of the variation and best describes the shape differences between Arctic charr and brown trout. CV2 (shown on the X axis) accounts for 18% of the variation and best describes the shape differences between Atlantic salmon and all other species, Wire frames depict body shape at outer most point of each axis. Ellipses represent 50% confidence.

European whitefish and Arctic charr (from parents of wild freshwater populations) both showed high levels of phenotypic plasticity which supports the theory that the frequency of occurrence of sympatric populations in the wild (Table 2.1. and 2.2.) that arise through the exploitation of new resources is likely related to the degree of plasticity. However, based on the number on sypmaptric polymorphic populations of lake dwelling trout (Table 2.3.) it was surprising to find that brown trout from wild freshwater parents were more plastic in their response compared to Arctic charr and European whitefish. This would suggest that the plastic ability of freshwater brown trout to diverge is present, but not necessarily expressed, or that the variation we do see is observed along a continuum. Difference in phenotype at tail ends of this range are thus diluted by intermediate phenotpyes and are therefore more cryptic.

Phenotypic plasticity allows adaptation to the environment; organisms with high levels of phenotypic plasticity have a significant fitness advantage in colonising or utilising new habitats or resources (West-Eberhard, 1989, Scheiner, 1993, Via *et al.*, 1995, Schlichting, 2004). Where brown trout co-exist with whitefish and Arctic charr they are usually the dominant species, often utilising the productive littoral zone which suits a generalist foraging strategy. Where sympatric polymorphic populations of whitefish or Arctic charr do occur it is frequently in sympatry with brown trout supporting the theory that high levels on interspecific competition to be fundamental in the development of resource specialists in sympatry as well as maintaining them. Brown trout are most commonly found in the littoral zone which is highly heterogeneous compared to the pelagic and benthic zones by comparison (Benson *et al.*, 1992). The littoral zone provides a continuum of foraging opportunities related to prey type and habitat whereas the pelagic and benthic zones are disjunct from one another. It could be speculated that the highly connective littoral zone creates a continuum of different habitats and thus brown trout express phenotypes suited to every different possible niche and is there for not ‘structured’ by definition (Ford, 1966). In contrast the benthic and pelagic environments, where polymorphic Arctic charr and European whitefish are most commonly seen, may due to occupying two very different and fragmented niches (pelagic and benthic) which permits the development of alternative phenotypes that are contrasting to one another.

It was interesting to find that offspring from freshwater populations were always more plastic than anadromous populations and that offspring from wild populations were more plastic than those from a hatchery origin.

It has been suggested that anadromy is a phenotypic response to environmental conditions such as low productivity and/or competition which ultimately have the same selection pressure (Appendix 1). Rather than adopting an alternative foraging tactic such as exploiting an alternative niche in a freshwater environment, individuals exploit an alternative niche in the marine environment. Brown trout and Arctic charr from the freshwater population had a Mahalanobis distance greater than the anadromous population, 40% and 28% respectively. This is supported by Appendix 1 which found freshwater brown trout to have more phenotypic flexibility than anadromous brown trout. The benefits of this increased plasticity would allow for adaptation to a more varying and competitive freshwater environment compared to the marine environment. In fact, both the anadromous groups tested in this study had the lowest and third lowest and least significant Mahalanobis distances.

Atlantic salmon showed the lowest levels of divergence which we expected based on there being no known foraging polymorphisms centred around benthic and pelagic specialisation in the wild. But the morphological response was still significant showing this species to have the plastic capabilities for divergence, but suggesting that due to other ecological factors they do not have to use it, however this was not tested. Explanations of why Atlantic salmon do not exhibit morphological plasticity in response to foraging could be due to habitat type, social position and life history. Atlantic salmon are often the dominant species and thus have very little competition (Armstrong *et al.,* 2003). High levels of competition have already been shown to play a fundamental role in sympatric speciation (Dieckmann and Doebeli, 1999). In addition, nearly all populations are anadromous (with some exceptions (Fenderson and Carpenter, 1971; Tessier and Bernatchez, 1999). The marine environment is highly productive thus suiting a generalist approach. The need to be able to adapt your phenotype to prevailing contemporary environmental conditions is likely to be of little benefit in the marine environment compared to freshwater.

Fish groups that came from a hatchery origin also had a low Mahalanobis distance. Phenotypic plasticity is a trait and therefore partly inherited. The loss of phenotypic plasticity could result from multiple generations in sterile and physically homogenous environments such as those experienced in hatchery environments. Habitat complexity has been shown to positively correlate with phenotypic variation (Garduno-Paz and Adams, 2010a). Retention of a trait that has no fitness benefit in such an environment would

eventually be lost over time and would support our findings. This could have severe repercussions for populations that are frequently stocked by hatchery populations to aid decreasing numbers by introducing individuals into wild populations that are less able to adapt to environmental changes.

2.6. Conclusions

Our results support the theory that plasticity is partly responcible for the frequency in which sympatric polymorphic populations arise. However, the frequency in which they occur is not only dependant on the level of plasticity the species possesses, but other environmental factors as well. For example, if alternative niches are available, how disconnected they are may be important to sympatric divergence. Species communities are also likely to be important as some species provide a form of competition forcing other species to adapt their foraging techniques, but may also act to fragment the primary habitat of other species.

2.7. Acknowledgments

I would like to thank Alex Lyle for his help in collecting and spawing broodstock, Travis Van-Leeuwen and Madeline Carruthers for their assistance with fish husbandry and Kathryn Elmer for her contributions towards sourcing livestock and advice regarding the experimental design. This work was supported by funding from the European Union's INTERREG IVA Programme (project 2859 'IBIS') managed by the Special EU programmes body.

CHAPTER 3.

THE PHYSIOLOGICAL COSTS OF PREY SWITCHING REINFORCE FORAGING SPECIALISATION.

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3.1. ABSTRACT

Sympatric speciation is thought to be strongly linked to resource specialisation with alternative resource use acting as a fundamental agent driving morphological divergence. However, resource driven adaptive radiation is dependent on foraging specialisation being consistent over space and time. Physiological adaptations to a specific diet may increase the efficiency with which it is utilized, but may have an increased cost associated with switching diets. Standard metabolic rate is the minimal maintenance metabolic rate of an ectotherm in a post-absorptive and inactive state and can constitute a significant portion of an animal's energy budget, thus standard metabolic rate and growth are two measures frequently used as an indication of the physiological performance of individuals. In this study we use the diet specialisation of polymorphic Arctic charr to look at the effects of early prey specialisation on standard metabolic rate and growth. We found a significant effect of diet type on standard metabolic rate and growth. Furthermore, we found evidence of diet specialisation with all fish maintaining a standard metabolic rate and growth rate lower than expected when fed on a diet different to which they were raised, possibly due to a maladaptation in digestion of alternative prey items. Our results show that early diet specialisation may be important in the process of speciation by increasing the costs of prey switching. In doing so it stabilizes alternative resource use by increasing the search time threshold during periods of low primary prey abundance, significantly contributing to ecological speciation through novel niche expansion.

3.2. INTRODUCTION

A major theme emerging from our understanding of how ecologically driven speciation occurs in sympatry is that it is frequently linked with resource specialization (Diekmann and Doebelli, 1999; Nosil, 2012). There is considerable evidence that intraspecific foraging specialisms are an important step driving the early stages of divergence in sympatry (Knudsen *et al*., 2006; Grant and Grant, 2011). Resource use specialization has very significant consequential effects on the ecology for the individual exhibiting a resource specialism (Skúlason and Smith, 1995). These effects include habitat use (Heithaus *et al*., 2002), fitness (Cucherousset *et al*., 2011), growth (Metcalfe, 1986) and reproduction (Dewsbury, 1982; Suryan *et al*., 2000). Individuals exhibiting different specialisms may also differ in some or all of these characteristics as a consequence. Such effects are the foundation of the concepts of ecological speciation (Skúlason and Smith, 1995; Skúlason *et al*., 1999). Examples of ecological speciation in sympatry have been shown in plants (Ostevik *et al*., 2011), insects (Grant, 1949; Coyne and Orr, 1997), birds (Smith and Skúlason, 1996) and fish (Adams *et al*., 1998; Hatfield and Schluter, 1999; Rogers and Bernatchez, 2007; Elmer *et al*., 2014).

Where examples of sympatric divergence exist they are thought to be the result of either strong intraspecific competition and/or the availability of new and often novel prey types. Sympatric divergence is more likely to occur when alternative resources are discrete and the behavioural skills and anatomical tools needed to efficiently exploit them are contrasting (Snorrason *et al*., 1994; Skúlason *et al*., 1999; Amundsen *et al*., 2004; Kahilainen and Østbye, 2006). Thus divergent selection can operate differentially on a diverse array of morphological, behavioural and physiological traits to increase foraging efficiency on alternative prey types (Svanbäck and Eklöv, 2003). Traits required for high foraging efficiency can be genetically inherited, or ontogenetic, arising through phenotypic plasticity (Via *et al*., 1995); or a combination of both (Adams and Huntingford, 2002). However, for evolved traits to manifest through plasticity alone, any foraging specialism must lead to increased fitness and be maintained over a significant portion of an animal's life. For example, learned behaviours that increase foraging efficiency may help to maintain long-term foraging specialization and as a consequence expose individuals specialising on different prey to different selection regimes. Mechanisms such as search image formation (Stanton 1984) and specific prey foraging techniques (Hughes and Seed, 1981; Guillemain *et al*., 2001) can increase the cost of prey switching for individuals specializing on a single prey type, and thus promote long term specialization.

Optimal foraging theory predicts that a decrease in foraging efficiency associated with switching prey increases with the level of behavioural difficulty in acquiring that prey (Hughes, 1979; Hughes and Seed, 1981). It has also been shown that subtle differences in morphology between individuals can help maintain specialization by increasing foraging efficiency on different prey types (Garduno-Paz and Adams, 2010). For example, differences in gill raker spacing and mouth shape in both the three-spined stickleback (*Gasterosteus* spp) and European white fish (*Coregonas laveratus*) (Kahilainen and Østbye, 2006) have been shown to have dramatic effects on their foraging efficiency on benthic or planktonic resources (Schluter, 1993).

Physiological adaptations to foraging specialization are less well understood and not as well documented, likely due to their cryptic nature (Van Leeuwen *et al.*, 2011). Standard metabolic rate (SMR) and growth rate are two frequently used measures of the physiological performance of individuals (Van Leeuwen *et al.*, 2011). Differences in food quantity (Van Leeuwen *et al.*, 2011; Auer *et al*., 2015a) and food type (McNab, 1986; McBride and Kelly, 1990; Yang and Joern, 1994; Starck, 1999; Rosenfeld *et al*., 2015; Van Leeuwen *et al*., 2015) have been shown to influence SMR, which can in turn affect the growth rate of the individual (Van Leeuwen *et al.*, 2011; Auer *et al*., 2015b). Therefore, physiological adaptation to increase conversion efficiency of novel prey types may manifest as differences in SMR and growth rate in response to varying food types.

Standard metabolic rate, which is equivalent to basal metabolic rate (BMR) in endotherms, is the minimal maintenance metabolic rate of an ectotherm in a post-absorptive and inactive state and can constitute a significant portion of an animal's energy budget (Finstad *et al*., 2007). Standard metabolic rate has been found to be consistent across an individual's life span, is thought to be inherited and there can be up to a three-fold difference among individuals (see review in: Metcalfe *et al.*, 2016). Comparative studies have shown that SMR is of ecological and evolutionary importance (Glazier, 2005; Steyermark *et al*., 2005; Careau, 2008; Artacho and Nespolo, 2009; Burton *et al*., 2011). Differences in physiology have been shown to be a result of ecologically driven selection pressures that can drive and maintain the coexistence of incipient species of lake fish (Dijkstra *et al*., 2011; Evans *et al*. 2012). Differences in SMR can be underpinned by environmental factors, such as resource acquisition (Steyermark, 2005; Alvarez and Nicieza, 2005), which can affect physiology and life history trajectories making SMR a

useful metric for understanding the role of physiology in the occurrence of resource specialization.

Variation in SMR and growth rate among contrasting genotypes is partly rooted in larger digestive tracts and maximum food rations that contribute to a higher SMR (Rosenfeld *et al*., 2015; Allen *et al.*, 2016). Digestive tracts have already been shown to be phenotypically plastic and respond to changes in food availability and prey nutrition (McNab, 1986; McBride and Kelly, 1990; Yang and Joern, 1994; Starck, 1999; Armstrong and Bond, 2013) with increased food rations leading to increased surface area and microtopography of the intestine (Rosenfeld *et al*., 2015). Studies investigating food quality have shown that animals eating a low quality diet may evolve lower metabolic rates in order to balance their energetic requirements (McNab, 1986). Because lipids have low metabolic activity, differences in SMR may reflect differences in lipid stores, with individuals that have greater lipid stores also having a lower mass specific SMR (Rosenfeld *et al*., 2015). Furthermore, McNab (1986) found that individuals fed low-quality diets (e.g. low protein and lipid content) also had reduced internal organ mass, potentially resulting in a lower SMR. In contrast McBride and Kelly (1990), Yang and Joern (1994) and Starck (1999) found a positive correlation between the amount of indigestible material in a diet and organ size with individuals that had larger organs having a higher metabolic rate.

The Arctic charr (*Salvelinus alpinus*) has been frequently recorded exhibiting sympatric resource polymorphisms most frequently occurring as foraging specialisms (Snorrasson *et al*., 1994). The two most commonly reported sympatric ecotypes are a pelagic form which specializes on zooplankton prey and a benthic form that specializes on macro invertebrate prey (Malmquist *et al*., 1992; Snorasson *et al*., 1994; Adams *et al*., 1998; Chapter 5).

In this study we use Arctic charr to investigate the potential effects of early prey specialization on SMR and growth rate using three different prey items, one from the pelagic environment and two from the benthic environment. Furthermore, we test if there were any effects on SMR and growth rate associated with diet switching. The main objectives of this study were 1) to compare the effect of different diets typical of specialization in the wild on SMR and growth rate, and 2) to establish the role of physiology and its effect on the development of resource specialization using SMR and

growth rate as a proxy in Arctic charr from a lineage that had not differentiated into planktonic and benthivorous specialists in the period since the last glaciation from anadromous ancestors.

3.3. Materials And Methods

3.3.1. LIVESTOCK CARE AND PREPARATION

Arctic charr were acquired in February 2014 as eggs from a cultured anadromous brood stock. This brood stock was chosen because the anadromous stock provided an undifferentiated lineage with which to test the diversification potential of diet specialization and to avoid potential confounding effects (genetic and non-genetic) as a result of using eggs from parents which were themselves benthic or pelagic specialists. Eggs were transported to the Scottish Centre for Ecology and the Natural Environment (SCENE, Loch Lomond, Glasgow). Upon arrival the eggs from six full-sib families were placed in separate meshed rearing baskets suspended in a holding tank in a constant temperature room. Constant temperature rooms were illuminated using fluorescent tubes on a 10L:14D cycle, controlled with a timer. Water was supplied directly from Loch Lomond and was maintained at 4.0 ± 0.5 ^{o}C. Developmental rate and time of sampling was measured in degree-days (dd; the cumulative water temperature for a known period of time in days), this commenced when all fish had hatched. Fish reached the 'first feed' stage at approximately 102dd when the yolk sac was almost exhausted and fish started to actively seek food. At this point chopped liver was introduced *ad libitum* as an exogenous food source. At 328dd 16 offspring from each of the six different families of Arctic charr were evenly mixed (N = 96) and then distributed across six aquaria (N= 16 fish per aquarium). All aquaria measured 48 x 30 x 22cm thus providing two replicates of each diet treatment. All aquaria were supplied with water from Loch Lomond on a flow through system at ambient temperature which ranged from 5^{o}C – 16^{o}C.

3.3.2. DIETARY TREATMENTS

From 328dd fish were fed one of three diet types; *Daphnia pulex*; bloodworm (*Chironomus sp.* larvae); or *Gammarus pulex*, all three prey are commonly found in the stomachs of sympatric polymorphic lake-dwelling Arctic charr in the wild (Adams and Huntingford 2002; Knudsen *et al*. 2006). *Daphnia pulex* represented a pelagic prey item, whereas bloodworm and *Gammarus pulex* represented benthic prey items. The mass and nutritional composition for each prey type is given in Table 3.1. During the rearing period, which ran from 12 March 2014 – 17 October 2014 (approximately 2303dd), fish were fed

to satiation three times daily at four-hour intervals. These groups are henceforth referred to as *Daphnia* fish, bloodworm fish or *Gammarus* fish (Figure 3.1.).

Table 3.1. Size and nutritional content of the different prey items. All nutritional inforamtion is provided by BCUK Aquatics, Lincolnshire, England.

Food	Mass (mg)	Protein (%)	Fat (%)	Fibre (%)	Moisture (%)
Daphnia	0.76 (SD ± 0.025)	5.0	0.7	1.0	90.0
Bloodworm	12.1 (SD ± 1.412)	5.0	0.5	0.9	89.0
Gammarus	15.92 (SD ± (2.79)	8.0	1.0	1.2	89.8

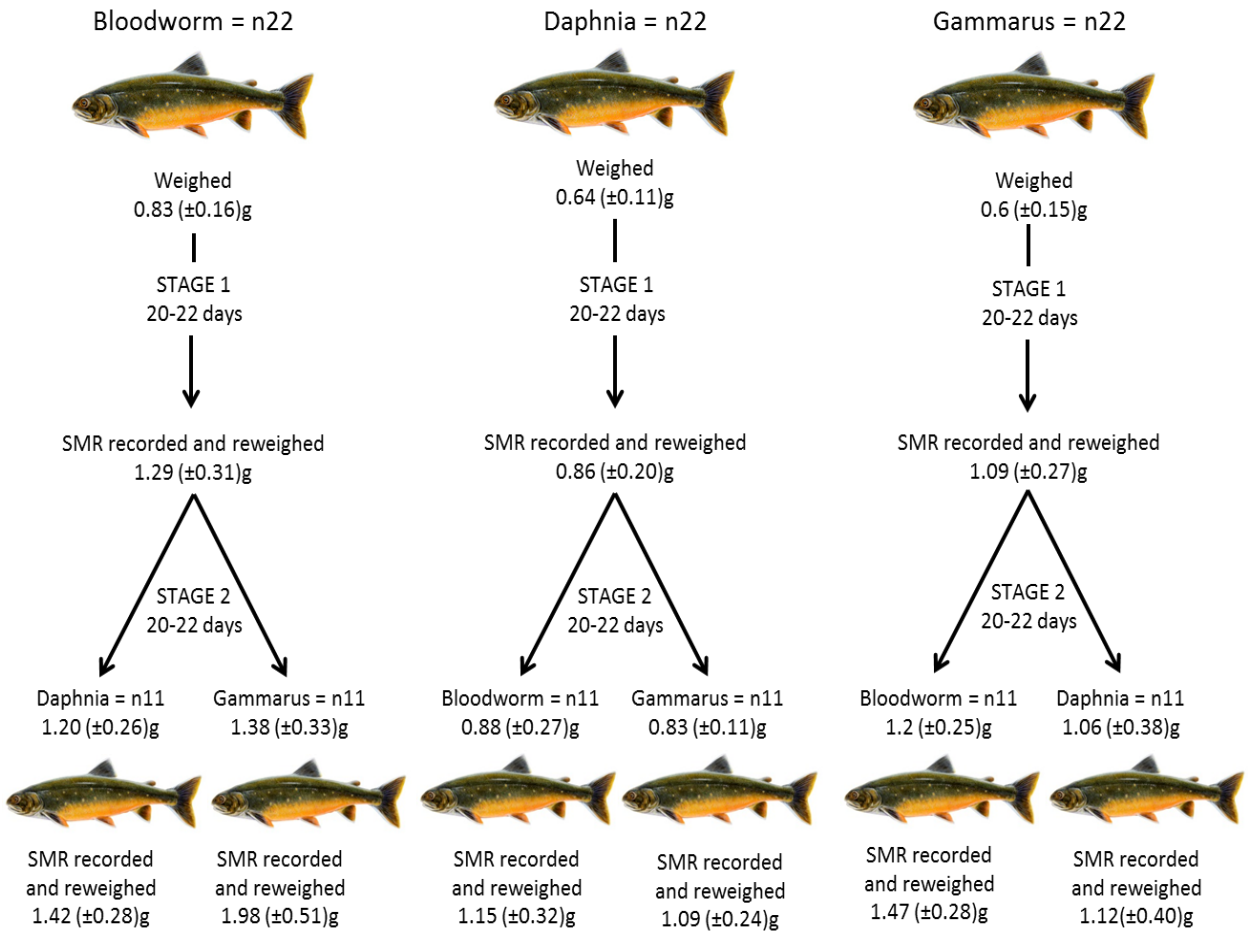

Figure. 3.1. Schematic showing experimental design, including sample size for each stage and mean weights and standard deviation for each sample group.

During the rearing period leading up to oxygen uptake and growth rate measures aquaria were supplied daily with 45g (wet weight) of food from one of the three diet types discussed above. Food items were thawed prior to feeding. To ensure that any observed changes in SMR and growth rate were the result of diet type and not simply quantity, fish were fed to satiation to help reduce differences in food acquisition as a result of dominance hierarchies. Food ration sizes large enough to ensure satiation were calculated prior to the experiment by placing known amounts of food into an aquarium and observing the point at which the addition of food resulted in no additional feeding response.

3.3.3. MEASUREMENT OF GROWTH AND SMR

Twenty two fish from each diet treatment were randomly selected from aquaria. At this stage each individual was marked with visible implant elastomer (Northwest Marine Technology, Inc.), and equally distributed across two replicate aquaria (11 fish per tank) measuring 48 x 30 x 22cm keeping diet type discrete. Remaining fish were used as part of an additional study. Two assessments of growth rate and SMR were then obtained for each of the marked fish. The first assessment of growth rate and SMR (Stage 1) were obtained for fish on their starting (initial) diet (Figure 3.1.). At the start of Stage 1, fish were weighed to ± 0.01g. At the end of this stage (20-22days) fish were starved for 24 hours, reweighed to ± 0.01g and subject to metabolic measurement. Starving fish for 24 hours allowed sufficient time for fish to evacuate their guts prior to oxygen uptake measurements. Individual fish were placed into one of 22 darkened glass respirometer chambers (30mm diameter, 80mm length, 56.6ml volume) to minimize fish activity during measurements (Cutts *et al.*, 2002) and allowed to settle for 18-20 hours to acclimatise and come to rest before oxygen consumption measures commenced. All oxygen uptake measurements were taken between 06:00 and 12:00 hours. Immediately after oxygen uptake measurement, the 22 fish from each starting diet were divided randomly into two groups of 11 fish. Each group of fish was then fed one of the two alternative diets, for example fish raised on bloodworm were fed either *Daphnia* or *Gammarus*; fish raised on *Daphnia* were fed either bloodworm or *Gammarus*; and fish raised on *Gammarus* were fed either bloodworm or *Daphnia* (Stage 2). These new diets continued for a further 20-22 days, after which fish were again starved for 24 hours, reweighed so that growth rate and SMR could once again be determined (Figure 3.1.).

Oxygen uptake was measured using flow-through respirometery (Steffensen, 1989) whereby the rate of oxygen consumption by the fish is measured as a reduction in oxygen

concentration between the water flowing into, and out of, the respirometer (holding the fish). By definition SMR should be measured on fish which are not growing, i.e. fish that are on a maintenance ration and therefore the term "apparent SMR" is more appropriate. For consistency throughout this manuscript the use of the term SMR is taken to mean "apparent SMR".

Water was supplied from a central header tank to the respirometry chambers using 4mm diameter tubing attached to a manifold. An air stone in the header tank of the respirometer apparatus kept inflow water fully saturated with oxygen. Water oxygen concentration exiting the chamber was measured using an oxygen meter (FireStingO_2 oxygen meter; PyroScience) fitted with 4 oxygen probes; each probe was calibrated daily. The average flow rate of the water was 0.07 l/hr (approximately 1 ml/min); this was adjusted (±) to ensure that there was at least a 10% drop in oxygen concentration between the inlet and outlet of each respirometer, although concentrations never dropped below 80% oxygen saturation. Flow was controlled using micro-valves positioned at the inlet of the respirometer chambers. The flow rate for each chamber was calculated by weighing the amount of water (to 0.01g) that exited the chamber in a 60s period and was measured at the same time as oxygen concentration measurements were taken. The respirometry apparatus was located inside a constant temperature room, held at 13.3ºC (± 0.1 ºC). The rate of oxygen consumption was determined using the following equation (Ege and Krough, 1914):

$$\mathbf{MO2(whole)=V_w\Delta C_w0_2}$$

Where *V*w is the flow rate (l/h) of water through the respirometer and ΔCwO_2 is the difference in the oxygen concentration between the inflow and outflow water (ml/l). The concentration of oxygen was calculated by correcting ppO_2 (partial pressure of oxygen) for barometric pressure and multiplying by αO_2 (umol L^{-1} torr-^{1}), the solubility coefficient at the observed temperature. SMR was determined using two oxygen uptake measurements taken three hours apart with the average of these two measurements used for statistical analysis. If the two measurements differed by greater than 10% a third measure was taken.

3.3.4. STATISTICAL ANALYSES

Instantaneous growth rates of fish (% bodyweight gain per day) were calculated as 100{[log (final mass) – log (initial mass)]/time elapsed (days)} (Ricker, 1975).

Given the increase in fish mass over the course of the experiment, and the effect of mass on metabolism and growth, we used size-corrected residual values for SMR (rSMR) and growth (rGrowth) in subsequent analysis. Size corrected values were calculated as residuals from the regression of absolute oxygen consumption (SMR) or growth on mass (g) (all log transformed) for all fish. However, the use of residuals in this way have recently been criticised because they remove the possibility of identifying potential effects where variable co-correlation occurs (Freckleton, 2002; McCoy *et al*., 2006). Both SMR and growth are notoriously variable in salmonid fishes (Cutts, Metcalfe and Caylor, 1998). One strength of the study design presented here is that we were able to track changes in metabolic rate and growth with diet change in individual fish, thereby reducing within group variation caused by individual differences. However, to ensure a robust analysis of SMR and growth between diet treatments and to ensure our analysis was not biased by the use of size corrected residuals, we first tested our data using a generalised linear mixed effect model (GLMM) with fish mass was a covariate and individual as random effect. A *post-hoc* test was used to extract all relevant comparisons. In addition, the same comparisons were further tested using the additional power of the paired T-test to reduce the effect of between individual variation. We tested for the effect of diet type (Stage 1) (bloodworm, *Daphnia*, and *Gammarus*) on SMR and growth using an ANOVA. To test for the effect of diet switching on SMR and growth we again used residuals (as described above) to compare the SMR and growth of individual fish before (Stage 1) and after (Stage 2) the diet switch using a paired t-test. Finally, residual values of SMR and growth of fish on their raised diets (Stage 1) were again used to compare against the two other groups that had been switched to that respective diet. For example, the SMR and growth of bloodworm fish being fed bloodworm (Stage 1) were compared to the SMR and growth of *Daphnia* fish and *Gammarus* fish being fed bloodworm (Stage 2) using a linear model. Models included raised diet (explanatory variable) and SMR or growth (response variables).

SMR data were log transformed to linearize the data and meet assumptions of normality and homogeneity of variance. Mass measurements were log transformed following an application of a constant of one to allow transformation of negative values, which occurred as a result of some individuals having a mass of less than one gram. All analyses were conducted using R version 3.1.0 statistical software (R Development Core Team, 2012).

3.4. RESULTS

3.4.1 STAGE 1 THE EFFECT OF INITIAL DIET

During Stage 1 there was a significant effect of prey type on SMR ($F_{2,63} = 86.73$, $P < 0.0001$) (Figure 3.2A.) and growth rate ($F_{2,63} = 10.68$, $P < 0.0001$) (Figure 3.2B.). Fish feeding on *Gammarus* had the highest SMR and growth, whereas fish feeding on *Daphnia* had the lowest SMR and growth (Table 3.2). A GLMM on raw data showed an identical effect (Table 3.3).

3.4.2. STAGE 2 THE EFFECT OF DIET SWITCHING

When diet type was switched there was a significant effect on individual SMR (Figure 3.3A.) and growth (Figure 3.3B.). Fish feeding on Bloodworm showed a significant decrease in SMR and growth when switched to *Daphnia* or *Gammarus*, however during Stage 1, fish feeding on *Gammarus* had a significantly higher SMR and growth. Contrastingly, fish feeding on *Daphnia* showed a significant increase in SMR but a significant decrease in growth when switched to bloodworm or *Gammarus*. Finally, a decrease in SMR and growth was observed when fish feeding on *Gammarus* were switched to *Daphnia* or bloodworm (Table 3.4). The same pattern was found using a GLMM analysis, however, as this test was not as sensitive as the paired t-test because of a high level of inter-individual variation in SMR, some results were not significant (Table 3.5).

Interestingly, the SMR of fish switched to an alternative diet (Stage 2) was always significantly lower compared to the SMR of the fish that were raised on that diet (Stage 1) (Figure 3.4A.). For example, fish fed on a *Daphnia* or *Gammarus* diet and switched to a bloodworm diet (Stage 2) had a significantly lower SMR and growth than fish feeding on bloodworm (Stage 1). Similarly, fish fed on a bloodworm or *Gammarus* diet and switched to *Daphnia* (Stage 2) had a significantly lower SMR and growth than *Daphnia* fish fed *Daphnia* (Stage 1). Finally, fish fed bloodworm or *Daphnia* had a significantly lower SMR when switched to *Gammarus* (Stage 2) than *Gammarus* fish fed on *Gammarus* (Stage 1). A similar trend was also observed for growth but was not statistically significant (Figure 3.4B.; Table 3.6.). Again the GLMM showed the same pattern in the results but some *post hoc* results were not significant (Table 3.7.)

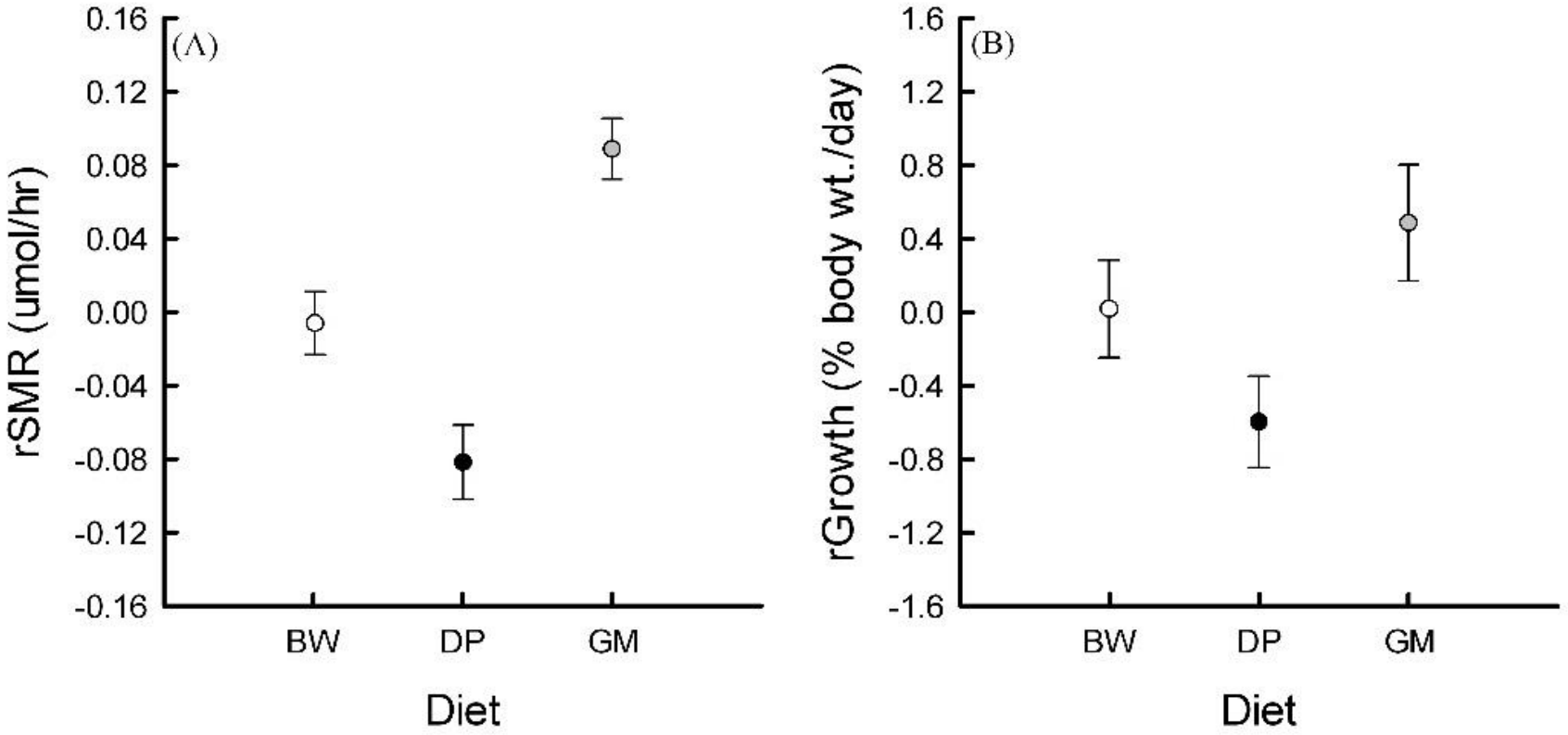

Figure 3.2. Body mass corrected rSMR (A) and rGrowth rates (B) for fish on each diet during Stage 1 (initial diet). BW = bloodworm, DP = *Daphnia*, GM = *Gammarus*). Measures of standard metabolic rate and growth are expressed as residuals, after correction for body mass. See text for statistical analysis.

Table 3.2. Between diet (Stage 1) pairwise post hoc differences in rSMR (overall *P* <0.0001) and rGrowth (overall *P* <0.0001). Residual SMR was compared as umol/hr per gram bodyweight (log), rGrowth was compared as percent bodyweight gain per day (log).

Diet comparison	rSMR	rGrowth
Bloodworm v *Daphnia*	Significantly higher t_{63} = 5.95, *p* = <0.001	Significantly higher t_{63} = 2.67, *p* = 0.03
Bloodworm v *Gammarus*	Significantly lower t_{63} = -7.20, *p* = <0.001	Lower t_{63} = -1.93, *p* = 0.14
Daphnia v *Gammarus*	Significantly lower t_{63} = -13.16, *p* = <0.001	Significantly lower t_{63} = -4.60, *p* = <0.001

Table 3.3. Between diet (Stage 1) pairwise post hoc differences from the GLMM in SMR (overall P <0.0001) and Growth (overall P <0.0001). Standard metabolic rate was compared as umol/hr per gram bodyweight (log), Growth was compared as percent bodyweight gain per day (log).

Diet comparison	T-test rSMR	T-test rGrowth
Bloodworm v *Daphnia*	Significantly higher t_{120} = 12.81, P <0.001	Significantly higher t_{120} = 4.49, P <0.001
Bloodworm v *Gammarus*	Significantly lower t_{120} = -6.3, P <0.001	Lower t_{120} = -1.844 1, P = 0.64
Daphnia v *Gammarus*	Significantly lower t_{120} = -19.82, P <0.001	Significantly lower T_{120} = -6.53, P <0.001

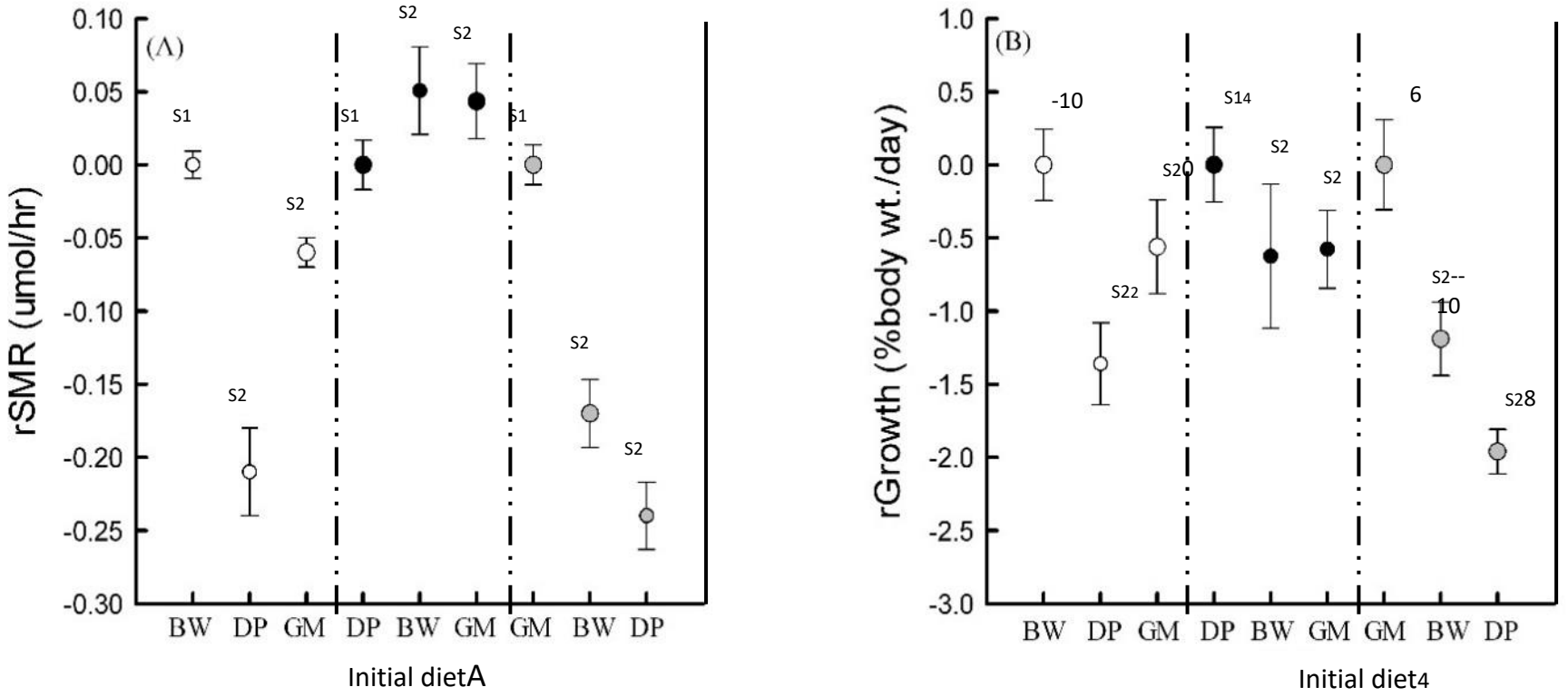

Figure 3.3. Body mass corrected rSMR (A) and the rGrowth (B) measured when fish were fed their inital diet (S1) and being fed alternative diets (S2). Each set of colured symbols depicts the same group of fish on their raised diet (S1) and the diet they were switched to (S2). Fish raised on bloodworm are depicted with white symbols, *Daphnia* with black and *Gammarus* with grey. Diets at the time the SMR and growth measurements were taken are represented by initals on the X axis (BW = bloodworm, DP = *Daphnia*, GM = *Gammarus*). Measures of standard metabolic rate and growth are expressed as residuals, after correction for body mass. See text for statistical analysis.

Table 3.4. Results from paired t-test's comparing individual's rSMR and rGrowth during Stage 1 and following a switch in diet, Stage 2. Residual SMR was compared as umol/hr per gram bodyweight (log), rGrowth was compared as percent bodyweight gain per day (log).

Diet change	rSMR Increase/decrease		rGrowth Increase/decrease	
Bloodworm raised fish switched to *Daphnia*	Significant decrease	$t_{(10)} = -13.51$, $p = <0.0001$	Significant decrease	$t_{(10)} = -7.02$, $p = 0.0011$
Bloodworm raised fish switched to *Gammarus*	Significant decrease	$t_{(10)} = -7.65$, $p =-)<0.0001$	Significant decrease	$t_{(10)} = -3.82$, $p = 0.022$
Daphnia raised fish switched to Bloodworm	Significant increase	$t_{(10)} = 2.98$, $p = 0.028$	Significant decrease	$t_{(10)} = 3.35$, $p = 0.018$
Daphnia raised fish switched to *Gammarus*	Significant increase	$t_{(10)} = 3.11$, $p = 0.022$	Significant decrease	$t_{(10)} = 2.26$, $p = 0.033$
Gammarus raised fish switched to Bloodworm	Significant decrease	$t_{(10)} = -11.81$, $p =)<0.0001$	Significant decrease	$t_{(10)} = -8.92$, $p = <0.0001$
Gammarus raised fish switched to *Daphnia*	Significant decrease	$t_{(10)} = -17.67$, $p =)<0.0001$	Significant decrease	$t_{(10)} = -13.412$, $p = <0.001$

Table 3.5. Results from GLMM comparing individual's SMR and Growth during Stage 1 and following a switch in diet, Stage 2. SMR was compared as umol/hr per gram bodyweight (log), Growth was compared as percent bodyweight gain per day (log).

Diet change	rSMR Increase/decrease	rGrowth Increase/decrease
Bloodworm raised fish switched to *Daphnia*	Significant decrease $T_{120} = -16.92$, P = <0.001	Significant decrease $t_{120} = -5.73$, P =-<0.001
Bloodworm raised fish switched to *Gammarus*	Significant decrease $t_{120)} = -4.88$, P =<0.001	Decrease $t_{120} = -1.655$, P = 0.77
Daphnia raised fish switched to Bloodworm	Significant increase $t_{120} = 4.79$, P =<0.001	Decrease $t_{120} = -0.44$, P = 0.99
Daphnia raised fish switched to *Gammarus*	Significant increase $t_{120} = 4.41$, P =<0.001	Decrease $t_{(10)} = -0.76$, P = 0.99
Gammarus raised fish switched to Bloodworm	Significant decrease $t_{120} = -11.3$, P =<0.001	Significant decrease $t_{120} = -5.96$, P = <0.001
Gammarus raised fish switched to *Daphnia*	Significant decrease $t_{120}= -19.08$, P =<0.001	Significant decrease $t_{120} = -9.61$, P = <0.001

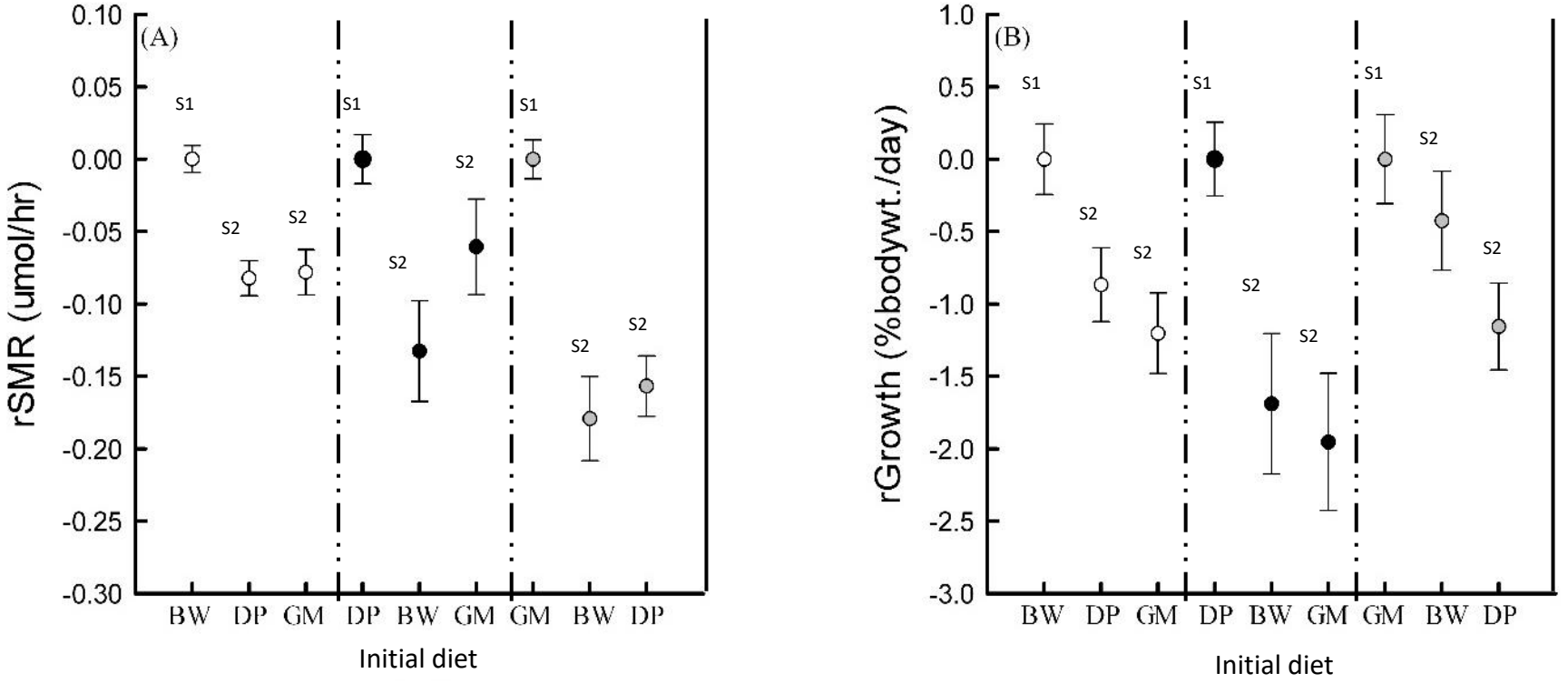

Figure 3.4. Body mass corrected rSMR (A) and rGrowth (B) measured when fish were fed their initial diet (S1) and fish from an alternative initial diet being fed the same diet (S2). Each set of colured symbols depicts the diet at the time the SMR and growth measurements were taken. Bloodworm diet is depicted with white symbols, *Daphnia* with black and *Gammarus* with grey. Initial diets (that fish were raised on) are represented by initals on the X axis (BW = bloodworm, DP = *Daphnia*, GM = *Gammarus*). Measures of standard metabolic rate and growth are expressed as residuals, after correction for body mass. See text for statistical analysis.

Table 3.6. Pairwise post hoc differences in rSMR and rGrowth of fish from different initial diets being fed the same diet. Residual SMR was compared as umol/hr per gram bodyweight (log), rGrowth was compared as percent bodyweight gain per day (log).

Diet comparison	SMR	Growth
Bloodworm raised fish v *Daphnia* raised fish fed on bloodworm	Significantly higher $t_{41} = 9.74$ $p = <0.001$	Significantly higher $t_{41} = 4.49$ $p = <0.001$
Bloodworm raised fish v *Gammarus* raised fish fed on bloodworm	Significantly higher $t_{41} = 9.24$ $p = <0.001$	Significantly higher $t_{41} = 6.23$ $p = <0.001$
Daphnia raised fish v bloodworm raised fish fed on *Daphnia*	Significantly higher $t_{41} = 7.27$ $p = <0.001$	Significantly higher $t_{41} = 6.39$ $p = <0.001$
Daphnia raised fish v *Gammarus* raised fish fed on *Daphnia*	Significantly higher $t_{41} = 3.32$ $p = 0.005$	Significantly higher $t_{41} = 7.38$ $p = <0.001$
Gammarus raised fish v bloodworm raised fish fed on *Gammarus*	Significantly higher $t_{41} = 12.85$ P <0.001	Higher $t_{41} = 1.78$ $p = 0.191$
Gammarus raised fish v *Daphnia* raised fish fed on *Gammarus*	Significantly higher ($t_{41} = 11.24$ $p = <0.001$	Significantly higher $t_{41} = 4.83$ $p = <0.001$

Table 3.7. Results from mixed model comparing individual's SMR and Growth of fish from different initial diets being fed the same diet. SMR was compared as umol/hr per gram bodyweight (log), Growth was compared as percent bodyweight gain per day (log).

Diet comparison	SMR	Growth
Bloodworm raised fish v *Daphnia* raised fish fed on bloodworm	Significantly higher $t_{120} = 6.48$, P <0.001	Significantly higher $t_{120} = 4.23$ P <0.001
Bloodworm raised fish v *Gammarus* raised fish fed on bloodworm	Significantly higher $t_{120} = 6.49$ P <0.001	Significantly higher $t_{120} = 5.015$ P <0.001
Daphnia raised fish v bloodworm raised fish fed on *Daphnia*	Significantly higher $t_{120} = 5.03$ P <0.001	Higher $t_{120} = 2.047$ P = 0.5
Daphnia raised fish v *Gammarus* raised fish fed on *Daphnia*	Higher $t_{120} = 2.34$ P =0.31	Significantly higher $t_{120} = 4.72$ P <0.001
Gammarus raised fish v bloodworm raised fish fed on *Gammarus*	Significantly higher $t_{120} = 9.01$ P <0.001	Higher $t_{120} = 12.71$ P =0.14
Gammarus raised fish v *Daphnia* raised fish fed on *Gammarus*	Significantly higher $t_{120} = 12.12$ P <0.001	Significantly higher $t_{120} = 5.99$ P <0.001

3.5. DISCUSSION

The type of diet juvenile Arctic charr were initially exposed to following first feeding had a significant effect on SMR and growth rate even after the effect of body mass was accounted for. Fish fed on *Daphnia* had the lowest SMR and growth rate with fish feeding on *Gammarus* having the highest.

One possible explanation could have been differences in intestinal length or surface area, which has been shown to be highly plastic in juvenile salmonids (Armstrong and Bond 2013). It is therefore plausible that differences in protein content, palatability and prey size between those prey used in this study may have led to differences in intestinal development.

Fish fed *Gammarus* exhibited the highest SMR and growth. Because all fish were fed *ad libitum*, our results are likely a developmental response as a result of prey type and not a result of differential food intake across diet types or those associated with behaviours within the aquaria. The higher protein and fat content of *Gammarus* relative to other prey types may provide one mechanism for growth and SMR differentials. However, a similar trend was also observed in fish fed bloodworm, with these individuals having a higher SMR and growth than those fish fed *Daphnia* despite having similar protein content. This suggests that protein content alone is likely not responsible for diet-related differences in SMR and growth observed in this study.

Daphnia and *Gammarus* have a hard exoskeleton which is likely costly to digest (Swaffar and O'Brien, 1996; Van Leeuwen *et al*., 2015) compared to soft-bodied bloodworm. This could explain the higher initial SMR and growth observed in fish feeding on bloodworm compared to those feeding on *Daphnia*. As the energy demand to breakdown the exoskeleton of the *Daphnia* increases without the added benefit of greater protein and fat content, the consumer is likely to respond in a similar way as individuals subjected to low food rations, leading to a decrease in SMR (Van Leeuwen *et al.*, 2011) and growth. However, this does not explain why fish fed *Gammarus* had a higher SMR than fish fed bloodworm. This could be partly explained by the ratio of exoskeleton to internal tissue which will be higher in *Daphnia*. Because they are smaller, the number needed to be consumed to equal that of a single *Gammarus* would be greater and therefore so would the amount of exoskeleton ingested.

Prey size may also be of relevance to the patterns observed for SMR and growth with larger prey (*Gammarus*) resulting in an elevated SMR and growth, as has been shown in other animals (Andrade *et al.*, 1997; Secor and Faulkner, 2002; Secor and Boehm, 2006; Millidine *et al.*, 2009). For example, Secor and Boehm (2006) found that larger meals increased the Specific Dynamic Action (SDA) of mole salamanders (***Ambystomatidae***) and is maintained in individuals with a higher metabolic rate (Andrade *et al.*, 1997; Fu *et al.*, 2005; Millidine *et al.*, 2009).

Interestingly, a switch in diet from one that maintained a low SMR and growth to a diet that maintained a higher initial SMR and growth did not always result in an increase in either. Instead SMR and growth decreased in fish feeding on bloodworm when they were switched to *Gammarus* despite a *Gammarus* diet maintaining the highest SMR and growth during the initial feeding stages of the experiment (Stage 1). *Daphnia* raised fish showed a slight increase in SMR when switched to bloodworm or *Gammarus* but in both cases growth and SMR remained lower than fish on a continuous bloodworm or *Gammarus* diet. In addition, growth decreased when *Daphnia* fish were switched to an alternative higher quality diet. These results suggest a maladaptation in the assimilation efficiency of the alternative prey type. Surprisingly, this apparent maladaptation seemed to persist regardless of whether the diet switch represented an increase or decrease in prey quality. This suggests that deviating from a familiar prey type to an alternative prey type results in a metabolic discontinuity and ultimately a growth cost even over the relatively short feeding trials in our study. Although the mechanism through which these growth and metabolic rate costs arise remains to be tested, fish in the wild are often exposed to periods of high and low seasonal abundance of a single prey type (Parnell *et al*., 2013).

Physiological or anatomical specialization to increase efficiency in converting a specific prey type to energy may involve differences in the digestive tract that arises during development and thus may not be reversible. That being said, the digestive anatomy of other taxa has been shown to be flexible (Piersma and Lindstrom, 1998; Starck, 1999; McWilliams, and Karasov, 2001) with some species adapting their anatomy on a seasonal basis (McWilliams, and Karasov, 2001). This may therefore be to some extent reversible. If the digestive tract were to "retool" itself in an attempt to adapt to different prey following a diet an increase in SMR would be expected, however this was not seen in our study. From this study it appears that any physiological adaptation is not immediately reversible as there was no evidence of this after three weeks (the duration of stage 2).

The decrease in growth after a change in diet that was observed in our study may have multiple causes. The decrease in growth may be caused by an increase in metabolic costs resulting in a drop in conversion efficiency of energy. However, SMR decreased making this an unlikely cause, thus a loss in conversion efficiency due to some form of maladaptation of the digestive tract to an alternative prey type is much more likely resulting in a reduction in growth. Reduced efficiency may be caused by the gut not being able to fully up-regulate to its maximum capacity due to a miss-match with the composition of the new diet. An alternative is that reduced anabolic metabolism caused by a decrease in growth resulting from less assimilated energy available. As a result of this down regulation in growth, a decrease in SMR occurs caused by a reduction in available energy.

Factors that may affect energy assimilation may be underpinned by differences in the gross anatomy as a result of being fed different diets during critical developmental periods. Digestive organs have been shown to adjust their size depending on the type of prey being consumed. This can affect SMR because digestive organs have a high mass specific SMR. Variation in SMR may also arise from differences in digestive chemicals and their quantities/ratios needed to break down prey as these will likely differ according to the prey type. If the digestive chemicals produced differ in their type and quantity so might the gland cells associated with their production. These can alter the molecular physiology caused by different diets thus contributing to variation in SMR (Burton *et al*., 2011).

Optimal foraging theory predicts significant advantages to individuals specialising in feeding on a small range of prey items but this is highly dependent on ecological context. The optimal foraging model estimates a search time threshold which defines when it becomes beneficial to start foraging on an alternative prey item (Pyke *et al.*, 1977; Pyke, 1984). If there are unaccounted digestive or metabolic costs of specializing on a single prey type that reduce growth efficiency following a prey switch, then this maladaptation to novel prey has the potential to stabilize foraging specializations by increasing the search time threshold above which prey switching is beneficial during periods of low primary prey abundance thus significantly contributing to ecological speciation through novel niche expansion.

Logically the physiological and growth costs of prey switching shown here should also decrease the fitness of intermediate phenotypes that display reduced foraging specialization. In addition to the individual fitness benefits, such resource specialisms may also have long term evolutionary consequences by providing an additional mechanism that re-enforces the benefits of diet specialization that drives evolutionary divergence.

Current conceptual models of ecologically driven evolution have resource specialisms and in particular foraging specialisms as the first step of early divergence (Skúlason *et al.*,1999; Parsons *et al.*, 2014). Quantitative modelling has reinforced differential resource specialisms within species as the most important ecological variable enabling divergence in symaptry (Diekmann and Dobelli, 1999) and empirical studies have demonstrated how early stable foraging specialization may arise (Malmquist *et al*., 1982; Robinson, 2000; Garduno-Paz and Adams, 2010; Siwertsson *et al*., 2013). Despite this, the study presented here is one of the first to show that in addition to the morphological and behavioural optimal foraging advantages to individuals that specialise, there can also be significant physiological advantages in maintaining foraging specialization once they have become established. These physiological advantages can provide further stability in the adoption of foraging specialisms. Our study elevates the physiological costs of diet specialization to the same level of significance in evolutionary divergence as phenotypic and behavioural foraging specilizations, supporting the importance of cryptic differentiation in digestive metabolism as an important dimension on the integrated phenotype.

3.6. ACKNOWLEDGMENTS

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CHAPTER 4.

TEMPERATURE MODULATES THE EXPRESSION OF PHENOTYPE IN A FRESHWATER FISH.

*Please note this chapter has been submitted to *The Journal of Experimental Biology*

4.1. ABSTRACT

Phenotypic plasticity is an organism's ability to express multiple phenotypes in response to the prevailing environmental conditions and may thus respond to anthropogenic modifications of that environment. The most current anthropogenic modification of the environment is climate change. We test the effect of an abrupt elevation in water temperature, in line with those observed in historic climate data, has on the expression of morphological phenotypes of known functional relevance for foraging in Arctic charr. Head shape differed between ambient and elevated temperature fish. The magnitude of shape difference increased over time but the strength of this effect diminished as fish got larger. Head shape of the elevated temperature fish differed significantly to that of their parents. Fish raised at an elevated temperature also exhibited significantly less within group phenotypic variation. Ambient temperature fish expressed a phenotype closer to the pelagic foraging specialists and elevated temperature fish a phenotype closer to benthic foraging specialists. This may result in a shift to greater use of macro-benthos foraging resources by more populations or a loss of fitness due to reduced plankton foraging efficiency. A decrease in between-individual variation would cause a loss of phenotypic diversity for selection, reducing the potential to evolve and adapt.

4.2. INTRODUCTION

In nature, the expression of intraspecific, discrete, alternative phenotypes can be modulated by the environment (Via and Lande, 1985; Smith, 1993; Adams and Woltering, 2003; Dawson, 2008) and by reproductive isolation (Gross, 1996; Taborsky *et al.*, 2008). One commonly reported pattern in nature is a link between specialisation by individuals in resource use, most frequently, but not exclusively, foraging resources, and the expression of phenotypes that have functions related to the utilisation of these resources. These are often referred to as resource polymorphisms (Skúlason and Smith, 1995). There is growing evidence that the environmental mechanisms that drive and maintain resource polymorphisms are broadly similar across species that are geographically and phylogentically distinct (Skúlason and Smith, 1995; Smith and Skúlason, 1996). One common pattern in resource polymorphisms is that they have arisen repeatedly in sympatry (West-Eberhard, 1989; Skúlason and Smith, 1995; Smith and Skúlason, 1996). The selection environment thought to have driven the emergence and maintenance of resource polymorphisms is a combination of discrete alternative resources (West-Eberhard, 1989) and high intraspecific competition (Svanback *et al.*, 2008). There is good evidence that expression of such discrete phenotypic variation is an important step that may ultimately lead to speciation (Wund *et al.*, 2008; Muschick *et al.*, 2011).

Phenotypic plasticity is the ability of a single genotype to express multiple alternative phenotypes (polyphenisms) in response to different environmental conditions (Pigliucci, 2004). Plasticity itself is often considered a trait, manifesting across a broad range of taxa (Lubchenco and Cubit, 1980; Newman, 1992; Hammond *et al.*, 2001; Corno and Jurgens, 2006). It can be instantaneous, anticipatory or delayed, permanent or reversible, adaptive or non-adaptive, beneficial or harmful, passive, discrete, continuous and generational (Whitman and Agrawal, 2009). It is known to facilitate the expression of novel phenotypic traits (West-Eberhard, 1989; Smith and Skúlason, 1996; Moczek *et al.*, 2011) upon which selection may then act (Parsons *et al.*, 2007). Thus phenotypic plasticity has an important role in the evolutionary processes of organisms that exhibit plasticity.

As the expression of discrete functional phenotypes in some species is plastic and by definition modulated by variation in the natural environment, it is logical that they may also respond to anthropogenic modifications of that environment. Plasticity induced modulation of phenotype in response to temperature within the natural range of some

species has been demonstrated (Harkey and Raymond, 1988; Chai and Srygley, 1990; Orizaolo and Laurilla, 2009; Kavanagh *et al.,* 2010). In addition, some studies have looked at the potential of temperature change to cause heterochrony of developmental and ontogenetic processes that modulate the expression of functional traits (Parmesan 2006; Charmantier *et al.,* 2008), whilst others have predicted what the morphological outcome may be (McPhee *et al.,* 2012). However, the ecological consequences that result from temperature induced phenotypic change has not been tested (Ramler *et al.,* 2014).

The most obvious and far reaching anthropogenic modification of the contemporary environment is that of climate change. Climate change is predicted to raise the average temperature of the Earth's surface between 2-5^{o}C in the next 85 years (e.g. IPCC 2007). Many ecological systems have the ability to adapt to gradually changing environments as these changes are less disruptive (Streets and Glantz, 2000). Abrupt climate changes are however more punishing and although abrupt climate changes have occurred in the past from natural causes, it is believed that global warming may increase the likelihood, frequency and magnitude of abrupt climate changes (Delworth and Knutson, 2000; Alley *et al.,* 2003; Hanson., *et al.,* 2012).

The Arctic charr (*Salvelinus alpinus*) is a species of freshwater fish with a highly variable phenotype (Klemetsen, 2013). At least some of this phenotypic variation is plastic in origin (Adams *et al.,* 2003; Garduno-paz and Adams, 2010a). The expression of discrete alternative phenotypes living in sympatry is relatively common. In nature frequently two (but up to four) sympatric ecomorphs have been described, differing in some or all of the following; head allometry, body shape, meristic counts, size, colour and life history (Jonsson and Hindar, 1982; Jonsson *et al.,* 1988; Snorrason *et al.,* 1994; Adams *et al.,* 1998; Fraser *et al.,* 2007; Corrigan *et al.,* 2011). Alternative phenotypes observed in Arctic charr are often functional adaptations to foraging on either littoral benthic macro-invertebrates or pelagic living plankton (but occasionally other) food resources (Bjøru and Sandlund, 1995; Adams, et al 1998; Skúlason *et al.,* 1989; Chapter 5), parallelisms of which can be seen across different species of fish (Schluter, 1993; Robinson and Wilson, 1996; Svanbäck and Eklöv, 2004; Siwertsson *et al.,* 2010).

A temperature change of 4^{o}C has been observed in sequential years during the 20th century (Delworth and Knutson, 2000; Alley et al., 2003). In this study we compare multiple effect of an elevated water temperature of 4^{o}C on the plastic expression of

morphological phenotypes in Arctic charr. Specifically, we investigate what these differences are, compare them to morphological phenotypes of ecological importance to foraging in Arctic charr and compare the amount of phenotypic variation between groups.

4.3. MATERIALS AND METHODS

4.3.1. FISH COLLECTION AND REARING

Nine full sibling crosses were produced from adult Arctic charr belonging to a morphologically uni-modal, plankton feeding population that inhabit Loch Clair, Wester Ross, Scotland. Fish were caught during spawning time from the in-flowing River Coulin (where they spawn) (57°32.648'N, 5°19.125'W). Fertilised eggs were water hardened and transferred to incubation facilities at the Scottish Centre for Ecology and the Natural Environment (SCENE), Loch Lomond. Eggs were acclimatised over a two-hour period to the new water supply and placed in mesh baskets suspended in a holding tank in a constant temperature room maintaining a water temperature of 4ºC (± 0.5ºC).

4.3.2 EXPERIMENTAL PROCEDURE

To control for differences in development due to different temperatures, the number of degree-days (dd) were used as a measure of developmental rate. Degree-days are the cumulative count of the water temperature for a known period of time in days. Eggs reached the eyed stage after 212dd at which point they were raised to a temperature of 6ºC (± 0.5ºC) for a further 89dd before being separated into two temperature treatment groups (n=480 per group), an ambient and an elevated temperature treatment. Eggs were held in equal numbers in eight replicate tanks per treatment group (n=60 per tank). Water temperatures were then lowered or raised by 2ºC to 4ºC and 8ºC (± 0.5ºC) respectively. Eggs exposed to the ambient temperature (4ºC) began hatching after 367dd, the hatching period lasted 73dd (total developmental time to 100% hatch 440dd). Eggs exposed to the elevated temperature (8ºC) began hatching after 388dd, the hatching period lasted 50dd (total incubation time to 100% hatch 438dd). When hatching was complete, temperatures were raised by 3ºC; the ambient temperature treatment to 7ºC and the elevated temperature treatment to 11Cº (± 0.5ºC). Fish became partially dependant on exogenous food at 505dd for the ambient temperature treatment and 504dd for the elevated temperature treatment. When the yolk sack was fully exhausted, 689dd for the ambient temperature treatment and 686dd for the elevated temperature treatment, temperatures were raised a further 2ºC for both treatments to 9ºC and 13ºC (± 0.5ºC). Fish were fed four times a day to satiation at

three hour intervals (± 0.5 hours) using a standard commercial 3mm hatchery sinking pellet.

4.3.3. DATA COLLECTION

Adult Arctic charr used as brood stock were photographed in a lateral position on the left side before spawning. Lateral view photographs of juveniles were taken at 700dd (N=130), 1000dd (N=80) and 1400dd (N=60) using a Cannon EOS 350D digital camera, for geometric morphometric analysis. Degree days were measured after hatching was 100% complete. For each photograph, a scale reference was added to allow for size correction (removal of size associated shape change). Nine consistently identifiable landmarks on the head (Figure 4.1.) were digitised in two dimensions on each fish image using tpsDig2 (Rohlf, 2006a) and tpsUtil (Rohlf, 2006b).

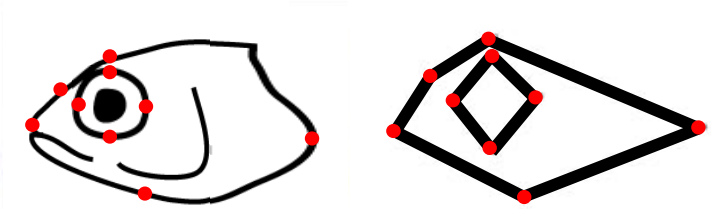


Figure 4.1. Nine landmarks were used to characterise the shape of the head; Landmark (LM) 1, the tip of the nose; LM2, the most posterior part of the upper jaw; LM3, edge of cranium directly above the eye; LM4, edge of cranium at the central point between LM3 and LM2 at a 90 degree angle; LM5-8, most upper, posterior, lower and anterior parts of eye respectively; LM9, most posterior part of the gill operculum.

4.3.4. DATA ANALYSIS

Prior to geometric morphometric analysis, landmark data were subject to a Procrustes superimposition using MorphoJ (Klingenberg, 2011) to remove variation in the data created by size, position and orientation (Rohlf and Slice, 1990; Mitteroecker and Gunz, 2009). The mean shape configuration was then computed and the variation around this mean calculated (Dryden and Mardia, 1998).

Following this, a single, pooled within-group regression of Procrustes coordinates on log centroid size was conducted using MorphoJ (Klingenberg, 2011) for samples collected at 700dd, 1000dd and 1400dd's. The residuals from this regression provide a

measure of shape free from allometric scaling (Klingenberg, 1998) associated with early ontogeny. The residuals from this regression were subsequently used for all further morphometric analysis.

A single Discriminant Function Analysis (1000 permutations) using MorphoJ (Klingenberg, 2011) was used to compare geometric morphometric data from three developmental stages (700dd, 1000dd and 1400dd) to test if the degree of shape difference between groups changed over time. Procrustes distance and Mahalanobis distance were used as pairwise measures of the magnitude of shape difference.

Using the scores generated in the Discriminant Function Analysis (described above) as a measure of shape, a generalised linear mixed effect model, fitted by maximum likelihood using the software R 3.1 for Windows (R Development Core Team, 2014) and the package lme4 was used to describe the effect of temperature, exposure time (the number of dd at each developmental stage), fish size (measured as centroid size) and replicate (tank) as a random effect, on the expression of shape. Model simplification was conducted by removing highest order least significant terms in order and then the exclusion of each term summarised (Crawley, 2012). Terms were discarded if after removal from the model they did not significantly increase the model's deviance using likelihood ratio tests (Chi).

Using the images collected at 1400dd a Canonical Variate Analysis (CVA) (1000 permutations) using MorphoJ (Klingenberg, 2011) was used to establish which group had been affected by the temperature treatment by comparing the head shape of individuals raised in ambient or elevated temperature conditions in the laboratory to their parents. Differnces in shape between adults and juveniles caused by allometirc scaling were removed using a single, pooled within-group regression of Procrustes co-ordinates on log centroid size as described previously in the methods (Klingenberg, 2011).

A variance ratio test using the software R 3.1 for Windows (R Development Core Team, 2014) on all raw Procrustes coordinates collected at 700dd, 100dd and 140dd was used to compare the amount of within-group phenotypic variation between groups.

To establish which temperature regime had stimulated expression of head shape closest to the wild type, head shape of juveniles at 1400dd was compared against the head

shape of two known ecologically divergent sympatric populations of Arctic charr, one from Loch Rannoch (Perthshire, Scotland) (Adams *et al.,* 1998) and one from Loch Dughaill (Strathcarron, Scotland) (Chapter 5) using a Canonical Variate Analysis (1000 permutations) in MorphoJ (Klingenberg, 2011). The same nine consistently identifiable landmarks used for analysing laboratory raised individuals (Figure 4.1.) were digitised in two dimensions on previously collected images (which included a scale for size correcting) of fish from Loch Rannoch and Loch Dughaill. Again, differnces in shape between adults and juveniles caused by allometirc scaling were removed using a single, pooled within-group regression of Procrustes co-ordinates on log centroid size as described previously in the methods (Klingenberg, 2011).

Residuals of all parameters were checked for deviation from normality and were found to satisfy the assumptions of parametric statistics.

4.4. RESULTS

The Discriminant Function Analysis (DFA) found head shape to differ across all sampling periods between ambient and elevated temperature fish (Table 4.1.) (Figure 4.2.). The DFA correctly assigned 73.8% of ambient temperature fish and 82.8% of elevated temperature fish (from all sampling periods combined) to the correct temperature exposure. In general, elevated temperature fish had a more rounded head and sub-terminal mouth than ambient temperature fish.

Table 4.1. Results from Discriminant Function Analysis at each sample stage.

Sample time in dd	Procrustes distance	Mahalanobis distance
700, 1000 and 1400 combined	0.028, p = <0.0001	1.43, p = <0.0001
700	0.0356, p = <0.0001	1.819, p = <0.0001
1000	0.0395, p = <0.0001	2.884, p = <0.0001
1400	0.0612, p = <0.0001	2.923, p = <0.0001

In a mixed model, using Discriminant Function Scores as a measure of shape, temperature had a highly significant effect (likelihood ratio χ^2 = 39.81, p = <0.001) (Table 4.2.). Interestingly there was a significant interaction between Exposure and Centroid Size (likelihood ratio χ^2 = 7.23, p = <0.01) (Table 4.2.). This interaction between exposure (number of degree days) and fish size (centroid size) is negative. Thus, the difference in

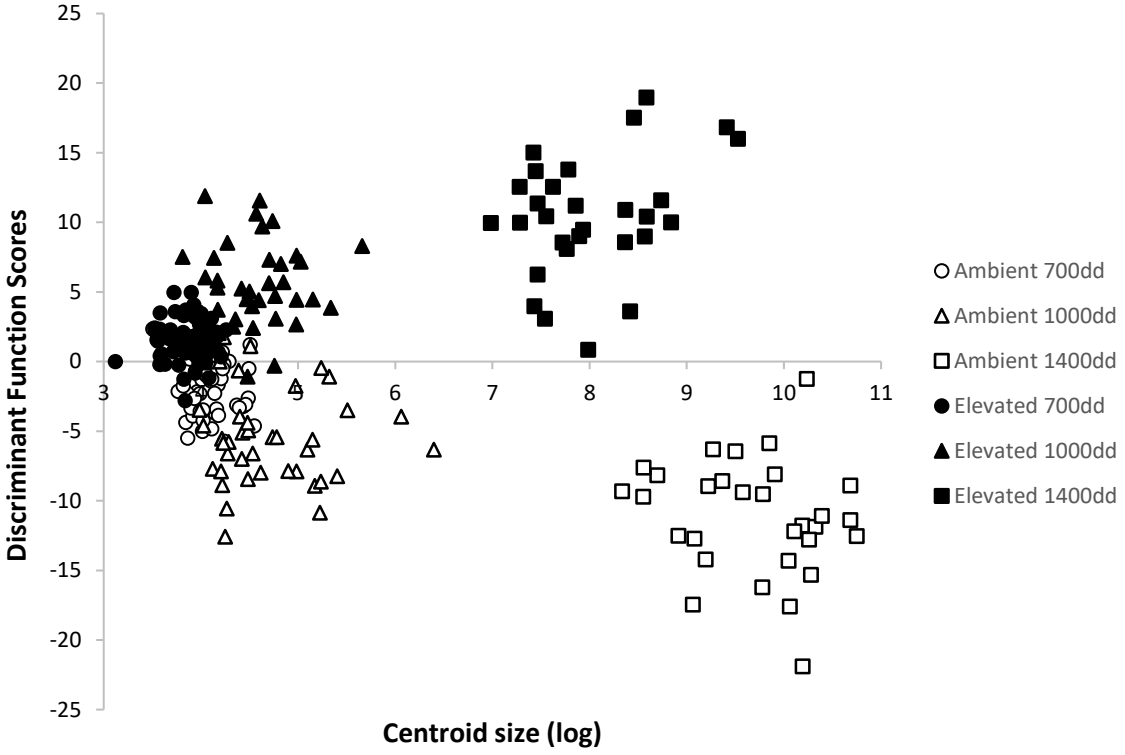


Figure 4.2. Individual Discriminant Function Scores for head shape of Arctic charr of different sizes (represented by centroid size). Three sample periods are given, 700dd are represented by circles, 1000dd by triangles and 1400dd by squares. Ambient exposed fish are denoted with open symbols; elevated temperature fish are denoted with closed symbols.

morphology between fish raised on each temperature regime was greater in larger fish, but the rate of divergence had decreased as fish got larger. This indicates that the rate of divergence caused by different temperature regimes is greater during early ontogenetic stages.

The variance ratio test found the phenotypic variation within groups to be significantly higher in ambient temperature fish compared with elevated temperature fish (F = <0.0001, df = 269, p = <0.0001).

Canonical Variate Analysis (CVA) found that the head shape of the ambient temperature fish was not significantly different to that of their parents (Figure 4.3.) (Procrustes distance = 0.0137, p = 0.3844; Mahalanobis distance = 0.8898, p = 0.8574), however elevated temperature fish were significantly different to their parents (Procrustes distance = 0.0439, p = <0.0001; Mahalanobis distance = 2.6838, p =<0.0001) showing that the elevated temperature had altered the expression of phenotype.

Table 4.2. Parameter estimates from the minimum adequate generalized linear mixed model describing the effect of temperature, exposure and centroid size on shape DF scores).

	Estimate	SE	X^2	*P*
Intercept (Ambient)	-6.84	1.91	-	-
Exposure time	0.002	0.002	-	-
Elevated temperature	2.36	0.21	39.81	<0.0001
Centroid size	1.63	0.48	-	-
Exposure : Centroid Size	-0.0012	0.0005	7.23	<0.01

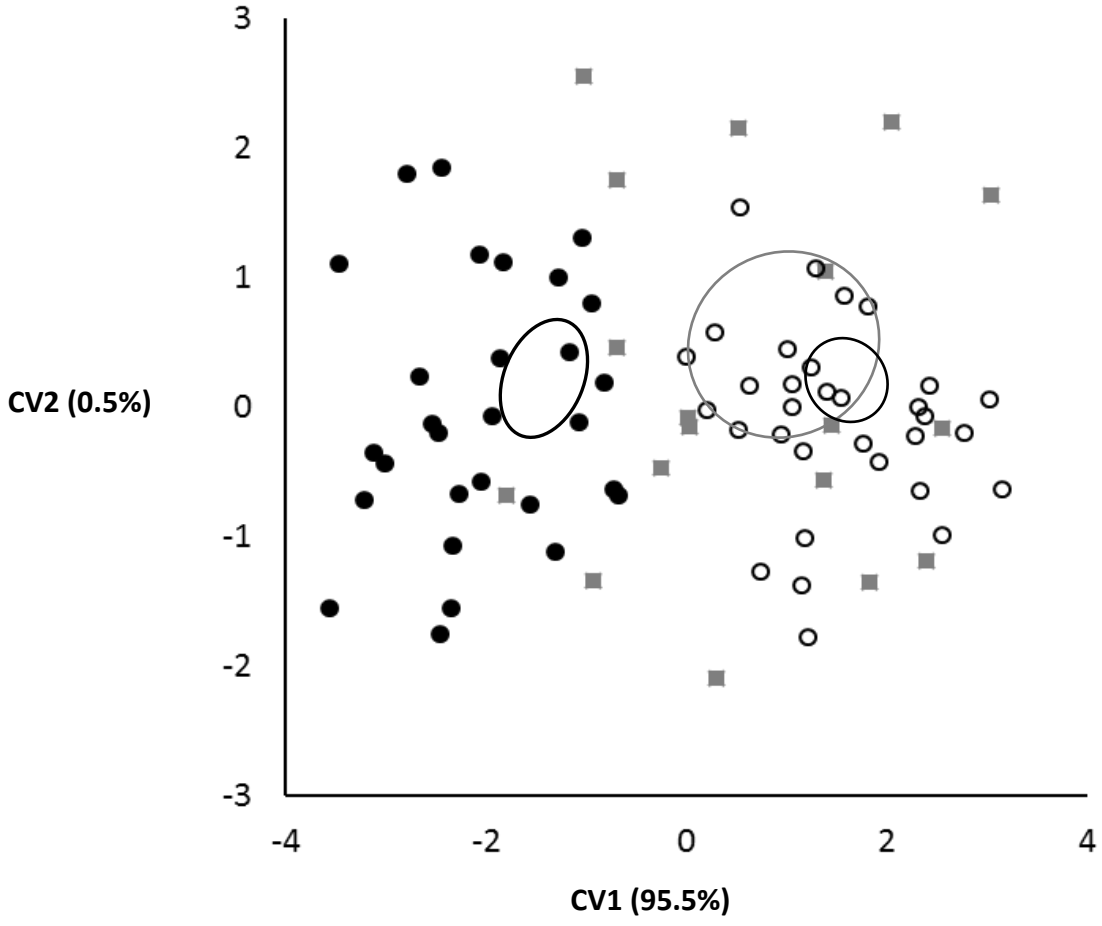


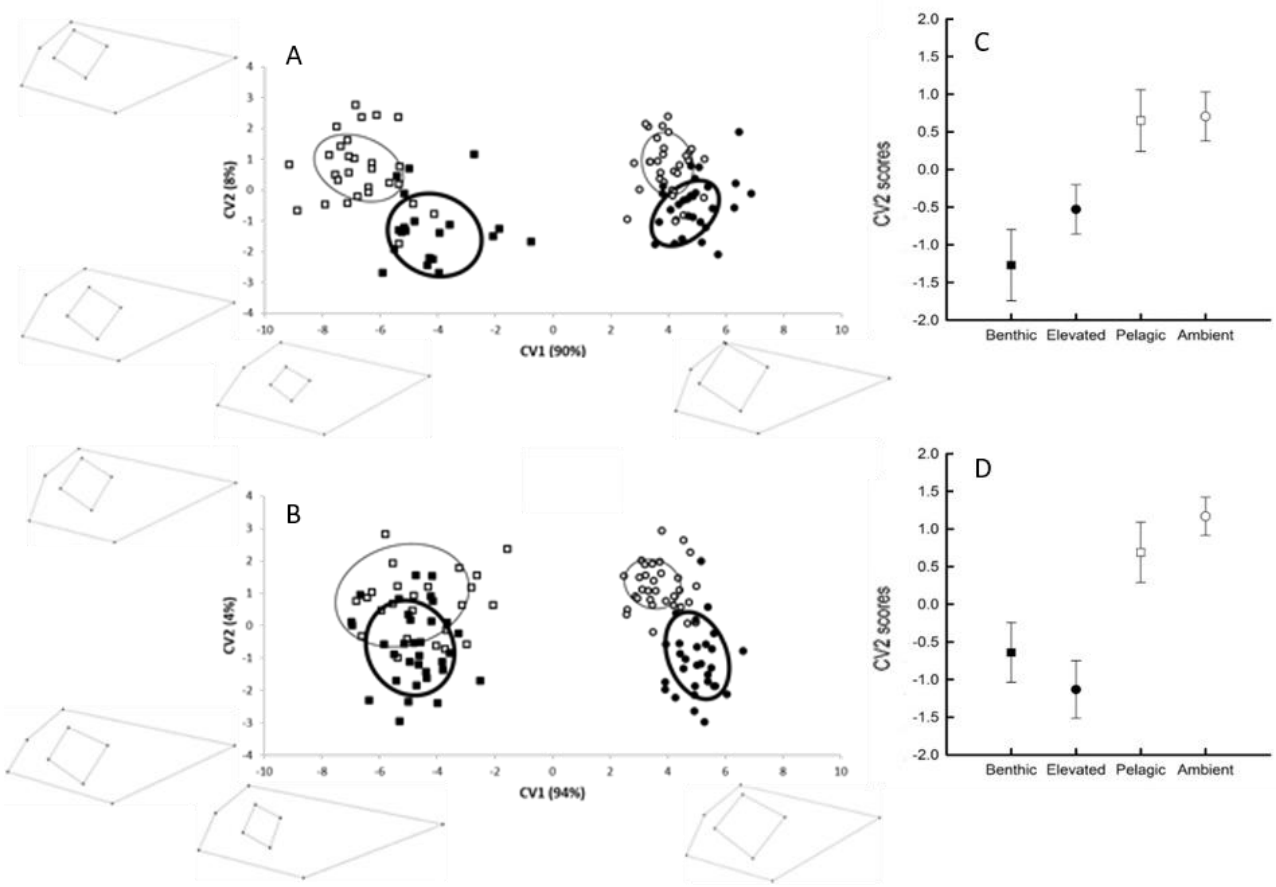
Figure 4.3. Canonical Variate Analysis of parent fish (grey squares), ambient exposed fish sampled at 1400dd (open circles) and warm exposed fish sampled at 1400dd (closed circles) with 95% confidence elipses. Percentage along the axis denotes the amount of variation explained by each canonical variate.

Using a CVA to compare experimental (Coulin) charr from both temperature groups to benthic and pelagic foraging specialists from Loch Rannoch and Loch Dughaill, in both cases we found ambient temperature fish expressed a phenotype closer to the pelagic specialists and elevated temperature fish a phenotype closer to benthic foraging specialists (Figure 4.4. and Table 4.3.). CV1 mostly captures shape differences between fish of different origin, experimental charr and Loch Rannoch charr (Figure 4.4. A) and experimental and Loch Dughaill charr (Figure 4.4. B). CV2 however captures within group shape differences between fish raised at either ambient or elevated temperatures and differences between benthic and pelagic fish from Loch Rannoch (Figure 4.4. A) and benthic and pelagic fish Loch Dughaill (Figure 4.4. B). This is shown clearly in Figure 4.4C. and 4.4D.

Table 4.3. Results from the CVA comparing ambient and elevated temperature raised fish to wild benthic and pelagic ectomorphs form Loch Rannoch and Loch Dughaill.

Comparison	Location	Mahalanobis distance	*p* value	Procrustes distance	*p* value
Pelagic v Ambient	Rannoch	10.95	<0.0001	0.157	<0.0001
Pelagic v Elevated	Rannoch	11.68	<0.0001	0.158	<0.0001
Benthic v Ambient	Rannoch	9.78	<0.0001	0.111	<0.0001
Benthic v Elevated	Rannoch	8.44	<0.0001	0.095	<0.0001
Pelagic v Ambient	Dughaill	8.36	<0.0001	0.121	<0.0001
Pelagic v Elevated	Dughaill	9.74	<0.0001	0.149	<0.0001
Benthic v Ambient	Dughaill	9.86	<0.0001	0.152	<0.0001
Benthic v Elevated	Dughaill	8.69	<0.0001	0.129	<0.0001

Figure 4.4. Canonical Variate Analysis of 1400dd ambient and elevated temperature Arctic charr and sympatric polymorphic populations of Arctic charr; A) Loch Rannoch, B) Loch Dughaill. Elipses represent 95% confidence limits for means. Polymorphic charr populations are represented by squares and experimental Arctic charr by circles. Open symbols denote wild plankton feeding Arctic charr and Arctic charr raised at an ambient temperature. Closed symbols denote benthic feeding Arctic charr and Arctic charr raised at an elevated temperature. Percentage along the axis denotes the amount of variation for each canonical variate. Wire frames on CV1 are scaled at -8 and +8, wire frames for CV2 are scaled at -4 and +4. C) and D) show mean CV2 scores with standard with 95% confidence intervals.



4.5. DISCUSSION

Our results demonstrate that exposure to elevated water temperature, in line with predications made by climate change models (Streets and Glantz, 2000; Alley et al., 2002), significantly affected head development in Arctic charr. This temperature effect on shape was cumulative with the fish on different treatments becoming more different over time (Figure 4.2.). However, the rate of change of the effect of temperature on development decreased with time suggesting that early life stages are more sensitive to this effect.

Offspring raised at an elevated temperature expressed a phenotype different to siblings raised at an ambient temperature, and their parents. When compared to polymorphic populations that show distinct and stable foraging specialisms fish raised in an elevated temperature expressed a phenotype more suited to benthic foraging (Adams *et al.*, 1998; Chapter 5). The head of elevated temperature fish was shorter, more robust, with a rounder snout. Features such as larger more robust heads, a blunt snout and a large gape are frequently seen in littoral macro-benthos feeding specialists. In contrast ambient temperature fish expressed a phenotype like that of their parents and more suited to pelagic foraging. Their heads were more delicate, elongated and pointed, similar to naturally occurring plankton feeding specialists which also express a more delicate head shape, finer jaw structure and smaller mouths, (Adams *et al.*, 1998; Skúlason *et al.*, 1999; Jonsson and Jonsson, 2001; Klemetsen *et al.*, 2002; Knudsen *et al.*, 2006; Klemetsen, 2013).

The expression of differing phenotypes of functional importance such as those associated with foraging, as seen in this study, have consequences for foraging success and ultimately fitness (Skúlason and Noakes, 1989; Malmquist, 1992; Snorrason *et al.*, 1994; Adams and Huntingford, 2002b). The phenotype responses to elevated temperature are in line with phenotypic differences seen in different foraging specialists in the wild (Skúlason and Noakes, 1989; Malmquist *et al.*, 1992; Adams and Huntingford, 2002b). This study indicates that a temperature change of the order of that predicted by global warming models will drive the expression of phenotypes with a higher efficiency for macro-benthos feeding in Arctic charr (Figure 4.4.) and a reduced efficiency for zooplankton feeding. Differences in the morphology associated with feeding can also modulate the type of food an organism feeds upon (Parsons and Albertson, 2009). Even subtle differences between individuals in morphology have been shown to promote differences in foraging specialisms (Garduno-Paz and Adams, 2010c) that may ultimately lead to foraging segregation (Skúlason *et al.*, 1999).

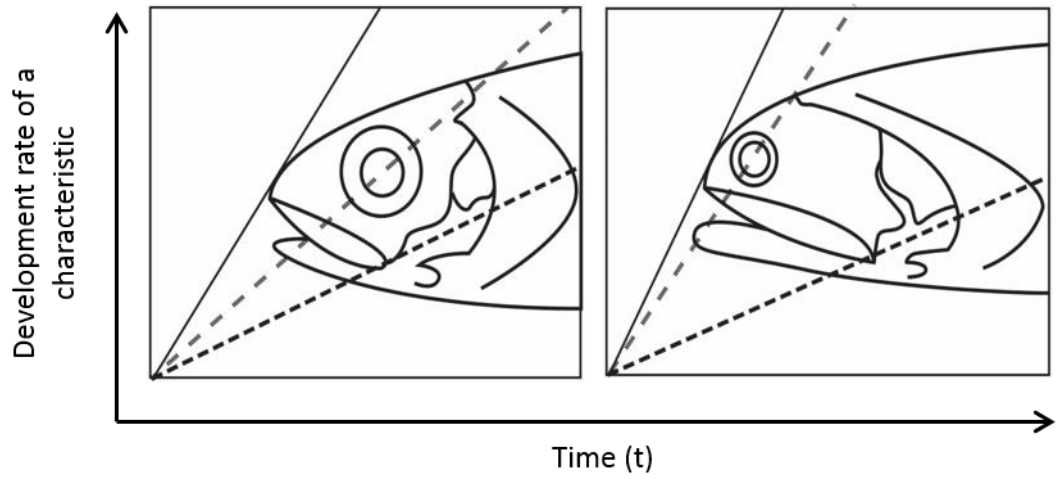
In fish, phenotypic differences can manifest as variation in meristic counts of scales, gill rakers, fin rays and vertebrae due to differences in developmental rate (Hubs, 1922; Barlow, 1961; Beacham, 1989). It has been speculated that fish exposed to increased temperatures (fast development) have lower meristic counts and would thus develop a thicker set body more characteristic of a benthic fish (McPhee *et al.*, 2012). Our results agree with this theory and show that elevated water temperature in line with climate change predictions caused offspring from a pelagic feeding population to develop head shape more suited to benthic feeding and are thus more likely to adopt a macro-benthic foraging strategy or lose efficiency in foraging on pelagic prey items.

In addition to the expression of morphological differences resulting from elevated temperature exposure there was also a significant reduction in the variation in the phenotypes expressed in individuals raised in the elevated temperature treatment compared to ambient temperature. This decrease in phenotypic variation can reduce the phenotypic range minimising the scope on which selection can act. It can also reduce the future evolutionary scope of a population in the face of changing selection regimes (Meyer, 1989; Griffiths, 1994; Wimberger, 1994; Biro and Ridgeway, 1995; Robinson *et al.*, 1996; Smith and Skúlason, 1996). One consequence of this effect is an increased probability of extinction (Chevin *et al.*, 2010).

The mechanism through which environmental temperature may drive the plastic expression of phenotypes was not directly tested in these experiments. However, several possibilities exist.

Phenotypic plasticity can be mediated through pathways such as heterochrony (Meyer, 1987). Differences in environmental temperature can cause heterochrony by disrupting the onset and termination of processes associated with development and the rate at which these processes occur. This process can also affect the magnitude of phenotypic variation that is expressed by reducing the potential for temperature to effect development (Ramler *et al.*, 2014). The elevated temperature treatment showed such an effect in this study. Thus temperature effects mediated through heterochrony has the potential to perturb both the rate and timing of developmental processes that have consequential effects for phenotypic expression across the whole organism (Figure 4.5.) (Klingenberg, 1998; Zelditch and Fink, 1996).

Figure 4.5. Schematic how an increase or decrease in rate for one characteristic can have an affect across the entire organism. Each line represents the rate of development for a characteristic.



The ontogenetic timing and rate of progression of chondrogenesis and ossification of bony structures in fish is known to be highly plastic (Campinho *et al.*, 2004). There is also evidence that exposure to different temperatures results in a plastic response of chondrogenesis in fish that can in turn lead to heterochrony during early development (Koumoundouros *et al.*, 2001; Sfakianakis *et al.*, 2004).

Temperature induced heterochrony may also be underpinned by modification of physiological processes. In amphibians the regulation of hormones specific to bone development has already been shown to be partly dependant on temperature (McWhinnie and Cortelyou, 1967). Differences in temperature can also affect gene expression (Dillen *et al.*, 1997; Karsenty and Wagner, 2002). It is therefore plausible that differences in temperature may affect the transcript genes responsible for calmodulin production (*CaM*) and bone morphogenetic protein 4 (*Bmp4*) which are known to be significant in the timing and rate of skeletal development of different craniofacial features in cichlid fish and finches (Parsons and Albertson, 2009).

4.6. CONCLUSIONS

In conclusion, the results of this experiment show that under climate change there are both short and potentially long term ecological and evolutionary consequences for Arctic charr. A majority of Arctic charr populations are currently planktivorous, phenotypes expressed under elevated water temperatures may result in a shift to greater use

of macro-benthos foraging resources by more populations or a loss of fitness due to reduced foraging efficiency. Shifts in the variance–covariance pattern of complex traits can influence a populations' response to selection in multiple ways and thus alter the evolutionary direction. A decrease in between-individual variation would cause a loss of phenotypic diversity for selection, reducing the potential to not only evolve, but adapt to an artificially and rapidly changing environment.

4.7. ACKNOWLEDGMENTS

I would like to thank Travis van Leeuwen, Luc Bussierre, Alex Lyle, Jennifer Dodd, Martin Hughes and Peter Cunningham (Wester Ross Fisheries Trust) for their contribution and The Coulin Estate where fish were collected. This work was supported by funding from the European Union's INTERREG IVA Programme (project 2859 'IBIS') managed by the Special EU programmes Body).

CHAPTER 5.

MORPHOLOGICAL, ECOLOGICAL AND BEHAVIOURAL DIFFERENTIATION OF SYMPATRIC PROFUNDAL AND PELAGIC ARCTIC CHARR (*SALVELINUS ALPINUS*) IN LOCH DUGHAILL SCOTLAND.

*Please note this chapter has been published in *Hydrobiologia*

5.1. ABSTRACT

Phenotypic variation in populations of fishes that inhabit postglacial lakes is often associated with trophic specialisations. A common sympatric foraging divergence seen in Arctic charr is into either plankton or littoral-zoobenthos feeding specialisms. In this study we report a sympatric polymorphic Arctic charr population which is not centred on this divergence but instead manifests as a plankton (pelagic) – profundal zoobenthos foraging specialisms. The head shape of profundal fish was round and robust, the body thick set and pectoral fins long. In contrast, the head of pelagic fish had a pointed and slender, the body fusiform in shape and with short pectoral fins. There was no difference between profundal and pelagic fish in gill raker number. Body lipid content was significantly higher in pelagic fish as were the number or *Diphyllobothrium* cysts. The carbon isotope ratio was more heavily depleted in profundal fish. There was no dietary overlap in the prey items recovered from stomach contents of profundal and pelagic fish. We suggest the proximate driver behind the sympatric divergence was the successful exploitation of the profundal zone. The consequences of this have led to the development of adaptations in morphology and behaviour to support and maintain this divergence.

5.2. INTRODUCTION

In some taxonomic groups, intraspecific genetic and phenotypic structuring within a population is common (Skúlason and Smith, 1995; Smith and Skúlason, 1996). This is particularly true for fishes in postglacial lakes (Taylor and McPhail, 1999; Jonsson and Jonsson, 2001; Østbye *et al.,* 2006) and results in alternative phenotypes living in sympatry within a single lake (Knudsen *et al.,* 2006). This is seen in Arctic charr, *Salvelinus alpinus* (Linnaeus 1758) in which the structuring is based on the adaptation of foraging specialisms to alternative food resources (Malmquist *et al.,* 1992; Adams *et al.,* 1998; Amundsen *et al.,* 2008; Garduño-Paz *et al.,* 2010). Referred to as resource polymorphisms, they are frequently identified by the expression of different morphological phenotypes, foraging ecology and differences in diet (Smith and Skúlason, 1996).

Arctic charr exhibits phenotypic variability in head and body morphology (Skúlason *et al.,* 1989; Adams *et al.,* 1998, 2003; Jonsson and Jonsson, 2001; Adams and Huntingford, 2002a; Klemetsen *et al.,* 2003), differences in growth (Jonsson *et al.,* 1988; Adams *et al.,* 1998), reproduction (Jonsson and Hindar, 1982; Jonsson *et al.,* 1988; Klemetsen *et al.,* 2003; Corrigan *et al.,* 2011; Garduño-Paz *et al.,* 2012), habitat use (Hindar and Jonsson, 1982; Jonsson *et al.,* 1988; Klemetsen *et al.,* 2003), and behaviour (Jonsson and Jonsson, 2001; Klemetsen *et al.,* 2003). Arctic charr sometimes exhibit clearly defined, discrete and alternative phenotypes, each adopting a different foraging specialism whilst living in sympatry e.g. Lake Thingvallavatn (Iceland) (Malmquist *et al.,* 1992) whereas elsewhere the difference in phenotype may be more subtle e.g. Loch Tay (Scotland) (Adams *et al.,* 2003; Garduño-Paz *et al.,* 2010). The most commonly reported foraging divergence seen in sympatric populations of Arctic charr is that of a divergence into planktonic and littoral-zoobenthos feeding (Malmquist *et al.,* 1992; Adams *et al.,* 1998, 2003; Adams and Huntingford, 2002a; Amundsen *et al.,* 2008, Corrigan *et al.,* 2011; Garduño-Paz *et al.,* 2012).

Parallelism in body shape associated with prey specialisation and associated habitat use (Malmquist *et al.,* 1992; Adams *et al.,* 1998; Jonsson and Jonsson, 2001; Klemetsen *et al.,* 2003; Knudsen *et al.,* 2006) is almost exclusively seen as either adaptations to planktonic or benthic foraging. Functional adaptations to feeding on planktonic prey in the pelagic zone results in a more streamlined body with a narrow, more pointed and delicate head and mouth structure, often with dorso-ventral countershading. Benthic foraging adaptations in the littoral or sub-littoral zones, often in deeper waters, results in thicker set

bodies with more robust deeper heads that aid consumption of larger macro invertebrates (Skúlason *et al.*, 1989; Malmquist *et al.*, 1992; Adams *et al.*, 1998, 2003; Jonsson and Jonsson, 2001; Klemetsen *et al.*, 2003; Knudsen *et al.*, 2006; Garduño-Paz *et al.*, 2012).

When sympatric populations occur, they provide models which help to elucidate the mechanisms that lie behind ecologically driven divergence and speciation (West-Eberhard, 1989; Bolnick and Fitzpatrick, 2007). Understanding the interaction between genetic, morphological, ecological, physiological, and behavioural drivers that can be observed in sympatric polymorphisms increases our ability to understand some of the causes and effects of divergence and thus the speciation process when it occurs in sympatry. In this study we report a previously undescribed and rare sympatric polymorphism in an Arctic charr population which is not centred on the usual divergence into planktivorous and littoral-zoobenthos foraging specialisms. We examine variation in the foraging ecology of individuals, relate this to head and body morphology and quantify the effect of different foraging specialisms (supported by stable isotope and stomach content analysis) on body lipid content, habitat use and parasite loadings.

5.3. Materials And Methods

Arctic charr were collected from Loch Dughaill, Strathcarron, Highland, Scotland (Lat 57.47^{o}N – Long 05.34^{o}W). Loch Dughaill has a surface area of 1.15 km^{2}, a mean depth of 20m and max depth of 62m and a total volume of 10^{-6}m^{3}. The littoral zone constitutes 27.3% of the surface area. It is situated at 24m above sea level, receives no ice cover during the winter months and is oligotrophic. In addition to Arctic charr, the fish community includes brown trout, *Salmo trutta* (Linnaeus 1758), Atlantic salmon, *Salmo salar* (Linnaeus 1758), European eel, *Anguilla anguilla* (Linnaeus 1758), flounder, *Platichthys flesus* (Linnaeus 1758), three-spine stickleback, *Gasterosteus aculeatus* (Linnaeus 1758), and European minnow, *Phoxinus phoxinus* (Linnaeus 1758). Arctic charr were sampled using Nordic multipanel gill nets, consisting of 12 panels each measuring 2.5m long and ranging from 5 - 55mm knot-to-knot mesh. These nets select impartially across size classes in the size range of 45 - 495mm fork length in salmonids (Jensen and Hesthagen, 1996). Benthic nets measuring 30m x 1.5m (depth) were set overnight on the bed of the lake at depths ranging from 5 - 60m. Pelagic nets measuring 30m x 6m (depth) were set overnight at the water surface over water depths ranging from 18 - 55m. For benthic set nets, the depth of each end of the net was measured by a hand-held sonar. The

capture depth of each fish was estimated by interpolation of the depth of each net panel in which it was caught.

A total of 57 fish were sampled in October 2013 and a further 42 in June 2014. During the June sampling period 30 of the 42 fish were released alive (as part of an acoustic telemetry study) with only non-lethal data collected on their morphology and ecology. For information on sample period and size for each ecological variable tested please refer to Table 5.1. All 99 fish were photographed, measured (fork length ±1mm) and weighed (±1g) after which 69 of the total fish sampled were dissected.

Table 5.1. Sample sizes of pelagic and profundal Arctic charr used in all statistical analyses and the relevant sampling period.

Ecological variable tested	Total number of fish included in analysis and sample period	Pelagic fish	Profundal fish
Capture depth during June	42	21	21
Capture depth during October	57	32	25
Lipid content	30 (all from June)	15	15
Body morphology	99 (42 from June and 57 from October)	53	46
Pectoral fin morphology	20 (all from October)	10	10
Diphyllobothrium cysts	69 (12 from June and 57 from October)	38	31
Stomach contents	34 (7 from June and 27 from October	19	15
Stable Isotope Analysis	69 (12 from June and 57 from October)	38	31
Gill raker number	40 (all from October)	20	20

Whole body tissue lipid content was measured on 30 live individuals using a Distell FM 692 fat meter. This meter is pre-calibrated (factory calibration) to the fat - water relationship specific to Arctic charr. The Distell fat meter has a microstrip sensor which

can measure the water content of a sample. The fat content of fish is correlated with the water content and thus the measurement of one can determine the other if the relationship between the two is known. Only live individuals were used due to the method in which the fat content is calculated. A mean was determined from four measurements, one taken on the anterior lateral surface of the body and one on the posterior lateral surface on both sides of the fish.

Lateral view photographs of fish were taken on a scale using a Canon EOS 350D digital camera to enable geometric morphometric analysis of shape for all of the 99 fish sampled. Twenty analogous landmarks were digitised in two dimensions using the software tpsDig (Rohlf, 2006a) and tpsUtil (Rohlf, 2006b). Landmarks were carefully chosen to clearly represent both head and body shape (Figure 5.1. A). Procrustes superimposition was then used to remove unwanted variation created by size, position and orientation (Rohlf and Slice, 1989; Mitteroecker and Gunz, 2009).

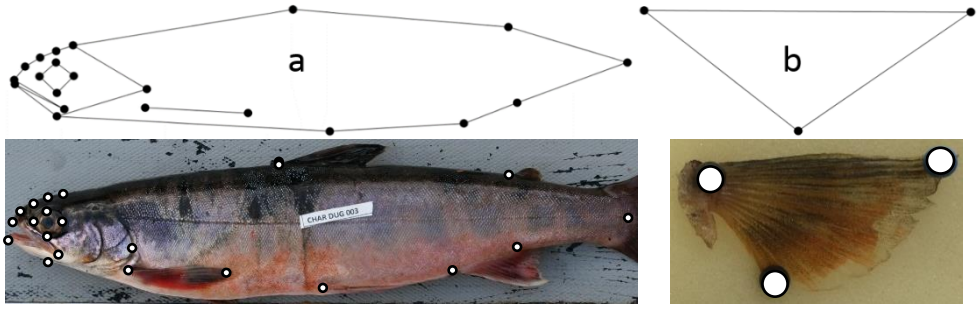


Figure. 5.1. Position of landmarks used for geometric morphometric analysis of Arctic charr for the body (a) and right pectoral fin (b).

Shape change associated with size (ontogenetic allometry) was removed (size corrected) by deriving residuals from a multivariate, pooled within-group regression of the Procrustes coordinates on the log centroid size (a robust measure of fish size) (Klingenberg, 1998).

Principal Component Analysis (PCA) of these residuals was performed to explore shape differences between groups. Principal Component 2 (PC2) was dominated by unwanted non-biological lunate distortion. This type of artefact from the image collection process is frequently reported in studies that involve fish and is caused by *rigor mortis* of

the body muscles (Siwertsson *et al.*, 2013). This shape artefact was removed by using the residuals from a regression of the raw Procrustes coordinates on PC2. This creates a new set of Procrustes coordinates which are independent of PC2 and thus free of any shape variation associated with the lunate bending effect. Although the loss of some variation from other parts of the anatomy can occur using this method, examination showed that landmark position not associated with bending in PC2 was minimal and thus removal of bending effects did not interfere with the overall results.

Discriminant function analysis (DFA) (1000 permutations) was used to test for and quantify the shape difference between fish groups (measured as Procrustes and Mahalanobis distance) Fish were assigned to one of two working class groups using data collected on their ecology; the approach used is described later in the methods. All morphometric analyses were carried out using the software MorphoJ v.1.06d (Klingenberg, 2011).

Dorsal view photographs of 20 pectoral fins from the left side were taken to compare the fin shape between fish. The pectoral fin was removed, fanned out and mounted on foam using pins and a scale reference added. Three landmarks were identified (Figure. 5.1.B), the upper most point at the base of the fin, the tip of the longest fin ray at the leading edge of the fin, and the tip of the longest ray towards the back of the fin. Damaged fins were not used. Fin shape was then analysed as described above.

The intensity of infection by *Diphyllobothrium* sp. (larvae), a parasitic cestode, was determined for 69 fish prior to dissecting stomachs by counting the number of *Diphyllobothrium* cysts attached to the stomach, gut and internal walls of the body cavity. *Diphyllobothrium* cysts are easily identifiable as opaque white nodules usually attached to the gut and swim bladder as well as other organs.

The stomachs of 69 Arctic charr were dissected of which only 34 contained prey items. These were preserved in 70% ethanol and the contents later identified to family and where possible, species level. Stomach contents were then dried at 48 ^{o}C for 48 hours in a drying oven to calculate relative and total prey dry weight.

Approximately 1cm^2 of white muscle tissue was removed by dissection from the lateral muscle below the posterior edge of the dorsal fin and above the lateral line for stable

isotope analysis for 69 fish. Tissue samples were initially frozen at -20^{o}C then later thawed and the epidermal layer removed. White muscle tissue was then dried at 48^{o}C for 96 hours and ground to a fine powder using a pestle and mortar. 0.7mg (±0.1mg) subsamples were loaded in to 5 X 5mm tin capsules, ready for stable isotope analysis. Samples were analysed for δ^{15}N and δ^{13}C, at the Natural Environment Research Council Life Sciences Mass Spectrometry Facility, East Kilbride, via continuous flow isotope ratio mass spectrometry (CF-IRMS). This system employs an Elementar Pyrocube elemental analyser interfaced with a Delta XP IRMS. The standard deviation of multiple analyses of the internal gelatine standard in each experiment was ~0.1‰ for both δ^{15}N and δ^{13}C.

The first gill arch from the left side of 40 fish was removed by dissection and the total number of gill rakers counted using a Brunel MONEX series AR Microscope illuminated with a EUROMEX LE 5210 external cold light source.

Morphological data (represented by the shape change associated with PC1 scores from the geometric morphometric analysis (hereafter, PC-morphology) was combined with parasite and stable isotope data in a Principal Component Analysis (PCA) to look for putative discrete groupings of Arctic charr from Loch Dughaill. PC-morphology scores were positive and large for fish with a long, more pointed snout and a fusiform body. This shape is one indicative of charr specialising in plankton feeding that inhabit the pelagic zone (Skúlason *et al.,* 1989; Adams *et al.,* 1998). The intermediate host of the trophically transmitted *Diphyllobothrium* parasite is a planktonic copepod (Knudsen *et al.,* 1996), thus a high parasite loading is indicative of planktivorous fish in the pelagic zone. δ^{13}C provides an indication of the ultimate carbon sources contributing to tissue formation. A high δ^{13}C (relatively low δ^{13}C content) in white muscle tissue is characteristic of fish that feed on organisms of a higher trophic position such as zooplankton (Vander Zanden and Rasmussen, 1999). Principal Component 1 of the PC-morphology weighted, parasite loading and delta δ^{13}C in the same direction (but negatively) thus fish with a highly negative score indicated fish with a strong affinity to planktonic feeding.

A second PCA was used to combine morphological (PC-morphology) and capture depth data for fish that were returned alive and thus for which there are no parasite or stable isotope data. As before, the scores for morphology PC1 (taken from the same PC-morphology used above) were positive and large for planktonic feeding fish. Capture depth (measured as negative deviations from the surface (which = 0)) was also more positive

(less negative) for fish that inhabit the surface of the water column (pelagic zone), typical of plankton feeding specialists. Both variables loaded in the same direction for this PC1 (but negatively) with individuals yielding highly negative scores indicative of fish with a plankton feeding-like morphology and inhabiting the pelagic zone.

PC scores from each of these two PCA's (the full PCA and the PCA constrained to only non-destructive data) were used to define putative ecomorph groups which were then used as a factor, with body length as a covariate, in a linear model to explore a number of between group differences. For lipid content, mass was used as a covariate. Each comparison initially included a two-way interaction between factors and covariates. Comparisons that included an interaction between factors and covariates were subject to model simplification with the removal of non-significant interactions (p = <0.05) (Crawley, 2007). Covariates were dropped in all models due to non-significance. Model diagnostics were assessed graphically by examining the residuals for heterogeneity. All analyses were conducted using R (R Development Core Team, 2011).

5.4. RESULTS

Principal Component Analysis (PCA) of body and head shape (represented by PC1-morphology from the geometric morphometric analysis), *Diphyllobothrium* infection rate and $\delta^{13}C$ stable isotope signature was thus carried out on 69 fish. PC1 explained 56% of the variation in these variables. PC1 coefficients indicate strong negative loadings for body and head shape -0.645, *Diphyllobothrium* infestation rate -0.426 and $\delta^{13}C$ stable isotope signature -0.635. On the basis of the distribution of these data, 38 individuals were given a working classification as belonging to planktonic feeding specialist group (negative PC1 score) and 31 as belonging to another feeding group (positive PC1 score) (Figure. 5.2. A).

In the PCA of non-lethal data, body and head shape (as described above) and net capture depth on 30 fish, PC1 explained 91% of the variation. PC1 coefficients indicate negative loadings for body and head shape -0.701 and capture depth -0.426. Again based on these variables, the results of the PCA found negative PC1 scores to be indicative of a planktonic feeding fish and positive PC scores indicative of fish feeding on an alternative food source. On the basis of this, an additional 15 individuals were given a working classification as planktonic feeding specialists (now referred to as pelagic fish) (negative PC1 score) and 15 as belonging to another feeding group (positive PC1 score) (Figure. 5.2. B).

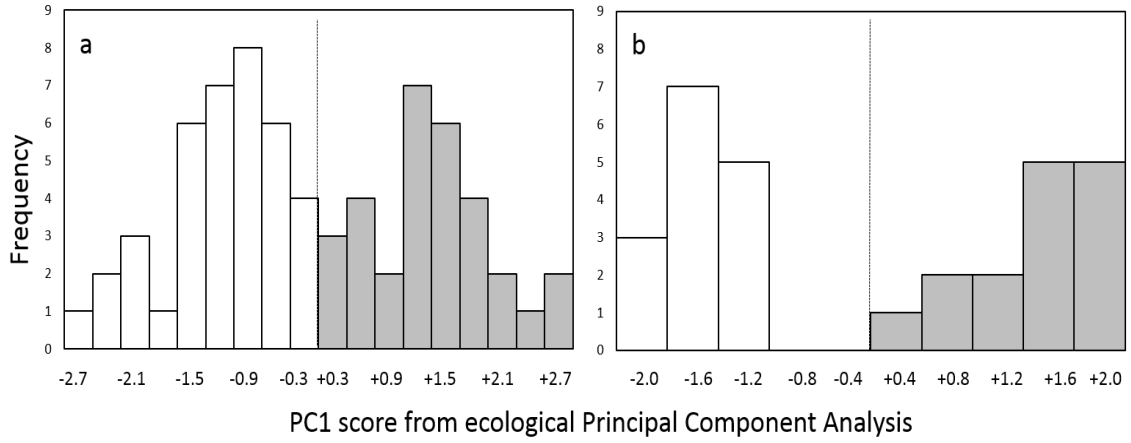


Figure 5.2. Distribution of individual Arctic charr assigned to either plankton feeding (pelagic) or non-plankton feeding (profundal) working classes using Principal Component Analysis of ecological variables. Fish were assigned to bins of 0.3 intervals for lethal (a) and 0.4 for non-lethal (b) variables to aid visualisation. Individuals assigned to a plankton feeding working class are shown in white and non-plankton feeding are shown in grey. Grey dashed line indicates 0.

During June the mean capture depth for the pelagic fish was significantly shallower (-2 m ± 0 SE) than the other group (-45.9 m ± 1.76 SE). This group was clearly occupying the profundal zone (now referred to as profundal fish) (t 25.184 $_{1, 41}$ p = <0.0001) (Figure 5.3.). However, both pelagic fish (-5.4 m ± 0.68 SE) and profundal fish (-7.6 m ± 0.9 SE) occupied shallow water during the sampling period in October (Figure 5.3.) although capture depth was still statistically different (t 2.656 $_{1, 56}$ p = 0.0103).

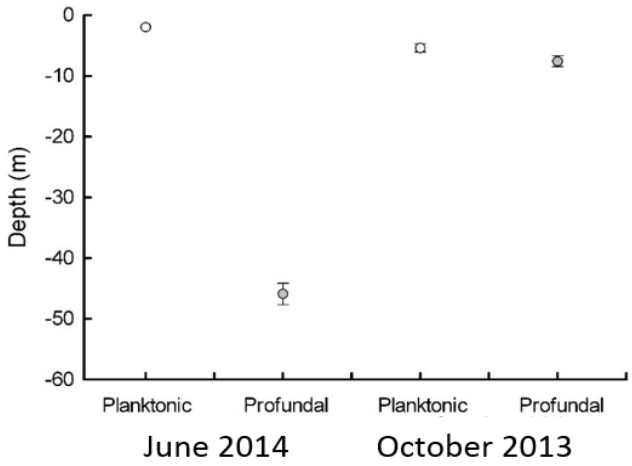


Figure 5.3. Capture depth of Arcitc charr for June 2014 (left) and October 2013 (right), white symbols represent pelagic fish and grey profundal fish, both with SE.

Profundal fish were on average slightly larger but this was not statistically significant for length (t = 0.732 $_{1, 98}$ p = 0.491) or weight (t = 0.691 $_{1, 98}$ p = 0.557). Profundal fish length ranged from 157-277 mm (213.45 ± 4.02 SE) and 44-247 grams (114.76 ± 5.85 SE) in weight, pelagic fish length ranged from 125-287 mm (203.06 ± 5.22 SE) and 19-251 grams (101.7 ± 7.01 SE) in weight. There was also no statistical difference in the relationship between length and weight for profundal fish compared with pelagic fish (t = 0.136 $_{2, 97}$ p = <0.892).

Whole body lipid content as a percentage of body mass (fat content) of pelagic fish (6.10% ± 0.43 SE) was significantly higher than that of the profundal fish (3.03% ± 0.12 SE) (t = 6.855 $_{1, 29}$ p = <0.0001).

Average morphology was highly significantly different in a Discriminant Function analysis between profundal fish (N=46) and pelagic fish (N=53) (Procrustes distance 0.0296, p = <0.0001, Mahalanobis distance 3.6411, p = <0.0001) (Figure 5.4.). Landmarks that showed the most variation between groups was associated with pectoral fin length, which was longer and body depth, which was deeper, in profundal fish. Pelagic fish were more fusiform and their head were more delicate and snouts more pointed. Profundal fish in contrast were more thick set in their body and their head shape more rounded and robust (Figure 5.4.). Geometric morphometric analysis of pectoral fin shape of 10 individuals from each group also showed significant differences between them (Procrustes distance 0.0981, p = <0.0001, Mahalanobis distance 2.2123, p = <0.0010) with fins of profundal fish being wider relative to fin length than the pelagic fish.

Mean *Diphyllobothrium* cyst count was significantly lower in profundal fish (0.22 ± 0.1SE) than the pelagic fish (43.84 ± 5.93 SE) (t = -5.073 $_{1, 68}$ p = <0.0001).

Prey items found in the stomachs of pelagic fish comprised only of *Leptidora kindtii,* a pelagic cladoceran. Stomachs of profundal fish comprised of *Pisidium* sp. and *Chironomid* sp., both of which are known to be deep water benthic organisms. There was no overlap in stomach contents between the two groups (Figure 5.5.).

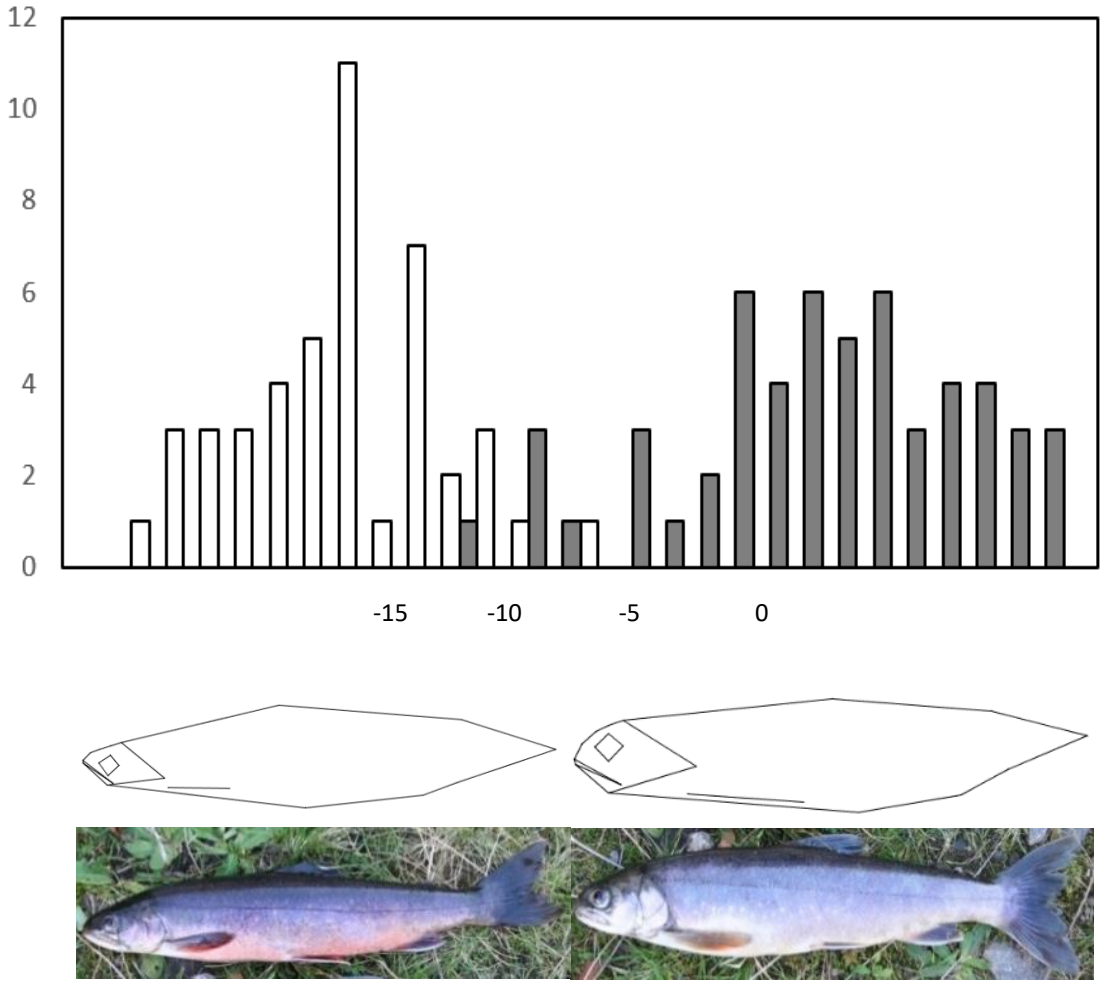


Figure 5.4. Distribution of pelagic (white) and profundal (dark grey) Arctic charr from the discriminant function analysis on body shape. Wireframes represent the shape at the outer most point of each distribution (both profundal and pelagic scaled at -15 and +15 respectively). Below are images of pelagic (left) and profundal (right) Arctic charr from Loch Dughaill.

Stable isotope analysis of white muscle showed profundal fish to have a significantly lower $\delta^{13}C$ value ($\delta^{13}C$ -29.38 ± 0.11 SE) than pelagic fish ($\delta^{15}C$ -28.76 ± 0.09 SE) (t = -6.388 $_{1, 68}$ p = <0.0001). No difference was found between the $\delta^{15}N$ values of profundal fish ($\delta^{15}N$ 7.49 ± 0.07 SE) and pelagic fish ($\delta^{15}N$ 7.47 ± 0.11 SE) (t = 0.882 $_{1, 68}$ p = 0.381).

The number of gill rakers of profundal fish (17.9 ± 0.28 SE) and pelagic fish (18.2 ± 0.6 SE) was not significantly different (t -0.455 $_{1, 39}$ p = 0.652).

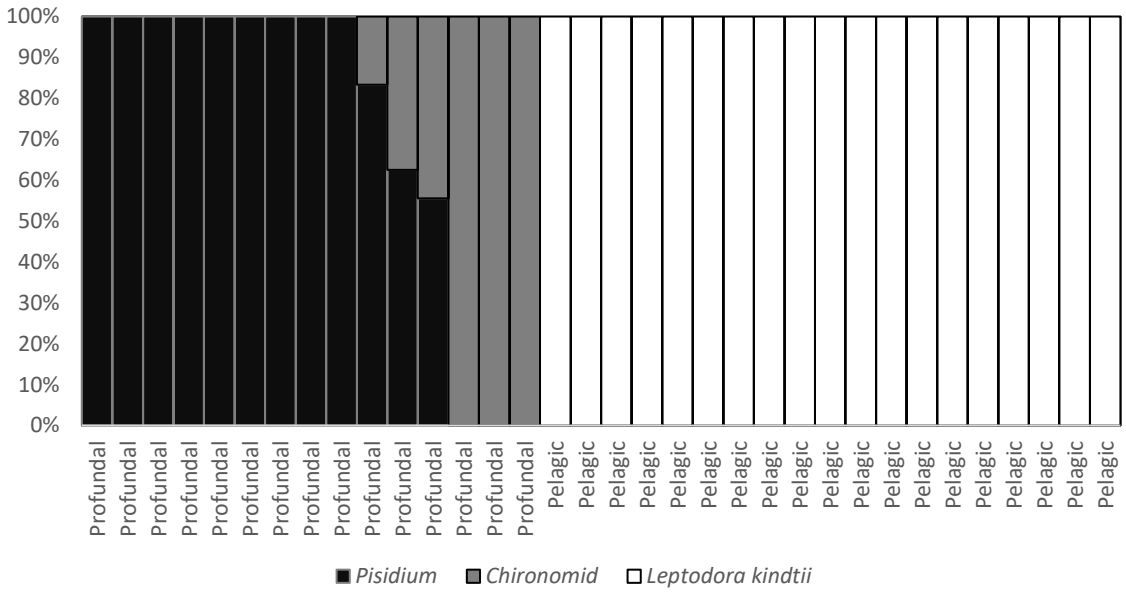


Figure 5.5. Stomach contents analysis of profundal and pelagic Arctic charr (indicated at the bottom of each bar). Benthic invertebrates are represented in dark and light grey shading, pelagic invertebrates in white.

5.5. DISCUSSION

This is the first description of sympatric profundal and pelagic habitat Arctic charr specialists in Scotland. This differential habitat use in sympatry has been documented relatively infrequently (we can find only 13 records previously) in the accessible literature, of which only eight populations still persist (Table 5.2.). The comparisons of morphology, ecology and behaviour between profundal and pelagic fish reported here support the hypothesis that the two populations of Arctic charr in Loch Dughaill have become isolated through the utilisation of two contrasting trophic niches.

Differences in morphology, size and colouration (Hesthagen *et al.,* 1995; Alekseyev and Pichugin, 1998; Knudsen *et al.,* 2006; Soreide *et al.,* 2006) and often temporal and spatial isolation in spawning behaviour (Klemetsen, 2010) are known to maintain genetic isolation in sympatric populations. However, direct interactions between morphs are less likely compared to other littoral-zoobenthos – pelagic sympatric populations as their habitats are more separated.

Differences in morphology can solely arise through the effect of plasticity, however, the differences seen in Loch Dughaill would appear too extreme (Figure 5.4.) to

Table 5.2. Known sympatric polymorphic Arctic charr systems of which one morph is profundal with additional information on lake surface area and current population status.

Country	Lake	Surface area	Recorded by	No. of morphs	Status
Austria	Attersee	46 km^2	Brenner, 1980	3	Only profundal
Canada	Gander	113 km^2	O'Connell and Dempson, 2002	2	Both persist
Germany	Constance	536 km^2	Dorfel, 1974	2	Only pelagic
Norway	Fjellfrosvatn	6.5 km^2	Knudsen *et al.,* 2006	2	Both persist
Norway	Selura	5.7 km^2	Hindar *et al.,* 1986	2	Both persist
Norway	Sirdalsvatn	19 km^2	Hesthagen *et al.,* 1995	2	Both persist
Norway	Skogsfjordvatn	13 km^2	Skoglund *et al.,* 2015	3	All persist
Norway	Tinnsjoen	51 km^2	Soreide *et al.,* 2006	2	Both persist
Norway	Vangsvatnet	7.7 km^2	Hindar and Jonsson, 1982	2	Both persist
Russia	Davatchan	16 km^2	Alekseyev and Pichugin, 1998	2	Both persist
Russia	Bol'shoe Leprindo[a]	66 km^2	Alekseyev, S. S. pers. comm.[b]	2	Both persist
Russia	Maloe Leprindo[a]	6.5 km^2	Alekseyev, S. S. pers. comm.[b]	2	Both persist
Scotland	Dughaill	1.2 km^2	This paper	2	Both persist
Switzerland	Neuchatel	218 km^2	Quartier, 1951	2	Only pelagic

be explained by plasticity alone and thus we speculate in the absence of any specific data, that at least some of the morphological characteristics are genetic in origin. The profundal and pelagic Arctic charr in Loch Dughaill show many of the parallelisms shared with other

polymorphic lakes systems that support pelagic and littoral-benthic foraging specialists of Arctic charr (Alekseyev and Pichugin, 1998; Adams *et al.,* 1998, 2003; Klemetsen *et al.*, 2002) as well as brown trout (Ferguson and Mason, 1981), whitefish (Amundsen *et al.*, 2004; Harrod *et al.,* 2010; Siwertsson *et al.,* 2013) and sticklebacks (McPhail, 1984). The profundal fish had much shorter, round and robust heads and large sub-terminal mouths (Figure 5.4.) which are suited to foraging from the substrate (Fugi *et al.*, 2000), a feeding behaviour characteristic of feeding on *Pisidium*, the main component of their diet (Figure 5.5.). The bodies of profundal fish were much deeper and cryptic in colour (Figure 5.4.), often seen in fish that inhabit deeper water (Jonsson and Jonsson, 2001; Klemetsen, 2010). Pelagic fish had more pointed, delicate heads in comparison (Figure 5.4.), again which is suited to catching smaller pelagic prey items (Adams and Huntingford, 2002b) such as *Leptidora kindtii*, the only prey item found in the stomachs of pelagic fish (Figure 5.5.).

Pelagic fish bodies were more streamlined in appearance (Figure 5.4.) (and it was noted during dissection that pelagic fish had noticeably thicker, tougher more muscular body walls than that of profundal fish which were in contrast extremely thin and required little force to make an incision) supporting the need for increased swimming activity associated with foraging in open water. The ventral side of pelagic fish had pale to dark red pigmentation in contrast to the profundal fish which had pale skin with some grey shading. Pectoral fins were much larger in profundal fish compared to the pelagic fish. Large fan like pectoral fins are characteristics of many benthic feeding fish that have to manoeuvre and orientate with accuracy in order to find, capture and manipulate prey. The narrower pectoral fins of the pelagic fish are more suitable in structure for greater swimming efficiency (Walker and Westneat, 2002). These are repeatedly reported features of benthic and pelagic feeding specialists in Arctic charr (Klemetsen, 2010).

The depth at which individuals were captured can be used to make inferences about habitat use. There was no overlap in the depth use for either morphs during summer (June). Only benthic set nets (set between 35 - 60m) caught profundal fish and pelagic nets set at the surface (0 - 6m) caught only pelagic fish, indicating very strong spatial depth segregation (Figure 5.3.). The overlap in depth use in October is probably a change in behaviour and habitat use associated with spawning. These data presented here indicate that at spawning time, spatial depth segregation between these forms is eroded and its probable there is a temporal overlap in spawning of both Arctic charr ecomorphs in Loch Dughaill. Locational data from the nets that caught fertile male and female fish of both

morphs would suggest that spawning in both morphs is likely to takes place in waters approximately 15 – 20m deep towards the north of the lake and at a similar time as both fecund male and female fish of both morphs were caught in the same nets. However, this is purely speculative. If genetic isolation between the morphs persists it would most likely be maintained by two factors. Either spatially, with each morph spawning in a different location, or, through positive assortative mating, with each morph showing a preference to spawn with conspecifics. Genetic data will confirm if the morphs are genetically isolated and positional information from an ongoing telemetry study will give more insight as to what degree their spawning habits overlaps both temporally, as well as spatially. This will allow a more precise explanation of the possible processes that are maintaining two sympatric morphs/populations in such a small lake.

Stomach content analysis suggests that profundal and pelagic fish have stable and precise foraging niches. There was no change or overlap in the prey items being consumed by profundal and pelagic fish during either sampling period. The diet of profundal fish consisted of items that were exclusively deep water benthic in their ecology by consuming predominantly *Pisidium* which contributed 95% of benthic prey items found in stomachs and larval chironomids the other 5%. Pelagic fish consumed exclusively *Leptidora kindtii*. More interestingly there was no stomach contents overlap in fish sampled during October where there is evidence of a temporary overlap in habitat with profundal fish inhabiting much shallower water. It would appear that the change in behaviour during the October sampling that causes a shift in habitat use does not influence the trophic ecology of profundal fish supporting a strong dietary segregation that still persists during the speculated spawning period.

Stable isotope ratios ($\delta^{15}N$ and $\delta^{13}C$) of an animal allow its trophic position and carbon source to be quantified (Kelly, 2000). Due to the difficulty in sampling the zoobenthos at >50m depth it wasn't possible to collect samples of dominant prey items for stable isotope analysis. In freshwater aquatic systems, the $\delta^{13}C$ signature of planktonic food items is more depleted than that of benthic invertebrates (Harrod *et al.,* 2010). This was supported in the stable isotope analysis of $\delta^{13}C$ which was significantly more depleted in the white muscle tissue of the profundal fish. This is characteristic of animals that forage in the benthic zone (Harrod *et al.,* 2010), providing evidence of long-term and temporally stable differences in trophic ecology between the two ecomorphs. Of the 15 profundal stomachs that contained identifiable prey items, six fish had consumed chironomid larvae

and three of had fed exclusively on chironomids indicating they contribute a significant proportion of the profundal diet. Some species of chironomid (larvae) harbour methanotrophic bacteria in areas of O_2 depletion. Since biogenic CH4 has exceptionally low $\delta^{13}C$ this can result in very low $\delta^{13}C$ for the chironomids (-20 to -70‰) and anything that consumes them (Jones *et al.*, 2008). Nitrogen stable isotope ratios of a consumer may become enriched by 3-4‰ (Vander Zanden and Rasmussen, 1999; Kelly, 2000) of their prey and thus are a good indicator of the trophic level at which at animal feeds. Profundal organisms tend to have enriched $\delta^{15}N$ as the profundal environment is dominated by detritus derived from species higher in the food chain (Vander Zanden and Rasmussen, 1999). However, stable isotope analysis did not find differences in $\delta^{15}N$ indicating that although the two morphs in Loch Dughaill feed on different prey items, the similar $\delta^{15}N$ signatures of the prey are maintained through different routes.

The first intermediate host of the trophically transmitted *Diphyllobothrium* parasite is a planktonic copepod (Knudsen *et al.*, 1996), thus a high parasite loading is indicative of fish that feed on plankton in the pelagic zone. In profundal fish the mean number of *Diphyllobothrium* cysts was significantly lower than in pelagic fish. *Diphyllobothrium* cysts were only present in six of the 31 profundal fish sampled (19%) compared 37 of the 38 pelagic fish (97%). This adds support to the stomach contents and stable isotope data that the specific niches both morphs exploit are stable over space and time and the different diets are not ontogenetic shifts. The only parasite data recorded was on *Diphyllobothrium* cysts as they can be can be easily identified in the body cavity. These cysts persist long after the prey that has resulted in the infection has been digested. Therefore, their presence/absence can be used to make inference about prey choices of individuals with empty stomachs making it a good identifier of long term niche exploitation. Due to the high specificity of some parasites with respect to their life cycle, information on parasite diversity and abundance can also provide information on niche width (Knudsen *et al.*, 1996). In some sympatric polymorphic populations, it has been suggested that parasitism may help maintain trophic segregation as the level of infection positively correlated with the degree of genetic segregation (Karvonen *et al.*, 2013).

The significantly higher lipid levels in pelagic fish suggest that the rate of accumulation of surplus energy is higher in this morph. Although benthic food items have been shown to contribute significantly to food webs in lakes (Jones *et al.*, 2008) differences in lipid levels could be reflecting a relatively more productive feeding resource

at the time of sampling due to the seasonal abundance of pelagic prey (Persson *et al.*, 1996). It is uncertain if the difference in lipid deposition rate between morphs remains stable throughout the year as lipid deposition can drop during less productive periods. Alternatively, it could be a reflection of the lipid levels in the prey items being consumed, rather than prey abundance itself (Eloranta *et al.*, 2013a).

There have been numerous accounts of differing numbers of gill rakers between benthic/profundal and pelagic ecomorphs, most notably in whitefish (Lindsey, 1981; Amundsen *et al.*, 2004; Kahilainen *et al.*, 2011) and to some lesser extent sticklebacks (Schluter, 1993) and Arctic charr (Sandlund *et al.*, 1992). Surprisingly we found no difference in the number of gill rakers between profundal and pelagic fish in Loch Dughaill. It would be reasonable to expect the profoundal specialist, described here as feeding predominantly on *Pisidium* which live buried in the deep water substrate, to have a lower gill raker count as this would benefit the feeding behaviour characteristic of fish that forage by sifting through sediment.

Greater lake surface area and depth are often seen as a driver behind sympatric divergence as it provides habitat heterogeneity (Nosil and Reimchen, 2005). The size of Loch Dughaill (1.15 km^2) is very small compared with other systems that support polymorphic populations, however, it is very deep by comparison (62m). Given this example of such an extreme difference in habitat use in what is a comparably small polymorphic system (Table 5.2.), it is surprising the level of habitat heterogeneity is great enough to support such a divergence. This shows that habitat structuring, even in small ecosystems, can promote and maintain divergence. The combination of a narrow niche and high intraspecific competition of the two forms described here means there is likely to be strong selection to evolve morphological and behavioural traits related to these foraging specialisms. It is likely that the profundal morph evolved to be an effective soft bottom feeder in sympatry with the pelagic morph by diverging from an ancestral form that is closer to the plankton feeding form described here (as the ancestral niche of Arctic charr does not include this type of soft bottom feeding (Knudsen *et al.*, 2006). Competition for available resources is an important driver behind ecological speciation (Rundle and Nosil, 2005). Thus, we speculate that high intraspecific competition in the pelagic zone of Loch Dughaill may have forced individuals to utilise an alternative niche. This alternative niche in a majority of polymorphic charr populations is the littoral-zoobenthos zone. A switch to the profundal zone is a more extreme foraging niche change

and arguably requiring a more significant divergence from an ancestral foraging form and thus less common (Klemetsen, 2010). For such a divergence to occur, a possible hypothesis could suggest either an unsuitable littoral- zoobenthos foraging zone at Loch Dughaill which is already dominated by a more aggressive conspecific, such as brown trout which are known to displace Arctic charr from shallow benthic habitats (Jansen *et al.*, 2002; Forseth *et al.*, 2003; Eloranta *et al.*, 2013b).

5.6. Conclusions

The results from the various comparisons in morphology, physiology, ecology and behaviour that have been presented and supported by parallelisms in the literature suggest the proximate driver behind the sympatric divergence was the successful exploitation of the benthic profundal zone, a previously untapped niche. It is essential in ecological speciation that a population both expand its current range and exploit a new stable resource successfully. This relies on a combination of morphological, physiological and behavioural adaptations that can arise through natural selection, disruptive selection, plasticity or a combination of selection and plasticity. Selection pressure would include changes in resource availability and the ability to forage at low temperatures. The pelagic resource (*Leptodora* sp.) is abundant during the summer but this decreases during winter, therefore it is likely that difference in foraging strategies between forms may not persist as clearly in winter as one food source declines in abundance. This transition to permanent profundal feeding >50m, in an almost lightless habitat, on food items with a hard shell and buried in the benthos would require the evolution of morphological and behavioural traits associated with this type of foraging. The consequences of this has driven functional adaptations in morphology and changes in behaviour to allow this divergence to become stable over time. This supports the theory of sympatric divergence through utilisation of profundal resources.

5.7. Acknowledgments

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CHAPTER 6.

EFFECTIVENESS OF LOCAL BIODIVERSITY ACTION PLANS TO IDENTIFY LOCALLY RARE AND ENDANGERED FRESHWATER FISH IN SCOTLAND.

Please note this chapter has been published in the *Scottish Geographical Journal*

6.1. ABSTRACT

Biodiversity loss is an important environmental issue globally. Local Biodiversity Action Plans (LBAPs) provide an important conservation tool and should represent national priority species where they are present. Although much effort has gone in to developing LBAPs, their value as a conservation tool has been questioned. Here the effectiveness of the LBAP system to raise awareness of the freshwater fishes of national and international priority in Scotland is tested. Inclusion of freshwater fish in the LBAP suite was evaluated using current distribution data taken from available literature sources. Of the 25 examined LBAPs in Scotland, there were 79 LBAP entries for the 12 priority freshwater fishes found in Scotland. This contrasts with an expectation of 139 entries. Nineteen authorities failed to represent all priority freshwater fish species extant in their geographical boundary. Fourteen authorities provided cover for 50% or less for the species distributed in their area and five authorities included no rare freshwater fish species where one or more was expected. Possible underlying reasons for this mismatch include: frequently changing conservation status and taxonomic blindness of this group. It is plausible to suggest that this low rate of inclusion will have a detrimental effect on the allocation of limited conservation resources.

6.2. INTRODUCTION

Biodiversity loss is one of the most important environmental issues facing our planet (Hooper *et al.* 2012). Climate change, invasive species, habitat loss, degradation and fragmentation and growing energy demands strain the environment and its ability to support diverse ecosystems. These threats have resulted in the development of both conservation policy and programme actions aimed at halting this decline (Cullen *et al.*, 2001). One extremely important strand of biodiversity action in many countries is the provision of a mechanism through which international and national conservation obligations and strategies are implemented effectively at local level. Signatory nations to the 1992 Convention on Biological Diversity (CBD) committed themselves to conserve threatened habitats and species at a local level (Hagvar, 1998). This paper examines the effectiveness of one such mechanism - Biodiversity Action Plans - through which broad scale national conservation policy is implemented at local level.

Among with many others, the Scottish Government is committed to halting biodiversity loss by 2020 as part of the "European Union (EU) Biodiversity Strategy" (European Commission, 2012) and the 2020 "Challenge for Scotland's Biodiversity" (Scottish Natural Heritage, 2013). The mechanism through which biodiversity is protected is highly complex and achieved using a hierarchical framework of commitments, and national and international legislation agreements to meet these commitments. In Scotland these commitments, many of which are driven by EU directives, are transposed into national legislation to facilitate delivery (Table 6.1.).

Local Biodiversity Action Plans (LBAPs) are used to aid the development of conservation strategies, practical conservation management and inform planning decisions at local level. Species included in LBAPs are those with some national and/or international conservation priority, where they are present in an LBAP area. In Scotland, LBAPs aim to provide conservation action plans for species listed on the UK Post-2010 Biodiversity Framework (formerly the UK Biodiversity Action Plan) and the Scottish Biodiversity List (SBL).

The SBL lists species identified on the UK Post-2010 Biodiversity Framework (referred to hereafter as the UKBF) which are extant in Scotland; the list was published to satisfy requirements under The Nature Conservation (Scotland) Act 2004. The SBL was developed in July 2012 using information from the UKBF and additional criteria (Table

Table 6.1. The hierarchical connectivity from global initiatives to local legislation of conservation commitments and legislation.

Level	Committee	Date
Global	International Union for Conservation of Nature (IUCN)	1948-present
	European Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)	1973-present
European	UN Convention on Biological Diversity	1992-present
	Habitat and Species Directive	2002-2012
National	UK Biodiversity Action Plan (UK-BAP)	1994-2012
	UK Post 2010 Biodiversity Action Framework	2012-present
Regional	Scottish Biodiversity Strategy (2004)	2004-present
	The Nature Conservation (Scotland) Act 2004	2004-present
	Species action framework	2007-2012
Local	Scottish Local Biodiversity Action Plans (LBAP's)	1995-present

6.2.) to help public bodies carry out their biodiversity protection responsibilities by identifying local species and habitats of high conservation value (Scottish Biodiversity Forum, 2012). There are 1150 species and 65 habitats listed on the UKBF priority list. Of these, the SBL lists 453 species and 37 habitats (Biodiversity Planning Tool Kit, 2012) which are considered important in both contributing to, and sustaining, biodiversity in Scotland. Species included on both the UKBF and SBL are those identified as being of international conservation concern.

There is no legal requirement for all UKBF or SBL listed species to be included on an LBAP where they exist. However, LBAPs are an important guide to assist decision makers, such as local authorities and national parks, when discharging their statutory duty in relation to the conservation of biodiversity and priority species (Biodiversity Planning Tool Kit, 2012). Thus inclusion on an LBAP should be the first step in acknowledging a national and international priority species at a local level.

The functions of LBAPs are thus to translate national, i.e. UKBF species and habitats, into effective action at a local level; identify conservation targets for species and habitats important to the local area; stimulate effective partnerships to ensure that

Table 6.2. Additional criteria used in conjunction with the SBL to aid decision makers in developing effective LBAPs for rare species in Scotland (Scottish Biodiversity Forum, 2012).

	Criteria used in identifying species in need of conservation action
1	All UK priority species present in Scotland
2	Species for which Scotland, through the UK, has international obligations to safeguard species
3	All species defined as nationally rare at a GB or UK level, which are present in Scotland
4	Species with populations present (resident, wintering or breeding) in five or fewer ten km squares or sites in Scotland
5	Species present in Scotland for which a decline of 25% or more in abundance or range (defined as the number of sites where appropriate) has occurred in Scotland over the 25 years or other appropriate time period
6	All species that are endemic to Scotland
7	Any sub-species or race, that is widely recognised and accepted by the scientific (or other relevant community) and that is endemic to Scotland, if it also meets one of the other criteria.

programmes for biodiversity conservation are developed and maintained in the long term; raise awareness of developers and the public of the need for biodiversity conservation; ensure that these are promoted and included in local policies (and decisions); and provide a basis for monitoring and evaluating local action for biodiversity priorities (at both a local and national level). Omissions from LBAPs may result in species with conservation needs being overlooked or ignored by policy makers.

Although much time and effort has been expended developing LBAPs, their value as a conservation tool has been questioned (White *et al.*, 2000; Evans, 2004; Laycock *et al.*, 2009). Of particular concern is the risk of taxonomic bias. Freshwater fishes as a group have been overlooked and underrepresented by conservation policy and action in the past (Maitland, 1995). This paper evaluates the representation of rare freshwater fish in the LBAP suite. Specifically, this study addresses the following questions 1) do Local Biodiversity Action Plans adequately represent the rare and endangered freshwater species extant within their geographic area? 2) if not, what factors underlie any limitations and

how can local and national conservation strategies for the protection of fish be strengthened?

6.3. METHODS

There are twelve priority freshwater fish species listed on the UKBF that are also contained in the SBL (Table 6.3.) and 25 active LBAPs in 2012 across Scotland (Table 6.4.). To test the level of representation of rare freshwater fish in the LBAP suite inclusion was compared to current distribution data for each species (Table 6.5.). This was drawn from easily accessible and authoritative publications providing data (Maitland, 2004; Maitland, 2007; Davies *et al.*, 2004; Maitland and Lyle, 2005). Data that was most current at the time LBAPs were written was used to ensure fair analysis.

The National Biodiversity Network (NBN) comprises of an online database providing distribution data on a wide range of species in the UK. A pilot comparison of these data with those available in published records showed that NBN data inaccurately reflected the 'current' known species distribution in the UK for priority fish species. The NBN lists as still present species on the basis of historic references for species in areas where they are no longer extant resulting in an unfair evaluation of LBAP accuracy. This source of distribution data was therefore not used in the analysis.

The total number of LBAP entries for threatened Scottish freshwater fish that should be included in LBAPs was calculated by totalling together the number of priority fish species found in each LBAP area.

To assess how well each priority species of freshwater fish was represented, the total number on LBAP inclusions for a species was divided by the total number of LBAP areas where that species was extant

The percentage cover provided by a LBAP was calculated by dividing the number of correct inclusions for priority fish species by the LBAP, by the number of priority fish species known to be extant in the LBAP area based on their known geographical distribution.

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Table 6.3. Freshwater fish species on the Scottish Biodiversity List, their IUCN status, the frequency of occurrence of each species in the 25 LBAP areas, the number of times each species was included (incorrectly and correctly) in an LBAP and the proportionate match (%) between species inclusion in a LBAP and its presence in the LBAP area (expressed as total coverage).

Common name	Scientific name	IUCN status	No. of LBAP areas where species are extant	Times included in LBAP for each species	Times included correctly in LBAP for each species	Total coverage %	Total correct coverage %
Sturgeon	*Acipenser sturio*	CE	1	1	1	100	100
Allis shad*^	*Alosa alosa*	LC	2	3	1	150	50
Twait shad*^	*Alosa fallax*	LC	2	5	2	250	100
European eel	*Anguilla Anguilla*	CE	25	7	7	28	28
Vendace*	*Coregonus albula*	LC	1	1	1	100	100
Powan	*Coregonus lavaretus*	V	4	1	1	25	25
River lamprey	*Lampetra fluviatalis*	LC	22	12	12	55	55
Smelt^	*Osmerus eperlanus*	LC	5	5	4	100	80
Sea lamprey	*Petromyzon marinus*	LC	17	9	9	53	53
Atlantic salmon	*Salmo salar*	LR/LC	25	17	17	68	68
Brown trout	*Salmo trutta*	LC	25	12	12	48	48
Arctic charr^	*Salvelinus alpinus*	LC	10	6	5	60	50
TOTAL			139	79	72	-	-

*Listed in the Species Action Framework for United Kingdom (Joint nature Conservation Committee, 2012) ^Miss-represented; on seven occasions there were LBAP entries for species in areas they were not extant. This occurred for four species; Allis and twaite shad (resulting in more LBAP entries than areas extant) and is likely to be caused by a lack of accurate data on their distribution; Smelt and Arctic charr, which were intentional pre-empting the possibility of future translocations/reintroductions.

Table 6.4. Public bodies in Scotland providing a LBAP, the geographical areas they cover, the frequency of occurrence for LBAP species extant in the geographical area covered by the LBAP, the number of inclusions in the LBAP for high conservation value fish species, the number of inclusions that match the distribution of these species, total species representation and total correct species representation.

LBAP authority	Area covered	No. of species distributed in LBAP area	No. of LBAP entries	No. of correct LBAP entries	Total represe-ntation %	Total correct represe-ntation %
Argyll and Bute (AS, TS)	Argyll and Bute	7	5	3	71	43
Ayrshire	East, North and South Ayrshire	6	0	0	0	0
Cairngorms (NP)	Aberdeenshire, Angus, Highland, Moray and Perth and Kinross	6	6	6	100	100
Clackmannanshire	Clackmannanshire	7	6	6	86	86
Dumfries and Galloway (AC)	Dumfries and Galloway	9	10	9	111	100
Dumbarton	East and West Dumbartonshire	6	3	3	50	50
East Lothian	East Lothian	5	4	4	80	80
Edinburgh	Edinburgh	4	3	3	75	75
Falkirk	Falkirk	7	6	6	86	86
Fife	Fife	6	0	0	0	0
Glasgow	Glasgow	4	1	1	25	25
Highlands (AS, TS)	Highland	7	9	7	129	100
Loch Lomond and Trosachs (NP)	Argyll and Bute, Dumbarton, Stirling and Glasgow	6	2	2	33	33
Midlothian	Midlothian	4	1	1	25	25
North East	Aberdeen City, Aberdeenshire and Moray	6	3	3	50	50
North Lanarkshire	North Lanarkshire	5	1	1	20	20
Orkney Isles	Orkney Isles	3	3	3	100	100
Renfrewshire	Renfrewshire, East Renfrewshire and Inverclyde	5	1	1	20	20
Scottish Borders	Scottish Borders	6	0	0	0	0
Shetland Isles	Shetland Isles	4	1	1	25	25
South Lanarkshire (EA)	South Lanarkshire	5	0	0	0	0
Stirling (S)	Stirling	6	6	5	100	80
Tayside (TS)	Angus, Dundee and Perthshire and Kinross	7	6	5	86	71
West Lothian	West Lothian	4	0	0	0	0
Western Isles	Western Isles	4	2	2	50	50
Total		139	79	72	-	-

Ecosystem approach = EA; Overrepresentation of Allis Shad (AS), Twaite Shad (TS), Smelt (S) and Arctic Charr (AC).

Table 6.5. Overlay of public bodies in Scotland and 12 listed freshwater fish species accurately or erroneously included in a LBAP, or missing from a LBAP where the species is extant.

LBAP authority	Sturgeon	Allis shad	Twaite shad	Eurpoean eel	Vendace	Powan	River Lamprey	Sparling	Sea Lamprey	Atlantic salmon	Brown trout	Arctic charr
Argyll & Bute		O	O	A		A	R		R	R	A	A
Ayrshire				A			A		A	A	A	A
Cairngorms NP				R			R		R	R	R	R
Clackmannanshire				R			R	R	R	R	R	A
Dumfries & Galloway		R	R	R	R		R	R	R	R	R	O
Dumbarton				A		A	R		A	R	R	
East Lothian				A			R		R	R	R	
Edinburgh				A			R			R	R	
Falkirk		A	R	R			R	R		R	R	
Fife				A			A	A	A	A	A	
Glasgow				A			A			R	A	
Highlands	R	O	O	R			R		R	R	R	R
Loch Lomond NP				A		R	R		A	A	A	
Midlothian				A			A			A	R	
North East				A			A		R	R	R	A
North Lanarkshire				A			A		A	R	A	
Orkney Isles				R						R	R	
Renfrewshire				A			A		A	R	A	
Scottish Borders				A			A		A	A	A	A
Shetland Isles				A						A	A	R
South Lanarkshire				A			A		A	A	A	
Stirling				R		A	R	O	R	R	R	
Tayside			O	A			R	R	R	R	A	A
West Lothian				A			A			A	A	
Western Isles				A						R	A	A

R = species accurately represented in the named LBAP; A = species is absent from the LBAP area where it is extant; O = species is over-represented due to an LBAP entry in an area the species is not extant.

6.4. RESULTS

Of the 25 LBAPs in Scotland, a total of 79 LBAP entries were found for the 12 priority fish species listed on the UKBF (Table 6.3.). This figure is lower than the expected number of 139 entries expected if the known distribution of these species is overlain against the geographic boundaries of the LBAPs. Five authorities included a total of seven entries for four species in geographic areas where the listed species was absent. These erroneous records, when removed reduced the number of appropriate species entries to 72.

The degree to which different species were represented across the suite of LBAPs in Scotland was highly variable (Table 6.3.). Of the 12 species examined only three species; twaite shad (*Alosa fallax*); sturgeon (*Acipenser sturio*); and vendace (*Coregonus albula*); showed a complete match with coverage in LBAPs across their entire distribution.

Twait shad (once) and allis shad (twice) were overrepresented in the LBAP suite. This might be due to uncertainty over recorded distribution. Both smelt (*Osmerus eperlanus*) and Arctic charr (*Salvelinus alpinus*) (the latter pre-empting the possibility of a future translocation into the authorities geographical boundary) were also overrepresented in LBAPs. Three species, brown trout (*Salmo trutta*), European eel (*Anguilla anguilla*), and powan (*Coregonus lavaretus*) showed a less than 50% match between distribution and coverage in LBAPs; European eels and powan achieving only 28% and 25% respectively.

There was considerable variation between individual LBAPs in their provision of cover for Scottish freshwater fish of high conservation value. Of the 25 LBAPs, 21 failed to represent all freshwater fish species known to be extant in the area (i.e. <100% coverage of actual distribution). A total of 14 LBAPs acknowledged less than 51% of the freshwater species distributed in their area. Five LBAPs included no freshwater fish species despite the expectation of 27 entries from the known distribution of the 12 species with the high conservation status evaluated here. Of these, one LBAP had adopted an ecosystem, rather than species approach, to local conservation (Table 6.4.), thus, although the five species extant in its area were not acknowledged, suitable protection was implicit in the protection of the habitat important to those species.

Conversely five LBAPs had over-represented against expectation with seven entries in place across four species not extant in their catchments. In two instances this resulted in a total coverage score greater than 100% (Table 6.3.). Only four LBAP

authorities included all species extant in their area. Just two of these authorities, Cairngorms National Park and Orkney (Table 6.4.), having an exact match of LBAP entries for freshwater fish species of high conservation value extant in their area, with distribution.

6.5. DISCUSSION

The conservation role of LBAPs is to ensure that practical conservation strategies are developed and executed at a local level. There is a multitude of different legislative and conservation strategies at many levels all providing awareness and highlighting the threats and conservation requirements of Scotland's 12 freshwater fish species with no need of conservation management. Despite this, overall coverage of these high conservation value freshwater fish species was poor. Three quarters of all species were underrepresented geographically and the two species with the highest level of protection at an international level, powan and European eel, showed the lowest match between LBAP coverage and distribution; 25% and 28% respectively.

Powan, Scotland's rarest freshwater fish with only two native populations, received the lowest coverage, only 25% cover comprising an entry from one LBAP of the four in which it is extant. Powan are listed on the SBL and the UKBF as a priority species of conservation concern; in Schedule 5 of the Wildlife and Countryside Act 1981; in Schedule 3 of the Conservation (Natural habitats etc.) Regulations 1994; the EU Habitats and Species Directive and in Appendix III of the Bern Convention. Powan have also been designated as "vulnerable" by the International Union for Conservation of Nature (IUCN). Their ecology in Scotland is well studied with considerable published and readily accessible scientific literature on their ecology, distribution and conservation (Etheridge *et al.*, 2010a, b, 2011, 2012).

European eels have the joint highest international conservation profile of the 12 priority species extant in Scotland but had only 28% coverage from seven LBAP authorities of the 25 in which it is found. European eels are listed on the SBL and the Salmon and Freshwater Fisheries Act (Consolidation) (Scotland) 2003. Also a UKBF priority species, they are recognised under the Natural Environment and Rural Communities Act 2006 as a species of principal importance for the purpose of conserving biodiversity (Natural England). The species has an IUCN status of "critically endangered", it is listed in Annex B of the European Convention on International Trade in Endangered

Species of wild fauna and flora (CITES) regulations with an EU ban on the export of the species outside the EU (Defra, 2011). There have been a considerable number of studies on the ecology and status of European eels across Europe (Dekker, 2003; Laffille, 2005) and Scotland (Maitland and Lyle, 1991; Chadwick *et al.,* 2007; Adams *et al.,* 2013) that highlight concerns about declining European eel numbers. Published data show that European eel numbers have declined by an estimated 95% in the past 25-30 years and across Europe the population is described as "being below ecologically sustainable limits" (Aprahamian and Walker, 2009). It could be argued that Scottish eel populations may be relatively strong (Adams *et al.,* 2013) and therefore do not require conservation action from local authorities where they are extant. However due to their mass spawning strategy, safeguarding strong Scottish populations is important in contributing to the European eel's global wellbeing.

There are several possible explanations for the mismatch between the presence of high conservation value fish in an LBAP area and their omission from the LBAP, none of which are mutually exclusive. The freshwater fish species listed on the SBL have recently been enlarged to 12 and the international conservation status of many species can, and has, changed rapidly. Notably the European eel has seen recent changes to it conservation status. Changes in the classification of some species makes keeping LBAP's up-to-date an extremely difficult task for the authorities responsible.

Mismatch may also stem from the need for protection being evaluated on a case by case basis by each LBAP. A species may be at risk at a national scale (such as the SBL) or international scale (i.e. the UKBF) but certain LBAP areas may have strong and abundant local populations. This potential explanation implies that LBAP inclusion is not recognising national and international imperatives but is responding to local data on population abundance and resilience. This explanation seems unlikely given the paucity of data for most of the species examined here. Unreliable distribution data on poorly studied or illusive species could result in under-representation; this may be the cause for the mismatch in representation of, for example, allis and twaite shad.

Under-representation of threatened freshwater fish species in Scotland may also be the result of a lack of awareness or of available local expertise which can influence decision-making authorities developing potential plans (Evans, 2004). However there is a considerable amount of general (advisory) literature available for freshwater fish species

listed on the UKBF and SBL (Maitland and Lyle, 1991; Kirchhofer and Hefti 1996; Maitland, 2007).

One finding of this study has been the surprising lack of cross referencing of LBAP's that share or overlap geographic boundaries. One example is that of Loch Lomond, half of Scotland's rarest freshwater fish species fish can be found here, including important populations of powan and an endemic river lamprey population (Adams *et al.* 2008). This site is bounded by LBAPs from four authorities; Argyll and Bute, Dumbarton, Stirlingshire and Loch Lomond and the Trossachs National Park. No LBAP included all six species, river lampreys were the only species to be included in all four of these LBAP's; Atlantic salmon were included in three, brown trout and sea lamprey only two and powan and European eels just once (Table 6.5.). Collaboration among relevant local authorities would ensure more consistent species coverage, utilisation of knowledge, expertise and use of monitary resources.

Species that have multiple and alternative life history strategies, for example brown trout and sea trout, can complicate the inclusion process for authorities. Brown trout achieved only 48% cover across their distribution and although 'resident' brown trout populations are under little threat, those that adopt a 'migratory' life history (sea trout) have seen a rapid decline in rod catches of approximately 75% in the west coast rivers and 60% in the east rivers of Scotland between 1952 and 2008 (Green *et al.,* 2012). The simple lack of knowledge regarding the biological complexities of a species may contribute to a species receiving poor or ineffective coverage.

It could be argued that it is correct for species to be included in LBAPs that cover areas where the species is no longer extant. By doing this the potential for rehabilitation of degraded habitats and the reintroduction of extinct populations can be developed and with it the restoration of local biodiversity.

A potential effect on LBAP inclusion of species is the level of their public profile. A recent poll to identify the five Scottish animals most important to the public comprised no fish and was dominated by birds and mammals; golden eagle (*Aquila chrysaetos*); red squirrel (*Sciurus vulgaris*); red deer (*Cervus elaphus*); harbour seal (*Phoca vitulina*); and otter (*Lutra lutra*); (Scottish Natural Heritage, 2013). Animals which are more charismatic

or have a high recreational value have a significantly greater public profile (Maitland, 1995) and are more likely to receive funding for conservation (White *et al.,* 2000). This effect may result in over and under representation between taxonomic groups. It is suggested that taxonomic blindness has, at least in part, resulted in the poor representation of the threatened freshwater fish fauna detailed here. Financial resources intended for nature conservation are limited (White *et al.,* 2000) therefore a taxonomic bias in the perception of conservation needs and thus the allocation of limited resources is of serious concern. Of the 25 LABPs examined 24% were out of date but were still classed as effectively operational although they had not been reviewed or reassessed to accommodate changes in the conservation requirements, abundance and distribution of the species that they are designed to protect. Bias may also arise due to personal preferences of contributing board members (Evans, 2004). Members involved in the finalisation of LBAPs and where attention should be concentrated are not always aware of the needs of protection, especially if they are not from a specialist background.

6.6. Conclusions

The mismatch between presence and inclusion in LBAP's for threatened Scottish freshwater fish species likely stems from the complicated classification of their conservation status and how the conservation needs for each species can differ across their national (Scotland), international (European) and global (worldwide) range. LBAP's are frequently based around a 3-5 year plan which makes adopting changes to coincide with the changing status and needs of a species, mid LBAP term, difficult. By increasing their flexibility in formation and addressing the cover of a species on a case by case basis coupled with cross authority collaborations (which have been seen in some but not all cases, Table 6.4.) would transfer valuable knowledge and expertise and increase the effectiveness and efficiency of local level conservation. LBAPs should be regarded as the beginning of the conservation process and not the end. The development of an LBAP involves an enormous amount of time, effort and paperwork and with it a large monitory cost. It is thus important that local plans adequately reflect real conservation resource needs.

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CHAPTER 7.

DISCUSSION

7.1. SUMMARY

West-Eberhard (2003) proposed that phenotypic plasticity is fundamental to incipient speciation. It provides a source of variation upon which diversifying selection pressures can act. The alternative phenotypes which emerge from this process and which are a product of these selection pressures are often the result of early ontogenetic processes and exposure to differing environmental conditions. Thus, phenotypic plasticity is essential to phenotypic structuring within a population. In the wild it is most often demonstrated as morphological adaptations associated with foraging and the associated resource. These adaptations are suggested to then feed the process of ecological speciation through channels (West-Eberhard, 1989) such as; changes in foraging behaviour associated with the acquisition of new prey; colonisation of new habitats associated with new prey; sexual selection processes such as assortative mating associated with the new morphological phenotypes that are expressed; changes in timing of spawning as a result of differing life history strategies; all of which have been shown to contribute to genetic isolation in sympatry (Smith and Skúlason, 1996). It is therefore plausable to suggest that the level of phenotypic plasticity a species or population possesses can determine its potential to diverge. This theory is partially supported in Chapter 2. But this only takes in to account plasticity and is slightly naïve to other factors and complex interactions associated with speciation. What this chapter does show is that not only plasticity is important in speciation events, but so are species interactions, resource availability and habitat type.

We know that different diets can have a diversifying effect among individuals of the same species that opt for alternative food sources. Frequently proposed as the initial stage in sympatric speciation (Smith and Skúlason, 1996). We also know that differences in diet can lead to significant variation in morphology within a single generation (as seen in chapter 2). Although this variation after a single generation can be statistically significant in a controlled environment, the differences are very slight and after one generation are likely too subtle for assortative mating or significant fitness gains for foraging (however this was not directly tested). Whatever the environmental conditions that new characteristics had originated from and then reinforced, the presence of said environmental conditions need to be persistent or repeated. For morphological phenotypic plasticity to trigger processes that underpin speciation events, new foraging specialisms

need to be adhered to over a significant portion of an organism's life. Given the stochastic nature of aquatic environments and the variability in resource abundance that can fluctuate from year to year, seasonally and even daily, for resource specialisms to be maintained over multiple generations it is likely that other forces are at work. This hypothesis was addressed in Chapter 3, showing that there are physiological benefits associated with specialising and these contribute to the cementation of specialisms by adding a cost associated with foraging on alternative prey. Although morphological adaptations are more frequently documented and often considered as the most important stage in incipient speciation because they are involved with foraging efficiency, prey choice, habitat use and sexual selection, physiological adaptations may be more constraining by imposing high fitness costs for those that deviate from their primary food type. This would make the physiological effect of prey specialisation an extremely important component to ecological speciation.

In Chapter 4 I showed that the physical property of water temperature can significantly affect the expression of phenotype. Furthermore, these alternative phenotypes that are expressed are of functional significance. Fish raised at an ambient temperature expressed a phenotype the same as their parents whilst those raised at an elevated temperature expressed a different phenotype. When compared to wild sympatric polymorphic populations it became clear that the elevated temperature fish had developed a phenotype much more suited to foraging on benthic prey. This study demonstrated that anthropogenic modifications to the environment have the potential to disrupt ecological and evolutionary processes. With the ominous onset of climate change, further work is needed to elucidate how highly plastic organisms will adapt if their natural phenotypic trajectory is altered. The results from this study would suggest populations of Arctic charr may have to switch either their foraging tactic to one more suited to their expressed phenotype, or in locations where this isn't possible, suffer loss of fitness associated with foraging on a prey not as suited to its phenotype.

There are many other fitness consequences associated with foraging. We know that the morphological component of the phenotype is quintessential to foraging capabilities. We also know diet type can affect your morphology (Chapter 2), your physiology and fitness (Chapter 3) and that it can be driven by other parameters that may have a consequence on your phenotype (Chapter 4). In Chapter 5, I used a rare extreme sympatric polymorphic population of Arctic charr to better understand the cause and effect of

selection pressures. Although this chapter highlights different ecological variables that can be used to identify alternative foraging specialism, it also shows that much of the phenotypic structuring that we see can in fact be an indirect effect of alternative foraging, with a change in foraging acting as the catalyst for these additional consequences. These consequences are important in providing additional types of phenotypic structuring which are not the direct product of handling prey, such as head morphology. Differences in body morphology, not associated with handling prey, were clearly different between morphs and more likely a response to different habitat types as individuals search out new prey items under different environmental conditions. The way individuals have to move can cause phenotypic responses in fin and body shape. Coloration can be directly affected by food type and cause visual difference that may pose crucial to assortative mating. Diet can also have effect on other aspects of an individual ecology. I found a significant difference in lipid content which has obvious fitness consequences especially in relation to gamete production which can influence the morph ratio in sympatric populations (Ide *et al.*, 2011) and may affect when fish spawn. I also found differences in parasite fauna which also effects fitness and in some fish populations has been shown to correlate with genetic structuring (Karvonen et al., 2013). Thus both these 'consequences' of alternative foraging strategies provide different types of population structuring.

The production of alternative phenotypes in response to differing environmental conditions can significantly contribute to biodiversity (Schmitz, 2003; Duffy, 2008; Mouritsen and Poulin, 2005). In more recent years' biodiversity has received more attention and the efforts to conserve it have been increased. Phenotypic structuring provides a valuable source of biodiversity which is not only often overlooked but also worthy of protecting. However, identifying and quantifying phenotypic structuring is complicated, especially in fish. Because the phenotype is composed of many different characteristics, a perturbation in any of these caused by the environment and mediated through plasticity results in phenotypic variation, thus many fish species show variation and structure in their phenotype within a single population. In chapter 6 of this thesis I evaluated how well fish are protected by Local Biodiversity Action Plans (LBAPS). Within the British Isles there are 12 freshwater fish species listed, varying in their needs for active conservation strategies to ensure their wellbeing. Some of their importance stems from how threatened these species are at a local level such as powan (*Coregonus laveratus*) and salmonid species such as Arctic charr (*Salvelinus alpinus*), Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*). Or species which are threatened at an

international level such as the European eel which it could be claimed have relatively strong populations within the British Isles, their numbers have been decreasing globally to the extent that the IUCN has them listed as critically endangered. Due to the high profile of these fish at a local and international level it was surprising that these species were the most poorly represented by LBAP's.

In this analysis (Chapter 6) I show that Powan only received effective cover from 25% of the total number of LBAP's that should have them on their agenda. European eel was only marginally better represented at 28% but given their global status, this figure was alarmingly low. Arctic charr which are well documented as having valuable and unique populations only received 50% cover.

I believe, along with other factors, that creating conservation policy to protect highly variable species is the most problematic. This becomes even more difficult when populations within species differ significantly because effective management or conservation policy designed for one population may not be appropriate for other poulations of the same species (Bush and Adams, 2006). Furhtermore, identifying populations that should be prioritised when deciding where to allocate limited conservation resources adds complications to this problem. Difficulties may also arise from a limited knowledge of policy makers whose experience may be in alternative fields of conservation and/or species. Brown trout are an exemplary example where life history variation complicated policy making, brown trout received 48% effective cover by LBAP's. With no clear distinction between potatdromous (resident brown trout) and anadromous (sea trout) phenotypes, implementing policy becomes difficult when using the common 'umbrella' approach. Whilst potadromous brown trout are unthreatened, anadromous brown trout numbers have been rapidly declining, but with catchements (and populations) contributing to both quite often sea trout are neglected as populations may be evaluated as low risk due to thriving resident populations.

7.2. Future work

As is often the case in science, and research, for every questions that is answered, more present themselves. In each chapter a better understanding of how phenotypic structuring may arise and be maintained, but each study but also presents more questions where valuable future work could be carried out. Below are what I feel the most important 'next' questions to be answered that have arisen form the studies presented here.

1) Chapter 2: If brown trout are so plastic, why do we not see more sympatric polymorphic populations? By answering this we will be able to gain a better understanding of selection pressures associated with sympatric divergence.
2) Chapter 3: Which has a greater associated fitness benefit, morphological adaptations associated with acquiring novel prey or the physiological adaptation associated with its digestion? This will provide insight as to which adaptation is more fundamental to ecological speciation.
3) Chapter 4: How will populations whose phenotype is artificially modified through plasticity in response to unnatural changes to the environment adapt. Will a shift in foraging patterns i.e. prey choice be adapted to better suit their morphology, or will a loss of fitness be seen associated with higher costs associated with handling and manipulating prey? It could be that different species accommodate changes in a different manner.
4) Chapter 5: With two very contrasting populations of the same species inhabiting the same water body, how is genetic isolation maintained? Furthering our understanding of their space use and when and where they spawn will provide valuable information on how speciation occurs in sympatry.
5) Chapter 6: What is the most effective way to protect species with highly variable phenotypes, particularly those that have contrasting life history strategies, as seen in brown trout, or those with complex life cycles, as seen in European eel. Improving these will not only safeguard these species by potential help in developing strategies applicable to other highly variable species.

In general, more research is needed in the field of environmentally driven phenotypic structuring. Understanding the effect of diet, habitat and species will not only provide valuable information which can aid the development of effective and efficient conservation strategies, it will better our understanding of the complex covariant processes involved in evolution.

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APPENDIX 1

OFFSPRING OF MIGRATORY TROUT SHOW LESS DIET-INDUCED FLEXIBILITY IN MORPHOLOGY AND GROWTH THAN OFFSPRING OF RESIDENTS

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A1.1. ABSTRACT

Partial migration, in which some individuals of a population migrate while other individuals remain resident, is generally associated with ontogenetic shifts to better feeding or as a response to adversity, but its underlying mechanisms remain relatively unknown. Brown trout (*Salmo trutta*) exhibit partial migration, with some individuals remaining in fresh water (freshwater-resident) while others undertake an anadromous migration, gain most of their adult size at sea, and then return to fresh water to spawn. The option adopted by an individual trout is thought to be partly determined by its growth performance in early life, which in the stochastic and dynamic environment of freshwater streams may be dependent on its flexibility. To examine potential effects of parent type on phenotypic flexibility, we measured the metabolism, growth, and morphology of full-sibling groups of offspring from freshwater-resident and anadromous parents both before and after a switch in diet. We found that fry had a higher growth rate and a more rounded head and body shape when reared on chironomid larvae compared with when they were reared on *Daphnia*, but diet had no effect on standard metabolic rate. Interestingly, offspring of anadromous parents were less able to maintain their growth rate when fed on *Daphnia* than were those of freshwater-residents and showed a correspondingly greater increase in growth following a switch from *Daphnia* to chironomid larvae. Offspring of anadromous parents also showed less morphological flexibility in response to diet than did the offspring of freshwater-residents. We discuss how the migration history of the parents might interact with phenotypic flexibility in early life to influence the migration probability of the offspring.

A1.2. INTRODUCTION

Phenotypic flexibility, an organism's ability to match its morphology and physiology to current environmental conditions, is fundamental to adaptability and occurs when complimentary combinations of traits change in response to environmental conditions to maximise the efficiency of resource exploitation. For example, within fish there may be changes within the lifetime of the individual animal in gill raker spacing and mouth shape to suit shifts in prey type (Schluter, 1993), changes in body shape that are related to parallel changes in the velocity of water in which the fish is living (Peres-Neto and Magnan, 2004) and more recently differences in SMR (the minimal maintenance metabolic rate of an ectotherm in a post-absorptive and inactive state) in response to local food availability (Van Leeuwen *et al.*, 2011; Auer *et al.*, 2015). While much of the research surrounding phenotypic flexibility has been focussed on explaining patterns of resource polymorphisms within species in the context of adaptive radiation and speciation, it may also help explain other ecological patterns of intraspecific variation, one of which is the phenomenon of partial migration.

Partial migration, in which members of a population differ in whether or not they undertake migrations, occurs across a wide range of taxa including invertebrates (Hansson and Hylander, 2009), fish (Dodson *et al.*, 2013), birds (Newton, 2008) and mammals (Ball *et al.*, 2001). The commonest form is non-breeding partial migration (*sensu* Chapman *et al.*, 2011), where migrants and residents breed sympatrically but overwinter apart. There have been many hypothesised explanations for this variation in migratory pattern, including competition for resources, differences in thermal tolerances and differences in arrival times/prior residence (see Chapman *et al.*, 2011). In all cases however, the migration can be viewed as a response to adversity (Taylor and Taylor, 1977), but the degree of adversity will depend on the particular environmental conditions that are experienced at the time. For example, individuals that are of a larger body size or experiencing a higher food supply may generally have less to gain from migration (Chapman *et al.*, 2011).

It is likely that both abiotic and biotic factors influence the decision to migrate or not, since it is potentially influenced by both genetic causes (i.e. determined by the parents through genetic or parental effects, so that offspring of migrants have a higher probability to migrate) and environmental factors (e.g. through condition-dependent migration; Brodersen *et al.*, 2008). Berthold (1988) and Berthold and Pulido (1994) provide support

for a genetic pre-disposition for migratory tendency and migration distance in the Blackcap. However, it has also been suggested that partial migration is driven by a complex interaction between the environment and genetics. In the "threshold model" the triggering of migration depends on whether or not a continuous character ("liability trait") exceeds a genetically predetermined threshold value (Chapman *et al.*, 2011; Dodson *et al.*, 2013). In this scenario, individuals physiologically self-evaluate their performance against this threshold (e.g. of growth rate, body size or physiological condition), with migration being dependent on whether or not the threshold is exceeded (Fleming, 1996; Pulido, 2011; Dodson *et al.*, 2013).

A well-documented example of a species exhibiting partial migration is the Brown trout, a polymorphic species that adopts a continuum of life history strategies, with the two most common being freshwater-resident and anadromous migrant (which migrates to sea as a juvenile and returns to fresh water to spawn). The two ecotypes can occur in sympatry, possibly derived from a single gene pool, with both anadromous and freshwater-resident adults having the ability to interbreed and produce offspring capable of adopting either life history (Wysujack *et al.*, 2009; O'Neal and Stanford, 2011). Freshwater-resident and anadromous trout appear indistinguishable during early life, and it is presumed that they only become separable when after one or more years the migrants turn silver in colour in preparation for entry to sea water ('smolting'; Jonsson, 1985). Jonsson (1985) proposed that migrant brown trout are made up of the slower growing individuals in a population, which migrate in search of more productive habitats. It has also been suggested that metabolic constraints play an important role in determining physiological state and thus migration probability. The fish are often found in oligotrophic habitats in fresh water (e.g. upland temperate lakes and streams), and in this low food environment individuals with a lower growth efficiency, higher food requirement and/or higher metabolic rate (i.e. energy maximisers) will become energetically constrained earlier in life compared to those with higher growth efficiency, lower food requirement and/or lower metabolic rates (efficiency maximisers; Metcalfe *et al*.,1995; Forseth *et al*., 1999; Morinville and Rasmussen, 2003; Rosenfeld *et al*., 2013). The individuals with the lower growth efficiencies and/or higher metabolic rates may therefore migrate in search of more productive habitats (lakes, oceans) to meet their outstanding metabolic needs.

It is likely that genetics interacts with growth history, current body size and physiological condition to determine whether or not the animal reaches the threshold that

triggers migration. However, there may also be a role for the morphological and physiological flexibility of the organism (i.e. its phenotypic flexibility). For example many species of fish adjust their body shape in response to diet type and water velocity to increase their efficiency of prey detection, capture and handling of prey items (Skúlason and Smith, 1995; Adams and Huntingford, 2002b) and to reduce swimming costs (Peres-Neto and Magnan, 2004). Furthermore, flexibility in physiology may be equally important since individual brown trout that showed the biggest change in SMR when food availability was altered (either upwards or downwards) were recently found to have the fastest growth under the new food regime (Auer *et al*., 2015). Given that freshwater fluvial ecosystems are often regarded as being stochastic and that the decision to migrate is likely based on a cumulative assessment of performance over a range of environmental conditions experienced to date (i.e. a timespan of several years), it is possible that differences in the phenotypic flexibility of the individual may be more important in determining growth performance, and thus explaining patterns of partial migration, than whether it has a consistently "high" or "low" value for traits or conditions of interest. Therefore individuals who are more able to match morphology and physiology to current environmental conditions, and therefore to maximise growth (or minimise their energetic costs), may be more suited to freshwater fluvial habitats compared to less phenotypically flexible individuals who may be more suited to more homogenous habitats such as large lakes and oceans. If true, then offspring of freshwater-resident parents might be more likely to exhibit plasticity in early life than those of anadromous brown trout.

To explore these issues, we reared brown trout offspring from eggs of known parentage (i.e. freshwater-resident or anadromous) under two diets of equal energy content but potentially differing ease of digestion (*Daphnia* and Chironomid larvae), which were then switched to test for phenotypic flexibility in both morphology and physiology, and the consequences for growth rate.

A1.3. METHODS

A1.3.1. BROODSTOCK COLLECTION

Twenty-four mature freshwater-resident (12 male and 12 female) and 14 anadromous (7 male and 7 female) brown trout were captured during the breeding season using electrofishing on 11 and 23 October 2013 from two neighbouring sub-tributaries of the River Tweed, Scotland. Freshwater-resident trout were collected from above an

impassable dam on the Whiteadder River (55° 88'N, 2°57'W) while the anadromous trout were collected from the College Burn (55° 77'N, 2°18'W). Adult fish were classified as freshwater-resident or anadromous based on size and colouration (Eek and Bohlin 1997): freshwater-resident fish were smaller and dark brown in colour with red spots, while anadromous fish were larger and silvery-grey in colour with black spots. Both ecotypes were transported to the Belhaven Trout Company, Scotland, where they were held separately (keeping parental ecotypes discrete) in two round 1530 L aluminum tanks supplied with 8.1 $\pm$ 0.4 °C (mean$\pm$SD) well water under ambient photoperiod and assessed every three days for ripeness.

Ripe fish were anaesthetised, blotted dry, and their eggs or sperm extruded by abdominal massage. Eggs were fertilised with sperm from a haphazardly-chosen male of the same life history origin to create 12 full sibling families from freshwater-resident parents and 7 full sibling families from anadromous parents. Freshwater-resident and anadromous fish were spawned from 3 November - 29 November and 17 November - 4 December 2013 respectively.

A1.3.2. EGG REARING, HATCHING AND EXPERIMENTAL PROCEDURES

Each family of eggs was housed separately in a plastic mesh egg basket, placed in one of two (1m X 3m X 0.4m) rearing troughs supplied with well water and covered with dark plastic sheeting to ensure eggs were in complete darkness. Water temperature during incubation was 8.1 $\pm$ 0.4 °C and was recorded daily along with any dead eggs which were carefully removed. Eggs were checked daily for hatching; those from freshwater-resident and anadromous parents hatched from 19 December 2013 - 17 January 2014 and 30 December 2013 - 24 January 2014 respectively. Once eggs began to hatch, the newly emerged offspring (alevins) were separated from the remaining eggs and gently placed into a small mesh basket (one per family) located in the same two troughs as the egg baskets.

On 31 January 2014 alevins were transported to the Scottish Centre for Ecology and the Natural Environment, Scotland and housed in 15 L (50cm X 30cm X 15cm) clear plastic aquaria on a partial recirculation system at a constant temperature of 9.2 $\pm$ 0.2 °C (mean$\pm$SD) and simulated ambient photoperiod. The aquaria each contained a single air stone and were supplied with water pumped directly from Loch Lomond, which was first treated with an ozone generator (Sander S1000, Germany) before being discharged into a large sump. Water from the sump was pumped through an in-line 110W UV steriliser

(Tropical Marine Center (TMC), Manchester, UK) before entering the aquaria. Return water was gravity fed into a large free standing filter before being discharged back into the main sump. Fish were monitored daily and any dead fish removed.

On 3 March 2014, at the time of first feeding, random selections of offspring from across families were haphazardly assigned into eight 15 L (50cm X 30cm X 15cm) clear plastic aquaria (keeping parental ecotypes discrete), with four aquaria per parental type and 10 fish per aquaria. The aquaria were placed inside a constant temperature room on a partial recirculation system at a temperature of 13.6 $\pm$ 1 °C (mean$\pm$SD), with a simulated ambient photoperiod. Fish were fed *ad libitum* several times daily by pipetting food onto the surface of each aquaria, with excess food (which was clearly visible on the bottom of the aquaria after every feed) being removed by vacuum siphon at the end of each day. Diet treatments consisted of *Daphnia* (BCUK Aquatics, Lincolnshire, England; composition: protein 5%, fat 0.7%, fibre 1%, moisture 90%) or Chironomid larvae (BCUK Aquatics, Lincolnshire, England; composition: protein 5%, fat 0.5%, fibre 0.9%, moisture 89%); diet types were supplied frozen from the manufacturer and thawed daily before feeding. It was presumed that, although the two diets had an almost identical nutritional and water content, fish would grow more slowly on the *Daphnia* treatment due to the extra costs associated with digesting and processing the hard exoskeleton of the *Daphnia* (Swaffar and O'Brien, 1996) in comparison with the soft body of the Chironomid larvae. Two replicate aquaria (i.e. 20 fish) per parental type were randomly allocated to each of the two diet treatments.

On 11 June 2014 fish were anaesthetised, measured (fork length $\pm$0.1mm; body mass $\pm$0.0001 g), and tagged with a visible implant elastomer (Northwest Marine Technology, Inc.). They were then anaesthetised, re-measured and photographed on 2 July 2014, so that their growth rate (from 11 June to 2 July 2014) and morphological shape on their initial diets (3 March 2014 to 2 July 2014) could be measured (interval one). The SMR of all fish was then measured (see below) once over the period from 2 July 2014 to 12 July 2014. Once all fish had been subjected to metabolic measurements the two diet types were switched (12 July 2014) so that all individuals previously fed *Daphnia* were switched to a diet of Chironomid larvae and *vice versa*. On 28 August 2014 all fish were again anaesthetised, re-measured and photographed, then their SMR recorded (measurements over the next 10 days), for assessment of growth rate, morphological shape change and metabolic rate following the diet switch (12 July to 28 August 2014; interval two). Fish were maintained on their switched diets to further evaluate the degree of shape

change through later ontogeny (through to 30 September 2014, when they were again anaesthetised and photographed; interval three).

A1.3.3. MEASURING STANDARD METABOLIC RATE

Aquaria were vacuum siphoned to remove food and debris the day before fish were placed in respirometry chambers. This ensured that fish were unfed for at least 28 h prior to oxygen uptake measurements, and had sufficient time to evacuate their guts; 28 h post-feeding has been shown to be adequate for the specific dynamic action (SDA) response to subside in salmonids (Cutts *et al.*, 2002). SDA is an elevation in metabolic rate due to the increased energy demands associated with digestion, immediately following a meal (Rosenfeld *et al.*, 2015), and is generally not considered part of SMR.

Oxygen uptake was measured over a 24 h period, from approximately 10.00 AM onwards, using intermittent flow respirometry. Individual fish were placed into 1 of 8 separate (8.0cm length, 3.4cm diameter) glass respirometry chambers. Chambers were submersed in a water bath housed inside a second constant temperature room kept at the same temperature (13.6 $\pm$ 0.5 °C across all measurements) as the tanks in which growth was measured. An air-stone in the water bath of the respirometer apparatus kept the water fully saturated with oxygen. Chambers were wrapped in dark plastic to prevent visual contact between individual fish during measurements, and all measurements were conducted in the dark to further minimise fish activity (Cutts *et al.*, 2002). Glass respirometers and tygon tubing were used to minimise potential issues with use of plastics and oxygen permeable materials (Stevens, 1992). Oxygen uptake was measured for 20 min every 45 min on a continuous 25 min "on" and 20 min "off" cycle. During the "on" cycle oxygenated water from the water bath was driven by a water pump (Eheim 300 universal, Deizisau, Germany) through each respirometer. Flow rate was regulated by adjusting the tension of a hose clamp on the outflow side of the pump tubing to prevent swimming and spontaneous behaviour during this period of flushing. After 25 minutes the pump creating the water turnover was automatically switched off (Superpro MFRT-1 timer, Somerset, England) allowing for a decrease in oxygen concentration to be measured during the 20 min "off" period, during which a peristaltic pump (Masterflex L/S, London, England) was used to ensure adequate mixing within each respirometer. Water oxygen concentration was measured every 1s for 20 min during this time period. Oxygen concentration within the respirometer was measured using one of two oxygen meters (FireStingO_2 oxygen meter; PyroScience) each fitted with 4 oxygen probes which were

placed in individual measurement chambers (Loligo systems, Tjele, Denmark) connected inline between the outlet side of each respirometer and the peristaltic pump; concentrations never dropped below 80% oxygen saturation in this experiment. Probes were calibrated daily, and rates of background oxygen consumption were subtracted from the observed values by measuring the oxygen concentration of water inside each of the respirometers in the absence of fish at the beginning and end of each measurement trial and assuming a linear decrease in oxygen concentration over the measurement period.

The rate of oxygen consumption was determined using the following equation (Ege and Krogh, 1914):

$$\mathbf{MO_2 = V_w(\Delta C_w 0_2)/\Delta t}$$

where V_w is the volume of water in the respirometer and associated tubing minus the volume of the fish and $\Delta C_w 0_2$ is the change in oxygen concentration of the water over time period Δt (Steffensen, 1989). Oxygen concentration was calculated by correcting PO2 (partial pressure oxygen) for barometric pressure and multiplying by αO2 (μmol L^{-1} $torr^{-1}$), the solubility coefficient at the observed temperature. Standard metabolic rate was estimated by using the average of the lowest 10% of values observed during the respirometry trial (Norin, 2014). Following respirometry measures all fish were anaesthetized, blotted dry and weighed to the nearest 0.0001g.

A1.3.4. MORPHOLOGICAL MEASURES

Lateral view photographs of all fish were taken using a Canon EOS 350D digital camera fixed to a camera stand and illuminated with two blue lights mounted on either side of the camera stand to ensure quality images for geometric morphometric analysis. For each photograph a scale reference was added to allow for the correction of shape change associated with changes in body size. Twenty consistently identifiable landmarks were digitised on each image (Figure A.1.) using tpsDig and tpsUtil software (Rohlf 2006 a,b).

Landmark configurations for each specimen were aligned, translated, rotated and scaled to a unit of centroid size by using a Procrustes superimposition using the mean shape of all the images as the starting form (Rohlf and Slice, 1990). Shape change due to differences in allometry and not a response to diet type were removed (size corrected) using a multivariate, pooled, within-group regression of the Procrustes coordinates on the

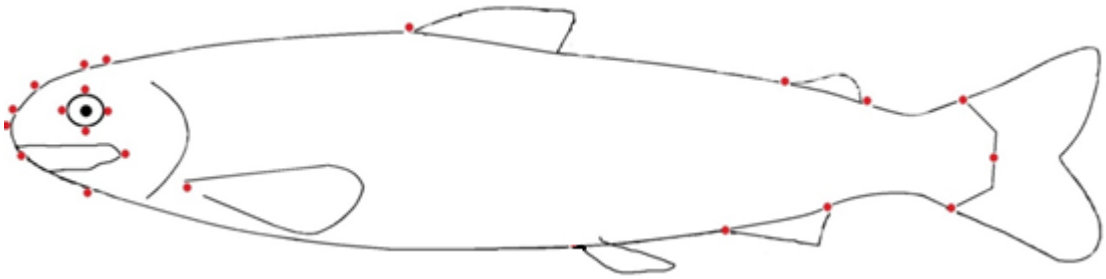

Figure A.1. Schematic diagram of the morphological landmarks used for analysis.

log centroid size of the individual (Klingenberg and McIntyre, 1998). The residuals of this regression were then used for all further analysis. Canonical variate analysis was undertaken in MorphoJ to assess the effect of diet on body shape, using the average Mahalanobis distance (D) between the two diet groups from a single parent type (freshwater-resident or anadromous) for each time interval. Comparison of the changes over time in the size of D for the offspring of freshwater-resident and anadromous fish indicates the relative degree of morphological flexibility of the two offspring types.

A1.3.5. CALCULATIONS AND STATISTICAL ANALYSES

Specific growth rates of fish (percent per day) were calculated as $100[\log_e(\text{final mass}) - \log_e(\text{initial mass})]/\text{duration}$, where duration refers to the interval between measurements (Ricker, 1975). Given the large variation in fish mass and the confounding effect of mass on metabolism and growth, we used residual SMR and residual growth in subsequent analysis. These residual values were calculated as residuals from the regression of absolute oxygen consumption or growth rate (SMR or Growth) on body mass (g); in order to standardise the results I used a reference of the combined data for offspring from freshwater-resident and anadromous fish habituated to the Chironomid larvae diet (i.e. during interval one; $\log10\ (\text{SMR}) = (1.02*\log_{10}\text{mass}) + 0.7576$; n=38); $\log10\ (\text{Growth}) = (0.0116*\log_{10}\text{mass}) + 0.619$; n=38), plotted on double logarithmic axes. Prior to being log transformed a constant of one was added to the growth data to allow transformation of negative growth values (since some fish on the *Daphnia* food treatments lost mass).

We used linear mixed effects models (LME) to test for the effects of diet and parental life history on growth and SMR. All LME models initially included all possible two way interactions, with aquarium tank and individual included as random factors to control for potential tank effects and non-independence of measures for individuals. Variance inflation factors (VIF's) for all explanatory variables were calculated prior to

analysis; all VIF's were less than 3, indicating that collinearity among explanatory variables was unlikely to have affected the analyses (Zuur et al. 2009). Furthermore, visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. Likelihood ratio tests comparing models with and without a given term were used to sequentially compare model fit; models were progressively simplified provided that any increase in the log-likelihood ratio statistic was non-significant (p = > 0.05). Tukey and LS means tests were used to compare treatment groups. All analyses were conducted using R version 3.0.1 statistical software (R Core Team, 2013) and the lme4 function (Bates *et al.*, 2011).

A1.4. RESULTS

A1.4.1. STANDARD METABOLIC RATE

There was no significant effect of parental type on SMR, nor of initial diet (Figure. A.2.A.) However overall there was a significant decrease in SMR when offspring were switched from their initial diets to their alternate diets (Figure.A.2.B; Tukey, all less than p = <0.001), with fish switching from Chironomid larvae to *Daphnia* showing a greater decrease in SMR compared to those individuals that switched from *Daphnia* to Chironomid larvae (Figure. A.2.C.; χ^2=5.51, df=1, p = <0.02).

A1.4.2. GROWTH RATE

The effect of diet on growth rate depended on the parental type (Figure A.2.D; χ^2=28.08, df=3, p = <0.001), with offspring of freshwater-resident parents having a higher growth rate than those of anadromous parents, but only if on a diet of *Daphnia* during the first time interval (LSMEANS, p = 0.04). There was no significant effect of parental type on growth when fry were feeding on Chironomid larvae (Tukey, p = 0.120), or on *Daphnia* having previously been fed Chironomid larvae (Tukey, p = 0.598). However fish grew faster on Chironomid larvae than on *Daphnia* (χ^2=293.1, df=3, p = <0.001), and the switch from *Daphnia* to Chironomid larvae produced a bigger increase in the growth of offspring from anadromous parents than those from freshwater-resident parents (Figure A.2.E; χ^2=6.51, df=1, p = 0.01).

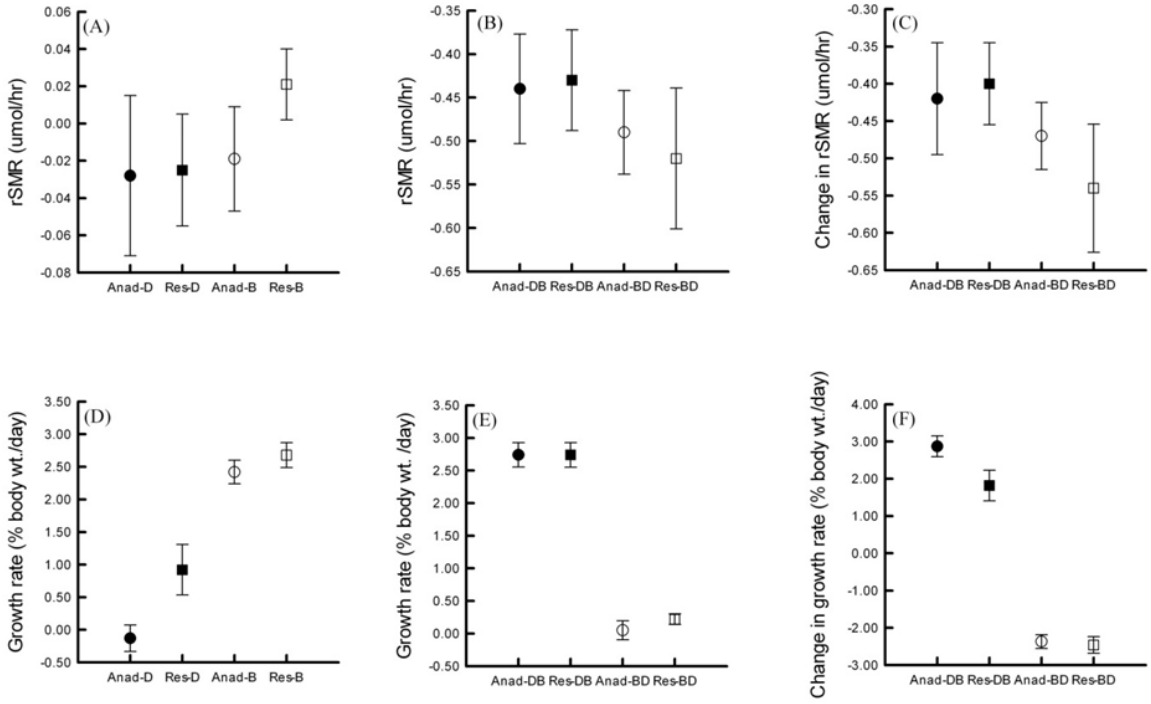

Figure A.2. The effect of diet and parental type (squares = offspring of freshwater-resident parents (Res); circles = offspring of anadromous parents (Anad)) on standard metabolic rate (SMR) and growth rate. (a) SMR at the end of interval one of fish that had been fed since first feeding on *Daphnia* (D) and Chironomid larvae (B); (b) SMR at the end of interval two of fish that had been switched at the end of interval one from a diet of *Daphnia* to Chironomid larvae (DB; closed) or from Chironomid larvae to *Daphnia* (BD; open); note change in scale of ordinate compared to previous graph; (c) Change in SMR after the change in diet (negative values indicating a lower SMR after the switch). (d-f) Corresponding data for growth rates over (d) interval one and (e) interval two, and (f) change in growth rate after the change in diet (negative values indicating a slower growth rate after the switch). SMR and growth rates are expressed as residuals to correct for body mass. Error bars represent 95% confidence intervals. See text for statistical analysis.

A1.4.3 MORPHOMETRICS

There was a similar significant morphological response to diet in offspring of the two parental types (Figure A.3.; Figure A.4.), with fish initially fed on Chironomid larvae developing a rounder body and head compared to those fed on *Daphnia*, which had a more slender body and head (Figure A.5.).

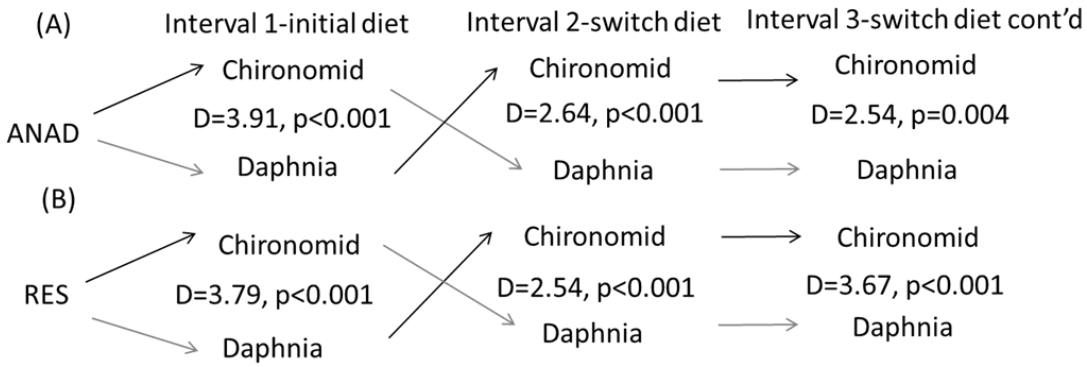

Figure A.3. Schematic diagram of the morphological shape response for offspring from anadromous (Anad)) and freshwater-resident (Res) parents during time periods when fish were reared on *Daphnia* (grey arrows) and Chironomid larvae (black arrows). Diets were switched at the start of interval two. Note the equivalent morphological responses to diet of the two offspring types during intervals one and two (i.e. a similar degree of initial morphological divergence between fry on the *Daphnia* and on the Chironomid larvae diets, and a similar effect of a diet switch, as measured by Mahalanobis (D) distance). However, by interval three the offspring of freshwater-resident parents showed a greater dietary-induced morphological divergence (D = 3.67) compared to those of anadromous parents (D = 2.54).

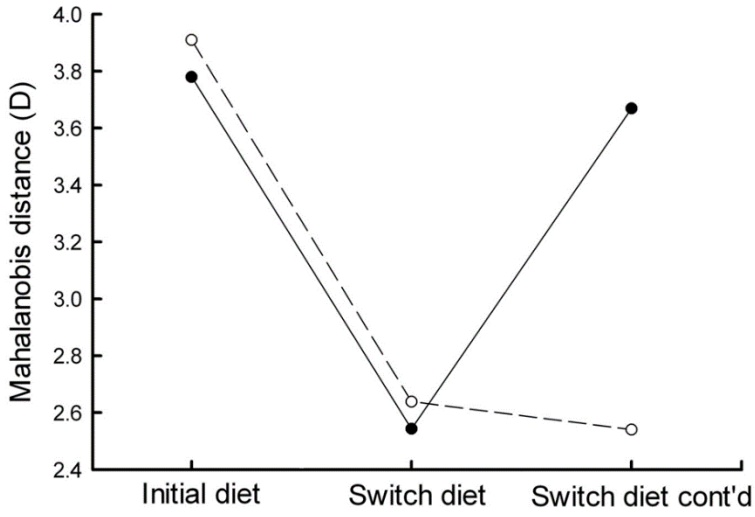

Figure A.4. The morphological difference in shape response for offspring from anadromous (open circles; dashed line) and freshwater-resident (closed circles; solid line) parents during time periods when fish were reared on *Daphnia* and Chironomid larvae. The Mahalanobis (D) distance quantifies the difference in body shape between fish of the same parentage that were reared on the two diets. See text for further description of the analyses.

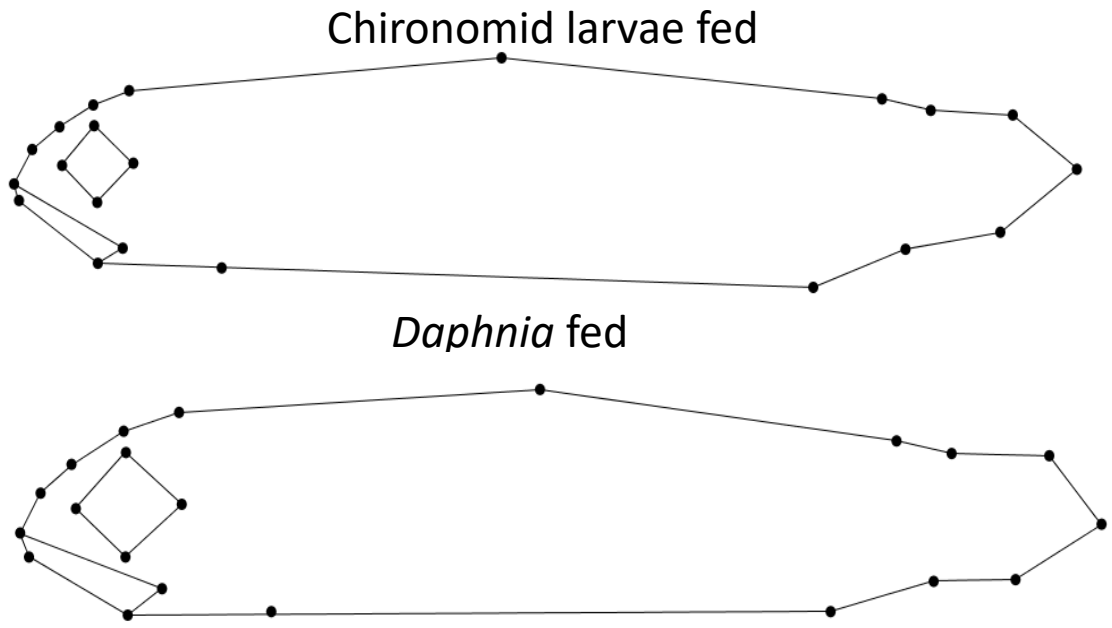

Figure A.5. Wire frame diagrams of the morphological shape change associated with canonical variance one (CV1) from the canonical variance analysis (CVA). CV1 split groups based on diet and accounted for 36.2% of the overall variation. Note the more rounded head and body shape in the fish fed Chironomid larvae compared to those fed *Daphnia*. Analysis is based on fish morphometrics averaged across all three intervals.

When the diet was switched, the fish responded by developing the appropriate morphology (i.e. those previously fed on *Daphnia* developed a rounder body and head when switched to Chironomid larvae, and *vice versa*). The extent of the diet-induced difference in morphology was similar for offspring of the two parental types during both interval one (anadromous: Mahalanobis distance = 3.91, p = <0.0001, freshwater-resident: Mahalanobis distance = 3.79, p = <0.0001) and interval two (anadromous: Mahalanobis distance = 2.64, p = <0.0001, freshwater-resident: Mahalanobis distance = 2.54, p = <0.0007; Figure A.3. and Figure A.4.). However, during interval three the offspring of freshwater-resident parents diverged more in morphology in response to diet than did those of anadromous parents (anadromous: Mahalanobis distance = 2.54, p = <0.004, freshwater-resident: Mahalanobis distance = 3.67, p = <0.0001), even though all fish had been on the same diets since the beginning of interval two (Figure A.3. and Figure A.4). This suggests a greater morphological flexibility in offspring of freshwater-residents than anadromous trout.

A1.5. DISCUSSION

The diet on which juvenile brown trout were reared had a significant effect on body (especially head) shape and growth, with fish fed on Chironomid larvae having a higher growth rate and developing a more rounded body shape compared to those fed on a *Daphnia* diet. There were initially no differences in SMR between fish on the two diets, nor between fish from different types of parent (i.e. freshwater-resident versus anadromous). However, given that SMR often differs by a factor of 2 or 3 between individual trout fry of the same age and size (Burton *et al*., 2011), this result may be due to low statistical power to detect differences among groups. Individual differences in SMR within salmon and trout populations have been linked to variation in individual growth and life history strategies (e.g. timing of subsequent smolt migration; McCarthy, 2000). Although we did not detect a difference in SMR between offspring type and diet type we did find a decrease in SMR when diets were switched (in either direction). Flexibility in SMR has been shown to occur in salmon and trout populations in relation to food availability, with individual SMR decreasing following a period of food restriction (Du Preez, 1987; Wieser *et al*., 1992) and increasing when food is supplied above baseline levels (O'Connor *et al.*, 2000; Van Leeuwen *et al*., 2011; 2012); moreover, growth is fastest in those individuals that show the biggest change in SMR in response to changing food availability (Auer *et al.*, 2015). However this doesn't explain the reduction in SMR in our study as it happened regardless of the direction of the diet switch and despite the fact that the fish were fed an equal ration (in terms of relative mass), calculated to be *ab libitum*, for each diet type. One possible explanation is an imbalance between new prey type, digestive tract performance and assimilation, producing a similar response to when food levels are changed. Vertebrate digestive tracts have been shown to respond over relatively short time scales to differences in prey consumption and food availability (Starck, 1999; Armstrong and Bond, 2013). For example, snakes can increase the capacity and activity of their digestive tract during a meal, and conversely decrease its capacity and activity during periods of food deprivation (Secor and Diamond, 2000). Similarly, juvenile salmonids can dramatically increase the length of their intestine during sustained periods of increased food availability (Armstrong and Bond, 2013). The switch in diet may have meant that the digestive system of the fish was initially imperfectly matched to the type of food, which might produce a similar response to a food shortage.

This idea of a difference in digestive requirements for the two food types is supported by the analyses of growth rate. We found a difference in growth rate between

diet types and this in turn was affected by the fish's parental type. *Daphnia*, although relatively similar in proximate composition (and hence energy content per unit wet mass) to Chironomid larvae (see methods section above), have a hard exoskeleton; this is likely harder to digest (Swaffar and O'Brien, 1996), compared to soft-bodied Chironomid larvae, so it was not surprising that fish grew faster on a diet of Chironomid larvae compared to those fed *Daphnia*. However, offspring of freshwater-resident brown trout were more able to maintain their growth on the *Daphnia* diet than were those of anadromous parents, so that the latter showed a greater fluctuation in growth rate following a switch in diet, indicating potential differences between offspring from the two types of parent in the ability to compensate for changes in diet type. Differences in growth efficiency between freshwater-resident and anadromous individuals have been demonstrated in previous studies. For example, Morinville and Rasmussen (2003) demonstrated that individual migrant brook trout had a lower growth efficiency in the year prior to migration compared to sympatric resident brook trout.

Lastly we found that the extent of the divergence in body shape induced by diet (as measured by Mahalanobis distance between individuals fed Chironomid larvae and *Daphnia*) was similar between offspring types for the first weeks of feeding (i.e. during interval one, the first ~111 days since first feeding, and interval two, the next ~56 days after the diet switch). However, while in offspring of anadromous trout the diet-induced change in shape was maintained at the same level (as indicated by a relatively constant Mahalanobis distance) for intervals two and three, the offspring of freshwater-resident trout fed on Chironomid larvae continued to diverge in shape over this time period from those fed on *Daphnia*. This suggests a greater plasticity in morphology in the offspring of freshwater-residents. Morphological flexibility in response to diet type is well documented and is generally related to an increase in efficiency of detection, capture and handling of prey items (Skúlason and Smith, 1995; Adams and Huntingford, 2002a) and is a primary driver behind the expression of alternative trophic phenotypes. For example Walls *et al*., (1993) demonstrated that larval eastern long-toed salamanders (*Ambystoma macrodactylum columbianum*) fed tadpoles and brine shrimp nauplii developed significantly broader and deeper heads compared to those only fed brine shrimp nauplii. While we cannot be sure that the morphological differences induced by the two diets in this study were adaptive, the fact that the type of offspring with the greater morphological flexibility (i.e. the offspring of freshwater residents) also showed a greater ability to maintain growth on the poorer prey type is suggestive of an adaptive response. One

potential explanation for the difference in morphological flexibility between offspring from alternative life histories is their contrasting requirements for niche shifts. Freshwater ecosystems are often regarded as being food-limited (Imre *et al.,* 2005), so requiring adaptability in diet choice; moreover, freshwater-resident trout tend to move into deeper and slower-flowing habitats as they get older (e.g. deeper pools in rivers, and often eventually lakes; Klemetsen *et al.*, 2003). These ontogenetic changes in diet and habitat likely both require changes to swimming capability and foraging mode (e.g. with the fish becoming less active as they increase in size), so selecting for the ability to remain morphologically flexible throughout ontogeny (to minimise energetic costs and maximise prey capture efficiency). Freshwater-resident individuals may thus benefit from morphological flexibility, since this would help maintain growth in the unproductive and changeable freshwater environment. In contrast, fish migrating to sea will continue to be actively swimming against strong currents and obtaining prey by pursuit foraging, in a highly productive environment that allows narrow dietary specialisations, so possibly selecting against morphological flexibility.

One potential caveat to our study is that we were unable to determine whether the differences between offspring phenotypic flexibility were primarily due to genetic or maternal effects, but this would be difficult to establish given that the resident-anadromous dichotomy by its very nature prevents the use of the standard approach of rearing the parents in a common garden to rule out maternal effects.

In conclusion, the results of this study suggest that offspring from freshwater-resident and anadromous parental life history strategies show some differences in phenotypic flexibility that may be consistent with the future habitats individuals may encounter, with offspring of migratory fish being apparently morphologically less flexible and less able to maintain growth on a poor quality diet. Therefore we suggest that genetic and parental effects affecting phenotypic flexibility may contribute to the differences in performance observed in a common environment and may play a role in the perpetuation of non-breeding partial migration within populations of brown trout.

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