# Environmental Variability and Intraspecies Diversification in Arctic Charr (*Salvelinus alpinus*)

by

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## Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners. I understand that my thesis may be made electronically available to the public.

## Abstract

Arctic charr (*Salvelinus alpinus*) exhibit what is arguably the greatest observed range of phenotypic and ecological diversity within a single vertebrate species. The patterns of adaptive diversity that have developed among populations over evolutionary timescales have been well studied in this species. However, much less is known about the patterns of diversification occurring within populations over contemporary timescales. To better understand the mechanisms involved in the processes of diversification and speciation, this thesis investigates the relationship between environmental variability and phenotypic diversity of Arctic charr over different spatial and temporal scales.

To examine the relationship between environmental variability and phenotypic diversity over contemporary time scales, we tested the prediction that shifts in the growth patterns of wild Arctic charr would coincide with temperature fluctuations over a 29-year period. A significant portion of the variation in size-at-age among cohorts of Arctic charr was explained by the cumulative lifetime temperatures, but temperature had mixed effects on growth observed within a single growing season. These changes in cohort growth patterns with temperature demonstrated that environmental fluctuations can have cumulative effects on growth dynamics over an individual's lifetime. However, the observed changes in growth were better explained by the combined coincident changes in species community dynamics, Arctic charr behavioral patterns, and exploitation rates and practices in the Northwest Atlantic region than by the direct effects of temperature fluctuations alone.

Phenotypic diversity over a small spatial scale was examined in the context of a polymorphic population of Arctic charr in a high-latitude lake. An unbiased, Bayesian analysis indicated the size-at-age data collected from Lake Hazen was best described by three discrete clusters of individuals. Life history, diet, and morphological characteristics observed for two of these clusters were consistent with previous descriptions of a cannibalistic and a benthivorous morph. The third cluster represented an early-maturing group that attained adult body sizes smaller than those reported for the previously identified forms. Although there are alternative explanations for the occurrence of distinct size-at-age groups within a fish population, polymorphism is the most consistent explanation for the combination of differences observed

in life history (growth and age-at-maturity), trophic ecology (diet), and morphology among the three groups of Arctic charr identified here. The unusual patterns of diversification observed within this population highlight some of the holes in our broader understanding of the mechanisms of diversification in Arctic charr and other species.

The effect of spatial variation in environmental characteristics on intrapopulation diversity was directly examined by testing the prediction that intraspecific competition and ecological opportunity promote morphological diversity within populations of Arctic charr. The relative importance of morphological variation to inter-individual differences in diet was also examined, by testing the prediction that the covariation between diet and morphological variation was greater in populations experiencing less interspecific competition and greater ecological opportunity. The patterns of morphological and diet variation observed in the eleven populations of Arctic charr examined here did not support the predictions tested, though the broader conclusions that can be drawn from this study are limited by the relatively small samples sizes available for analysis. This chapter is best viewed as a pilot project to developing a larger study to test these predictions, as it revealed a suite of potentially interactive effects on the extent of ecological and phenotypic variation within populations of Arctic charr.

Phenotypic diversity in Arctic charr and other northern fish species is typically linked with ecological diversity, which is increasingly being quantified using stable isotope data. The final data chapter included in this thesis is a methods-based study meant to compliment the use of stable isotopes as a tool for quantifying diet diversity within and among populations of Arctic charr. Specifically, this chapter tests whether the effects of ecological factors on the differences in $\delta^{13}C$ and $\delta^{15}N$ values between muscle and liver tissues were large enough to affect the biological interpretation of isotope data when reconstructing temporal diet patterns. Our results demonstrated that life history, diet specialization, reproductive status and gender had significant effects on the difference in stable isotope values between tissues. These factors appear to affect $\delta^{13}C_{(M-L)}$ and $\delta^{15}N_{(M-L)}$ values indirectly, due to their physiological impacts on metabolism and tissue composition. Most of the ecological factor effects observed here were not large enough to cause misinterpretation when examining major changes in diet, but could confound studies concerned with more subtle changes in omnivory.

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# Chapter 1 - Introduction

One of the central aims of evolutionary biology is to better understand the mechanisms involved in the process of speciation. A number of different models have been proposed to explain this process (Coyne & Orr 2004), but the work presented in this thesis will focus on mechanisms associated with ecologically-based models of speciation. Fundamental to ecological speciation models is diversification due to natural selection on traits associated with resource use and competition (Mayr 1942; Schluter 1996). In ecological modes of speciation, reproductive isolation is postulated to develop as a by-product of selection associated with differential resource use (Schluter 1996; Schluter 2000). One example of this would be assortative mating based on adaptive morphological differences, such as body size, that develop between groups that utilize different ecological niches (e.g. Schluter 1996).

Ecological speciation differs from other speciation models because it emphasizes the role of environmentally-based mechanisms in the processes of diversification and speciation (Schluter 1996; Schluter 2000). Therefore, an important step in understanding the process of ecological speciation is clarifying what environmental conditions support the creation and maintenance of intraspecific diversity. Several fish species found in recently deglaciated northern lakes exhibit high levels of intraspecific diversity (Robinson & Wilson 1994; Schluter 1996; Skúlason et al. 1999; Robinson & Parsons 2002), but include few examples of full speciation (e.g. Schluter & McPhail 1992). Because they appear to be in the early or intermediate stages of the speciation process, these fish have been used as a model system to study the effects of environmental mechanisms on diversification within and among populations (Schluter 1996; Skúlason et al. 1999).

The research presented in this thesis examines patterns of intraspecific diversity in one of these fish species found at high latitudes, the Arctic charr (*Salvelinus alpinus*). Among northern fishes, Arctic charr exhibit what is arguably the greatest observed range of phenotypic and ecological diversity within a single species (Klemetsen 2013). Arctic charr have a large geographical range, with a Holarctic distribution and a wider latitudinal range than any other northern fish species (Reist et al. 2006; 2013). Due to the range of environmental conditions experienced by these fish across their global distribution, populations of Arctic charr in different

lake systems can be thought of as replicated natural experiments exposed to different temporal and spatial patterns of environmental variation. Arctic charr are, therefore, an excellent species to use in testing hypotheses about the role of environmental conditions in the processes of diversification and speciation.

## INTRASPECIES DIVERSITY IN ARCTIC CHARR AND OTHER NORTHERN FISH SPECIES

Arctic charr exhibit a relatively wide range of phenotypic diversity for a single vertebrate species (Klemetsen 2010; 2013). This includes intraspecific variation in morphological, life history, and behavioral characteristics; which have all been shown to vary with ecological traits such as diet and habitat use (reviewed in Skúlason et al. 1999; Jonsson & Jonsson 2001; Klemetsen et al. 2003; Klemetsen 2010; Reist et al. 2013). Within this species, phenotypic differences are observed among populations from different lake systems (Alekseyev et al. 2002; Adams et al. 2007; Kristjánsson et al. 2011; Woods et al. 2013), as well as within polymorphic populations comprised of phenotypically and ecologically distinct morphotypes that coexist in the same lake (Skúlason et al. 1999; Jonsson & Jonsson 2001).

Intraspecies diversity is not uncommon in freshwater fish species inhabiting recently deglaciated northern lakes (Robinson & Wilson 1994; Schluter 1996; Skúlason et al. 1999; Robinson & Parsons 2002). Resource polymorphisms are especially common among these species (Skúlason & Smith 1995; Smith & Skúlason 1996), and similar patterns of resource-based diversification have been observed within and among populations of northern fish species (Robinson & Wilson 1994; Robinson & Parsons 2002). One of the most common patterns of diversification observed in northern lake systems occurs along a pelagic – littoral (or benthic) habitat axis. Distinct pelagic and littoral morphotypes have been documented in threespine stickleback (*Gasterosteus aculeatus:* Schluter & McPhail 1992; McPhail 1993), lake whitefish (*Coregonus clupeaformis:* Lindsey 1981), European whitefish (*Coregonus lavaretus:* Harrod et al. 2010), Eurasian perch (*Perca fluviatilis:* Svanbäck & Eklöv 2002), pumpkinseed sunfish (*Lepomis gibbosus:* Robinson et al. 1996; Gillespie & Fox 2003) lake charr (*Salvelinus namaycush*: Blackie et al. 2003; Chavarie et al. 2014; Muir et al. 2014), brook charr (*Salvelinus fontinalis:* Dynes et al. 1999), and Arctic charr (Jonsson & Jonsson 2001; Klemetsen et al. 2003). Common patterns of morphological differentiation associated with prey handling and foraging

in the pelagic and littoral habitats are also well documented among morphotypes and geographically isolated populations of these fish species (reviewed in Robinson & Wilson 1994; Skúlason & Smith 1995; Schluter 1996; Jonsson & Jonsson 2001; Robinson & Parsons 2002). Morphological differentiation in these fish species typically includes body and fin shapes common to individuals that forage in similar habitats, and head shape features associated with capturing and handling similar prey types.

Diversification along the pelagic-littoral axis is postulated to be a product of the geology and associated food web structure of recently deglaciated lakes (Smith & Skúlason 1996; Robinson & Parsons 2002). Pelagic and littoral areas are the dominant habitat types in most post-glacial lakes, with the exception of a few very large and very deep lakes that include a substantial profundal habitat (Robinson & Wilson 1994; Snorrason & Skúlason 2004; Klemetsen 2010). This common axis of divergence may also reflect the observation that the primary sources of productivity in most northern lakes occur in the pelagic and littoral habitats (Vadeboncoeur et al. 2002). It has been proposed that diversification of species into pelagic and littoral morphotypes is maintained by selection associated with inter- and intraspecific competition for prey resources between the two habitats (Robinson & Wilson 1994; Skúlason & Smith 1995; Skúlason et al. 1999; Robinson & Parsons 2002).

Less common than divergence along the pelagic–littoral axis is diversification associated with water column depth. Examples of deep-water morphotypes have been documented in lake whitefish (Lindsey 1981), European whitefish (Kahilainen et al. 2004; Harrod et al. 2010), lake charr (Muir et al. 2014), and Arctic charr (Klemetsen & Amundsen 1997; O'Connell & Dempson 2002; Knudsen et al. 2006). The few truly profundal morphotypes of Arctic charr that have been documented exhibit similarities in diet and adaptive morphological and life history traits (Klemetsen 2010; Knudsen et al. 2016). Profundal Arctic charr typically feed on invertebrates in the soft substrate of the profundal habitat, and exhibit the relatively robust head shapes consistent with bottom-feeding fish (Klemetsen 2010). These deep-water morphotypes also have the relatively drab or pale coloring typically associated with low-light habitats (Klemetsen 2010; Kekäläinen et al. 2010). Profundal Arctic charr reach relatively small adult body sizes, which is likely an energetic consequence of foraging in a relatively low-

productivity habitat (Klemetsen 2010; Fraser et al. 2008). Similar to the pelagic-littoral morphotypes, diversification into the profundal habitat is postulated to be maintained by inter- and intraspecific resource competition across different lake habitats (Knudsen et al. 2006).

Unlike many of the fish species mentioned above, diversification in Arctic charr also extends to the freshwater-marine axis. Anadromous and freshwater forms can be found within and among populations of a number of northern fish, though these are mainly limited to salmonid species (e.g. broad whitefish, *Coregonus nasus*: Harris & Taylor 2010; Atlantic salmon, *Salmo salar*: Verspoor & Cole 1989; brown trout, *Salmo trutta*: Hindar et al. 1991; Sockeye salmon, *Oncorhynchus nerka*: Wood & Foote 1996; brook charr: Thériault et al. 2007; lake charr: Swanson et al. 2010; and Arctic charr: Nordeng 1983; Rikardsen & Elliott 2000; Loewen et al. 2010). Though lacustrine and anadromous forms often occur in different systems, polymorphic populations containing anadromous and freshwater resident morphotypes have been observed in most of these species (see references above). The anadromous and resident forms differ consistently in some life history traits, with the resident morphotype reaching a smaller adult body size and maturing at a younger age than the faster growing anadromous form (Rikardsen & Elliott 2000). Among Arctic charr, anadromous populations show considerable variation in age at migration and time spent in the marine habitat (Tallman et al. 1996; Loewen et al. 2010). Anadromous populations occur throughout the distributional range of Arctic charr, though they occur less frequently at lower latitudes (Klemetsen et al. 2003; Reist et al. 2013).

The coexistence of anadromous and resident individuals within a population is predicted to be maintained by one of two alternative mechanisms. One prediction is that anadromous-resident polymorphisms are maintained by selection on traits associated with growth and heterochrony (Gross 1996). The second prediction is that anadromous and resident morphotypes are the products of a variable life history strategy among members of a single interbreeding group (Fleming 1998; Hendry et al. 2004). In the latter case, becoming an anadromous or resident type is conditional on individual juvenile growth rate, which may be closely associated with environmentally induced changes in prey availability (Byström 2006). Among the few studies that have tested these predictions using Arctic charr, two of them

support the prediction that anadromy is a conditional strategy (Nordeng 1983; Moore et al. 2014). Support for the prediction that resident and anadromous morphotypes represent distinct breeding groups has been observed in other species, including Sockeye salmon (Wood & Foote 1996), Atlantic salmon (Verspoor & Cole 1989), and threespine stickleback (Karve et al. 2008).

Another example of life history based diversification in Arctic charr is the occurrence of cannibalistic morphotypes. Cannibalistic forms of Arctic charr typically occur in species depauperate lakes with a smaller, early-maturing morphotype that serves as the primary prey source for their larger conspecifics (Amundsen 1994; Hobson & Welch 1995; Amundsen 2016). Cannibalism has also been observed in wild populations of other northern fish species, including European perch (*Perca fluviatilis*; Persson et al. 2004), northern Pike (*Esox lucius*; Persson et al. 2006), European whitefish (Skurdal et al. 1985), and brown trout (Vik et al. 2001). In Arctic charr, polymorphisms including a cannibalistic form typically occur in higher latitude lakes, where prey resources are less diverse and less abundant than those observed in more temperate systems (Amundsen et al. 1999; Amundsen 2016). The proposed mechanisms for the maintenance of co-occurring large cannibalistic and smaller, typically benthivorous Arctic charr are similar to those proposed for anadromous-resident polymorphisms. Cannibalistic Arctic charr are either maintained by divergent selection on life-history traits, or the product of a conditional strategy associated with growth dynamics within a single population (Hobson & Welch 1995; Byström 2006; Finstad et al. 2006). Although these predictions have not been tested directly, the small numbers of cannibalistic individuals observed in some populations and changes in the frequency of cannibals within populations over time provide indirect support for the latter prediction (Johnson 1983; Hobson & Welch 1995; Klemetsen et al. 2002; Byström 2006; Amundsen 2016). Common rearing and feeding trials, however, suggest the feeding behavior of cannibalistic individuals likely has a genetic basis (Amundsen et al. 1999; Svenning & Borgstrøm 2005).

Although many northern fish species exhibit considerable intraspecific diversity, Arctic charr are arguably the most diverse of these species (Klemetsen 2010). Not only have Arctic charr diversified along all the habitat and life history axes described above, but this species also

includes regionally and lake-specific morphotypes that may be unique to Arctic charr or, at the least, have yet to be documented in other species. One example is the small benthivorous form of Arctic charr observed in Icelandic lakes, that feeds primarily on gastropods (*Limnaea* spp.) that it forages for in the crevices created by the spatially complex lava substratum found in Icelandic lakes (Snorrason et al. 1994; Sigurdsteindottir & Kristjánsson 2005). This small morphotype is thought to be maintained by divergent selection on resource use and, because it co-occurs with a larger benthivorous morph in some lakes, spatial segregation of the benthic foraging habitat into surfaces and crevices (Snorrason et al. 1994; Skúlason et al. 1999). Another specialized form of Arctic charr seen in Iceland is a small, invertebrate-feeding form in groundwater springs formed by the porous rock of the neo-volcanic zone (Kristjánsson et al. 2012). Lake Aigneau, located in eastern Canada, supports a deep-water piscivorous morphotype of Arctic charr that is unusual in its feeding behavior (Power et al. 2009). Although it is morphologically similar to profundal and cannibalistic forms of Arctic charr observed elsewhere, the deep-water morphotype in Lake Aigneau fasts during the summer and feeds on fish that overwinter in the deeper waters of the lake (Power et al. 2009).

## WHY ARE ARCTIC CHARR SO DIVERSE?

Examples of ecological diversity in freshwater fish have been documented throughout the globe, and include such extreme examples as the fish species flocks found in African rift lakes (Kornfield & Smith 2000). By our current understanding and definitions of speciation (Coyne & Orr 2004), most of the ecological diversity observed at latitudes closer to the equator occurs among separate species (Hillebrand 2004; Mittelbach et al. 2007; Schluter 2016). In contrast, northern fish species exhibit a relatively high degree of intraspecific diversity in functional ecological roles without undergoing full speciation (though evidence of speciation has been observed in threespine stickleback; Schluter & McPhail 1992).

The phenotypic and ecological diversity observed in Arctic charr is associated with a range of genetic diversity and reproductive isolation within and among populations. Genetic analyses using microsatellite loci indicate that the degree of genetic differentiation among morphotypes of Arctic charr varies considerably within polymorphic populations (Gislason et al. 1999; Westgaard et al. 2004; Adams et al. 2006; Conejeros et al. 2008; Power et al. 2009), and among

geographically isolated populations within a region (Bernatchez et al. 1998; 2002; Adams et al. 2006; Gordeeva et al. 2010). There are also examples of genetically indistinct morphotypes observed within a population (Gislason et al. 1999; Arbour et al. 2011), as well as genetically distinct groups that have similar functional ecological roles within an ecosystem (Corrigan et al. 2011b).

Several hypotheses have been proposed to explain the relatively high degree of intraspecific diversity observed among freshwater fish species at higher latitudes. One prediction is that intraspecific diversity in high-latitude fish species is facilitated by high levels of ecological opportunity in recently deglaciated regions (Skúlason & Smith 1995; Schluter 2016). Recently deglaciated lakes tend to have a variety of unoccupied niches, and reduced interspecific competition for resources due to the low species diversity and relatively short time that the lakes have been open to invasion (Skúlason & Smith 1995; Skúlason et al. 1999; Schluter 2016). These conditions have likely allowed the few fish species in the region to diversify into different functional roles within ecosystems, leading to the development of polymorphisms in many of these northern lakes (Skúlason & Smith 1995; Smith & Skúlason 1996).

The availability of underexploited resources suggests a reason for greater diversification opportunities in northern lake systems (Schluter 2016), but this explanation does not address the mechanisms by which species were able to successfully invade and adapt to using a variety of different niches (Skúlason & Smith 1995; Skúlason et al. 1999). Several fish species that occur in northern lake systems show considerable phenotypic diversity, but exhibit relatively low amounts of genetic diversity (Bernatchez & Wilson 1998; Robinson & Parsons 2002). This led to the conclusion that the high levels of morphological and ecological variation observed within these species were due to unusually high levels of phenotypic plasticity (Robinson & Parsons 2002). Meta-analyses of morphological plasticity in northern freshwater fish support the hypothesis that phenotypic plasticity is adaptive in postglacial lakes, and has evolved in several fish species to maximize fitness under environmentally variable conditions (Robinson & Parsons 2002). Related to this, several sources proposed that phenotypic plasticity is the key mechanism by which northern freshwater fish species were able to successfully invade new

habitats and undergo rapid diversification (West-Eberhard 1989; Skúlason et al. 1999; Robinson & Parsons 2002). Specifically, variability in prey choice associated with plasticity in feeding behavior could be the first step in ecologically-based population diversification and the development of resource polymorphisms (Skúlason & Smith 1995; Snorrason & Skúlason 2004; Pfenning et al. 2010). Experimental studies using Arctic charr, specifically, revealed evidence of plasticity in morphological (Adams & Huntingford 2004; Peres-Neto & Magnan 2004; Alexander & Adams 2004; Parsons et al. 2011), behavioral (Andersson 2003; Svenning & Borgstrøm 2005; Corrigan et al. 2011a), and life history (Nordeng 1983; Klemetsen et al. 2002) traits in populations found throughout the range of this species.

These observations raise the question of what role the environment plays in the relatively high levels of phenotypic variability found in northern freshwater fish species. One general hypothesis is that intraspecies diversity increases with environmental variability. Support for this hypothesis has been suggested using the pattern of decreasing species richness and increasing intraspecies diversity as one moves away from the equator (MacArthur 1972; Dynesius & Jansson 2000). Several sources have suggested that one reason for the increase in species diversity as one moves towards the equator is the product of greater long-term climatic stability in the region, which allowed more time for speciation to occur (Fischer 1960, Ricklefs & Schluter 1993; Jansson 2003; Mittlebach et al. 2007). For instance, Dynesius & Jansson (2000) proposed that the latitudinal pattern in species richness could be attributed to differences in long-term environmental instability associated with Milankovitch climate oscillations. They postulated that the more frequent climatic fluctuations in Polar Regions resulted in stronger selection for generalism and dispersal ability, while specialization and more complex species interactions were favored in the more stable equatorial climate (Dynesius & Jansson 2000). This suggests that long-term climatic fluctuations influence the latitudinal gradient in species diversity by effectually interrupting the speciation process at high latitudes, as environmental instability leads to constant shifting of the phenotypic optima in populations at higher latitudes. Environmental variability over shorter time scales has also been predicted to favor the evolution and maintenance of phenotypic plasticity for similar reasons (Via & Lande 1985; Via 1993; Travis 1994; de Jong 1995; Robinson & Parsons 2002). Therefore, high levels of

contemporary environmental instability and long-term climatic fluctuations could at least partially explain the relatively high levels of phenotypic plasticity and intraspecific diversity in northern fish species.

Short-term environmental variability, historical climatic fluctuations, and spatial environmental differences could explain why Arctic charr exhibit such a wide range of phenotypic and ecological diversity. Arctic charr inhabit a wider variety of ecosystems across a larger part of the world than any other northern freshwater fish species (Klemetsen 2010). The Arctic charr has a circumpolar distribution and a wide latitudinal range, which extends further north than that of any other freshwater fish species (see map in Reist et al. 2013). If environmental and climatic variability increase with latitude (Dynesius & Jansson 2000; Robinson & Parsons 2002; Mittlebach et al. 2007), then Arctic charr should experience some of the highest levels of environmental and climatic instability among northern freshwater fish species. Similarly, Arctic charr should encounter a wide range of environmental diversity across ecosystems because of their large distributional range. This scenario can, therefore, be utilized as a natural experiment in diversification, with replicates (populations in different lakes) occurring along a spectrum of contemporary environmental conditions, historical climatic changes, and timescales of diversification.

Furthering our understanding of the effects of environmental variation on Arctic charr and other northern species has become more important in light of recent climate changes. Observations suggest species found at higher latitudes currently, and will continue to experience greater short-term climatic variation than those nearer the tropics (IPCC 2013). Since the 1950's, unprecedented rises in global air and sea surface temperatures, precipitation, sea level, and the concentrations of greenhouse gasses have been recorded throughout the globe (IPCC 2013). These climatic changes have escalated more rapidly in the Polar Regions, and are associated with observable decreases in the depth and duration of annual snow and ice cover, the shrinking of glaciers, and a marked decrease in the amount of multi-year sea ice in Arctic and sub-Arctic Canada (Brown & Lemay 2012; IPCC 2013). In lacustrine systems, increases in average annual temperatures are correlated with shorter periods of ice cover, a longer summer growing season, and stronger thermal stratification patterns (Rühland et al. 2003; Smol

et al. 2005). These limnetic changes are also predicted to have indirect physiological effects on the growth, metabolism, and timing of life-history events in northern fish species, and could lead to changes in species community and food web dynamics (Reist et al. 2006; Power et al. 2012).

Arctic charr have the potential to be a useful study species for modelling the implications of climate change, but also for learning more about the early stages of diversification and speciation. Differences in environmental conditions over the spatial distribution of Arctic charr can be used to model the temporal environmental changes predicted to occur as a result of the climatic changes described above. At the same time, the rapid environmental changes currently occurring in the Arctic and sub-Arctic regions present an opportunity to explore the very beginning stages of diversification.

## RESEARCH OBJECTIVES

The main objective of this thesis was to investigate the relationship between environmental variability and phenotypic diversity of Arctic charr over different spatial and temporal scales. The patterns of adaptive diversity that have developed among populations over evolutionary timescales have been well studied in this species. However, much less is known about the variation in phenotypic traits that exists within populations, and how patterns of intrapopulation variation are influenced by ecosystem-specific characteristics. Similarly, few studies have examined the cumulative effects of contemporary environmental changes on the population dynamics of Arctic charr. The following data chapters address these gaps in our knowledge about the extent of diversity in this species, and the environmental factors that influence it.

The research presented in this thesis is divided into four core data chapters (Ch. 2-5). Chapters 2,3, and 5 were prepared independently as manuscripts suitable for submission to peer-reviewed journals. Prior to completion of this thesis, Chapter 2 was published in volume 650 of the journal *Hydrobiologia*, Chapter 3 has been accepted for publication pending revision in the journal *Arctic*, and Chapter 5 was published in volume 22 of the journal *Ecology of Freshwater Fish*. The specific hypotheses addressed in each chapter are outlined below.

Chapter 2: *Changes in growth patterns of wild Arctic charr (*Salvelinus alpinus *(L.)) in response to fluctuating environmental conditions.*

The goal of this chapter was to test the prediction that shifts in the growth patterns of Arctic charr would coincide with temperature fluctuations over a contemporary time period. Several experimental studies have established relationships between temperature and growth in juvenile Arctic charr (Jobling 1983; Larsson & Berglund 1998; Larsson et al. 2005) and other salmonid species (Elliot 1994). However, few have examined the effect of environmental conditions on growth patterns in wild populations of these species. In Chapter 2, a cohort approach was used to examine the effect of annual changes in mean summer temperature on the growth of Arctic charr sampled from the Nain stock complex in northern Labrador over a 29-year period. This study examined temperature and growth changes over two time scales: (1) inter-annually, by testing the hypothesis that annual cohort growth rates were positively related to annual summer temperatures; and (2) cumulatively, by testing the hypothesis that size-at-age was positively related to the cumulative summer temperatures experienced by a cohort at a given age. The effects of coinciding changes in regional prey availability and fishing pressure on the growth of Arctic charr were examined indirectly, by testing the hypothesis that cohorts experiencing the regional shift in capelin distribution during the early 1990s were significantly smaller at age than cohorts experiencing similar temperature regimes in the years prior to this shift.

Chapter 3: *Evidence of a third morph of Arctic charr (*Salvelinus alpinus*) in Lake Hazen, Nunavut, Canada*

The study presented in this chapter examines the extent of phenotypic diversity in a high-latitude population of Arctic charr. Lake Hazen is one of the few sites in North America where a polymorphic population of lacustrine Arctic charr has been identified, and this chapter re-examines the question of how many distinct morphs of Arctic charr are present in Lake Hazen. A Bayesian cluster analysis was first used as an unbiased test of the prediction that more than one morph of Arctic charr was present in Lake Hazen. We hypothesized that if more than one morph is present, then variation in the size-at-age data of sexually mature Arctic charr

would be best described by multiple clusters. We also predicted that if the groups identified in the cluster analysis represented different morphs, they would also exhibit group differences in trophic ecology and morphological traits. Based on this prediction, we tested three hypotheses: 1) mean $\delta^{13}C$ and $\delta^{15}N$ stable isotope values would differ among the groups identified in the cluster anlaysis; 2) the average frequency with which prey types occurred in individual stomach contents would differ among the groups; and 3) average head, body, and fin morphology would also differ among the groups identified in the cluster analysis.

Chapter 4: *Does ecological opportunity and the absence of interspecific competitors promote intrapopulation diversity in Arctic charr?*

The purpose of this chapter was to examine the effect of different environmental conditions across a latitudinal gradient on the extent of diversity observed within populations of Arctic charr. Specifically, we tested the prediction that intraspecific competition and ecological opportunity promote diversity within populations (Araújo et al. 2011), focusing on two environmental factors that influence ecological opportunity. The first was habitat heterogeneity, which was approximated using lake size and access to the marine environment. The second was interspecific competition, qualified here as the presence or absence of other fish species. Three hypotheses regarding the effect of ecological opportunity on intrapopulation morphological variation in Arctic charr were tested: 1) populations of Arctic charr in lakes with no other fish species will exhibit greater variation in morphology than populations of Arctic charr that coexist with other fish species; 2) the magnitude of intrapopulation variation in morphology will increase with lake size; and 3) populations of Arctic charr in lakes with access to the marine environment will exhibit greater morphological variability than those in landlocked lakes. We also examined the effect of ecological opportunity on the relationship between diet and morphological variation within populations by testing three similar hypotheses: 1) the covariation between morphology and diet will be greater in populations of Arctic charr that do not coexist with other fish species; 2) the intrapopulation covariation between diet and morphology will increase with lake size; and 3) populations of Arctic charr

with access to the marine environment will exhibit stronger covariation between diet and morphology than those in landlocked lakes.

Chapter 5: *Ecological influences on the differences in $\delta^{15}N$ and $\delta^{13}C$ values between fish tissues: implications for studies of temporal diet variation.*

This chapter presents a methods-based study related to the overall theme of diversity in Arctic charr. Phenotypic diversity in Arctic charr and other northern fish species is typically linked with ecological diversity. Differences in niche use and ecological diversification within and among populations are primarily detected using dietary data. This includes the increased use of $\delta^{15}N$ and $\delta^{13}C$ values to quantify longer-term patterns of diet diversity in this, and other species. However, some aspects of the use of stable isotopes in ecological studies are not well understood. This chapter focuses on the limitations of $\delta^{13}C$ and $\delta^{15}N$ data obtained from muscle and liver tissue for use in studies of temporal diet changes. The primary goal of this chapter was to determine whether the effects of ecological factors on the differences in $\delta^{13}C$ and $\delta^{15}N$ values between muscle and liver tissues were large enough to affect the biological interpretation of isotope data when reconstructing temporal diet patterns. Specifically, the effects of the following factors on stable isotope differences between tissues were investigated using the ecological diversity found among Arctic charr in North America: 1) life history type (anadromous or lacustrine); 2) gender (male or female); 3) reproductive status (spawning or not spawning); 4) diet (piscivorous or not piscivorous); and 5) feeding status (actively feeding or fasting). The effect of differences in lipid content between tissues on $\delta^{13}C$ values was also tested, since there is a well-established relationship between tissue lipid content and $\delta^{13}C$ value, and several of these factors have been shown to affect lipid storage and metabolism in fish.

# Chapter 2 - Changes in growth patterns of wild Arctic charr (*Salvelinus alpinus* (L.)) in response to fluctuating environmental conditions

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## INTRODUCTION

As evidence supporting the occurrence of contemporary rapid climate change continues to grow, more attention is being focused on the response of organisms and ecosystems to climatic changes (McCarty 2001; Walther et al. 2002; Parmesan 2006). One particular area of concern is how changes in climate might affect organisms exploited for human use, such as the many aquatic species harvested for consumption as part of commercial and subsistence fisheries (McLean et al. 2001; Vilhjálmsson et al. 2005; Fischlin et al. 2007). Research in this area tends to focus on the long-term effects of temperature increases on the distribution or productivity of various fisheries stocks (e.g. Wood & McDonald 1997; Drinkwater 2005; Perry et al. 2005); however, short-term environmental variation related to global climate change may not always be associated with warmer temperatures. For example, although regional climate patterns may follow a general warming trend, the amplitude of regional temperature variability about the mean could also increase, resulting in anomalously cold periods along with warmer years (Furevik et al. 2002; Vilhjálmsson et al. 2005). The potential impacts of short-term temperature fluctuations on regional ecosystems should not be overlooked, as they could be as important to determining the viability of a fishery as those caused by systematic long-term trends.

The potential for short-term, regional, climatic variation to have significant ecosystem impacts has been well demonstrated by changes occurring in the Northwest Atlantic over the last 30 years. During this time, the Newfoundland and Labrador coastal region experienced some of the most dramatic temperature fluctuations since recording in the area began in the 1940s (Colbourne & Anderson 2003). This included a period of anomalously cold temperatures during the early 1990s followed more recently by some of the warmest conditions recorded for

the area (Drinkwater 1996; Colbourne 2004). Coincident with extreme fluctuations in oceanographic conditions, including temperature and salinity (Colbourne et al. 1997a; Hakkinen 2002; Belkin 2004), significant changes to the biota of the region also occurred. Many of the changes observed were unprecedented and are reviewed in several papers (Parsons & Lear 2001; Lilly & Carscadden 2002; Greene & Pershing 2007; Greene et al. 2008) including the Arctic Climate Impact Assessment (Vilhjálmsson et al. 2005). Briefly, significant changes occurred at several ecosystem levels, including a shift in the zooplankton community composition and biomass (Colbourne & Anderson 2003; Frank et al. 2005), as well as a shift in distribution and abundance of several key fish and invertebrate species, including Atlantic cod (*Gadus morhua*) (deYoung & Rose 1993; Rose et al. 1994; 2000), capelin (*Mallotus villosus*) (Frank et al. 1996; Carscadden et al. 2001; Rose 2005), snow crab (*Chionoecetes opilio*) (Frank et al. 2005) and northern shrimp (*Pandalus borealis*) (Lilly et al. 2000; Parsons & Colbourne 2000).

In addition to the impacts on marine species, changes were also observed in stock characteristics of anadromous Arctic charr (*Salvelinus alpinus*) in the Labrador region (Dempson 1995; Power et al. 2000; Dempson et al. 2002; 2008). In particular, significant relationships between climatic variables and the mean size and age of Arctic charr captured on the Labrador shelf were noted for exploited stock complexes in the region (e.g. Dempson 1995; Power et al. 2000; Dempson et al. 2008). Trends were established primarily between year-to-year variability in temperature and the mean size of charr in annual catches. Longer-lived species like the Arctic charr, however, may experience periods of both above and below average temperatures during their lifetimes, which can have differing cumulative impacts on growth (e.g. Jobling 1997; Larsson & Berglund 1998). Therefore, examining relationships between temperature and growth patterns over several years should provide further insight into the collective effects of climate change on growth, as well as the ability of Arctic charr to mitigate the effects of annual fluctuations in temperature.

The intent of this study is to use a cohort approach to examine the relationship between annual changes in mean summer temperature and the growth of Arctic charr sampled from the Nain stock complex in northern Labrador. Specifically, data assembled for the period 1977 to 2005 were used to compare relationships between temperature fluctuations and growth on

two time scales: (1) inter-annually, by testing the hypothesis that annual cohort growth rates were positively related to summer temperatures; and (2) cumulatively, by testing the hypothesis that size-at-age was positively related to the cumulative temperature experienced by a cohort at a given age. We also indirectly examined the effect of changes in prey type and availability on Arctic charr growth by testing the hypothesis that cohorts experiencing the regional shift in capelin distribution during the early 1990s were significantly smaller at age than cohorts experiencing similar temperature regimes in the years prior to this shift.

## METHODS

*Fishery and temperature data*

Arctic charr data examined in this study were obtained from the commercial fishery operating in the Nain region of northern Labrador (Nunatsiavut) during the period 1977-2005 (described in Dempson 1995; Dempson et al. 2008). The majority of charr captured as part of this fishery come from inshore areas near the community of Nain and its adjacent bays, and the fishing season generally runs from mid-June until mid-August of each year (Dempson 1995). Landings are comprised of a regional stock complex of anadromous Arctic charr (Dempson & Kristofferson 1987), which are recruited to the fishery beginning at age 6 (Dempson 1995). All landings are processed at the fish plant located in the community of Nain, where basic biological information, including length (mm), weight (g), and age (otoliths), are collected throughout the season. The data used in this study were collected from a subsample of the total annual catches for which otoliths were taken for aging, details of which are described in Dempson (1995) and Dempson et al. (2008). Since these fish are typically landed in the head-on, gutted form, all weight data presented here will be for gutted fish, rather than whole or round weights. The following analyses were limited to the 6-12 year age-classes as they were the only ages for which sufficient data were consistently available during the years 1977-2005.

Temperature fluctuations for the area encompassed by the fishery were approximated using average monthly sea-surface temperature (SST), measured at 10 m depth, obtained from the oceanographic database for Station 27 (47º31’50” N; 52º35’10” W). Located in the inshore branch of the Labrador Current, hydrographic information recorded at this monitoring station has been shown to reflect general trends and large-scale climatic variation in the northwest

Atlantic, including the Nain region (Petrie et al. 1991; Colbourne et al. 1994; 1997a). The Station 27 database is one of the most consistent, long-term time series of marine climatic information available for the coastal Labrador region, and has been used in numerous other studies to investigate responses of biota in the northwest Atlantic to variations in climatic and oceanographic conditions (e.g. de Young & Rose 1993; Hutchings & Myers 1994, Montevecchi & Myers 1997; Colbourne et al. 1997b; Power et al. 2000; Carscadden et al. 2001; Colbourne & Anderson 2003)

Anadromous Arctic charr obtain the bulk of their annual energy intake while in the marine environment, where they undergo a period of rapid growth that nearly ceases before returning to freshwater (Johnson 1980; Berg & Berg 1989; Rikardsen et al. 2000). Consistent with this, Power et al. (2000) noted that for the Nain stock complex, summer temperatures explained more of the variation in mean size of charr in annual catches than winter temperatures. Reflective of these observations, the following analyses consider only the effect of temperatures experienced by Arctic charr while at sea.

Arctic charr in the Nain region typically make their first marine migration at the age of 4, leaving inland waters during late May and early June and returning in late July and early August (Dempson 1995; Power et al. 2000). Accordingly, at-sea temperatures were quantified for the period of May 16 through August 15 for each year, beginning at age 4 for each cohort. As the Station 27 temperature database used here contained only mean monthly temperatures, mean annual summer temperatures were calculated as the weighted average of the mean monthly temperatures for May-August. A weighted average was used in order to account for Arctic charr moving between the freshwater and marine environment during May and August (weights of 0.5 were applied to May and August mean temperatures, while the full mean monthly temperatures (weight of 1) were used for June and July). Summer cumulative degree-days (CDD) > 0°C were computed as mean monthly temperatures multiplied by the number of relevant days considered for each month, (i.e. 15 for May and August, 30 for June, and 31 for July). Cohort-specific CDD data were then summed across all relevant years to obtain cumulative temperatures experienced at sea for each age-class within a cohort.

*Statistical analyses*

Three different metrics were used to investigate the relationship between temperature and growth over different time scales. First, to explore the influence of summer temperature on growth within a season, age-specific growth rates were examined in relation to the mean summer temperatures experienced over the same growth period for each cohort. Mean specific growth rate (SGR) was calculated using the mean size-at-capture data for ages 6-12 in each cohort:

$$SGR_{it} = \log_{10} S_{it} - \log_{10} S_{it-1}$$

where $S_{it}$ is the mean size (weight or length) at capture of Arctic charr in cohort *i* at age *t*, and $S_{it-1}$ is the mean size at capture for the previous age-class within the same cohort. The relationship between SGR and mean summer temperature was then assessed with covariance analysis, using age as a covariate to account for age-related effects on growth rate, as well as an interaction term to investigate differences in the effect of temperature on growth rates among age-classes:

$$SGR_{it} = \beta_0 + \beta_1(T_{it}) + \beta_2(t) + \beta_3(T_{it} \times t) + \varepsilon_{it}$$

where $T_{it}$ is the mean summer temperature during the year in which individuals belonging to cohort *i* were captured at age *t,* and the *β*s are coefficients of the fitted model. Bonferonni-corrected significance values were used for post-hoc comparisons of growth rate among the age-classes.

Cumulative effects of temperature on growth were assessed by examining the relationship between size-at-age (weight or length) and cumulative degree-days. Again, covariance analysis was used to assess this relationship. Age was again included as a covariate in the model to account for age-related differences in mean size, and an interaction term was included to investigate differences in the relationship between size and cumulative temperature among the age-classes:

$$\log_{10} S_{it} = \beta_0 + \beta_1(\log_{10} CDD_{it}) + \beta_2(t) + \beta_3(\log_{10} CDD_{it} \times t) + \varepsilon_{it}$$

where $S_{it}$ is the predicted mean size (weight or length) of Arctic charr in cohort *i* captured at age *t*, $CDD_{it}$ is the cumulative degree-days experienced by cohort *i* at age *t*, and the *β*s are

coefficients of the fitted model. $Log_{10}$ transformed values for size and CDD were used to improve the fit of the model.

The effects of prolonged periods of anomalous temperatures on cohort growth patterns were examined by comparing growth functions among cohorts experiencing different cumulative temperature regimes. To simplify the analysis, cohorts were first split into three temperature groups based on their experienced cumulative degree-days at age 12 ($ECDD_{12}$), the oldest age-class considered here. Temperature groups were divided as follows: "average" cohorts were within (±) one standard deviation of the mean number of $ECDD_{12}$ for all cohorts combined (mean ± SD = 5646.37 ± 301.41); "cold" cohorts had $ECDD_{12}$ values more than one standard deviation below the overall mean number of $ECDD_{12}$ (< 5343.96); and "warm" cohorts had $ECDD_{12}$ values greater than one standard deviation above the overall mean number of $ECDD_{12}$ (> 5946.79). Von Bertalanffy growth functions were fitted to the size-at-age data for each temperature group using the nonlinear regression function in SPSS, version 14 (2005) as follows:

$$S_t = S_\infty \left(1 - e^{-k(t-t_0)}\right)$$

where $S_t$ is the predicted size (length or weight) at age $t$, $S_\infty$ is the asymptotic size, $k$ is a growth coefficient that determines how fast the asymptotic size is obtained, and $t_0$ is the hypothetical age at which size is equal to zero (Ricker 1975). Differences in growth functions among the temperature groups were assessed using the residual sums of squares method described by Chen et al. (1992). In addition, variation in mean size-at-age among temperature groups was examined using covariance analysis of the form:

$$\log_{10} S_{gt} = \beta_0 + \beta_1(\log_{10} t) + \beta_2(g) + \beta_3(\log_{10} t \times g) + \varepsilon_{it}$$

where $S_{gt}$ is the mean size (weight or length) of fish in temperature group $g$ at age $t$, and the $\beta$s are coefficients of the fitted model. Again, Bonferonni-corrected significance values were used for post-hoc comparisons of mean size-at-age among the temperature groups.

Finally, to investigate the impact of ecosystem shifts that occurred in the northwest Atlantic during the early 1990s, growth patterns of cohorts within the average temperature group were compared. Cohorts within the average group were split into those having natal dates prior to 1980, which did not experience the anomalously cold period, and those born

after. Fitted von Bertalanffy growth curves and mean size-at-age were compared between the two groups as described for the temperature group comparison above.

All statistical analyses were performed using SPSS, version 14 (2005), and significance was established at $\alpha = 0.05$, unless otherwise noted.

## RESULTS

Using data from 16577 Arctic charr sampled from the Nain fishery, mean size-at-age histories were reconstructed for 23 cohorts with natal dates ranging from 1971-1993. Of these, 6 cohorts were assigned to the "cold" temperature group (mean ± 95% CI: $ECDD_{12}$ = 5299.99 ± 10.56), 4 cohorts were included in the "warm" group ($ECDD_{12}$ = 6145.28 ± 31.12), and the remaining 13 cohorts made up the "average" group ($ECDD_{12}$ = 5650.96 ± 29.12) (Fig. 2.1). The average summer sea-surface temperatures recorded at Station 27 for the period during which these cohorts were in the marine environment (1971-2005) fluctuated over a range of 5.07°C (Fig. 2.2).

A positive relationship between mean summer SST and mean SGR-at-age was observed, but only for growth measured as annual changes in weight (Table 2.1, Fig. 2.3). The interaction term included in this model was not significant, ($F_{(5,126)}$ = 0.23; $P$ = 0.950) indicating the relationship between $SGR_{(weight)}$ and mean summer temperature was similar among age-classes. The ANCOVA model fitted without the interaction term explained 39% of the variation observed in $SGR_{(weight)}$. Post-hoc comparisons revealed no significant differences in $SGR_{(weight)}$ among the 9-12 age-classes for all cohorts (Table 2.2) ($P > 0.05$ for all pairwise tests among ages 9-12), and a significant decline in $SGR_{(weight)}$ from age 7 to 9.

A significant positive relationship was observed between CDD and mean length-at-age, as well as mean weight-at-age (Table 2.3, Fig. 2.4). The slopes of these relationships did not differ significantly among age-classes, as indicated by the interaction terms (length: $F_{(6,147)}$ = 0.45, $P$ = 0.85; weight: $F_{(6,147)}$ = 0.35, $P$ = 0.91), suggesting the effect of temperature on size was similar across the range of ages examined here. After the interaction terms were removed from the models, age and CDD accounted for over 80% of the variation in length and weight (length: $R^2$ = 0.89; weight: $R^2$ = 0.81) (Table 2.3).

Von Bertalanffy growth functions fitted to the weight data differed significantly between the warm and average ($F_{(3,11598)}$ = 2.61, $P$ = 0.002) and the cold and average groups ($F_{(3,15113)}$ = 2.61, $P$ < 0.001), with the warm group having the highest asymptotic weight value while the cold group had the lowest (Table 2.4). When von Bertalanffy growth functions were fitted to the length data, the functions for the cold and average groups differed significantly ($F_{(3,15113)}$ = 2.61, $P$ < 0.001), but those fitted for the warm and average groups did not ($F_{(3,11598)}$ = 2.61, $P$ = 0.28) (Fig. 2.5). For this set of growth functions, the cold group had the lowest parameter estimate for asymptotic length. The relationship between mean size and age also differed among the three temperature groups, as indicated by the significant interaction term in the ANCOVA model (Table 2.5). Post-hoc comparisons among all three groups revealed the warm and average groups did not differ significantly in terms of length- ($P$ = 0.115) or weight-at-age ($P$ = 1.000) over the range of age-classes examined here. Consistent with the results of the von Bertalanffy growth function analyses, the average fish in the cold group was significantly shorter ($P$ < 0.001; $P$ = 0.001) and weighed less ($P$ < 0.001; $P$ < 0.001) at age than fish from either the average or warm groups.

Within the average temperature group, cohorts with natal dates before 1980 did not experience a significantly different number of mean CDD by age 12 than those with natal dates after 1980 ($F_{(1,89)}$ = 2.57, $P$ = 0.11), indicating both sets of cohorts experienced similar temperature regimes during their periods of marine residency. Nevertheless, the von Bertalanffy growth functions fitted to size-at-age data for these two sets of cohorts differed significantly (length & weight: $F_{(3,10140)}$ = 2.61, $P$ < 0.001), with the group of cohorts emerging after 1980 having lower estimated $S\infty$ values. In fact, the $S\infty$ values for the post-1980 emergent cohorts were lower than those estimated for the cold temperature group as a whole (Fig. 2.6). The mean size-at-age of the post-1980 set of cohorts was also significantly less than that for the pre-1980 set for the range of ages considered here (Table 2.6).

## DISCUSSION

The results of this study demonstrate short-term temperature fluctuations can have significant impacts on the growth patterns of an exploited anadromous Arctic charr stock. Within a single growing season, temperature had mixed effects on growth. Mean summer

temperature was not significantly related to inter-annual changes in length, and, combined with age, explained only 39% of the variation in inter-annual weight changes among cohorts. However, cumulative temperatures, again combined with age, explained over 80% of the variation in length- or weight-at-age among cohorts. These relationships suggest the influence of past environmental conditions on growth history may have a more important impact on stock characteristics than temperatures experienced in one growing season alone. Comparisons of growth functions among cohorts experiencing similar temperature regimes suggested differences in growth patterns could also be linked to ecosystem shifts associated with climatic changes.

The relationship between energy intake, growth, and temperature has been well established for juvenile salmonids in experimental situations (Elliot 1994; Jobling 1997; Larsson & Berglund 1998). These studies indicate there is a commensurate increase in the scope for growth with temperature, up to the temperature at which optimum growth occurs. Given that the sea-surface temperatures off the coast of Labrador (Colbourne & Foote 1997) are well below the optimum growth temperatures reported elsewhere for this species (13-18°C: Jobling 1983; Larsson & Berglund 1998; Larsson et al. 2005), Arctic charr exposed to warming temperatures in the Labrador region should have the potential to increase growth when there is sufficient prey (ration) available. However, the mean size-at-age observed for the warm temperature group was not significantly larger than the average group, despite a greater deviation in mean $ECDD_{12}$ between the warm and average groups than between the cold and average group. The cold temperature group, on the other hand, was significantly smaller in mean size-at-age than the average group. This marked difference in the response of growth patterns to the opposing cumulatively cold and warm temperatures is, therefore, unlikely solely reflective of physiological effects of temperature-driven changes in the scope for growth. Rather, differences in observed growth patterns are likely to be associated with a combination of factors that may also interact with temperature, such as prey availability.

From an energetics perspective, Arctic charr in the warm cohorts would only have been able to realize the expanded scope for growth associated with higher temperatures if energy in excess of basic metabolic and reproductive needs were available (Elliot 1994). In this case,

failure to realize the full potential of a temperature-driven increase in the scope for growth could reflect changes in the quality of prey types available and/or their relative abundances. Although we have no specific data on relative prey abundance for the Labrador region, feeding studies by Dempson et al. (2002; 2008) noted significant temporal shifts in the relative quantities of different prey items found in the stomachs of charr subsampled from the Nain fishery, which correspond with the observed changes in regional temperature and Arctic charr growth patterns. Capelin is the dominant prey item used by Arctic charr captured from the inshore area around Nain, comprising 52% of the total weight of all prey items found in the stomachs of charr sampled over the years 1974-1999 (Dempson et al. 2002). Beginning in 1991, however, capelin were all but absent from the stomachs of Arctic charr captured in this region, making up less than 10% of the weight of all food items found in charr sampled during 1991-1999. The disappearance of capelin from the diet of Arctic charr is coincident with a decline in capelin in the diet of seabirds in the region, including common (*Uria aalge*), and thick-billed murres (*Uria lomvia*) (Carscadden et al. 2002), as well as a southward shift in capelin distribution (Frank et al. 1996; Carscadden et al. 2001).

In place of capelin, Arctic charr fed mainly on sandlance (*Ammodytes* spp.), sculpin (*Triglops* and *Myoxocephalus* spp.), and hyperiid amphipods (Dempson et al. 2002). The use of alternative prey items by Arctic charr during this time may have also led to a change in energy budget, as capelin have a relatively high lipid content and energy density in comparison with other fish species, such as sandlance (Lawson et al. 1998). Coupled with possible changes in prey quality, the quantity of available prey may have also been reduced, as evidenced by a decline in the mean total stomach fullness index for Arctic charr reported by Dempson et al. (2002) for charr captured during the late 1980s through 1999. Recently, capelin returned to the diet of Arctic charr in the Labrador region, accounting for 40% (by weight) of the stomach contents sampled from fish caught inshore of Nain during 2003-2005 (Dempson et al. 2008). Dempson et al. (2008) also found that the stomach partial fullness index calculated for capelin (which relates the weight of a given prey item consumed to predator size) explained 66% of the variation in mean weight of charr caught during the period 1982-1999, thereby illustrating a potentially strong link between capelin consumption and the growth of these Arctic charr.

As a result of the temporal pattern in temperature fluctuations and capelin distributional shifts, cohorts from the cold and average temperature group that lived through the 1990s would have experienced a coincident decline in capelin abundance and temperature, while those in the warm group would have avoided the cooler marine temperatures but only experienced an increase in capelin abundance at older ages. The significant difference in mean size-at-age between cohorts in the average group born prior to 1980 and those born after, suggests the decline in capelin availability had a strong influence on Arctic charr growth independent of temperature. Therefore, the similarity in mean sizes between the warm and average groups is likely to be at least partially reflective of a similar energetic constraint. In this case, charr may have been unable to consume sufficient resources to meet the joint demands of higher metabolic costs associated with warmer temperatures and investment in growth and reproduction (Elliot 1994). The lack of increased growth during warmer conditions could also be explained by a trade-off between present reproduction and future growth in the face of energetic constraints (Roff 1992). Maturation occurs at approximately 7 years of age for at least one population included in the Nain stock complex (Dempson & Green 1985), suggesting the majority of individuals included in this analysis are sexually mature. However, because charr are landed in the head-on gutted form, there is insufficient data on gonad investment with which to test such a trade-off hypothesis using this stock complex.

The similarity in growth patterns observed between charr exposed to warm and average temperatures could also be explained behaviourally, in terms of thermal habitat selection. In experimental studies, juvenile Arctic charr demonstrate clear temperature preferences, moving into areas closer to their preferred temperature when given the opportunity (Peterson et al. 1979; Larsson 2005). A tagging study reported by Rikardsen et al. (2007) suggested anadromous Arctic charr also show some degree of temperature preference in the wild, as fish maintained a relatively narrow range of mean daily temperatures while in the marine environment (mean ± SD: 10.7°C ± 0.66). The temperatures experienced by Arctic charr, therefore, may not be reflective of those measured at a fixed point in the environment. As a result, studies like this one may be overestimating the variation in temperatures that fish are exposed to, especially during warmer years. If the preferred temperature for the Arctic charr in the Nain stock

complex is closer to that experienced by the average cohorts, for example, the impact of warmer sea-surface temperatures on growth may be less extreme than predicted as charr could seek refuge in cooler, deeper, waters closer to their preferred temperature. Under this scenario, the measured difference in $ECDD_{12}$ between the average and warm groups would be considerably less than that between the average and cold groups, as fish would be unable to move into warmer water during cold periods. A reduction in experienced temperature differences would partially explain why cumulatively cooler temperatures appear to have comparatively greater impacts on growth.

Since the fishery is size-selective, the effects of fishery-induced selection pressures on growth within the stock complex should not be ignored (i.e. Reznick & Ghalambor 2005; Conover & Munch 2002). As noted by Dempson et al. (2008), the mean weight of Arctic charr caught annually declined steadily from the mid-1970s to the mid-1990s, but has been increasing since. Fishing pressure for Arctic charr in this region also declined dramatically by the 1990s (Dempson et al. 2008). The long period of steady decline in mean weight could be a response to size-selective fishing mortality (Conover & Munch 2002), while the increase in size-at-age among later cohorts could be explained by the subsequent reduction in fishing pressure (Conover et al. 2009). However, significant temperature and ecosystem shifts occurred coincidently with these changes in fishing effort, and have similar predicted impacts on growth patterns. For example, the colder years coincided with the end of a long period of intense fishing pressure, which are both conditions expected to result in reduced growth. Considering the close association of these events, and the large degree of variation in the dataset, it is unlikely that shifts in mean size-at-age over time are due to changes in fishing pressure alone, but rather interactions among the coinciding changes in temperature, ecosystem dynamics, and fishing pressure.

Although there is a significant relationship between temperature and growth for the Nain Arctic charr stock complex, differences in growth patterns among cohorts are likely reflective of coincident changes in prey availability, fish behaviour, exploitation, and climate shifts. Indeed, various combinations of these factors have been associated with changes in the distribution and productivity of several other species in the Northwest Atlantic region (Mann &

Drinkwater 1994; Atkinson et al. 1997; Rose et al. 2000; Carscadden et al. 2001; Sinclair et al. 2002). Collectively, this suggests future climatic changes will have both direct physiological impacts on the growth patterns of Arctic charr, as well as indirect impacts on growth associated with ecosystem responses to climate-driven changes in environmental variables. These results also highlight the fact that significant changes in growth can occur in a relatively short period of time. As the management implications of the various factors considered here are vastly different, it is clear that more research is needed to understand the complex interactions among the various factors that influence the growth patterns of Arctic charr in the wild.

## TABLES & FIGURES

**Table 2.1**: Analysis of covariance results for the effect of age and mean summer temperature on specific growth rate (length and weight), including the common slope (*β*) of each model, as well as the *F*-values and significance (*P*) associated with each parameter.

| | *β* | *F(df)* | *P* |
|---|---|---|---|
| $\underline{SGR}_{(length)}$ | | | |
| *Temperature* | 0.00 | 2.45 *(1,131)* | 0.120 |
| *Age* | | 20.35 *(5,131)* | < 0.001 |
| $\underline{SGR}_{(weight)}$ | | | |
| *Temperature* | 0.01 | 9.04 *(1,131)* | 0.003 |
| *Age* | | 15.06 *(5,131)* | < 0.001 |

**Table 2.2:** Mean $SGR_{(weight)}$ for each age class (± 95% CI). Pairwise comparison results are indicated by superscripts, with age classes that do not significantly differ in mean $SGR_{(weight)}$ having the same letter.

| **Age Class** | **Mean $SGR_{(weight)}$** |
|---|---|
| 7 | $0.11 \pm 0.04^{a}$ |
| 8 | $0.09 \pm 0.02^{a,b}$ |
| 9 | $0.05 \pm 0.02^{b,c}$ |
| 10 | $0.02 \pm 0.02^{c}$ |
| 11 | $-0.01 \pm 0.02^{c}$ |
| 12 | $0.01 \pm 0.03^{c}$ |

**Table 2.3:** Analysis of covariance results for the effect of age and CDD on mean cohort length and weight, including the common slope ($\beta$) of each model, as well as the *F*-values and significance (*P*) associated with each parameter.

| | $\beta$ | *F(df)* | *P* |
|---|---|---|---|
| Length | | | |
| $log_{10}CDD$ | 0.20 | 27.00 *(1,153)* | < 0.001 |
| *Age* | | 12.15 *(6, 153)* | < 0.001 |
| Weight | | | |
| $log_{10}CDD$ | 0.82 | 35.50 *(1, 153)* | < 0.001 |
| *Age* | | 10.51 *(6, 153)* | < 0.001 |

**Table 2.4:** Von Bertalanffy growth function parameters (± 95% CI) estimated for the average, cold, and warm temperature groups, and for cohorts in the average temperature group with natal dates before and after 1980.

| **Group** | $L_\infty$ | $k$ | $t_0$ |
|---|---|---|---|
| Average | 565 ± 4 | 0.51 ± 0.05 | 3.33 ± 0.29 |
| <1980 | 569 ± 5 | 0.50 ± 0.06 | 3.22 ± 0.37 |
| >1980 | 535 ± 9 | 0.62 ± 0.14 | 3.63 ± 0.57 |
| Cold | 562 ± 10 | 0.43 ± 0.08 | 2.73 ± 0.56 |
| Warm | 571 ± 12 | 0.46 ± 0.10 | 3.15 ± 0.64 |

| **Group** | $W_\infty$ | $k$ | $t_0$ |
|---|---|---|---|
| Average | 1701 ± 34 | 0.55 ± 0.08 | 4.84 ± 0.24 |
| <1980 | 1728 ± 38 | 0.55 ± 0.09 | 4.80 ± 0.30 |
| >1980 | 1440 ± 57 | 0.71 ± 0.22 | 4.94 ± 0.42 |
| Cold | 1616 ± 69 | 0.48 ± 0.11 | 4.55 ± 0.38 |
| Warm | 1831 ± 105 | 0.47 ± 0.13 | 4.95 ± 0.38 |

**Table 2.5:** *F*-values and significance (*P*) for each factor and interaction term included in the covariance analysis used to test for differences in mean size-at-age among temperature groups.

| | *F(df)* | *P* |
|---|---|---|
| Length | | |
| *Group* | 14.87 *(2,16571)* | < 0.001 |
| *Age* | 2985.29 *(1,16571)* | < 0.001 |
| *Group x Age* | 11.90 *(2,16571)* | < 0.001 |
| | | |
| Weight | | |
| *Group* | 22.07 *(2,16571)* | < 0.001 |
| *Age* | 1746.27 *(1,16571)* | < 0.001 |
| *Group x Age* | 21.14 *(2,16571)* | < 0.001 |

$R^2$: length = 0.23; weight = 0.15

**Table 2.6:** *F*-values and significance (*P*) for each factor included in the covariance analysis used to test for differences in mean size-at-age between the sets of cohorts within the average temperature group with natal dates before and after 1980.

| | *F(df)* | *P* |
|---|---|---|
| Length | | |
| *Group* | 16.94 *(1,88)* | < 0.001 |
| *Age* | 276.75 *(1,88)* | < 0.001 |
| | | |
| Weight | | |
| *Group* | 11.87 *(1,88)* | 0.001 |
| *Age* | 147.56 *(1,88)* | < 0.001 |

$R^2$: length = 0.77.; weight = 0.64

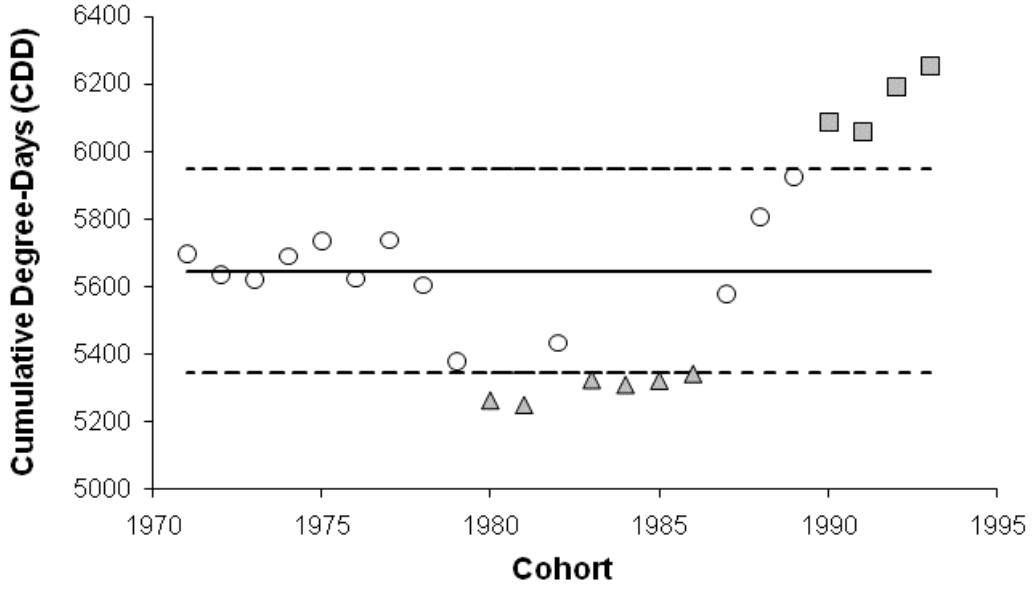


**Figure 2.1:** Cumulative degree days experienced at sea for each cohort at age 12, grouped as: average = O, cold = ▲, and warm = ■. Horizontal lines depict the mean $ECDD_{12}$ value for all cohorts ± one SD. Cohorts are plotted by natal year.

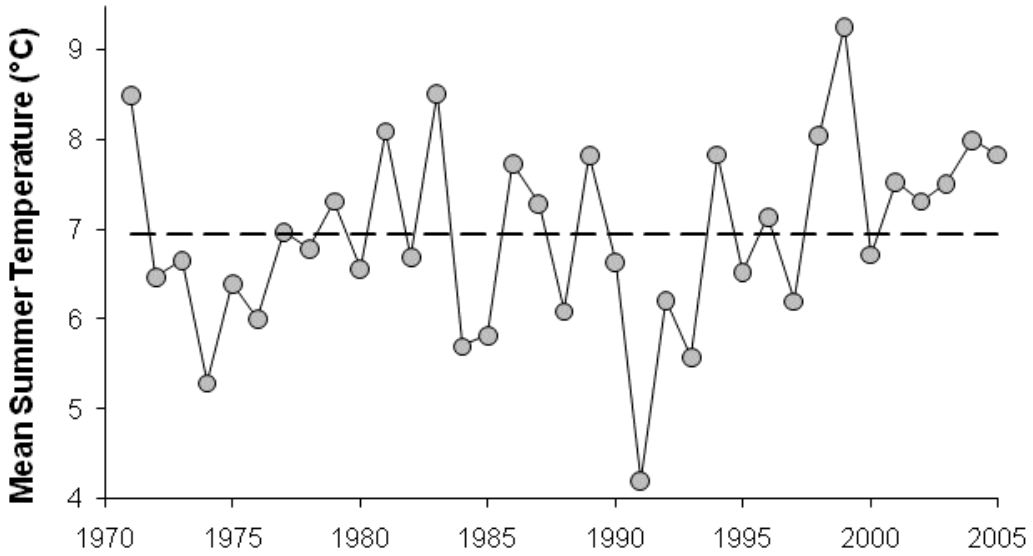


**Figure 2.2:** Mean summer sea surface temperatures (measured at 10m depth), recorded at Station 27. The horizontal line depicts the overall mean SST (6.96°C) recorded during the period shown.

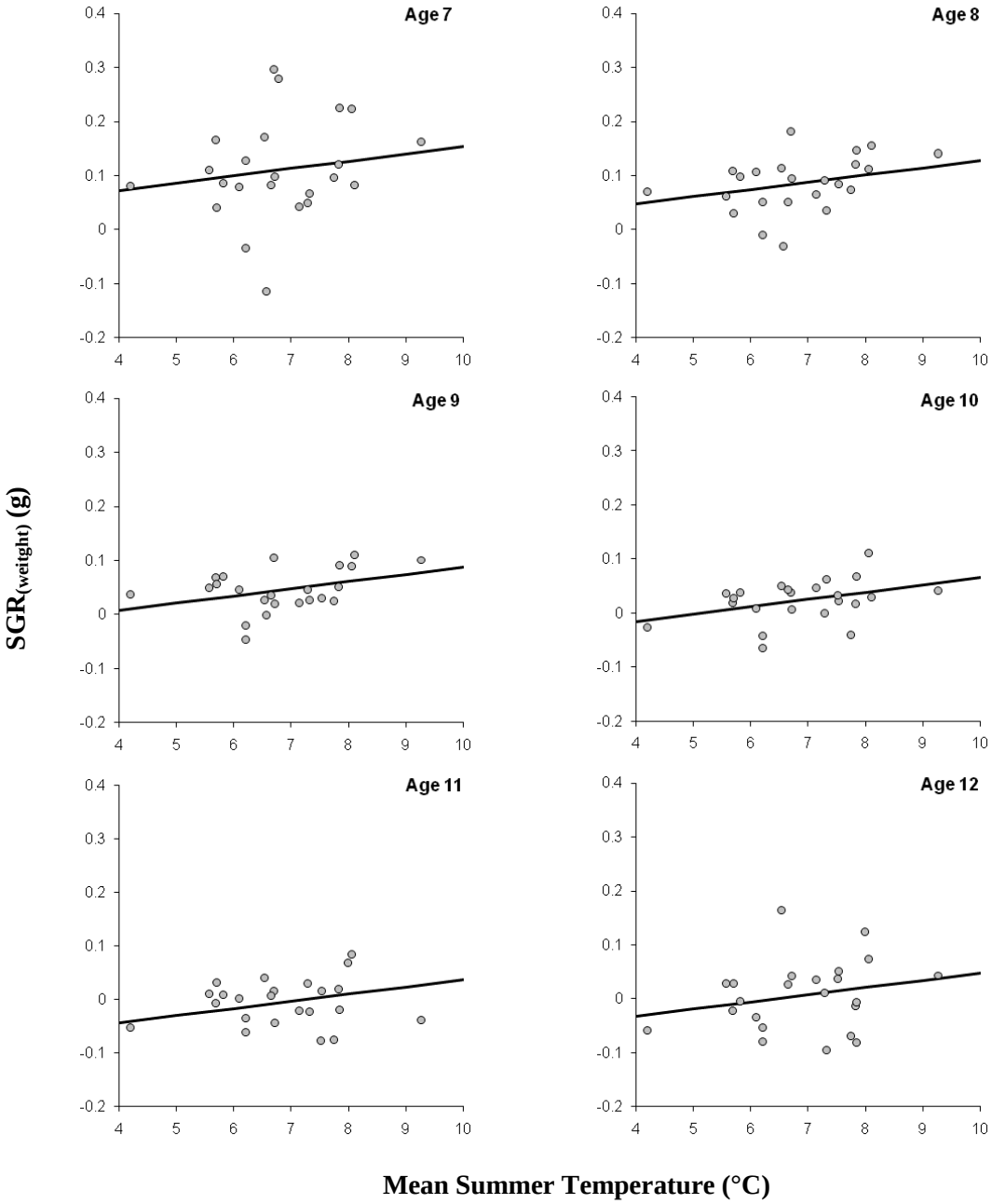


**Figure 2.3:** Specific growth rate (weight) at age for the mean summer temperature during the year over which growth occurred. Lines depict the predicted values of $SGR_{(weight)}$ over mean summer temperatures observed for the six age classes considered.

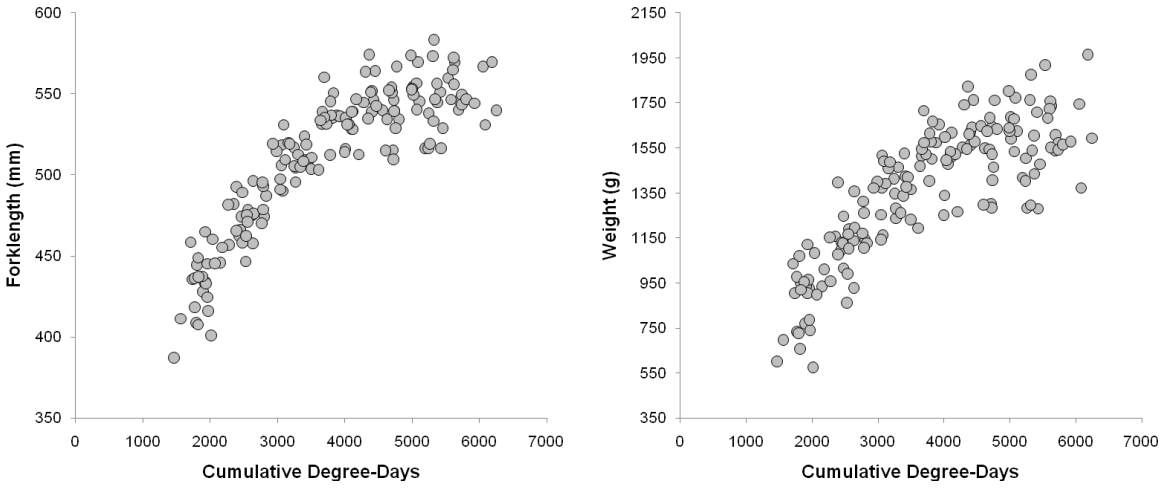


**Figure 2.4:** Mean cohort size-at-age and associated cumulative degree-days for all cohorts.

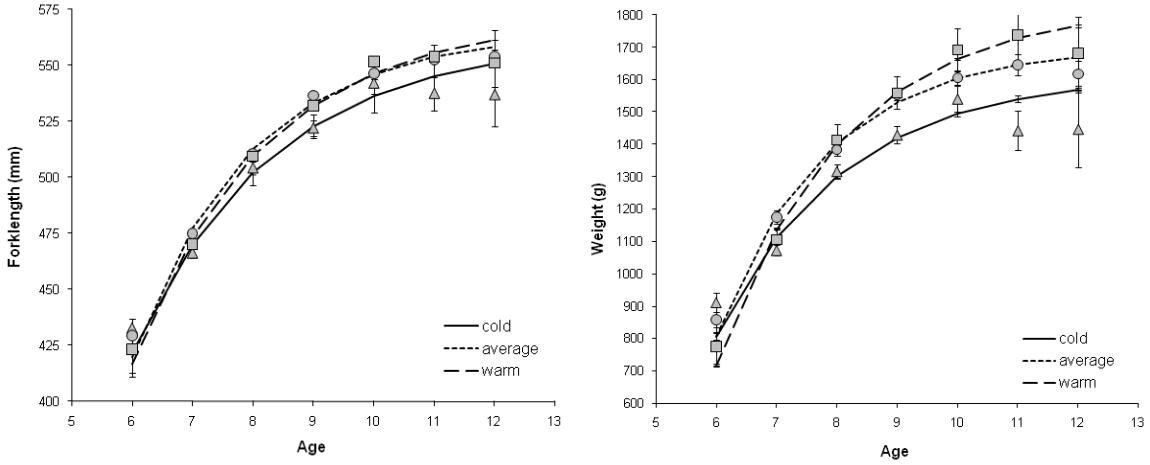


**Figure 2.5:** Mean size at age ± 95% CI for each temperature group (▲ = cold, ● = average, and ■ = warm), overlaid with the fitted von Bertalanffy growth functions.

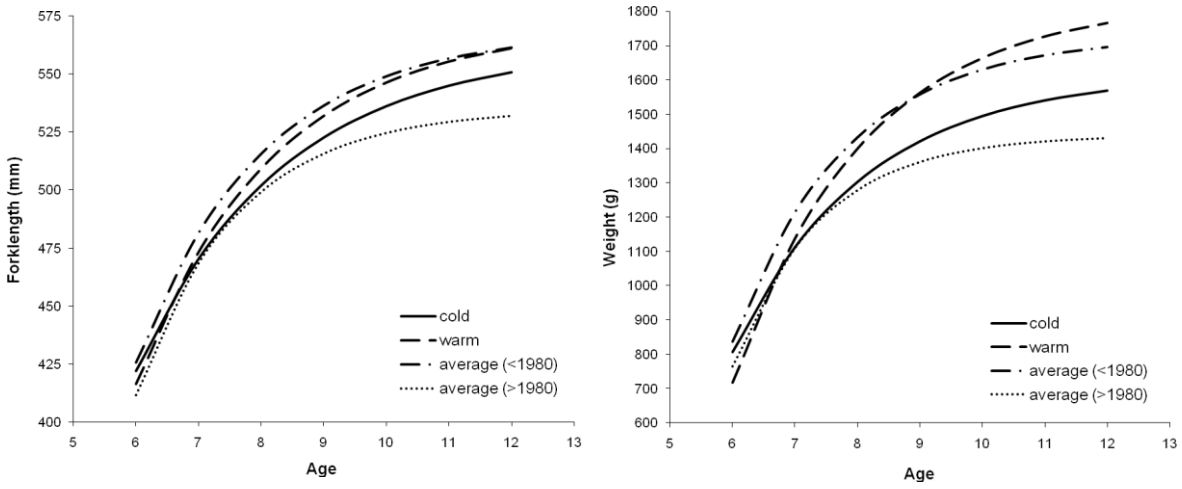


**Figure 2.6:** The fitted von Bertalanffy growth functions for the average group split into cohorts with natal dates before 1980 (<1980) and after (>1980), plotted with the functions for the warm and cold temperature groups.

# Chapter 3 - Evidence of a third morph of Arctic charr (*Salvelinus alpinus*) in Lake Hazen, Nunavut, Canada

## INTRODUCTION

Resource polymorphisms are defined as the occurrence of discrete phenotypic groups within a population that exhibit differential resource use (Skúlason & Smith 1995; Smith & Skúlason 1996). Polymorphic populations have been observed in many taxa, including freshwater and anadromous fish species in recently (~10-15 ka BP) deglaciated regions of the Northern Hemisphere (Robinson & Wilson 1994; Skúlason & Smith 1995; Robinson & Parsons 2002). Intraspecific competition in systems where resources are limited and alternative resources are available for exploitation is postulated to be an important factor in maintaining polymorphisms (Smith & Skúlason 1996; Bolnick 2004; Martin & Pfenning 2009). Phenotypic differentiation among morphs is maintained by divergent selection on traits that allow individuals to exploit alternative resources within the same system (Robinson & Parsons 2002; Adams et al. 2003; Garduño-Paz & Adams 2010). Population divergence may then occur if there is assortative mating among groups that use different resources (West-Eberhard 1989; Dieckmann & Doebeli 1999; Skúlason et al. 1999).

Among fish species in recently deglaciated northern lakes, polymorphic populations tend to differentiate along axes defined by foraging habitat (typically benthic or limnetic), and prey size or hardness (typically vertebrate or invertebrate prey) within those habitats (Skúlason & Smith 1995; Robinson & Parsons 2002). Although typically referred to as "morphs," the phenotypic differences among fish that utilize distinct dietary niches include morphological, life history, and behavioral traits. Differences in head shape, mouth orientation, fin lengths, and relative size of the caudal portion of the body have been linked to functional trade-offs in habitat-specific foraging; while gape size, head depth, gill raker length, and gill raker spacing are associated with the relative efficiency of consuming differently sized prey (Adams & Huntingford 2002; Svanbäck & Eklöv 2003; Garduño-Paz & Adams 2010). Differences in growth patterns and the timing of sexual maturation among morphs are thought to develop due to the per capita energy gain from feeding on vertebrate or invertebrate prey, and are reinforced by

selection associated with size-related foraging efficiency (Finstad et al. 2006; Fraser et al. 2008). Morphs that occupy benthic and limnetic niches also exhibit differences in foraging behavior (Adams et al. 2003; Garduño-Paz & Adams 2010).

Among northern fish species, the Arctic charr (*Salvelinus alpinus*) exhibits a high degree of intraspecific phenotypic diversity, including populations containing as many as four distinct morphs (reviewed in Jonsson & Jonsson 2001; Klemetsen 2010; Reist et al. 2013). In North America, populations containing two lacustrine morphs have been documented in Gander Lake, Newfoundland (Power et al. 2005; Gomez-Uchida et al. 2008), Lake Aigneau, Québec (Power et al. 2009), Lower Tazimina Lake, Alaska (Woods et al. 2013; May-McNally et al. 2015), and Lake Hazen, Nunavut, Canada (Reist et al. 1995; Arbour et al. 2011). Of these lakes, Hazen is the most recently deglaciated at approximately 5 ka BP (Smith 1999), and Arctic charr are the only fish species reported to occur in this lake (Reist et al. 1995; Christiansen & Reist 2013)

Past studies of Arctic charr from Lake Hazen suggest there are at least two phenotypically distinct groups within the population (Reist et al. 1995; Guiguer et al. 2002; Gallagher et al. 2009; Arbour et al. 2011). Based on morphological, diet, and life history data, this polymorphic population includes a cannibalistic and a benthivorous morph. These morphs were referred to as the "large" and "small" morphs by Reist et al. (1995), in reference to the larger size-at-age of the cannibalistic Arctic charr. Examination of individual diet and morphological characteristics suggested the large morph adults in Lake Hazen fed on conspecifics in the pelagic habitat, while the small morph fed mainly on chironomid larvae and pupae in the benthic habitat (Reist et al. 1995; Guiguer et al. 2002; Gallagher et al. 2009; Arbour et al. 2011).

The present study re-examines the question of how many morphs of Arctic charr are present in Lake Hazen, using a larger dataset of morphological, life history, and diet data than has been published previously. First, we used a Bayesian cluster analysis to test the prediction that more than one morph of Arctic charr was present in Lake Hazen. We hypothesized that if more than one morph is present, then variation in the size-at-age data of sexually mature Arctic charr would be best described by multiple clusters. We then predicted that if the groups identified in the cluster analysis represented different morphs they would also exhibit group

differences in trophic ecology and morphological traits. Hence, we hypothesized that differences in mean $\delta^{13}C$ and $\delta^{15}N$ stable isotope values, and the average frequency with which prey types occurred in individual stomach contents would differ among the groups. We also tested the hypothesis that groups identified in the cluster analysis would differ in average head, body, and fin morphology.

## METHODS

### *Site Description*

Lake Hazen (81°50'N, 70°25'W) (Fig. 3.1), is the largest lake in North America located completely north of the Arctic Circle. It has a surface area of approximately 538 $km^2$ (Inland Waters Directorate 1973) and a maximum observed depth of 267 m (Köck et al. 2012). The lake is fed primarily by glacial meltwater streams along its northwestern shore (France 1993), and has one main outlet, the Ruggles River, which flows into the Arctic Ocean. The diversity of organisms in Lake Hazen is relatively low compared to temperate and subarctic lakes, as there is little phytoplankton or zooplankton productivity within the lake, no macrophytes (McLaren 1964; Johnson 1990; Keatley et al. 2007), and few recorded macroinvertebrate species (mainly chironomids; Oliver 1963). The Arctic charr is the only fish species known to occur in Lake Hazen and in northern Ellesmere Island (Christiansen & Reist 2013). Although Lake Hazen has an outlet to the sea (Ruggles River, Fig. 3.1), it is thought that all Arctic charr in the lake are non-anadromous (Babaluk et al. 1997; Babaluk et al. 2001).

### *Sample Collection*

The data included in this study were obtained from Arctic charr collected during seven sampling events in Lake Hazen between 1981 and 2008 (Table 1). Sampling conducted in 1990 and 1992 occurred during May and June, while the lake was still fully ice-covered. During the remaining years, sampling took place in July and August, after ice breakup. Arctic charr were captured at several sites along the shoreline of Lake Hazen using gillnets or by angling (Fig. 3.1; 3.2). Multifilament nylon gillnets with mesh sizes ranging from 19 mm to 70 mm bar mesh were used in 1981, whereas multi-panel, multifilament nylon gillnets with mesh sizes ranging from 10 mm to 60 mm bar mesh were used from 1992 to 2008.

*Life History*

Individual ages were estimated by examination of sagittal otoliths prepared using either a break and burn technique (1981-2001 samples), or by thin-sectioning (1981, 2007, 2008 samples) (Chilton & Beamish 1982) (these techniques produce comparable age estimates: Campana et al. 2008; R. Wastle, Fisheries and Oceans Canada, personal communication 2013). Gender was assessed by visual inspection of the gonads, and maturity status categorized according to the index presented in McGowan (1987). This study was limited to individuals identified as being sexually mature, in order to avoid confusing group differences associated with polymorphism with ontogenic changes.

A model-based, Bayesian cluster analysis of the size-at-age data was used to test the prediction that more than one morph of Arctic charr occurred in Lake Hazen. The model-based cluster analysis in the R (v. 2.15.1, R Core Team 2012) package Mclust (v. 4.4, Fraley et al. 2012), was chosen for this analysis because it is unbiased to preconceived groupings. Mclust uses the Bayesian Information Criterion (BIC) to identify the number and shape of clusters that "best" describe variation in the dataset, rather than constraining the number of groups *a priori*.

*Diet*

Diet data were obtained for a subset of the Arctic charr collected during 1992 ($n$ = 49), 2007 ($n$ = 26), and 2008 ($n$ = 201). Individual $\delta^{13}C$ and $\delta^{15}N$ stable isotope values were obtained from dorsal muscle tissue removed from the left side of each fish, just posterior to the dorsal fin and above the lateral line. All Arctic charr stable isotope samples collected in 2007 and 2008 were processed as described in Chapter 5 (Michaud et al. 2013), and those obtained in 1992 were processed as described in Guiguer et al. (2002). For comparison with the Arctic charr isotopic data, $\delta^{13}C$ and $\delta^{15}N$ values were obtained for a representative set of invertebrate prey items. Due to low abundances and limited sampling opportunities, not all of the common prey types could be collected on site. Therefore, all stable isotope values reported here are from prey organisms collected from Arctic charr stomach contents and aggregated to produce samples large enough for analysis. Only minimally digested specimens were selected from the

foregut to reduce the impact of fractionation due to digestion on $\delta^{13}C$ and $\delta^{15}N$ values (Guelinckx et al. 2008). All samples removed from stomach contents were rinsed with deionised water prior to drying to minimize contamination by other digestive tract material (Ponsard & Averbuch 1999). Analyses of all carbon ($^{13}C$:$^{12}C$) and nitrogen ($^{15}N$:$^{14}N$) isotopic ratios were completed at the University of Waterloo Environmental Isotope Lab, using a Finnigan Deltaplus continuous flow stable isotope ratio mass spectrometer (Thermo Finnigan Scientific, Bremen, Germany) coupled to a Carlo Erba Elemental Analyzer (CHNS-O EA1108, Carlo Erba, Milan, Italy). Working internal laboratory standards were calibrated against the International Atomic Energy Agency standards $CH_6$ for carbon and $N_1$ and $N_2$ for nitrogen and run as controls throughout the analysis to ensure the continued accuracy of all measurements (± 0.2 ‰ for $\delta^{13}C$ and ± 0.3 ‰ for $\delta^{15}N$).

To test the hypothesis that the three groups of Arctic charr identified in the cluster analysis differed trophically, analysis of variance (ANOVA: Fisher 1918; Zar 1999) was used to determine whether the mean $\delta^{13}C$ and $\delta^{15}N$ values were similar among the groups. To account for the potentially confounding effects of multiple sampling years and sites on stable isotope values, a three-way ANOVA including group, site, and year of collection as factors was run in SPSS (v. 17.0, SPSS Inc. 2008). If the interactions between group and year, or group and site did not explain a statistically significant portion of the variation in stable isotope values, the confounding factors were removed from the model in a stepwise fashion. Tukey's pairwise comparisons (Tukey 1949; Zar 1999) were then used to test for differences in stable isotope values between groups. When Levene's test (Levene 1960) indicated heteroscedasticity among the groups, Tamhane's T2 multiple comparisons tests (Tamhane 1977) were used instead of the Tukey's pairwise comparisons.

Stomach contents were examined to substantiate diet differences suggested by the stable isotope data. The most common prey items observed in stomach contents were divided into five taxonomic categories: Copepoda, Chironomidae larvae, Chironomidae pupae, Hydracarina, and Arctic charr (fish remains). Other prey items, including cladocera, ostracods, as well as terrestrial invertebrates and plant material were observed, but consumed by fewer than 5% of all individuals and, therefore, considered incidental and not included in the

comparisons among groups. Individuals with empty stomachs were not included in the analyses. The relative abundance of specific prey types in the stomach contents of individual Arctic charr could not be quantified due to the partial degradation of smaller organisms, particularly zooplankton. Instead, only the presence or absence of each prey type was recorded for individual fish. The relative reliance on different prey organisms by the Arctic charr morphs was then quantified as the frequency of occurrence of each prey type in the stomach contents of individuals from the groups identified in the cluster analysis. Differences in the frequency of occurrence of each prey type among the groups were tested with Fisher's exact probability tests of 5x3 contingency tables (Fisher 1922) using the "R Commander" statistical package in R (Rcmdr: Fox 2005). When significant differences were noted among groups for a given prey category, pairwise comparisons adjusted using Holm's procedure (Holm 1979) were made between groups.

*Morphology*

The hypothesis that the three groups of Arctic charr identified in the cluster analysis were morphological distinct was tested using morphological data obtained from photographs taken of a subset of Arctic charr captured in 1992 ($n$ = 49), 2007 ($n$ = 23) and 2008 ($n$ = 192). Two-dimensional head and body shape were summarized using coordinate values for 16 homologous landmarks (Bookstein 1991) and 14 semi-landmarks (Bookstein 1997) obtained from digital images using the program TpsDig (version 2.16, Rohlf 2010). To obtain coordinate values for the semi-landmarks, the outline of the head was traced between homologous landmarks 2 and 9, and 10 and 19 (Fig. 3.3A) using the "draw curves" tool in TpsDig. The "resample curve" module in TpsDig was then used to resample the drawn curves to set semi-landmarks at equidistant intervals between the homologous landmarks. The XY coordinate values for the semi-landmarks were then obtained using the program TpsUtil (version 1.52, Rohlf 2012). Landmarks 1, 2, 9, and 19-31 (Fig. 3.3A) were used to define body shape, while landmarks 1-19 (Fig. 3.3A) were used to define head shape. Prior to any analyses, the "unbend specimens" procedure in TpsUtil was used to remove some of the shape variation attributable to dorsal-ventral curvature of the fish caused by post-mortem stiffening of the body (the "arching effect" described in Valentin et al. 2008). Landmarks 2, 31, 32, 33, and 34 (Fig. 3.3A)

were used to define the curve along which the individual specimens were "unbent" in this procedure.

Relative warp scores and centroid sizes were calculated separately for head and body morphology in TpsRelw (version 1.49, Rohlf 2010). Discriminant functions analyses of the relative warp scores were then conducted in SPSS to summarize differences in head and body morphology among the Arctic charr groups identified in the cluster analysis. To test for differences in allometry and sexual dimorphism among the groups, an ANCOVA model (Bailey 1931; Zar 1999) of the following form was run in SPSS:

$$DF_{ijk} = \beta_0 + \beta_1(G_i) + \beta_2(S_i) + \beta_3(C_{ijk}) + \beta_4(G_i \times S_i) + \beta_5(G_i \times C_{ijk}) + \varepsilon_{ijk}$$

where $DF_{ijk}$ is the discriminant function score of individual $i$ in group $j$ and sex $k$, $G$ is the categorical factor group, $S$ is the categorical factor sex, $C$ is the covariate centroid size, and $\varepsilon_{ijk}$ is the error term. Tukey's adjusted pairwise comparison tests were performed on the discriminant function scores to test for differences in head and body morphology between the groups (Tukey 1949; Zar 1999). Thin-plate spline transformation images depicting shape changes along each discriminant function were created by regressing the individual discriminant function scores onto the partial warp scores using tpsRegr (version 1.38, Rohlf 2011).

Several morphological traits not accounted for in the analysis of two dimensional head and body morphology were analyzed separately. These included measurements of pectoral (PEC), dorsal (DOR), pelvic (PEL), anal (ANL), and lower caudal (LCL) fin lengths (Fig. 3.3B). Four individuals were missing at least one set of fins and not included in this part of the morphological analysis. Each morphological trait was adjusted to account for differences in allometry as recommended by Reist (1986), using the distance from the center of the eye to the end of the caudal peduncle (BDL) (Fig. 3.3B) as a measure of body size. Differences in morphological characteristics among the Arctic charr groups were tested in SPSS using ANOVA models that included gender as a factor to test for sexual dimorphism:

$$T_{ijk} = \beta_0 + \beta_1(G_i) + \beta_2(S_i) + \beta_3(G_i \times S_i) + \varepsilon_{ijk}$$

where $T_{ijk}$ is the size-adjusted univariate trait being examined for individual $i$ in group $j$ and sex $k$, $G$ is the categorical fixed factor group, $S$ is the categorical fixed factor sex, and $\varepsilon_{ijk}$ is the error term. Tukey's (Tukey 1949; Zar 1999) or Tamhane's T2 (Tamhane 1977) adjusted pairwise tests

were used for post-hoc comparisons of mean trait values between groups, or between groups within each sex when the degree of sexual dimorphism differed among groups for that trait.

## RESULTS

### *Life History*

The clustering model with the lowest BIC value had four ellipsoidal shaped clusters with varying volume, shape, and orientation (model VVV in Fraley et al. 2012). Two of the clusters in this model overlapped completely, with one smaller cluster fully inset inside a larger cluster. The inset cluster defined a relatively high concentration of individuals around an age of 22 years and a forklength of 356 mm. It was concluded that this smaller, inset cluster represented a sampling artifact of a relatively large number of individuals captured around a particular age and size, and was not a biologically meaningful grouping. Therefore, the two overlapping clusters were combined and treated as one larger cluster (group 2; Fig. 3.4), leaving three distinct size-at-age groups identified among the Arctic charr from Lake Hazen. For the purposes of reporting the following results the group containing the largest individuals will be referred to as group 1, the group with the intermediately sized individuals will be group 2, and the group containing the smallest individuals will be referred to as group 3.

Group 1 included individuals from 41 different cohorts (i.e. individuals born in the same year) (Fig. 3.5). The earliest size- and age-at-maturity observed for this group were at 385 mm and 9 years, while the oldest and largest individuals had a forklength of 787 mm and an age of 35 years. Group 2 included 43 cohorts (Fig. 3.5), with an earliest size- and age-at-maturity of 269 mm and 8 years, while the largest individual had a forklength of 409 mm and the oldest fish was 36 years old. The third group included 24 cohorts (Fig. 3.5), but the earliest size- and age-at-maturity observed in group 3 was over 100 mm smaller (120 mm) and 4 years younger (4 years) than those in the other two groups. Individuals in group 3 also remained smaller than those in groups 1 and 2, reaching a max observed forklength of 262 mm, and reached a max observed age of only 18 years. The proportions of male and female Arctic charr were similar among the three groups (%M:%F, 1 = 58:42, 2 = 56:44, 3 = 51:49).

*Diet*

Group differences accounted for a statistically significant portion of the variation in $\delta^{13}C$ and $\delta^{15}N$ values among the Arctic charr from Lake Hazen ($\delta^{13}C$: $F_{(2,273)} = 17.03$, $P < 0.001$; $\delta^{15}N$: $F_{(2, 273)} = 820.64$, $P < 0.001$) (Fig. 3.6). These group differences could not be attributed to isotopic variation among sample collection years ($\delta^{13}C$: $F_{(3,256)} = 0.65$, $P = 0.586$; $\delta^{15}N$: $F_{(3, 256)} = 0.40$, $P = 0.753$) or sites ($\delta^{13}C$: $F_{(7,261)} = 1.24$, $P = 0.281$; $\delta^{15}N$: $F_{(7,261)} = 1.82$, $P = 0.083$). Group 1 fed at a higher trophic level (mean differences in $\delta^{15}N$: groups 1-2 = 3.99 ‰, $P < 0.001$; 1-3 = 3.81 ‰, $P < 0.001$), and had more negative $\delta^{13}C$ values (1-2 = 2.12 ‰, $P < 0.001$; 1-3 = 1.32 ‰, $P < 0.001$) than the two other Arctic charr groups. The second and third groups fed at a similar trophic level ($P = 0.143$), and exhibited a small but statistically significant difference in mean $\delta^{13}C$ values ($P = 0.019$).

A small portion of charr in each life history group had empty stomachs (1 = 17%, 2 = 21%, 3 = 1%). The majority of Arctic charr in group 1 had fish remains in their stomach contents, while the stomach contents of individuals in groups 2 and 3 included mainly invertebrate prey (Fig. 3.7). Chironomid larvae and pupae were the most frequently observed prey type in the stomach contents of individuals in groups 2 and 3. The only significant difference in stomach contents between groups 2 and 3 was a higher frequency of copepods in the stomachs of individuals in group 3. Although hydracarina were found in the stomach contents of nearly half the individuals in groups 2 and 3, it is unlikely these were targeted prey items, but rather organisms that were ingested incidentally along with the chironomid pupae serving as hosts for the parasitic hydracarina (Smith & Oliver 1986).

*Morphology*

The body shape variation of the Arctic charr sampled from Lake Hazen was summarized by two discriminant functions (Fig. 3.8). The first explained 83% of the variation in body shape among the groups identified by the cluster analysis (DF1: *Wilks'* $\lambda = 0.12$, $P < 0.001$), while the second explained the remaining 17% (DF2: *Wilks'* $\lambda = 0.57$, $P < 0.001$) (Fig. 3.8). Reclassification rates for the discriminant functions indicated that groups 1 and 3 exhibited distinct differences in body morphology (% correctly classified: 1 = 97%, 3 = 97%). The second group had a less distinct body shape (68% correctly classified), with most of the incorrectly classified individuals

(25%) assigned to group 3. The relationship between body shape and size (DF1: $F_{(2,255)} = 2.27$, $P = 0.106$; DF2: $F_{(2,255)} = 0.22$, $P = 0.802$) and the extent of sexual dimorphism (DF1: $F_{(2,255)} = 2.53$, $P = 0.081$; DF2: $F_{(2,255)} = 0.24$, $P = 0.790$) did not differ among groups for either discriminant function. Pairwise comparisons of average scores for both discriminant functions support the conclusion that all three groups differ in body shape (all $P < 0.001$).

Head shape differences were also summarized by two discriminant functions, which explained 87% (DF1: *Wilks'* $\lambda = 0.15$, $P < 0.001$) and 13% (DF1: *Wilks'* $\lambda = 0.66$, $P < 0.001$) of the variation in head shape among the groups identified in the cluster analysis (Fig. 3.9). Reclassification rates for head shape showed similar patterns to those for body shape. Groups 1 and 3 again exhibited distinct head morphologies (% correctly classified: 1 = 90%, 3 = 95%), while head shape for group 2 was less distinct (70% correctly classified), and the majority of incorrectly classified individuals from group 2 were again assigned to group 3 (28%). The relationship between head shape and size was similar among the groups (DF1: $F_{(2,255)} = 1.62$, $P = 0.200$; DF2: $F_{(2,255)} = 2.62$, $P = 0.074$), but differences in the degree of sexual dimorphism in head shape were detected (DF1: $F_{(2,255)} = 5.18$, $P = 0.006$; DF2: $F_{(2,255)} = 3.49$, $P = 0.032$). Pairwise comparisons indicated head shape along the first discriminant function differed between groups for both male and female Arctic charr (all $P < 0.001$). Head shape differences along the second discriminant function differed between groups for male Arctic charr (all $P < 0.001$). Female head shape along the second discriminant function differed between groups 1 and 2 ($P < 0.001$), and 2 and 3 ($P < 0.001$), but was similar between groups 1 and 3 ($P = 0.927$).

All three groups of Arctic charr identified in the cluster analysis had similar average caudal fin lengths (Table 2). The other fin lengths differed among groups, but the pattern of sexual dimorphism within groups differed for pectoral, pelvic, and anal fin lengths (Table 2). Pairwise comparisons indicated that males in group 2 had longer fins than males in either of the other two groups, while males in groups 1 and 3 had similar relative fin lengths (Table 3). Average pectoral fin lengths were similar for females in all three groups, but females in group 2 had longer dorsal and anal fins than those in groups 1 and 3, which had similar dorsal and anal fin lengths.

## DISCUSSION

Historically, polymorphic populations of Arctic charr were identified based on observed phenotypic differences among individuals captured in one location (e.g Walker et al. 1988; Snorrason et al. 1994; Reist et al. 1995). Bayesian approaches, however, are unbiased to preconceived ideas of grouping characteristics and, therefore, present a new and potentially powerful method for identifying morphs within populations that may otherwise go unnoticed. For example, Woods et al. (2012a) used a similar Bayesian-type analysis of size-at-age data to reveal previously unknown polymorphisms among Icelandic populations of Arctic charr

However, the inherent differences in life history patterns represented by clusters identified by a Bayesian analysis of size-at-age data (e.g. growth rates, adult body size, and age-at-maturity), could be explained by phenomena other than polymorphism. For example, sex-specific life history strategies, such as precociousness in males or anadromy in females (Jonsson & Jonsson 1993), are common in salmonid species and can manifest as distinct size-at-age clusters. Clustering in size-at-age data can also result from plastic responses in growth to anomalous events, such as extreme environmental variation or anthropogenic influences (e.g. Klemetsen et al. 2002). Variation in the size-at-age data of sexually mature Arctic charr from Lake Hazen was best described by three distinct clusters. These clusters included approximately equal proportions of males and females, and were identified in at least 24 different cohorts. Therefore, it is unlikely that the clusters of Arctic charr identified in Lake Hazen are the product of a sex-specific life history strategy or a brief change in cohort-specific growth patterns, but rather represent different morphs.

Polymorphic populations of Arctic charr are typically classified as resource polymorphisms, in which morphs are defined by a combination of group differences in life history and morphological characteristics as they relate specifically to differential resource use (Skúlason & Smith 1995; Smith & Skúlason 1996). The differences in diet observed among the size-at-age clusters identified in Lake Hazen provide support for the conclusion that there are at least two groups of Arctic charr in this population that exhibit differential prey resource use. In this case, one group feeds on smaller conspecifics while the other feeds primarily on benthic invertebrates. These explicit differences in prey resource use are accompanied by differences in

morphology that appear to be consistent with adaptations observed in Arctic charr and other fish species that utilize different habitats or specialize on different prey types (Jonsson & Jonsson 2001; Webb 1984; Langerhans & Reznick 2010). For example, the cannibalistic group of Arctic charr exhibited a more pointed snout shape and terminal mouth, which are characteristic of piscivorous fish that capture prey items out of the water column (Webb 1984; Jonsson & Jonsson 2001). In contrast, the benthivorous Arctic charr in Lake Hazen exhibited the characteristically rounded snouts and more subterminally-oriented mouths of fish that forage for prey along benthic or littoral surfaces (Webb 1984; Jonsson & Jonsson 2001).

The differences in body morphology among the three clusters identified from the size-at-age data also support the conclusion that these groups represent distinct morphs. The cannibalistic group exhibited the largest adult body sizes, a more fusiform body shape, a smaller caudal region, and shorter fins. This morphology is typical of fish that use a steady-swimming foraging strategy in open-water habitats (Webb 1984; Langerhans & Reznick 2010). Thus, the diet, morphological and life history characteristics of the cannibalistic Arctic charr observed here are consistent with previous descriptions of a cannibalistic morph in Lake Hazen (i.e. the large morph described in Reist et al. 1995; Guiguer et al. 2002; Gallagher et al. 2009; Arbour et al. 2011). Both benthivorous groups of Arctic charr identified here had longer and deeper caudal regions compared to the cannibalistic morph. This is a body shape typically associated with maneuverability and non-steady swimming in the more structurally complex benthic and littoral habitats (Webb 1984; Langerhans & Reznick 2010). However, the larger-bodied benthivorous group had a body shape that was deeper dorso-ventrally compared to the smaller-bodied benthivorous group. The smaller-bodied benthivorous individuals also tended to have relatively small fins for their body size compared to the larger-bodied benthivorous group. Individuals having a deep body shape and relatively long fins are similar to previous descriptions of a large benthivorous morph of Arctic charr in Lake Hazen (i.e. the small morph in Reist et al. 1995; Guiguer et al. 2002; Gallagher et al. 2009; Arbour et al. 2011). This previously identified benthivorous morph also had diet and life history characteristics similar to the intermediately-sized group of Arctic charr identified by the cluster analysis.

The third, small-bodied, early-maturing group identified by the cluster analysis has not been previously documented in Lake Hazen. However, this early-maturing group is similar to small, early-maturing morphs that have been observed in other polymorphic populations of Arctic charr (e.g. Alekseyev et al. 2002; Jonsson & Jonsson 2001; Finstad et al. 2006; Berg et al. 2010). These morphs are characterized by adult body sizes that do not exceed 250 mm in length and a diet consisting of invertebrate prey (Alekseyev et al. 2002; Jonsson & Jonsson 2001; Berg et al. 2010). The differences in life history traits among large and small-bodied morphs of fish have been associated with energetic constraints imposed by feeding on prey organisms of different sizes (Finstad et al. 2006; Fraser et al. 2008), and the physical limitations imposed by gape size on the maximum size of prey that can be ingested (Persson et al. 1996; Mittelbach & Persson 1998; Classen et al. 2002). Due to their similarity in diet, the differences in growth and maturity patterns observed between the early-maturing and large benthivorous Arctic charr in Lake Hazen cannot be explained by gape-size limitations or the energetic constraints associated with their prey resource.

Polymorphic populations of northern fish species rarely include two morphs that feed on the same type of prey. Instead, morphs typically differentiate into habitat-specific niches (Robinson & Wilson 1994; Skúlason & Smith 1995; Robinson & Parsons 2002). However, there are polymorphic populations with morphs that exhibit differential resource use by foraging for the same prey type in different locations. For example, the small- and large-bodied benthivorous morphs in Thingvallavatn exhibit spatial resource partitioning by feeding on gastropods in different microhabitats (Snorrason et al. 1994). In this case, the larger benthivorous morph forages epibenthically, while the smaller morph consumes prey found within crevices in the bottom of the lake too small for the larger morph to access (Snorrason et al. 1994). These differences in habitat use are accompanied by differences in growth, age-at-maturity, and morphology, leading to the conclusion that these two benthivorous groups are distinct morphs (Sandlund et al. 1992; Snorrason et al. 1994; Skúlason et al. 1989).

The large benthivorous and early-maturing groups of Arctic charr in Lake Hazen exhibit significant differences in morphology and life history traits, which suggest that they represent different morphs (Skúlason & Smith 1995; Smith & Skúlason 1996). However, it is not yet clear

if, or how they differ in resource use. One possibility is that they are partitioning the chironomid prey resource in a similar way to the two benthivorous morphs in Thingvallavatn; by foraging in different parts of the benthic habitat accessible to individuals with different body sizes (Snorrason et al. 1994).

The differences in adult body size and earliest age-at-maturity observed between the early-maturing and large benthivorous morphs are characteristic of the life history trade-off between survival and reproduction (Roff 1992; Stearns 1992). According to life history theory, early maturation increases the probability of surviving to reproduce, at the cost of increased fecundity (Roff 1992; Stearns 1992). Delayed maturity allows for increased fecundity and post-maturation survival as individuals attain larger body sizes, but comes at the cost of an increased risk of mortality prior to reproduction (Roff 1992; Stearns 1992). This trade-off is dependent on a difference in pre- and post-maturation survival rates, which is often associated with a size-dependant mortality factor, such as predation (Hutchings 2002). In Lake Hazen, the cannibalistic morph would present a significant size-dependant mortality risk for smaller Arctic charr.

Due to gape size restrictions (Persson et al. 1996; Mittelbach & Persson 1998; Classen et al. 2002), the maximum size of fish prey Arctic charr can consume is estimated to be between 40% (Damsgård 1995) and 45% (Amundsen 1994) of the predator's body length, while the average is estimated to be between 28%-32% of the predator's body length (Amundsen 1994). Using these factors, the maximum threshold of vulnerability to cannibalism among the Arctic charr examined here would be 315-354 mm, and the window of highest vulnerability would be at body sizes between 116-236 mm in length (Fig. 3.4). Given these approximations, the early-maturing morph adults would be within the range of body sizes subject to the highest predation pressure from the cannibalistic morph, while the large benthivorous morph begins to mature at the maximum limits of the window of vulnerability to cannibalism in Lake Hazen (Fig. 3.4). Therefore, the differences in growth and maturity patterns observed between the early-maturing and large benthivorous morphs could be explained by opposing life history trade-offs in response to size-selective predation pressure from the cannibalistic morph.

Similar to descriptions of the small, early-maturing morphs that have been observed in other polymorphic populations of Arctic charr, the morphological characteristics of the early-

maturing morph in Lake Hazen are reflective of paedomorphism (Skúlason et al. 1999). Typical of juvenile fish, a body shape with a longer caudal region and shorter fins increases the efficiency and speed of maneuvers associated with predator evasion (Webb 1984; Langerhans & Reznick 2010). The retention of morphological traits associated with predator avoidance in the early-maturing morph further suggests that predation pressure from the cannibalistic morph may play a role in maintaining the differences in life history and morphology observed between the early-maturing and large benthivorous morphs in Lake Hazen.

In natural and experimental environments, juvenile Arctic charr and European perch (*Perca fluviatilis*) that were subject to cannibalism used the more structurally complex near-shore benthic and littoral habitats more often than open off-shore littoral or pelagic areas (Persson & Eklöv 1995; Byström et al. 2004; Eklöv & Svanbäck 2006). Given their higher vulnerability to cannibalism, the early-maturing Arctic charr in Lake Hazen would similarly be expected to preferentially forage in parts of the benthic habitat that offer greater refuge from predation. The large benthivorous adults, which are outside the window of vulnerability to predation, could then escape competition for chironomid prey by foraging in areas of the lake that offer less protection from the cannibalistic morph. Therefore, if areas within the benthic habitat of Lake Hazen offer different levels of protection from predation, the early-maturing and large benthivorous morphs in Lake Hazen could exhibit spatial resource partitioning in response to size-selective predation pressure from the cannibalistic morph. Such a difference in microhabitat use is speculative based on the available data, and the role that cannibalism plays in maintaining differences between the early-maturing and large benthivorous morphs needs to be explored further.

*Future Work*

To better understand the mechanisms responsible for the maintenance of the three Arctic charr morphs in Lake Hazen, more information is needed concerning their spatial distribution, growth patterns, and genetic relationships. To date, only a small portion of Lake Hazen has been sampled using equipment appropriate for capturing all three morphs. To determine whether the early- and late-maturing benthivorous morphs are utilizing different

areas of the lake, more extensive sampling at different depths or tracking of individual movement patterns is needed.

Phenotypic differences within polymorphic populations of Arctic charr result from a combination of genetic variability and phenotypic plasticity, but the relative importance of these two factors varies widely among populations (Skúlason et al. 1996; Adams & Huntingford 2002; Adams et al. 2003; Klemetsen 2010; Andersson et al. 2003). The only conclusive way to determine whether the morphs in Lake Hazen represent different outcomes of a conditional life history strategy (all differences resulting from phenotypic plasticity), or have been influenced by divergent selection (genetic differences), is to rear offspring from each morph in a common environment. Unfortunately, it would be both costly and logistically difficult to obtain fertilized eggs of Arctic charr from Lake Hazen and transport them to a suitable facility in which to rear them.

Alternatively, molecular techniques could be used to test for genetic differences among the three morphs, but some of the most commonly used methods may not be suitable for detecting genetic differentiation among the Lake Hazen Arctic charr. If the three morphs diverged from a single invading population, genetic differences may not have had enough time to accumulate at neutral loci given the relatively short time since Lake Hazen was deglaciated (~ 5 ka BP; Smith 1999) (Thibert-Plante & Hendry 2010). This was noted in a recent study examining variation at five microsatellite loci between the cannibalistic and large benthivorous morphs in Lake Hazen (Arbour et al. 2011). The authors of that study concluded the cannibalistic and large benthivorous morphs were not genetically distinct populations, but also noted that further studies were needed to confirm this finding as the amount of genetic divergence between morphs could be under-represented by the selectively neutral loci (Arbour et al. 2011). Major histocompatibility (MH) genes have shown promise for differentiating among morphs of Arctic charr found in different lakes and different habitats (Conejeros et al. 2014). However, MH genes did not significantly differ between other morphs of Arctic charr that occupied the same habitat and fed on similar prey (Kapralova et al. 2010), so they may be not be suitable to use in testing for divergence between the early- and late-maturing benthivorous morphs in Lake Hazen. A potential option for molecular-based tests of genetic

differentiation between the three morphs would be quantitative trait loci associated with growth and age at maturity (e.g. Moghadam et al. 2007). This is because growth and age at maturity are the primary phenotypic differences observed among the morphs in Lake Hazen, and traits likely to be subject to divergent selection pressures if they play a role in differentiation among the three morphs.

*Conclusions*

The polymorphic population of Arctic charr in Lake Hazen includes three distinct morphs: the previously described large cannibalistic and large benthivorous morphs, and a third, smaller, early-maturing, benthivorous morph (Fig. 3.10). Although there are alternative explanations for the occurrence of distinct size-at-age groups within a fish population, polymorphism is the most consistent explanation for the combination of differences observed in life history (growth and age-at-maturity), trophic ecology (diet), and morphology among the three groups of Arctic charr identified here. The differences in head and body morphology observed among the groups of Arctic charr in Lake Hazen are consistent with the adaptations for capturing different types of prey in different habitats identified in other polymorphic populations of Arctic charr (Malmquist 1992; Adams & Huntingford 2002; Garduño-Paz & Adams 2010). However, it is unclear how resource use differs between the two benthivorous morphs. The occurrence of two distinct morphs that share a common diet is unusual among polymorphic populations, and deviates from the pattern of habitat-specific differentiation typically observed among morphs in northern lacustrine fish species. The pattern of differentiation between the two benthivorous morphs identified in Lake Hazen suggests size-selective predation associated with cannibalism plays a role in the maintenance of this polymorphic population, but more information on the spatial distribution of the three morphs in the lake is needed to support this idea.

## TABLES & FIGURES

**Table 3.1:** The number of individuals from the three morphs captured in seven sampling events between 1981 and 2008.

| | 1: Cannibalistic | 2: Large Benthivorous | 3: Early-Maturing | Total |
|---|---|---|---|---|
| **1981** | 21 | 149 | 0 | 170 |
| **1990** | 21 | 18 | 0 | 39 |
| **1992** | 73 | 54 | 7 | 134 |
| **1998** | 21 | 17 | 3 | 41 |
| **2001** | 30 | 29 | 14 | 73 |
| **2007** | 4 | 6 | 17 | 27 |
| **2008** | 13 | 8 | 180 | 201 |
| **Total** | 183 | 281 | 221 | 685 |

**Table 3.2:** Results of the two-way ANOVAs used to test for differences in morphological traits among morphs as identified by the cluster analysis and between male and female charr. The *P*- and *F*-statistic values associated with the fixed factors group and sex, and their interaction are reported for each size-adjusted trait.

| | Group | | Sex | | Group x Sex | |
|---|---|---|---|---|---|---|
| **Trait** | $F_{(2,254)}$ | *P* | $F_{(1,254)}$ | *P* | $F_{(2,254)}$ | *P* |
| Pectoral Fin Length | 11.87 | <0.001 | 40.79 | <0.001 | 3.63 | 0.036 |
| Dorsal Fin Length | 4.67 | 0.010 | 7.81 | 0.006 | 0.17 | 0.846 |
| Pelvic Fin Length | 12.48 | <0.001 | 40.79 | <0.001 | 3.40 | 0.035 |
| Anal Fin Length | 12.51 | <0.001 | 16.78 | <0.001 | 4.66 | 0.010 |
| Caudal Fin Length | 1.65 | 0.194 | 0.21 | 0.647 | 0.24 | 0.791 |

**Table 3.3:** Mean size-adjusted trait values (mm) calculated for male (M) and female (F) Arctic charr in each morph. Superscripts indicate groups with similar ($P > 0.05$) trait values based on post-hoc multiple comparisons.

| **Trait** | **1: Cannibalistic** | | **2: Large Benthivorous** | | **3: Early-Maturing** | |
|---|---|---|---|---|---|---|
| | M | F | M | F | M | F |
| Pectoral Fin Length | $38^{a}$ | $35^{b}$ | 42 | $37^{b}$ | $38^{a}$ | $37^{b}$ |
| Dorsal Fin Length | $31^{a}$ | $30^{a}$ | $34^{b}$ | $32^{b}$ | $32^{a}$ | $31^{a}$ |
| Pelvic Fin Length | $28^{a}$ | 25 | 31 | $27^{b}$ | $28^{a}$ | $27^{b}$ |
| Anal Fin Length | $26^{a}$ | $25^{b}$ | 30 | $27^{c}$ | $27^{a}$ | $26^{b,c}$ |

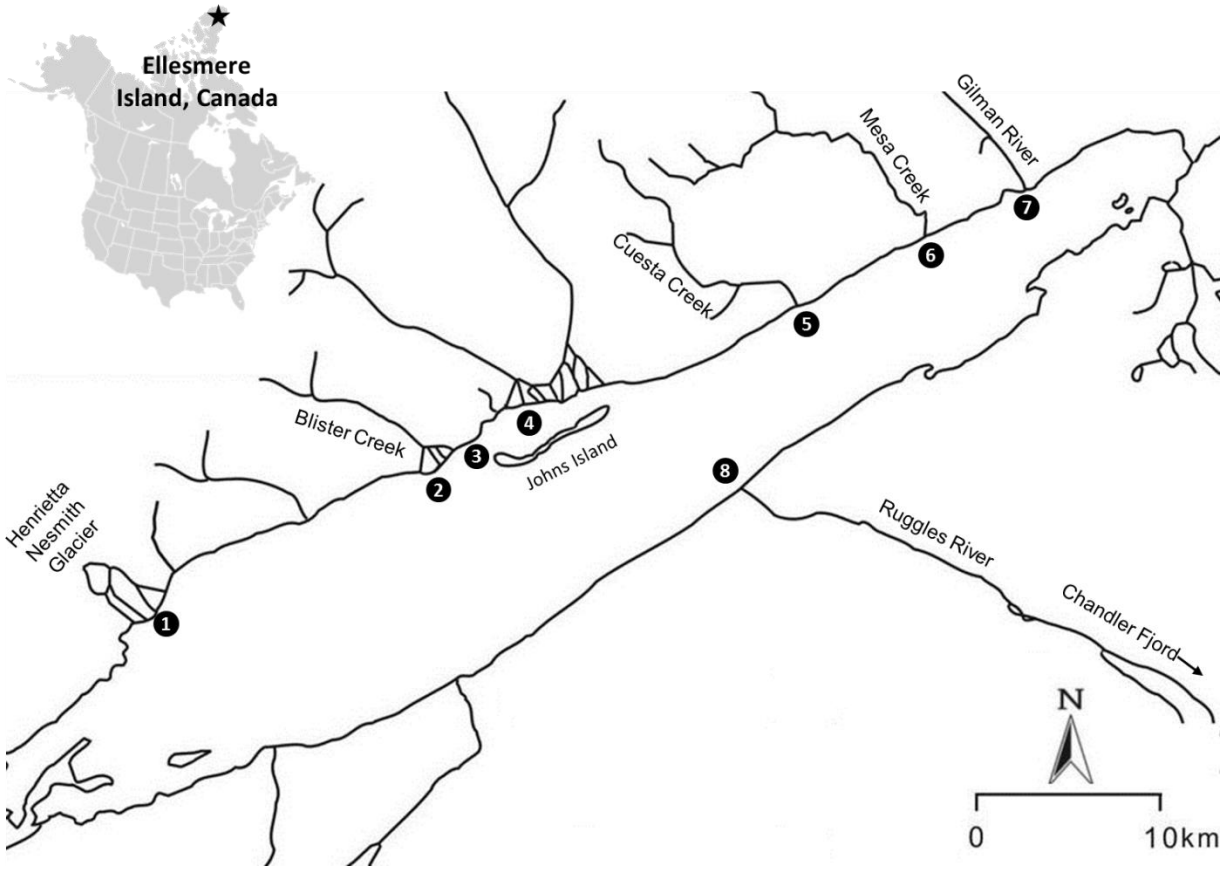


**Figure 3.1:** Map of Lake Hazen. Numbered circles indicate sampling locations for Arctic charr.

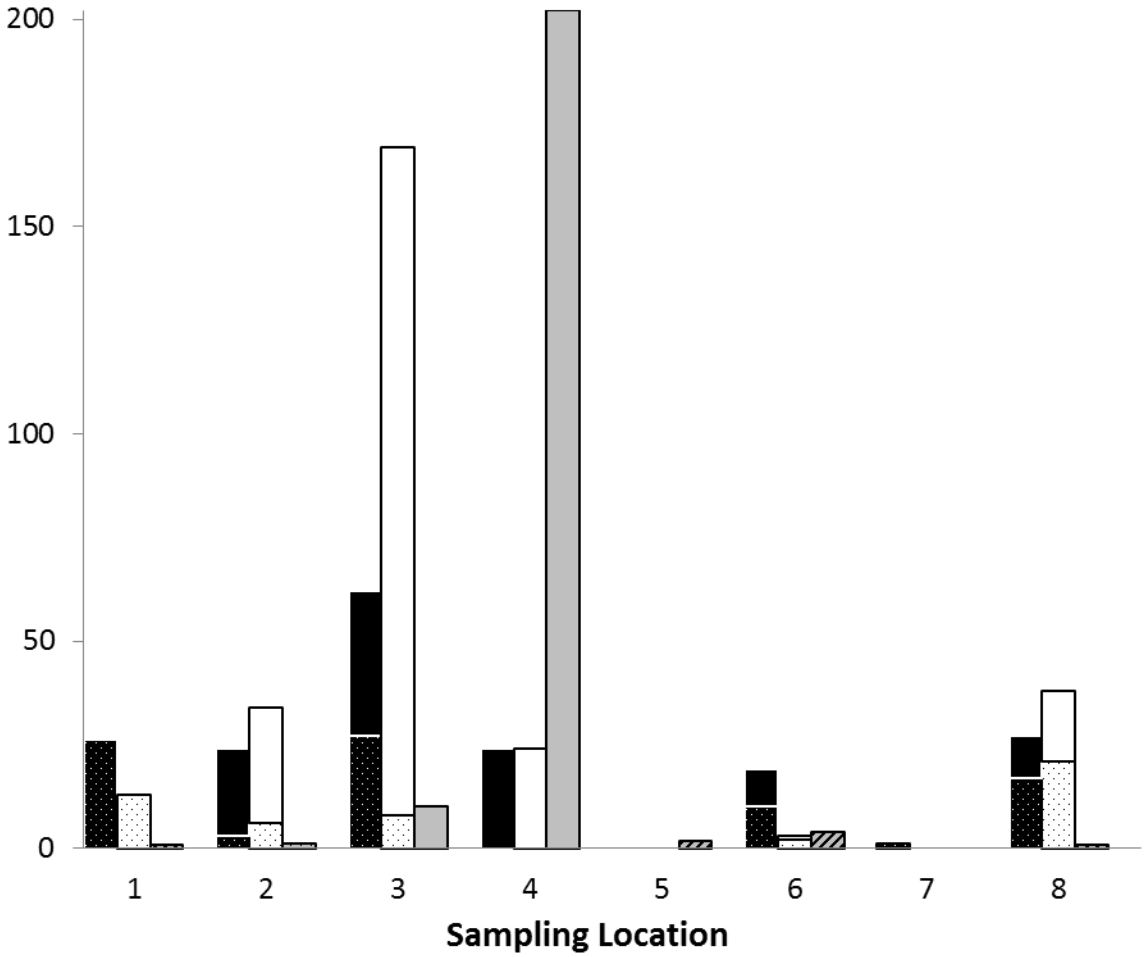


**Figure 3.2:** Number of individuals from life history groups 1 (cannibalistic, ■), 2 (large benthivorous, □), and 3 (early-maturing, ▩) captured at the sampling locations identified in Fig. 3.1. Method of capture is indicated by shading pattern, and includes gillnetting (solid □), angling (spotted ⊡), or removed from the stomach contents of larger Arctic charr (diagonal lines ◪).

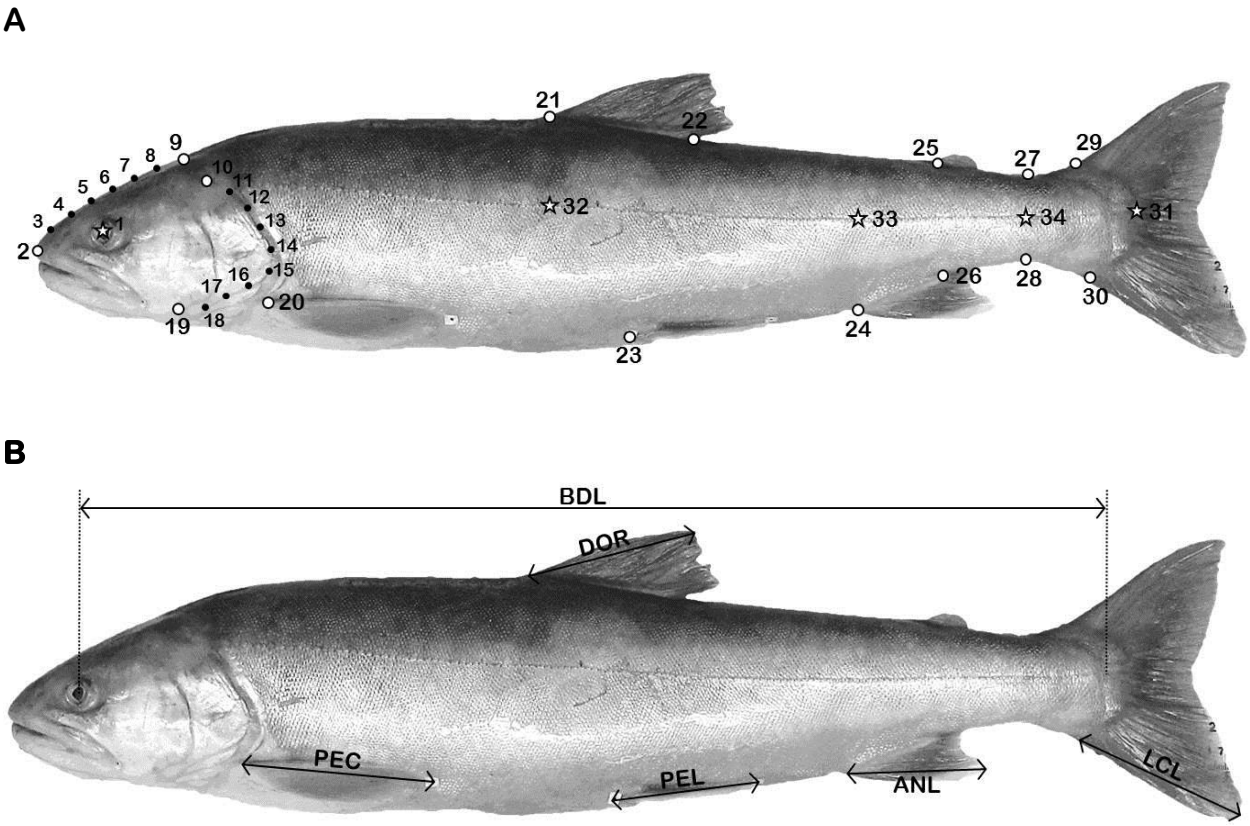


**Figure 3.3:** A) Anatomical landmarks used for geometric morphometric analyses. Homologous landmarks are indicated by white dots, while the smaller, black dots represent sliding semilandmarks used to outline head shape. Stars denote landmarks used to define the line along which specimens were straightened in the "unbending" procedure. B) Univariate morphological trait measurements obtained using digital images (abbreviations explained in text).

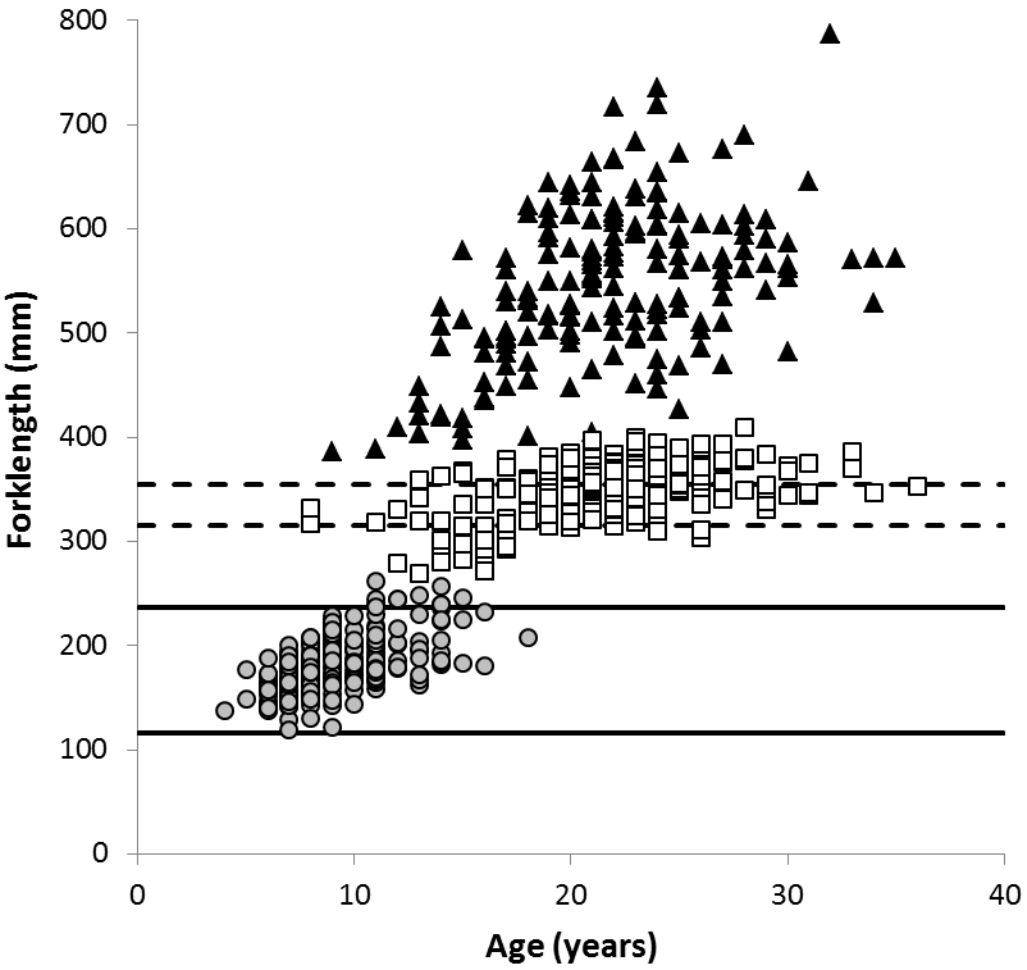


**Figure 3.4:** Individual size-at-age values of Arctic charr in the three groups identified by the cluster analysis (1: cannibalistic = ▲, 2: large benthivorous = □, 3: early-maturing = ○ ). Horizontal lines indicate the estimated window of greatest vulnerability to predation (——) and the maximum threshold of vulnerability to predation (– – –), as explained in the text.

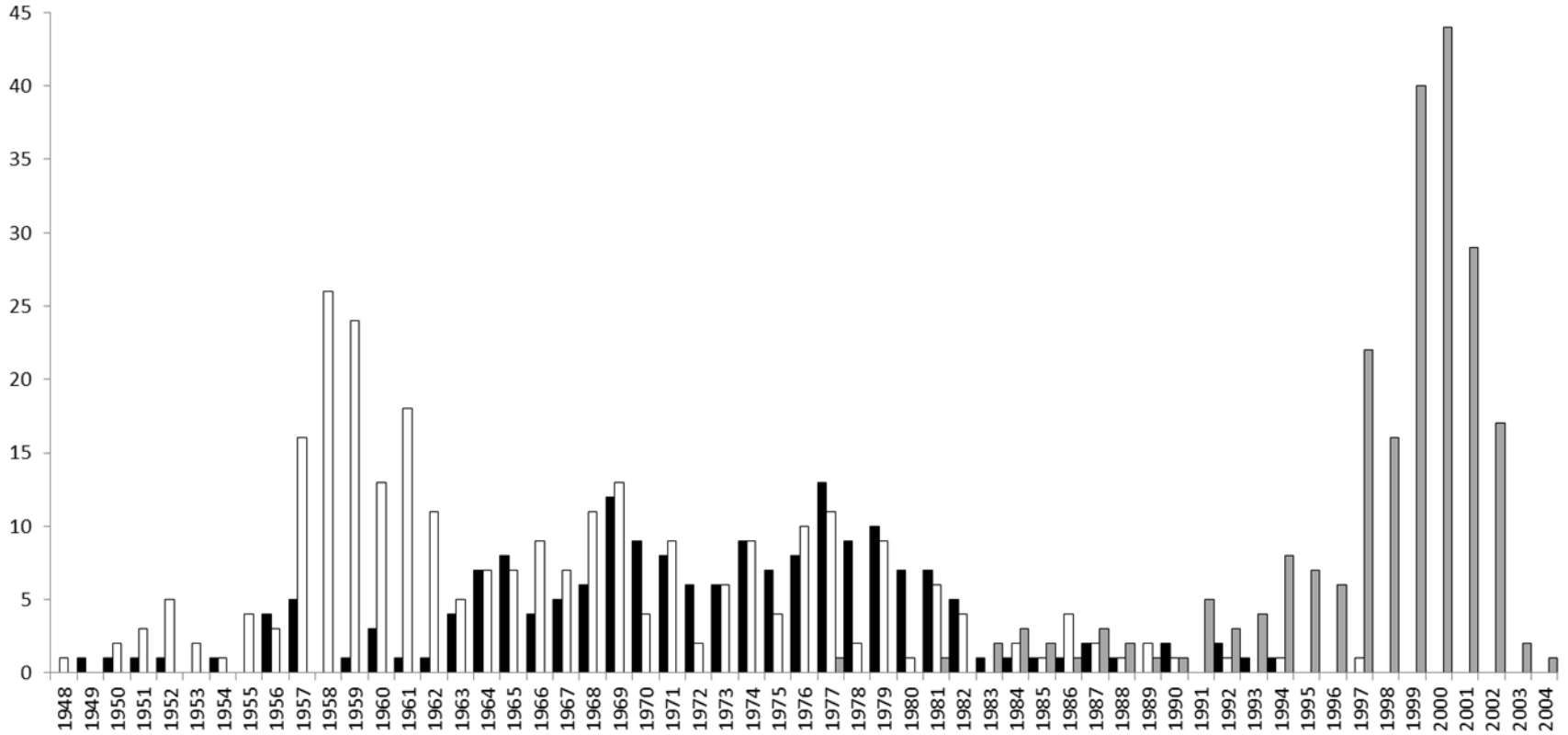


**Figure 3.5:** Number of Arctic charr from cohorts born in 1948-2004 included in life history groups 1 (large cannibalistic, ■), 2 (large benthivorous, □), and 3 (early-maturing, ▩).

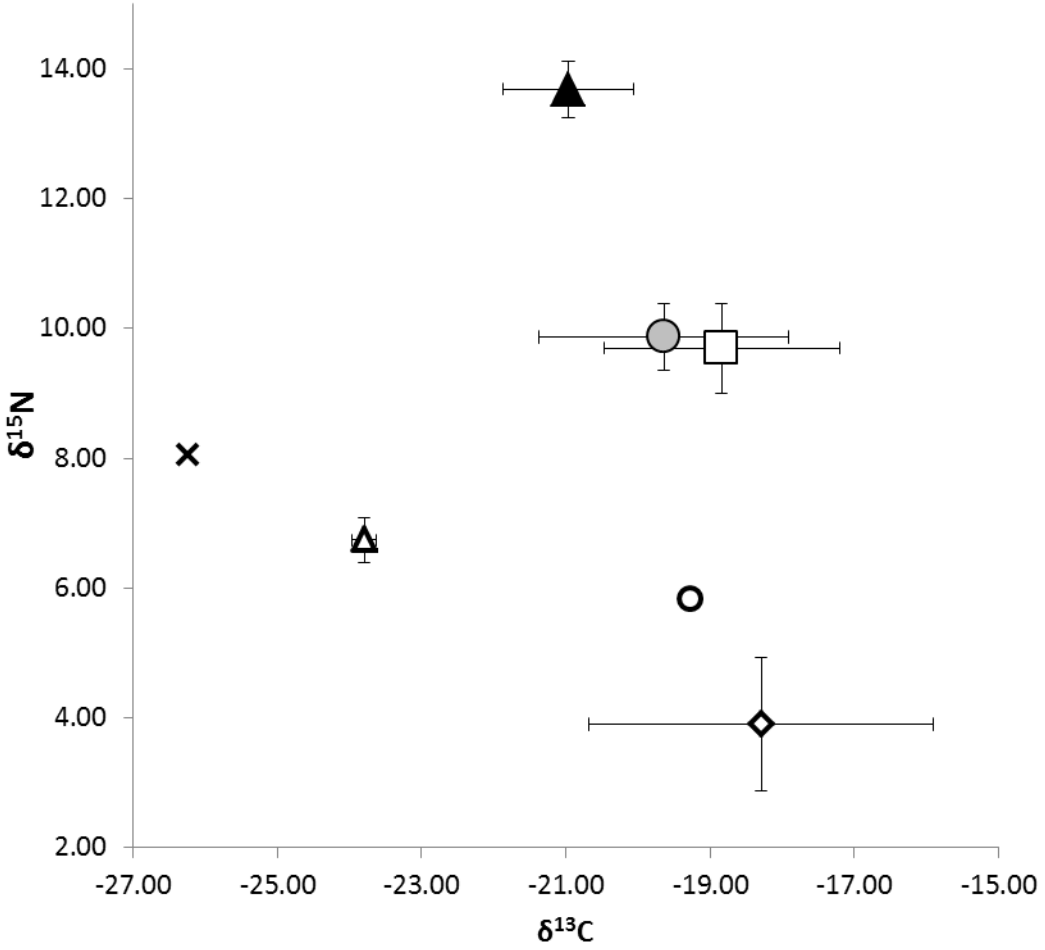


**Figure 3.6:** Mean (± SD) muscle tissue $\delta^{13}C$ and $\delta^{15}N$ values for each morph of Arctic charr (1: cannibalistic = ▲, 2: large benthivorous = □, 3: early-maturing = ● ), as well as common prey items taken from stomach contents (chironomid pupae - ◇, chironomid larvae - △, hydracarina - ○, and copepods - ×).

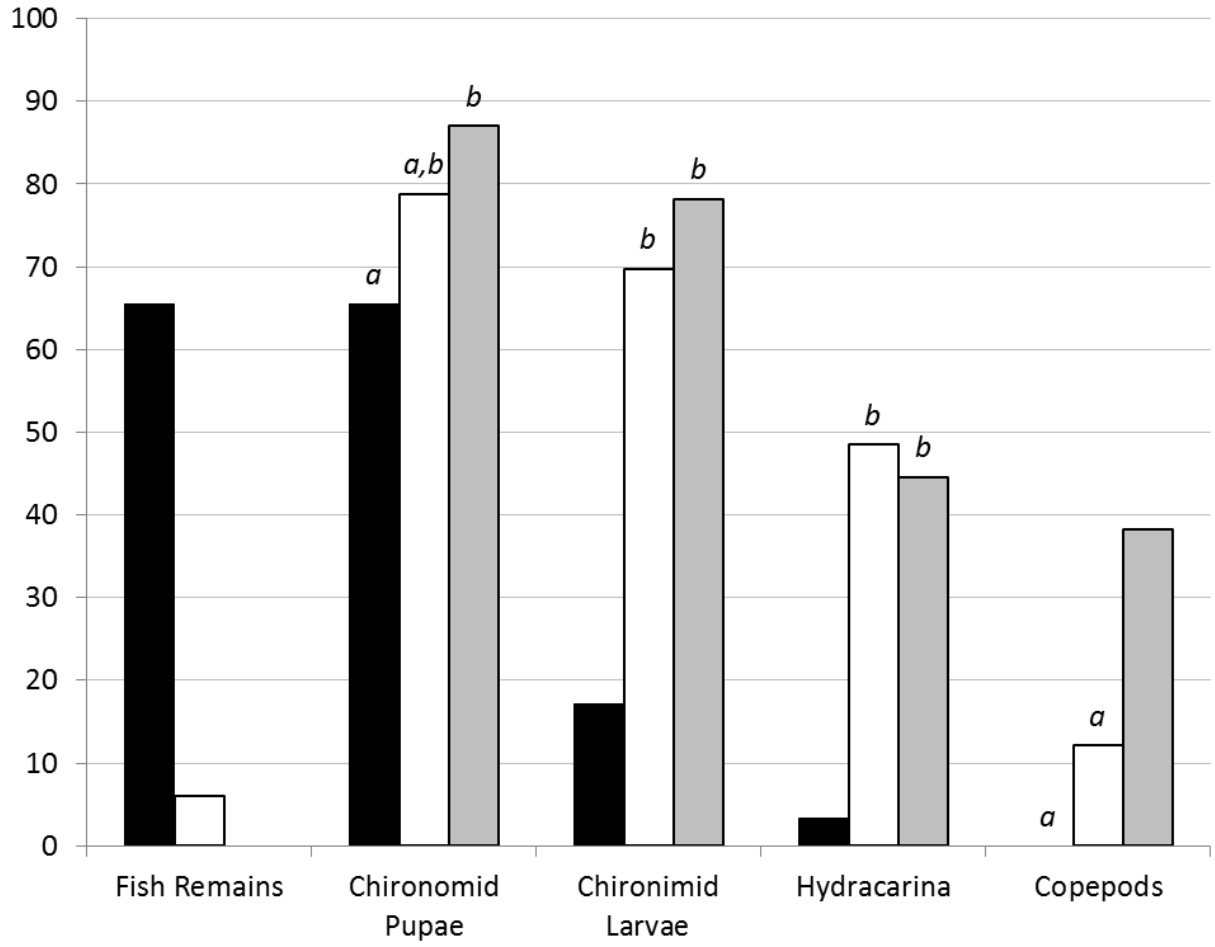


**Figure 3.7:** The observed percentage of individuals from each Arctic charr morph (1: cannibalistic = ■, 2: large benthivorous = □, 3: early-maturing = ▒) with prey types in their stomach contents. Superscripts indicate groups that consumed types of prey with similar ($P$ > 0.05) frequencies.

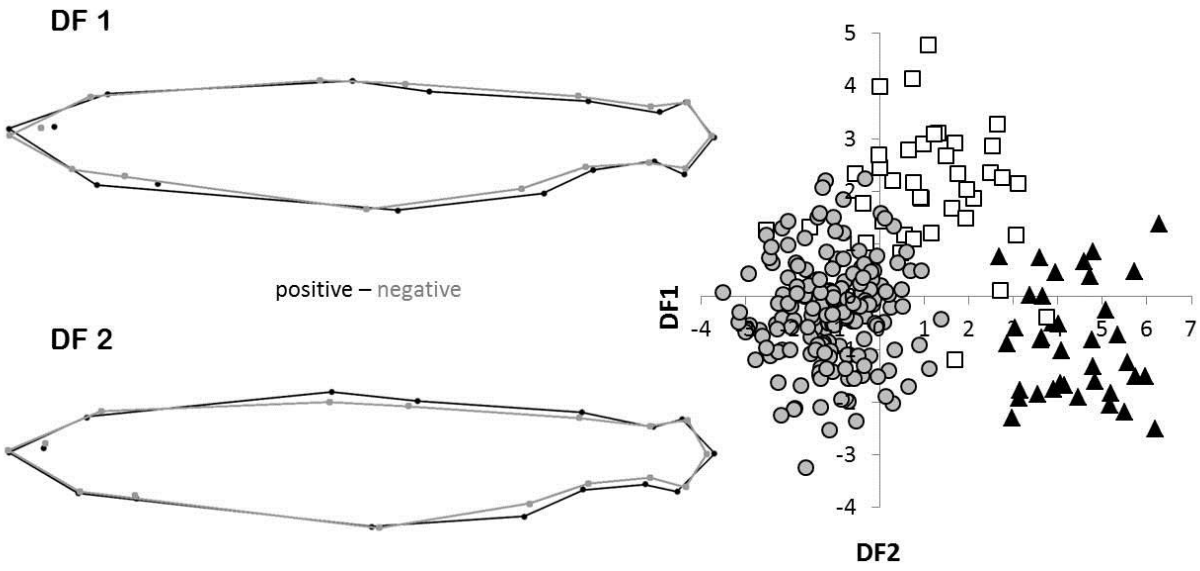


**Figure 3.8:** The scatter plot depicts individual body shape discriminant function scores for the three Arctic charr morphs (1: cannibalistic = ▲, 2: large benthivorous =□, 3: early-maturing = ○). Thin-plate spline images overlaid at left depict the shape deformations associated with individuals at the positive and negative extremes of each discriminant function.

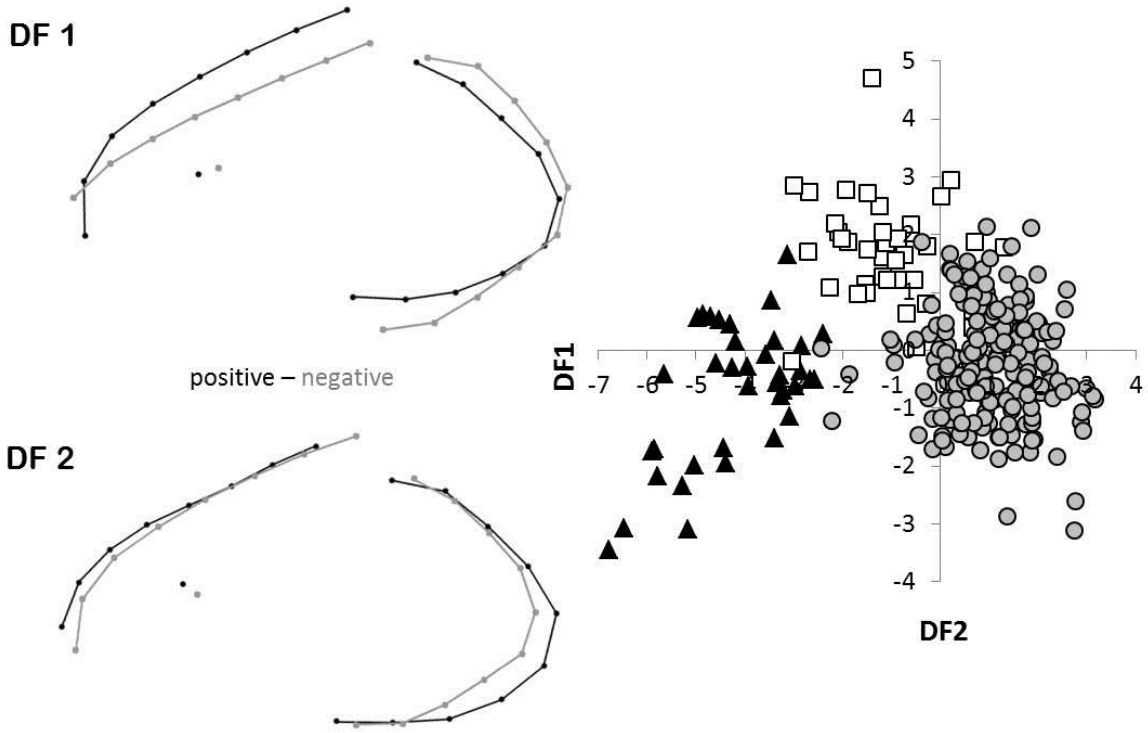


**Figure 3.9:** The scatter plot depicts individual head shape discriminant function scores for the three Arctic charr morphs (1: cannibalistic = ▲, 2: large benthivorous = □, 3: early-maturing = ○). Thin-plate spline images overlaid at left depict the shape deformations associated with individuals at the positive and negative extremes of each discriminant function.

**Figure 3.10:** Examples (from top) of the cannibalistic (group 1), large benthivorous (group 2), and early-maturing (group 3) Arctic charr from Lake Hazen. The black bar under each fish represents 5cm.

# Chapter 4 - Does ecological opportunity and the absence of interspecific competitors promote intrapopulation diversity in Arctic charr?

## INTRODUCTION

Individuals within a population can vary widely in terms of resource use. In many populations, for example, individuals differ in the relative amounts and types of prey they consume within the same ecosystem. The phenomenon of individuals using only a subset of the population's total resource base has been termed "individual specialization" and it has been observed in many different animal species (Bolnick et al. 2002; 2003). However, the extent to which individuals within a population differ in resource use varies widely among natural populations (Bolnick et al. 2002; 2003; Araujo et al. 2011).

Individual resource use variation within a population is postulated to be associated with competition for a limited set of resources (Roughgarden 1972; Taper & Case 1985; Bolnick et al. 2003). The niche variation hypothesis (NVH), for example, predicts that when interspecific competition is reduced, populations will exhibit greater among-individual variation in resource use (Van Valen 1965). This is based on the inference that in systems with fewer competitor species, intraspecific competition favors the diversification of individuals to use resources that would otherwise be used by competitors (Simpson 1953; Roughgarden 1972; Slatkin 1980; Bolnick 2001). Therefore, the relative amount of variation in resource use observed within a population is at least partly associated with the opposing effects of inter- and intraspecific competition. Intrapopulation variation would be constrained by interspecific competition as species gain fitness advantages by specializing on different resources, while intraspecific competition promotes diversification as it provides individual fitness advantages and reduces competition within the population (Roughgarden 1972; Taper & Case 1985; Bolnick et al. 2003).

The extent to which individuals can vary in resource use also depends upon the diversity of resources available to them. Intrapopulation resource use variation has been shown to increase with ecological opportunity, or the relative availability and diversity of resources within an ecosystem (reviewed in Yoder et al. 2010; Araújo et al. 2011). Many factors influence

ecological opportunity; including habitat heterogeneity, patch size, microhabitat diversity, resource diversity, and environmental stability (Yoder et al. 2010). Alternative resources must be available for ecological release to occur within populations (Simpson 1953; Hedrick 1986), creating an interactive relationship between ecological opportunity and resource competition and their influence on intrapopulation variation. Interspecific competition effectively reduces the abundance and diversity of resources available to a given population, while the extent of diversification that can occur in response to intraspecific competition is limited by ecological opportunity.

The ability of individuals within a population to successfully exploit alternative resources is at least partly attributable to their phenotype (Bolnick et al. 2003; Yoder et al. 2010; Araujo et al. 2011). Among-individual phenotypic differences, including behavioral (Werner & Sherry 1987; Bolnick et al. 2007), morphological (Van Valen 1965; Boag & Grant 1984; Simberloff et. al. 2000), or physiological (Lister 1976) traits, can influence intrapopulation variation in resource use as a result of functional trade-offs (Taper & Case 1985; Wilson & Turelli 1986; Ackermann & Doebeli 2004). These trade-offs occur when variation in a specific trait increases the efficiency with which individuals can utilize one type of resource, while decreasing their efficiency at utilizing others (Roughgarden 1972; Bolnick et al. 2003, Araújo et al. 2011). When resources are limited, individuals tend to exploit the narrower range of resources they are more efficient at utilizing, thus increasing their individual fitness (Schoener 1971; Werner 1974). Changes in relative resource availability over time provide a selective advantage to individuals who can exploit underused resources, thus maintaining a degree of phenotypic variability within the population over time.

Several studies have shown a positive relationship between diet and morphological variation within populations of fish that is consistent with the prediction that morphology plays a role in resource use variation within populations. For example, in three-spine stickleback (*Gasterosteus aculeatus*), populations exhibiting greater diet variation among individuals also exhibited greater variation in body morphology (Snowberg et al. 2015). Theoretical models indicate that the relationship between diet and morphological variation may be influenced by environmental factors (Svanback & Bolnick 2005). This was supported by observations of

increased correlations between diet and morphological variation with increases in intraspecific competition in Eurasian Perch (Svanback & Persson 2004) and three-spine stickleback (Svanback & Bolnick 2007).

Similarly, the correlation between diet and morphology was also greater in populations of Eurasian perch (*Perca fluviatilis*), and three-spine stickleback having wider niche widths (Svanback & Bolnick 2005;2007; Bolnick et al. 2007). Based on these observations, the correlation between diet and morphology within a population would be expected to be greater when intraspecific competition is high and individuals shift to feeding on the alternative prey types they are more efficient at utilizing due to their morphological differences (Schoener 1971; Werner 1974). Therefore, the correlation between diet and morphology should also increase with ecological opportunity if intraspecific competition and morphological trade-offs play important roles in resource use diversification within populations.

*Diversity in Arctic Charr*

The extent of morphological and resource use variability observed within populations of Arctic charr (*Salvelinus alpinus*) varies widely across the species' geographical range. This includes many polymorphic populations, which can contain up to four distinct morphs (see reviews in Skúlason et al. 1999; Jonsson & Jonsson 2001; Klemetsen 2010; Reist et al. 2013). As a species, Arctic charr provide several opportunities to expand on tests of the prediction that intrapopulation variation increases with ecological opportunity.

The amount of interspecific competition experienced by populations of Arctic charr varies with species community diversity along the wide latitudinal range of the species. This includes lakes in which Arctic charr have no interspecific fish competitors, because the species' range extends further north than any other freshwater fish species (see species distribution map in Reist et al. 2013). In these high-latitude systems, Arctic charr have the potential to diversify and exploit niches that would otherwise be used by competitor species in lakes at lower latitudes (Simpson 1953; Roughgarden 1972; Slatkin 1980; Bolnick 2001). This situation provides a rare opportunity to test the NVH in the extreme case of the presence and absence of interspecific competitors in natural systems.

The potential for diversification in response to ecological opportunity may also be more pronounced in Arctic charr compared to other fish species. Arctic charr can invade a wide variety of niches available in aquatic ecosystems, including multiple niches within the littoral, pelagic, and profundal habitats of lacustrine systems, as well as feeding niches in the marine habitat (Klemetsen et al. 2003; Klemetsen 2010; Reist et al. 2013; Chapter 3). Therefore, ecological opportunity for Arctic charr should be greater in larger lakes, where a greater diversity of habitats and microhabitats are available (Barbour & Brown 1974; Tonn & Magnuson 1982). In addition, anadromous fish species like the Arctic charr (Klemetsen et al. 2003) should also experience greater ecological opportunity in lake systems with access to the marine environment.

The relationship between trophic resource use and morphology has also been demonstrated in Arctic charr (see reviews in Skúlason et al. 1999; Jonsson & Jonsson 2001; Klemetsen et al. 2003; Reist et al. 2013). For example, common rearing studies have shown functional trade-offs in prey use efficiency associated with morphological differences among morphs within polymorphic populations (Malmquist 1992; Adams & Huntingford 2002; Garduño-Paz & Adams 2010). Common patterns between body shape and habitat use have also been established among fish (Webb 1982; 1984), and within Arctic charr (Jonsson & Jonsson 2001). Correlations between diet and morphological trait variation have also been observed within four populations of Arctic charr (Woods et al. 2013; Knudsen et al. 2014), however, the strength and significance of these correlations were not consistent across populations or different trait measures.

*Hypotheses*

The goal of this study was to further investigate the prediction that intraspecific competition and ecological opportunity promote diversity within populations (Araújo et al. 2011). Here, we focused on two environmental factors that influence ecological opportunity. The first was habitat heterogeneity, which was approximated using lake size and access to the marine environment. The second was interspecific competition, qualified here as the presence or absence of other fish species. Three hypotheses regarding the effect of ecological opportunity on intrapopulation morphological variation in Arctic charr were tested: 1)

populations of Arctic charr in lakes with no other fish species will exhibit greater variation in morphology than populations of Arctic charr that coexist with other fish species; 2) the magnitude of intrapopulation variation in morphology will increase with lake size; and 3) populations of Arctic charr in lakes with access to the marine environment will exhibit greater morphological variability than those in landlocked lakes. We also examined the effect of ecological opportunity on the relationship between diet and morphological variation within populations by testing three similar hypotheses: 1) the covariation between morphology and diet will be greater in populations of Arctic charr that do not coexist with other fish species; 2) the intrapopulation covariation between diet and morphology will increase with lake size; and 3) populations of Arctic charr with access to the marine environment will exhibit stronger covariation between diet and morphology than those in landlocked lakes.

## METHODS

*Site Data*

Arctic charr included in this study were collected from eleven lakes in Eastern Canada (Fig. 4.1, Table 4.1), including four in northern Ellesmere Island and seven in northern Labrador. Though difficult to quantify in natural systems, relative ecological opportunity has been approximated in freshwater systems using measures of lake size (Robinson et al. 2000; Svanbäck & Persson 2004; Nosil & Reimchen 2005). Here, we approximate differences in habitat heterogeneity among sites using lake size and access to the marine habitat. Relative lake size was approximated using surface area values obtained from the freely available "Global Lakes and Wetlands Database" (http://worldwildlife.org/pages/ global-lakes-and-wetlands-database) (Table 4.1). Access to the marine environment was inferred by the presence of anadromous individuals within populations, which were identified using stable isotope data as described in Chapter 5 (Michaud et al. 2013).

All of these sites are located in remote areas, and what is known about the fish species community in each lake is limited mainly to the species captured during the sampling opportunities described below. The only freshwater fish species reported to occur in northern Ellesmere Island is the Arctic charr (Christiansen & Reist 2013), and the only fish species captured in the lakes on Ellesmere Island included here. Therefore, we believe it is reasonable

to conclude Arctic charr do not experience interspecific competition with other fish in the four lakes on Ellesmere Island included in this study. In the Labrador lakes, Arctic charr were captured along with several other fish species, including lake charr (*Salvelinus namaycush*), brook charr (*Salvelinus fontinalis*), threespine stickleback (*Gasterosteus aculeatus*), and lake chub (*Couesius plumbeus*). However, given the diversity of fish species found in the lakes surrounding the Labrador sites (Black et al. 1986; Crossman & McAllister 1986), it is possible additional fish species were present in these lakes that have not yet been captured. Due to this lack of information, we infer that Arctic charr in the seven Labrador sites experience interspecific competition, but cannot account for differences in competition associated with different fish species and species community structures in the Labrador lakes.

*Arctic Charr Collection and Processing*

Arctic charr from all sites were captured during the late summer and early fall (July 16 - September 12) of 2007 and 2008, except for 55 individuals from Lake Hazen that were captured during June 15-21, 1992. The majority of individuals were captured using multi-mesh, multi-filament nylon gillnets with panels ranging from 10mm to 130mm bar mesh sizes. In addition, 26 Arctic charr from Lake Hazen were captured by angling, nine individuals from Tasisuak Lake were captured using dipnets, and two Arctic charr from Lake C were captured by electrofishing. All Arctic charr were frozen within 24 hrs of capture.

Digital photographs were taken of the left side of each Arctic charr after thawing. In cases where the left side of a fish was damaged, a photo of the right side was substituted. Gender and maturity status were determined by visual assessment of the gonads and categorized according to the index presented in McGowan (1987). Only sexually mature individuals were included in this study, in order to reduce the influence of ontogenic changes in morphology (e.g. Parsons et al. 2010) on the calculated amount of morphological variation within populations.

*Morphological Variation*

The magnitude of intrapopulation variation in morphology was quantified using multivariate geometric morphometric techniques. First, XY coordinate values for 16

homologous landmarks (Bookstein 1991) and 14 semi-landmarks (Bookstein 1997) were obtained from digital images of individual Arctic charr using the program TpsDig (version 2.16, Rohlf 2010) (Fig. 4.2), as described in Chapter 3. These coordinates were then divided into two sets, representing two-dimensional body shape (landmarks 1, 2, 9, and 19-31) and head shape (landmarks 1-19). Each set of coordinate data was subject to a Generalized Least-Squares (GLS) Procrustes superimposition to remove non-shape variation due to specimen position, orientation and scale (Rohlf & Slice 1990). Residuals from the regression of shape on centroid size were used in the following analyses in order to remove variation in morphology associated with allometry within each population. For the sets of coordinates used in the calculation of disparity, these corrections were made using CoordGen 8 and Standard 8, included in the IMP series of programs created by H.D. Sheets (2014, available at http://www.canisius.edu/~sheets/morphsoft.html). The same corrections were made in the program MorphoJ v. 1.06d (Klingenberg 2011) prior to calculation of the RV coefficients.

The magnitude of variation in two-dimensional head and body shape within each population was quantified as multivariate group disparity (*D*), after the method of Foote (1993):

$$D = \sum(d_i^2)/(n\text{-}1)$$

where $d_i$ is the Procrustes distance between the head or body shape coordinate centroid of individual *i* and the centroid of all *n* individuals (consensus shape) within a given population (Bookstein 1991). Head and body shape disparity values for each population were calculated using the IMP series program DisparityBox 8 (2014). All disparity values were reported as 1000x the observed values.

*Morphology & Diet*

Intrapopulation variability in diet was quantified using $\delta^{13}C$ and $\delta^{15}N$ values obtained from individual dorsal muscle tissue samples. Stable isotopes were chosen as an indicator of diet variation because they reflect cumulative dietary differences among individuals over a period of weeks to months (Dalerum & Angerbjörn 2005). All samples collected in 2007 and 2008 were processed as described in Chapter 5 (Michaud et al. 2013), while those for Arctic charr captured in Lake Hazen in 1992 were obtained as described in Guiguer et al. (2002).

The relationship between morphological and diet variation within populations was examined using a partial least squares analysis (PLS: Abdi & Williams 2013; Rohlf & Corti 2000). Here, we focus on the covariation between blocks of multivariate shape data, comprised of the adjusted body or head coordinate values, and multivariate diet data, which included individual $\delta^{13}C$ and $\delta^{15}N$ values. The amount of intrapopulation variation in morphology associated with diet was quantified using the *RV* coefficient, which is a multivariate analog of the $R^2$ correlation coefficient (Escoufier 1973; Klingenberg 2009). The *RV* coefficients for each population were calculated using MorphoJ, which also uses a permutation test to approximate *P*-values for the *RV* coefficient.

*Statistical Analyses*

Prior to examining the relationship between ecological opportunity and intrapopulation morphological variation, we tested for the potentially confounding effects of sample size and sexual dimorphism on shape disparity values. Spearman's rank-order correlation (Spearman's rho: Spearman 1904; Zar 1999) was used to test whether differences in head or body shape disparity among populations were independent of sample size or the ratio of male to female individuals in the sample. These tests were performed in SPSS (v. 17.0.1, 2008).

Multiple regression models were used to test the hypotheses concerning intrapopulation morphological variation and ecological opportunity. The full models were of the form:

$$D_i = \beta_0 + \beta_1(C_i) + \beta_2(\log_{10}SA_i) + \beta_3(M_i) + \beta_4(C_i \times \log_{10}SA_i) + \beta_5(C_i \times M_i) + \beta_4(\log_{10}SA_i \times M_i) + \varepsilon$$

where *D* is the disparity in head or body shape of Arctic charr collected from site *i*; *C* is the presence or absence of interspecific competitors in site *i*; *SA* is the estimated surface area of lake site *i*, and *M* is a bivariate factor indicating whether or not Arctic charr from site *i* utilize the marine environment. Non-significant interactions between the various aspects of ecological opportunity were removed from the model using a backward elimination procedure in which the term with the greatest *P*-value was removed from the model until all remaining terms had *P*-values < 0.05.

A similar multiple regression model was used to test the hypotheses concerning the effect of ecological opportunity on the covariation between morphology and diet. In these

tests, $RV_i$, the *RV* coefficient for Arctic char collected from site *i*, was substituted for $D_i$ in the above model.

## RESULTS

The eleven lakes included in this study covered a variety of sizes, with surface areas ranging from 0.63 $km^2$ to 527 $km^2$, and were located at altitudes between 0.65 km and sea level (Table 4.1). Population sample sizes ranged from 28 to 264 sexually mature individuals though most samples were relatively small, with samples from six sites including fewer than 50 individuals (Table 4.1). The ratio of male to female Arctic charr in the samples was approximately 1:1 for most of the populations, but ranged from 35% - 87% male across all sites (Table 4.1). Stable isotope data indicated that four of the sites located at altitudes of less than 0.05 km included Arctic charr that foraged in the marine environment (Table 4.1), indicating that individuals in these lakes had access to the marine habitat.

### *Morphological Variation*

Head and body morphology disparity values were independent of sample size differences among sites (body: $\rho$ = 0.20; $P$ = 0.563, head: $\rho$ = 0.07; $P$ = 0.832). The rank correlation tests indicated that disparity in head morphology decreased as the percentage of male Arctic charr in the samples increased ($\rho$ = 0.63; $P$ = 0.039), but disparity in body morphology was independent of sample sex ratios ($\rho$ = 0.15; $P$ = 0.668). The percentage of males in each population sample (%M) was, therefore, added as a factor in the multiple regression models for head disparity to account for the confounding effect of sexual dimorphism.

Intrapopulation variation in head and body morphology among the populations of Arctic charr examined here did not increase with ecological opportunity as predicted (Table 4.2, Fig 4.3). All interactions between the ecological factors were removed from the head and body shape disparity models through the backwards selection procedure (see Table 4.3). Based on the reduced models, the average variation in head morphology within populations was similar among sites with (mean disparity ± standard deviation: 5.03 ± 0.67) and without interspecific fish competitors (3.85 ± 1.04). Counter to our prediction, intrapopulation variation in body

morphology was greater among populations of Arctic charr that coexisted with other fish species (0.87 ± 0.12) than among populations that did not (0.63 ± 0.17). Average intrapopulation morphological variation was similar among sites with (head: 4.99 ± 0.47; body: 0.86 ± 0.17) and without (head: 4.38 ± 1.15; body: 0.73 ± 0.17) access to the marine environment. Intrapopulation variation in head and body morphology also did not increase with lake size as predicted. Combined, ecological opportunity accounted for a relatively small portion of the differences in morphological variability among these eleven populations of Arctic charr (head: adjusted $R^2$ = 0.25; body: adjusted $R^2$ = 0.45).

*Morphology & Diet*

The covariance between intrapopulation variation in body morphology and diet was statistically significant in five of the eleven populations of Arctic charr examined here, while head morphology and diet covaried in only three of these populations (Table 4.4). Of the relationships that were statistically significant, the covariation between diet and morphological variation was not high. All *RV* values associated with body morphology were less than 0.44, while the relationship between head morphology and diet was much weaker, with a highest *RV* value of 0.17. Because morphology and diet did not covary significantly in most of these populations, we were unable to test the second set of hypotheses predicting that the covariation between morphology and diet would also increase with ecological opportunity

## DISCUSSION

Intraspecific competition and ecological opportunity are predicted to promote phenotypic diversity within populations (Van Valen 1965; Roughgarden 1972; Bolnick 2001; Araujo et al. 2011). However, the patterns of intrapopulation variation observed among the eleven Arctic charr populations examined in this study do not support the prediction that morphological variation, specifically, increases with increased habitat heterogeneity or decreased interspecific competition. The extent of morphological variation within these populations of Arctic charr did not increase with lake size, and average morphological variation was similar among populations with and without access to the marine habitat. Average head shape variation was also similar between populations that did and did not experience

interspecific competition, while body shape variation tended to be greater among populations of Arctic charr that coexisted with other fish species.

Ideally, the hypotheses examined in this study would have been tested using a much larger dataset of Arctic charr populations collected from a more diverse set of locations. Due to sample sizes and number of locations available for analysis, the present study is limited in the scope of the conclusions that can be drawn from the results obtained here. However, testing for differences in morphological variation in relation to ecological opportunity among these eleven populations of Arctic charr provided clarification on the limitations presented by the dataset and analytical methods, and as well as the applicability of the conclusions. The patterns observed here do provide some insight into the relationship between morphological and diet diversity in Arctic charr, but no conclusions were reached about the role ecological opportunity and interspecific competition play in morphological diversity among Arctic charr. Therefore, this study is best viewed as a pilot project to developing a larger study to test the predictions that intrapopulation morphological and diet variation increases with ecological opportunity.

*Intrapopulation Morphological Variation and Interspecific Competition*

Intrapopulation variation in morphology is expected to be greater in populations that coexist with fewer competitor species (Van Valen 1965). However, studies that test this specific prediction from the NVH have produced mixed support for the hypothesis (Bolnick et al. 2007; Araujo et al. 2011). Among studies that have examined the effect of interspecific competition on morphological diversity in Arctic charr, Knutsen et al. (2007) observed that of two neighboring Arctic charr populations, the population in the lake containing fewer competitors exhibited greater among-individual variation in diet and morphology. Two larger scale studies of polymorphism in Arctic charr observed a similar trend, as the number of morphs observed in a population tended to decrease as the total number of species in the lake increased (Claessen et al. 2008; Woods et al. 2012b). One of the main differences between these and the study conducted here is that the other studies examined variation among populations within a relatively small region.

The NVH is based on the inference that intraspecific competition will favor the diversification of individuals in systems with fewer competitors, because they are able to utilize

resources that would otherwise be used by competitors (Simpson 1953; Roughgarden 1972; Slatkin 1980; Bolnick 2001). Therefore, a key assumption of the NVH is that resource availability is similar among populations in different systems. When comparing populations from geographically distant locations, this assumption may not hold due to climatic differences between regions. Although we do not have direct measurements of productivity in the lakes studied here, there is a general trend of reduced nutrient availability, primary productivity, and biomass in high Arctic lakes as they experience colder temperatures and longer durations of ice cover compared to those at lower latitudes (Karlsson et al. 2005; Vincent et al. 2008). There is also a general trend towards reduced biodiversity and more simplified foodwebs at higher latitudes (Christoffersen et al. 2008), and in more recently deglaciated regions (MacArthur & Wilson 1967; Bernatchez & Wilson 1998; Robinson & Schluter 2000). This reduction in biodiversity means that there are fewer (or no) interspecific competitors in the lakes on Ellsemere Island compared to those in Labrador, but it also has the simultaneous effect of reduced prey species diversity in the high Arctic lakes. Therefore, despite the lack of interspecific competition, reduced prey diversity and abundance may limit the opportunities for diversification in the populations of Arctic charr from Ellesmere Island. Conversely, the populations of Arctic charr in Labrador may experience greater interspecific competition, but this restriction on diversification may be mitigated by coincidently greater prey abundance and diversity.

In addition to resource availability, the extent of phenotypic variation within a population may also be limited by the time available for diversification to occur (Coyne & Orr 2004). For example, Siwertsson et al. (2010) observed a decrease in the number of morphs within polymorphic populations of European whitefish (*Coregonus lavaretus*) following the path of glacial retreat among three watercourses in Fennoscandia (lakes that were deglaciated more recently tended to have fewer morphs). This trend was observed in a relatively small region, however, the effect may be compounded when comparing populations from regions like Ellesmere Island and Labrador, which have very different glacial histories. The lakes on Ellesmere Island were open to invasion approximately 5 ka BP (Smith 1999), following the last glacial period, while in Labrador the glaciers retreated much earlier at approximately 8 ka BP

(Clark & Fitzhugh 1990; Dyke 2004). Although detectable phenotypic divergence can occur over very short time scales in Arctic charr (e.g. Michaud et al. 2008, Klemetsen et al. 2002), the difference in the time of glacial retreat means the populations in Labrador potentially had a much longer time for diversification to occur compared to those on Ellesmere Island. The trend toward greater diversification in lakes that have been open to invasion for a longer time period could explain why average intrapopulation variation in body morphology was observed to be greater in the Labrador populations.

The extent of morphological variation within populations is also constrained, to some degree, by genetic factors. For example, bottlenecks and founder effects that may occur during colonization can result in a loss of genetic variation which, in turn, could constrain the amount of phenotypic variation within a population (Barrett & Schluter 2008; Caldera & Bolnick 2008; Schluter & Conte 2009). To support this, a pattern of fewer morphs within polymorphic populations of European whitefish with reduced genetic variability was observed by Siwertsson et al. (2010). Similar issues may arise when dealing with populations from distant geographical regions. Although we do not have specific data on the genetic variability within the populations of Arctic charr examined here, the populations in Ellesmere Island and Labrador are part of distinct genetic lineages (Wilson et al. 1996; Brunner et al. 2001). Therefore, it is possible that genetic differences between these lineages include different genetic constraints on the potential for diversification in the populations from the two different regions.

*Intrapopulation Morphological Variation & Habitat Heterogeneity*

Morphological variation within populations was predicted to increase with habitat heterogeneity, as a greater diversity of habitats is expected to increase the number of alternative resources available for ecological release to occur (Simpson 1953; Hedrick 1986; Yoder et al. 2010; Araújo et al. 2011). As a species, Arctic charr have been observed to utilize a wide variety of habitats and micro-habitats in the freshwater and marine environment (Skúlason et al. 1999; Jonsson & Jonsson 2001; Klemetsen 2010; Reist et al. 2013). This suggests that Arctic charr have a high potential for diversification when alternative habitats are available, but we did not observe an increase in morphological diversity with lake size or access to the marine habitat among the populations examined here.

Increased intrapopulation variation in some morphological traits was observed with increased lake volume among Threespine stickleback (Nosil & Reimchen 2005). Regional studies of polymorphism in Arctic charr and European whitefish also found that the number of morphs in a population tended to increase with lake depth or volume (Claessen et al. 2008; Siwertsson et al. 2010; Woods et al. 2012b). However, we were only able to obtain lake surface area measurements for the lakes examined here, which is likely a poor way of quantifying relative habitat heterogeneity in lakes as it does not account for habitat diversity associated with depth.

We also did not have information available on the number of species present in the Labrador lakes. Larger lakes are expected to offer greater resource diversity and, because of this, are also expected to support a greater diversity of species (Barbour & Brown 1974; Tonn & Magnuson 1982; Ricklefs & Lovette 1999). Therefore, competition for resources by an increasing number of other fish species may have cancelled out the potential for diversification offered by greater habitat heterogeneity in the larger Labrador lakes. Although there were no fish species other than the Arctic charr in lakes on Ellesmere Island, greater habitat heterogeneity may be confounded by a lack of productivity in some habitats in these high Arctic lakes.

We predicted that access to the marine habitat would also offer greater habitat heterogeneity to a population of Arctic charr, since the species has both freshwater resident and anadromous forms (Klemetsen et al. 2003; Reist et al. 2013). We also predicted that diversification into this habitat would result in greater intrapopulation morphological variability because of the observed morphological differences between freshwater resident and anadromous forms of Arctic charr and other northern fish species (Nordeng 1983; Jonsson & Jonsson 1993; Morinville & Rasmussen 2008; Loewen et al. 2009). We observed no difference in average morphological variation between populations of Arctic charr with and without access to the marine habitat. However, the sample size for testing this hypothesis was too small to account for the interactive effects of other factors associated with ecological opportunity. Therefore, this hypothesis needs to be tested with a larger data set before any conclusions can be made.

*Morphology & Diet*

The covariation between diet and morphology was predicted to be greater in populations that experienced no interspecific competition and greater habitat heterogeneity. However, these two factors will only covary in populations where morphological traits confer a competitive advantage to individuals competing for limited resources (Svanback & Bolnick 2005; Bolnick et al. 2007; Taper & Case 1985; Wilson & Turelli 1986). Originally, the NVH proposed that diet variation would increase with niche expansion in response to reduced interspecific competition (VanValen 1965)**.** Because diet variation is difficult to measure directly in wild populations, the hypothesis was extended to predict that morphological variation would increase with reduced interspecific competition (VanValen 1965). However, the use of variation in morphology to test the NVH has led to mixed support for the hypothesis (reviewed in: Bolnick et al. 2007; Araujo et al. 2011), and recent studies indicate that this may be because morphology is a poor proxy for diet variation among individuals (Araújo et al. 2011; Snowberg et al. 2015).

Individual differences in diet are influenced by a variety of factors other than morphology. These include external factors, such as the relative abundance, distribution, and competition for different prey types (Svanbäck & Bolnick 2005). Phenotypic differences other than morphology, including behavioral and physiological traits, also influence the level of intrapopulation diet variation (Van Valen 1965; Roughgarden 1972; Bolnick et al. 2003, Araújo et al. 2011). In particular, individual differences in behavior may play a larger role than morphology in mitigating intraspecific competition, especially in response to short-term changes in prey availability (Swanson et al. 2003; Svanbäck & Bolnick 2007; Bolnick & Paull 2009).

The covariation between morphology and diet within the populations of Arctic charr examined here were relatively weak. These weak covariances are consistent with the diet-morphology relationships observed in other populations of Arctic charr (Woods et al. 2013a; Knudsen et al. 2014), threespine stickleback (*Gasterosteus aculeatus*) (Bolnick et al. 2007; Svanbäck & Bolnick 2007; Bolnick & Paull 2009; Snowberg et al. 2015), and Eurasian perch (Svanback & Bolnick 2005). Unfortunately, because we could not quantify diet variation, it is

impossible to determine whether the low covariances observed between diet and morphology are simply due to a lack of variability in diet or because other phenotypic traits are more important to diet variation in these populations. This also has implications for the previous hypotheses tested here, as the hypotheses were based on the assumption that morphological traits were important to resource use variation in these populations.

One of the key limiting factors of this study was the inability to quantify diet variability within populations. Ideally, tests of the NVH would directly examine the effects of ecological opportunity on intrapopulation variation in diet (e.g. Bolnick et al. 2007; Snowberg et al. 2015). Stomach contents are insufficient to encompass variation in diet that extends over a feeding season because they reflect only prey consumed over a period of days. Stable isotope values obtained from muscle tissue account for individual diet variability over a period of weeks to months (Tieszen et al. 1983; Hobson & Clark 1992). Stable isotopes provide a convenient tool for detecting diet differences, but variation in $\delta^{13}C$ and $\delta^{15}N$ values among individuals is also affected by a wide variety of factors not associated with prey consumption (Chapter 5: Michaud et al. 2013).

Omnivorous predators, like Arctic charr, feed on a variety of prey from different habitats (Amundsen 1995; Klemetsen et al. 2003; Amundsen & Knudsen 2009; Eloranta et al. 2011). Therefore, intrapopulation variation in $\delta^{13}C$ and $\delta^{15}N$ values should not be compared among populations without first accounting for differences between the baseline stable isotope values of benthic and pelagic rooted foodwebs from different sites (e.g. VanderZanden & Vadeboncoeur 2002; Post 2002). This is because the magnitude of intrapopulation variation in stable isotope values would be expected to be greater in populations from lakes with larger differences between the $\delta^{13}C$ and $\delta^{15}N$ values of prey from different habitats, even if the functional variation in prey choice is similar. Differences in the variation of stable isotope values of comparable prey organisms among lakes must also be accounted for. Among populations that feed on the same prey organisms, intrapopulation variation in $\delta^{13}C$ and $\delta^{15}N$ values would be expected to be greater in lakes where the variation in the stable isotope values of those prey organisms is also greater. Both of these conditions would result in increased variation in the

measured predator stable isotope values that does not reflect an increase in functional diet variation.

*Future Work*

The data available here was insufficient for a robust test of the prediction that morphological and diet diversity within populations increases with ecological opportunity. However, given their large geographic distribution and potential for ecological diversification, Arctic charr provide a rare opportunity to test various aspects of the NVH using a single species that experiences a wide range of factor levels associated with ecological opportunity. This includes the extreme situation of populations that experience no competition with other fish species, which may further our understanding of the relative roles of inter- and intraspecific competition in diversification and evolution. Ideally, we would have directly examined the effects of ecological opportunity on dietary resource use variation within populations, and the low covariations between diet and morphological variation observed here support the case for testing the NVH directly using diet data. During the process of developing the present study, we also learned that a more thorough collection of foodweb data is needed to address the relationship between diet and morphological variation within populations and compare among sites. Comparing populations of Arctic charr from two distinct geographic regions has also highlighted the need for collecting data from a greater number of sites within and across regions with different glacial histories and environmental conditions.

The variability in the covariance values observed here also raises questions of why the relationship between diet and morphology differs among populations of Arctic charr. Under what conditions is morphology more important to diet variability among individuals, and what environmental factors influence this relationship? Investigating this could provide more information on the role of environmental variation and different phenotypic traits in the process of ecological diversification in Arctic charr and other species. Other studies have found positive relationships between ecological opportunity and intrapopulation variation using the number of morphs in polymorphic populations as a measure of morphological and diet variation (Claessen et al. 2008; Siwertsson et al. 2010; Woods et al. 2012b). Here, we used the magnitude of among-individual variation in morphology to quantify intrapopulation variation.

However, it is interesting to note that of the populations examined here, those known to be polymorphic (e.g. Lake Hazen, Chapter 3), or contained a mix of anadromous and lacustrine individuals were not necessarily more morphologically variable than the other populations. This raises the question of whether this was a product of the way morphological variation was quantified, or if it is reflective of the proportion of individuals from each morph included in the sample. Alternatively, the differences between morphs within these populations may be based on other phenotypic traits, like behavior or life history, and not necessarily reflected in morphological differences.

## TABLES & FIGURES

**Table 4.1:** Sampling sites and summary of Arctic charr used in this study. Information for each lake site includes: the estimated lake surface area and altitude; the total number (*N*), proportion of males, age range, and size range (measured as forklength, FKL) of Arctic charr included in each sample; and whether or not individuals that foraged in the marine habitat were present at each site. Sites are divided according to the presence (shaded) or absence of interspecific fish competitors. Asterisks (*) indicate lakes that were given arbitrary names, as they do not have officially recognized names.

| Site | Abb. | Lake SA ($km^2$) | Altitude (km) | *N* | % Male | Age (years) | FKL (mm) | Marine |
|---|---|---|---|---|---|---|---|---|
| Fish Competitors: Absent | | | | | | | | |
| Lake C* | LKC | 0.90 | 0.46 | 98 | 52 | 5-29 | 149-702 | N |
| Lake G* | GTL | 2.70 | 0.18 | 28 | 64 | 10-24 | 236-292 | N |
| Lake Hazen | LHZ | 527.00 | 0.25 | 264 | 54 | 4-35 | 122-668 | N |
| Clements Markham Lake | CML | 5.80 | 0.03 | 41 | 56 | 5-28 | 170-497 | Y |
| Fish Competitors: Present | | | | | | | | |
| Hebron Lake #2* | HB2 | 4.10 | 0.65 | 44 | 50 | 3-25 | 96-505 | N |
| Upper Nakvak Lake* | UNL | 5.50 | 0.35 | 30 | 87 | 3-25 | 96-546 | N |
| Tasialuk Lake | TLL | 0.63 | 0.04 | 29 | 55 | 3-25 | 92-488 | N |
| Esker Lake | ESK | 37.50 | 0.34 | 91 | 53 | 3-30 | 92-537 | N |
| Tom's Pond* | TOM | 2.00 | 0.01 | 30 | 57 | 5-13 | 208-522 | Y |
| Hebron Lake #3* | HB3 | 2.40 | 0.03 | 69 | 35 | 3-17 | 96-488 | Y |
| Tasisuak Lake (Fraser River) | FRT | 60.50 | 0.01 | 41 | 51 | 3-13 | 86-493 | Y |

**Table 4.2:** The *F*- and *P*-values for each term included in the reduced models used to test for differences in mean body and head shape disparity among sites with and without interspecific competitors.

| | Body | | Head | |
|---|---|---|---|---|
| Model Terms | $F_{(1,11)}$ | *P* | $F_{(1,11)}$ | *P* |
| C | 7.45 | 0.029 | 4.70 | 0.073 |
| $Log_{10}SA$ | 2.13 | 0.188 | 0.65 | 0.451 |
| M | 1.19 | 0.312 | 0.09 | 0.779 |
| %M | | | 1.12 | 0.331 |

**Table 4.3:** Backwards elimination procedure for the multiple regression models predicting intrapopulation body (A) and head (B) shape disparity. Columns list the *P*-values associated with the terms included in the full and reduced models (term abbreviations are explained in the text). The adjusted $R^2$ values for each model are listed in the last row of the table.

A) Y = Body Shape Disparity

| | Models | | | |
|---|---|---|---|---|
| Model Terms | Full | 2 | 3 | 4 |
| C | 0.092 | 0.050 | 0.028 | 0.029 |
| $Log_{10}SA$ | 0.073 | 0.060 | 0.061 | 0.188 |
| M | 0.613 | 0.456 | 0.653 | 0.312 |
| C x $Log_{10}SA$ | 0.422 | 0.405 | | |
| C x M | 0.436 | | | |
| $Log_{10}SA$ x M | 0.148 | 0.124 | 0.152 | |
| Adj. $R^2$ | 0.52 | 0.55 | 0.56 | 0.45 |

B) Y = Head Shape Disparity

| | Models | | | |
|---|---|---|---|---|
| Model Terms | Full | 2 | 3 | 4 |
| C | 0.094 | 0.101 | 0.169 | 0.073 |
| $Log_{10}SA$ | 0.284 | 0.490 | 0.378 | 0.451 |
| M | 0.777 | 0.445 | 0.543 | 0.779 |
| %M | 0.242 | 0.251 | 0.227 | 0.331 |
| C x $Log_{10}SA$ | 0.187 | 0.272 | | |
| C x M | 0.272 | 0.265 | 0.273 | |
| $Log_{10}SA$ x M | 0.352 | | | |
| Adj. $R^2$ | 0.42 | 0.39 | 0.31 | 0.25 |

**Table 4.4:** Calculated *RV* coefficients and their associated *P*-values for the tests of covariation between morphological and diet data. Shaded rows indicate sites where interspecific competitors are present.

| | Body - Diet | | Head - Diet | |
|---|---|---|---|---|
| | *RV* | *P*-value | *RV* | *P*-value |
| LKC | 0.23 | <0.001 | 0.09 | 0.002 |
| GTL | 0.08 | 0.926 | 0.06 | 0.608 |
| LHZ | 0.03 | 0.018 | 0.01 | 0.186 |
| CML | 0.06 | 0.868 | 0.06 | 0.380 |
| HB2 | 0.12 | 0.145 | 0.10 | 0.084 |
| UNL | 0.39 | <0.001 | 0.17 | 0.039 |
| TLL | 0.12 | 0.175 | 0.02 | 0.869 |
| ESK | 0.03 | 0.270 | 0.01 | 0.807 |
| TOM | 0.08 | 0.584 | 0.14 | 0.078 |
| HB3 | 0.21 | <0.001 | 0.17 | 0.001 |
| FRT | 0.43 | <0.001 | 0.05 | 0.469 |

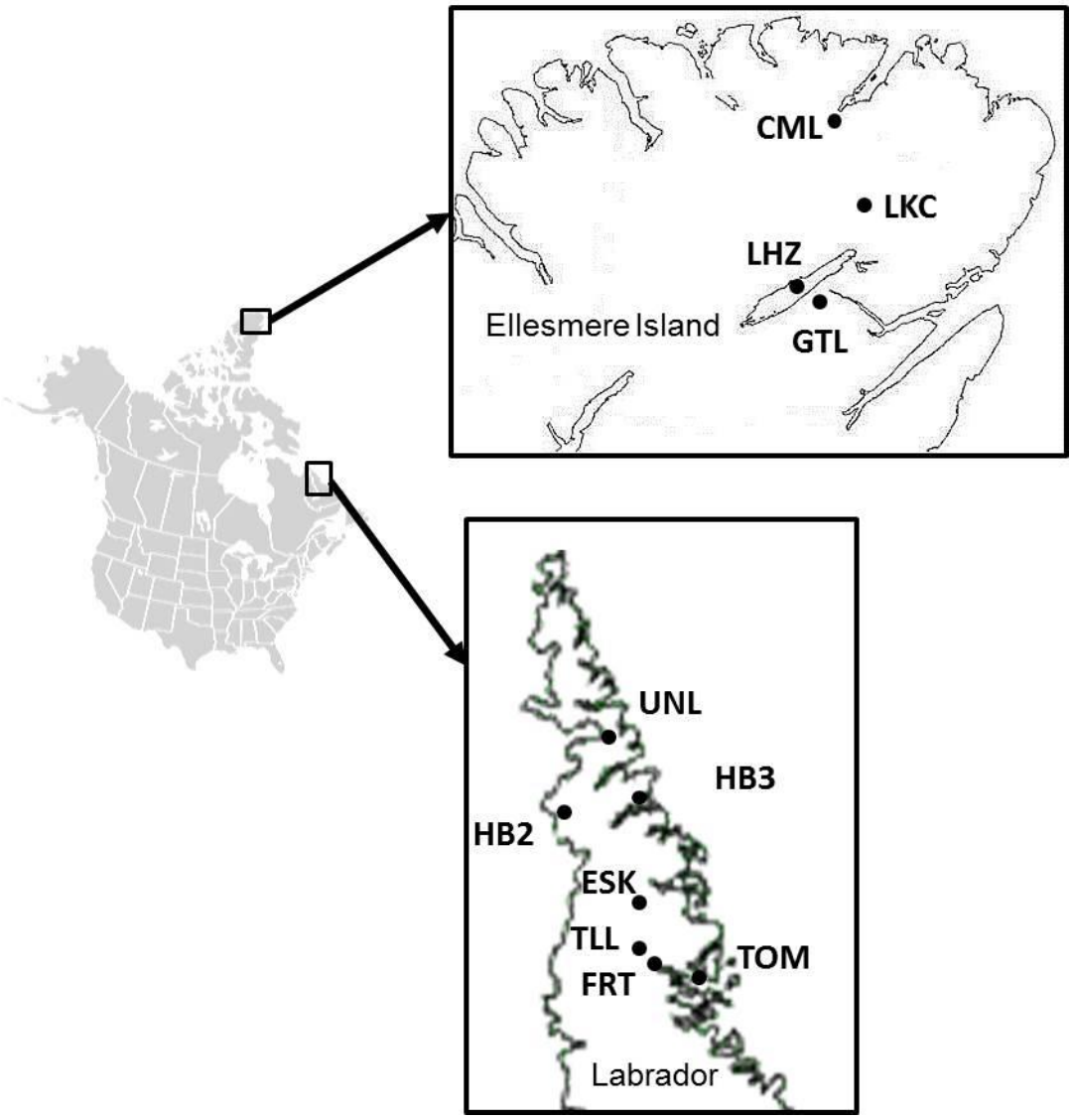


**Figure 4.1:** Sampling locations (see Table 4.1 for full lake names), including four lakes on Ellesmere Island where Arctic charr are the only known fish species and seven lakes in northern Labrador where Arctic charr coexist with other fish species.

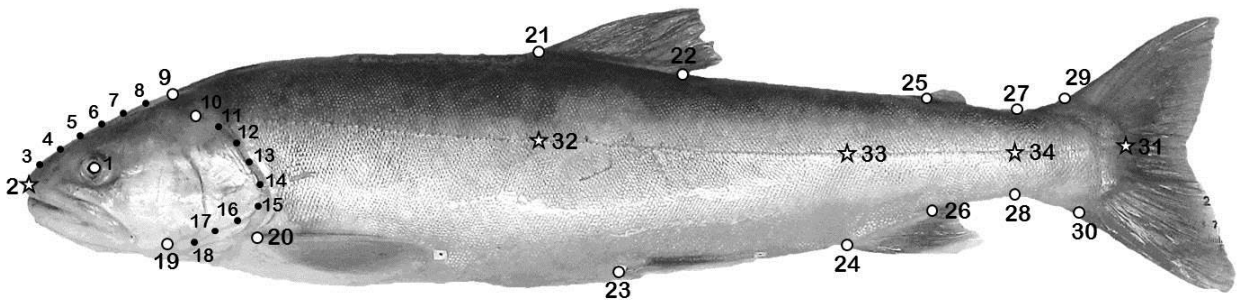


**Figure 4.2:** Anatomical landmarks used to define two-dimensional head and body shape. Homologous landmarks are indicated by white dots while the smaller, black dots represent sliding semi-landmarks used to outline head shape. Stars denote landmarks used to define the line along which specimens were straightened in the "unbending" procedure (described Chapter 3).

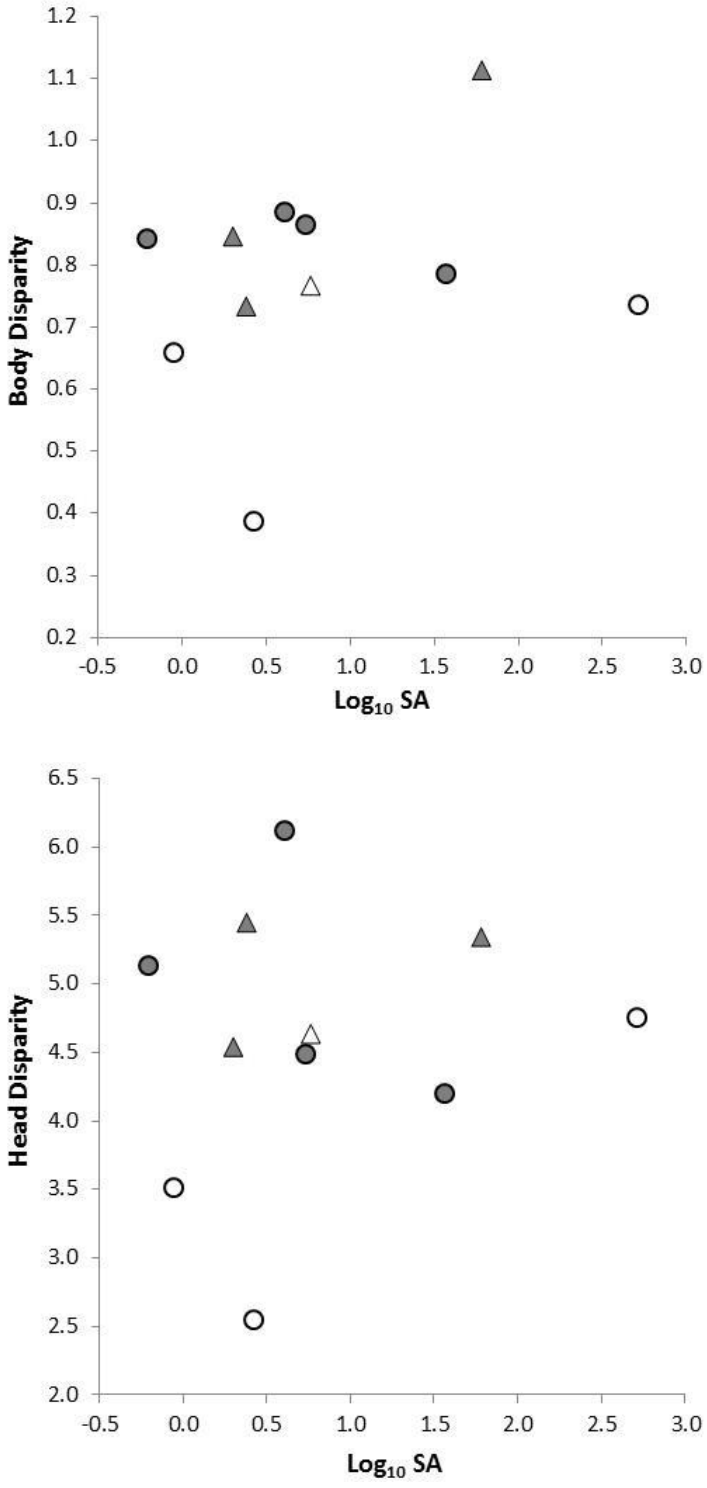


**Figure 4.3:** Morphological disparity values plotted against lake size for each population sample. Triangles (△) indicate sites with access to the marine habitat and circles (○) indicate landlocked sites, while shaded symbols indicate sites with (●) and without (○) interspecific fish competitors.

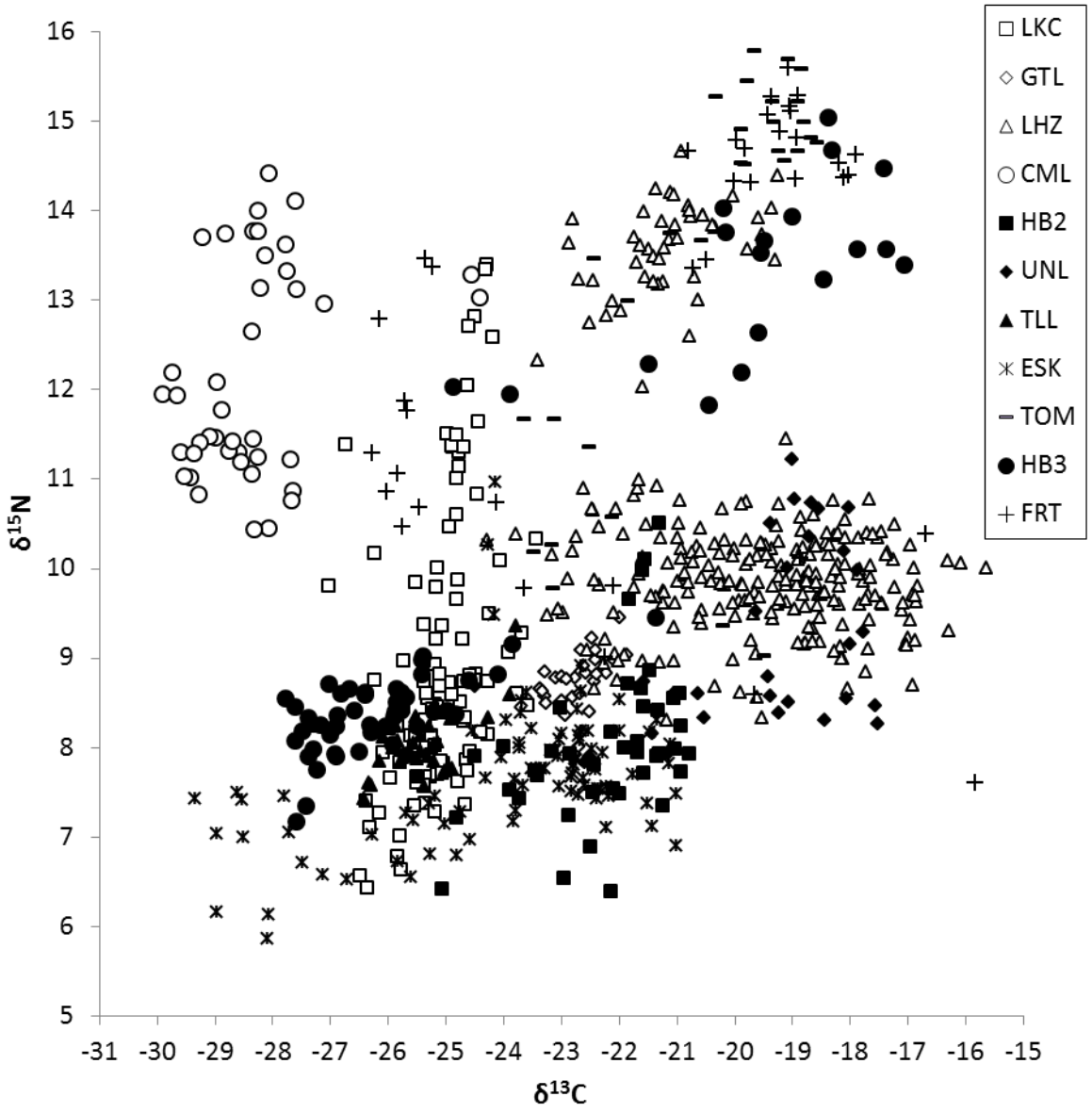


**Figure 4.4**: Individual $\delta^{13}C$ and $\delta^{15}N$ values for all populations.

# Chapter 5 - Ecological influences on the difference in $\delta^{15}N$ and $\delta^{13}C$ values between fish tissues: implications for studies of temporal diet variation

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## INTRODUCTION

Stable isotopes have become an important tool in ecological research, with $\delta^{13}C$ and $\delta^{15}N$ data being used increasingly to examine trophic patterns in a variety of organisms and ecosystems (reviewed in: Peterson & Fry 1987; Gannes et al. 1997; Post 2002; Martínez del Rio et al. 2009). Among their many applications, stable isotopes are used to examine individual diet changes over time. A method often employed to detect shifts in diet when serial sampling is not possible, is the comparison of stable isotope values from different tissues within the same organism that differ in their isotopic turnover times (Tieszen et al. 1983; Hobson 1999; Dalerum & Angerbjörn 2005; Suzuki et al. 2005; Phillips & Eldridge 2006). The differences in turnover times among tissues are related to the physiological roles of each tissue. More metabolically active tissues, such as blood or liver, generally have faster turnover rates than less metabolically active tissues, like muscle or bone (Tieszen et al. 1983; Hobson & Clark 1992). As a result of these metabolic differences, the $\delta^{13}C$ or $\delta^{15}N$ value of a tissue with a long isotopic turnover time will reflect the prey items that were assimilated over a relatively longer time period than the isotope value of a more metabolically active tissue. Disparities in the $\delta^{13}C$ and $\delta^{15}N$ values among tissues are, therefore, typically interpreted as resulting from differences in the stable isotope values of recently consumed prey items compared to the long-term diet of the consumer. There are, however, factors other than a change in diet that can affect differences in $\delta^{13}C$ and $\delta^{15}N$ values between tissues.

Metabolic routing, nutritional stress, age, growth, ontogenic changes, and the relative protein content of prey items could potentially influence differences in stable isotope signatures among tissues (see Dalerum & Angerbjörn 2005; Suzuki et al. 2005; Phillips &

Eldridge 2006). These factors can alter tissue lipid and protein composition, which, in turn, affect the $\delta^{13}C$ and $\delta^{15}N$ values of those tissues and the differences between them (Tieszen et al. 1983; Pinnegar & Polunin 1999; McCutchan et al. 2003). Although the effects of various ecological factors on the fractionation of $^{13}C$ and $^{15}N$ in specific tissues have been investigated, the impact these have on difference in $\delta^{13}C$ and $\delta^{15}N$ values among tissues has not been directly addressed. More importantly, it is unclear whether the effects of these factors are large enough to result in the misinterpretation of stable isotope data in studies of temporal diet change.

Accordingly, the primary goal of this study was to determine whether the effects of ecological factors on the differences in $\delta^{13}C$ and $\delta^{15}N$ values between muscle and liver tissues are large enough to affect the biological interpretation of isotope data when reconstructing temporal diet patterns. Muscle and liver $\delta^{13}C$ and $\delta^{15}N$ values obtained from wild Arctic charr (*Salvelinus alpinus (L.)*), and ecological factors purposefully chosen because of their previously demonstrated impacts on protein and/or lipid dynamics in fish (see Love 1980; Henderson & Tocher 1987; Shearer et al. 1994; Jørgensen et al. 1997; Jonsson & Jonsson 1998; Doucett et al. 1999a; Dempson et al. 2004) were used to address this question. Specifically, we test the hypotheses that the following ecological factors have statistically and biologically significant effects on the differences between muscle and liver $\delta^{13}C$ and $\delta^{15}N$ signatures: 1) life history type (anadromous or lacustrine); 2) gender (male or female); 3) reproductive status (spawning or not spawning); 4) diet (piscivorous or not piscivorous); and 5) feeding status (actively feeding or fasting). We also test whether interactions among the factors are important to explaining variation in the differences between muscle and liver $\delta^{13}C$ and $\delta^{15}N$ signatures, as these factors are unlikely to act independently in wild organisms. Finally, as several of the chosen factors have been shown to affect lipid storage and metabolism in fish (Love 1980; Henderson & Tocher 1987; Shearer et al. 1994; Jørgensen et al. 1997; Jonsson & Jonsson 1998; Doucett et al. 1999a; Dempson et al. 2004), and there is a well-established relationship between tissue lipid content and $\delta^{13}C$ (DeNiro & Epstein 1977, Focken & Becker 1998, Post et al. 2007), a subset of muscle and liver samples were used to determine if there were significant differences in lipid content between tissues associated with differences in $\delta^{13}C$ values.

## METHODS

*Sample collection and processing*

Arctic charr were collected from 18 sites in eastern Canada, ranging from the Labrador coast to Ellesmere Island (Table 5.1). Individuals were captured during the late summer and early fall (July 16 - September 12) using multifilament, multimesh gillnets (10-130 mm bar mesh sizes), or by angling. All fish were frozen within 24 hrs of capture and kept frozen until they could be processed further. After thawing, dorsal muscle and liver samples were removed for stable isotope analyses and individual stomach contents were examined for evidence of piscivory. All dorsal muscle tissue samples were taken from the left side of the fish, just behind the dorsal fin and above the lateral line, with the skin removed prior to drying. Reproductive status was determined by examination of the gonads. Individuals categorized as "spawning" were fish with gonad development sufficient to indicate they were likely to have spawned during the year of capture, while immature or resting individuals were classified as "not spawning."

All Arctic charr captured in marine or estuarine environments were classified as "anadromous", while those captured in landlocked sites were considered to be "lacustrine". For Arctic charr captured in the four freshwater sites with access to an ocean (Clements Markham Lake, Tasisuak Lake, Hebron Lake #3, and Tom's Pond), sulphur stable isotope ($\delta^{34}S$) data were used to distinguish anadromous from resident lacustrine individuals (*sensu* Peterson & Fry 1987; Hesslein et al. 1991; Hobson 1999; Doucett et al. 1999b).

Lacustrine charr were further categorized by diet as "piscivorous" or "not piscivorous" based on stomach contents and $\delta^{15}N$ data. All Arctic charr with fish remains in their stomachs were considered to be piscivorous. Individuals without fish remains in their stomach contents were classified as "piscivorous" if their muscle $\delta^{15}N$ values fell within the range of individuals from the same site that did have fish remains in their stomachs, and "not piscivorous" if they were outside of this range. The effect of diet was not considered for anadromous Arctic charr as all anadromous charr showed evidence of piscivory.

To test the effect of feeding status, anadromous Arctic charr were separated into "actively feeding" and "fasting" groups. Anadromous individuals captured in lakes were

expected to have begun the overwinter fasting period that is typical of anadromous Arctic charr (Dutil 1986; Boivin & Power 1990), while those captured in the marine environment were considered to be actively feeding. This was supported by examination of stomach contents, as nearly all (98%) of the anadromous individuals captured in freshwater were found to have empty stomachs. The effect of feeding status was tested using only anadromous Arctic charr because there was little evidence of fasting among the lacustrine fish, as very few (30) lacustrine charr had empty stomachs when they were captured.

*Stable isotope analyses*

All muscle and liver samples were dried at 50°C for at least 48hrs, then ground to a fine homogenate using either a Retsch MM 301 ball mill grinder (Retsch GmbH, Haan, Germany) or a mortar and pestle. All stable isotope analyses were conducted by the Environmental Isotope Laboratory (EIL) at the University of Waterloo, Ontario, Canada. Analyses of carbon ($^{13}C$:$^{12}C$) and nitrogen ($^{15}N$:$^{14}N$) isotope ratios were completed using a Finnegan Deltaplus continuous flow stable isotope ratio mass spectrometer (Thermo Finnigan Scientific, Bremen, Germany) coupled to a Carlo Erba Elemental Analyzer (CHNS-O EA1108, Carlo Erba, Milan, Italy) with an analytical precision of ±0.2‰ for $\delta^{13}C$ and ±0.3‰ for $\delta^{15}N$. Analyses of sulphur ($^{34}S$:$^{32}S$) stable isotope ratios were completed using a Micromass IsoChrom continuous flow stable isotope ratio mass spectrometer (GV Instruments, Manchester, UK) coupled to a Costech Elemental Analyzer (ECS 4010 CHNSO, Costech Analytical Technologies Inc., California, USA) with an analytical precision of ±0.5‰.

Stable isotope values are expressed in standard delta (δ) notation, corrected to the appropriate international standard defined for carbon (Peedee Belemnite; Craig 1957), nitrogen (atmospheric air; Mariotti 1983), or sulphur (Canyon Diablo meteorite; Rees et al. 1978). The international (IAEA, USGS, NIST) and internal (EIL) laboratory standards (calibrated against the international standards), used during the analyses included: ammonium sulphate (IAEA-N1 and IAEA-N2) for nitrogen; sugar (IAEA-CH6) and cellulose (EIL-72) for carbon; and AgS (IAEA-SI, IAEA-S2, and IAEA-S3), CuS (EIL-40), ZnS (EIL-43), Sphalerite ZnS (NBS-123), and bovine liver and muscle tissue (NIST) for sulphur.

Individual differences between muscle and liver $\delta^{13}C$ and $\delta^{15}N$ values were calculated by subtracting the stable isotope value of liver from that of muscle, hereafter denoted as $\delta^{13}C_{(M-L)}$ or $\delta^{15}N_{(M-L)}$.

*Lipid extraction*

To explore the relationship between differences in tissue lipid content and $\delta^{13}C_{(M-L)}$, a subset of individuals with high (N = 10) and low (N = 10) $\delta^{13}C_{(M-L)}$ values were selected from the pool of available Arctic charr tissue samples. The total lipid content of previously dried muscle and liver tissue was determined gravimetrically, using a modified version of the procedure published by Folch et al. (1957), as recommended by Iverson et al. (2001), and included additional modifications used by Kaufman et al. (2007). Briefly, 3 ml of chloroform-methanol solvent were added to approximately 0.10 g of dried and pulverized tissue. The mixture was allowed to soak overnight (>12 hrs) before centrifuging for 10 min at approximately 1000x gravity. The resulting supernatant was transferred to a separate vial, followed by an extra 1 ml of solvent that was rinsed through the pipette and added to the supernatant. An additional 1 ml of solvent was then added to the remainder of the original tissue sample, the mixture was centrifuged again, and the supernatant drawn off as above. The solvent-lipid solution was then washed by adding 1.50 ml of 0.88% KCl solution. After separation, the lipid-solvent layer was transferred to a pre-weighed aluminum dish and another 1 ml of solvent used to rinse the vial and added to the dish. Each dish was left to dry in a fume hood at room temperature overnight (>12 hrs), then weighed to within 0.0001 g using a Mettler-Toledo UMX2 ultra-microbalance (Mettler-Toledo AG, Greifensee, Switzerland). Individual differences in % lipid values between muscle and liver tissues (hereafter % $lipid_{(M-L)}$) were calculated using the average of three replicate lipid extractions for each tissue.

*Statistical analyses*

Linear mixed models were used to examine the differences in $\delta^{13}C_{(M-L)}$ and $\delta^{15}N_{(M-L)}$ related to five categorical ecological factors, including: life history type (anadromous or lacustrine), gender (male or female), reproductive status (spawning or not spawning), diet (piscivorous or not piscivorous), and feeding status (actively feeding or fasting). Site was

included as a random factor in each of the models to account for variation in $\delta^{13}C_{(M-L)}$ or $\delta^{15}N_{(M-L)}$ associated with site-specific variation, such as that due to environmental differences among sites and physiological differences among populations. Owing to the unequal distribution of anadromous and lacustrine Arctic charr among sites, the effects of the ecological factors were analyzed separately for anadromous and lacustrine fish. The effect of life history strategy on $\delta^{13}C_{(M-L)}$ and $\delta^{15}N_{(M-L)}$ was, therefore, tested independently of the other factors using a mixed model of the form:

$$\delta^{13}C_{(M-L)ij} \text{ or } \delta^{15}N_{(M-L)ij} = \beta_0 + \beta_1 (H_{ij}) + S_i + \varepsilon_{ij}$$

where $H$ is the fixed-factor life history strategy of individual $i$ from site $j$, the $\beta$ terms are the estimated model coefficients, $S_i$ is the site-specific random effect, and $\varepsilon_{ij}$ is the individual-specific random error term.

The effects of ecological factors on $\delta^{13}C_{(M-L)}$ and $\delta^{15}N_{(M-L)}$ for anadromous Arctic charr were examined using a full factorial linear mixed model including site as a random factor, gender, reproductive status and feeding status as fixed factors, and all respective two-way and three-way interaction terms. The full factorial mixed model used for lacustrine Arctic charr also included site as a random factor, but gender, reproductive status and diet were the fixed factors, and all respective two-way and three-way interaction terms were included in this model. The most parsimonious model in both cases was derived by removing non-significant terms ($P > 0.05$) in a sequential fashion, beginning with the highest order interactions and terms with the largest $P$-values. Remaining interactions that were significant at the $P = 0.05$ level were investigated post-hoc, using a Bonferroni multiple comparisons procedure to compare the mean $\delta^{13}C_{(M-L)}$ or $\delta^{15}N_{(M-L)}$ values between groups.

Levene's test (Levene 1960) was used to determine whether the variance in $\delta^{13}C$ and $\delta^{15}N$ values differed between muscle and liver tissues. A one-way analysis of variance was used to test for differences in mean % $lipid_{(M-L)}$ values between the subsets of Arctic charr grouped as having high or low $\delta^{13}C_{(M-L)}$ values.

All statistical analyses were performed using SPSS, version 17 (IBM Corp., New York, USA, 2008).

## RESULTS

Of the 714 individuals included in the analyses, 243 were classified as "anadromous" Arctic charr, leaving 471 "lacustrine" individuals. Anadromous charr captured in lakes containing both life history types had $\delta^{34}S$ values ranging from 11.43‰ to 17.76‰. Muscle stable isotope signatures were more positive than liver for the majority of individuals (87% for $\delta^{13}C$, and 96% for $\delta^{15}N$), and the overall variances in stable isotope values were similar between tissues ($\delta^{15}N$: $W_{(1,1426)} = 1.55$, $P = 0.214$; $\delta^{13}C$: $W_{(1,1426)} = 3.62$, $P = 0.057$).

*Ecological factor effects*

The largest ecological factor effect observed was for life history strategy on $\delta^{13}C_{(M-L)}$, when random effects due to site differences were controlled (Fig. 5.1, Table 5.2). Anadromous Arctic charr had a larger mean $\delta^{13}C_{(M-L)}$ value (estimated marginal mean (EMM ± SE: 2.77 ± 0.17) compared with lacustrine charr (1.11 ± 0.15) ($F_{(1,212)} = 116.48$, $P < 0.001$), but the two life history groups had similar $\delta^{15}N_{(M-L)}$ values ($F_{(1,210)} = 2.96$, $P = 0.087$) (EMM ± SE: anadromous = 1.11 ± 0.10; lacustrine = 0.95 ± 0.09).

All of the two- and three-way interaction terms were removed during the process of simplifying the initial model of ecological effects on the $\delta^{13}C_{(M-L)}$ values of anadromous Arctic charr. Of the main factors remaining in the simplified model, none of them explained significant portions of the variation in $\delta^{13}C_{(M-L)}$.

The simplified model of ecological factor effects on $\delta^{15}N_{(M-L)}$ values of anadromous charr also did not include any significant interactions. There was a significant effect of gender on $\delta^{15}N_{(M-L)}$ values (Table 5.3), with anadromous females having a greater mean difference in $\delta^{15}N$ values between muscle and liver compared to males (Fig. 5.1). However, the magnitude of this effect was less than the analytical precision of ±0.3‰ associated with the determination of $\delta^{15}N$ values (Table 5.3).

There was a significant difference in $\delta^{13}C_{(M-L)}$ values between piscivorous and non-piscivorous lacustrine Arctic charr (Table 5.2), with piscivorous individuals having a greater average difference in $\delta^{13}C$ values than non-piscivorous fish (Fig. 5.1). The interaction between gender and reproductive status was the only interaction in the simplified model of ecological effects on the $\delta^{13}C_{(M-L)}$ values of lacustrine charr (Table 5.2). Post-hoc tests revealed a

significant effect of reproductive status on female Arctic charr ($P$ = 0.013, effect size = 0.32), with non-spawning females having greater $\delta^{13}C_{(M-L)}$ values (EMM ± SE: 1.39 ± 0.20) than spawning females (1.07 ± 0.20). No other statistically significant effects were found among the pairwise comparisons (all $P > 0.114$).

Only gender and diet explained significant portions of the variation in $\delta^{15}N$ values between tissues of lacustrine Arctic charr (Table 5.3). Similar to anadromous charr, lacustrine females had a significantly greater mean difference in $\delta^{15}N$ values between muscle and liver compared to males (Fig. 5.1). Lacustrine Arctic charr that were not piscivorous also had, on average, larger values of $\delta^{15}N_{(M-L)}$ compared to piscivorous individuals (Fig. 5.1). The estimated factor effects associated with gender and diet were, however, less than the analytical precision associated with determination of the $\delta^{15}N$ values.

*Lipid content*

Mean % lipid values for muscle and liver in the high $\delta^{13}C_{(M-L)}$ group were 11.48% (± SE = 1.07) and 54.78% (± 4.32), respectively, while the low $\delta^{13}C_{(M-L)}$ group mean lipid content for muscle and liver tissues were 14.85% (± 2.39) and 19.60% (± 1.35). Muscle lipid content was similar between the high and low $\delta^{13}C_{(M-L)}$ groups ($F_{(1,18)}$ = 1.66; $P$ = 0.214) but the mean % lipid content of liver was significantly greater for fish in the high $\delta^{13}C_{(M-L)}$ group ($F_{(1,18)}$ = 60.44; $P <$ 0.001). The difference between the % lipid values of muscle and liver tissues was greater in the high $\delta^{13}C_{(M-L)}$ group (mean ± SE: 43.64% ± 3.84) than the low $\delta^{13}C_{(M-L)}$ group (4.50% ± 1.81) ($F_{(1,18)}$ = 85.17, $P <$ 0.001) (Fig. 5.2).

## DISCUSSION

Some of the ecological factors considered here had significant effects on the differences in $\delta^{13}C$ and $\delta^{15}N$ values between muscle and liver tissue of Arctic charr. Life history had a relatively large effect on the $\delta^{13}C_{(M-L)}$ values of Arctic charr, but only diet explained a significant portion of the variation in $\delta^{13}C_{(M-L)}$ values within the life history groups. Although several factors had statistically significant effects on $\delta^{15}N_{(M-L)}$ values, only the effect of reproductive status on female lacustrine arctic charr was greater than the analytical precision associated with collection of the $\delta^{15}N$ values. Differences in % lipid content between muscle and liver tissues

also accounted for some of the observed variation in $\delta^{13}C_{(M-L)}$ values, suggesting differences related to lipid storage and use between tissues may be part of the reason $\delta^{13}C_{(M-L)}$ values vary among individuals. Overall, the results of these analyses demonstrate that ecological factors can influence the differences in $\delta^{13}C$ and $\delta^{15}N$ signatures between muscle and liver tissues. However, the magnitude of the ecological factor effects varied in terms of their biological significance and potential impacts on the interpretation of stable isotope data in studies of diet change.

*Ecological factor effects & tissue physiology*

The $\delta^{13}C$ and $\delta^{15}N$ signatures reported for liver tend to be more negative than those reported for muscle tissue in fish, even among captive individuals fed constant diets under controlled conditions (e.g. Hesslein et al. 1993; Pinnegar & Polunin 1999; Suzuki et al. 2005; Trueman et al. 2005; Sweeting et al. 2007). This disparity has been attributed to differences in the composition and metabolic function of each tissue type, especially as they relate to lipid and amino acid content and composition (Pinnegar & Polunin 1999; Dalerum & Angerbjörn 2005; Suzuki et al. 2005). The two tissues differ metabolically, with the liver being involved in more metabolic processes than muscle tissue, including the break down and synthesis of lipids and proteins (Henderson & Sargent 1985; Henderson & Tocher 1987). Liver tissue also contains a relatively higher proportion of essential amino acids than white muscle tissue, which makes up most of the dorsal muscle in salmonids (Pinnegar & Polunin 1999; Jobling 1995). Essential amino acids derived from prey are typically incorporated directly into consumer tissues, undergoing little or no isotopic fractionation and, therefore, are less enriched in $^{13}C$ or $^{15}N$ than those made in vivo (Hare et al. 1991; Fantle et al. 1999; McMahon et al. 2010). This may be part of the reason liver tissue tends to have more negative $\delta^{13}C$ and $\delta^{15}N$ values than muscle. Differences in $\delta^{13}C$ values could also be related to differences in lipid storage dynamics between the tissues, as muscle tissue often serves as a long-term lipid storage site and the liver stores lipids only for short periods of time (Henderson & Tocher 1987; Tocher 2003).

The effects of the ecological factors on $\delta^{13}C_{(M-L)}$ and $\delta^{15}N_{(M-L)}$ values could be explained by the physiological changes or differences in muscle and liver tissue associated with each factor. Differences in $\delta^{13}C_{(M-L)}$ and $\delta^{15}N_{(M-L)}$ values associated with gender and reproductive

status, for example, could be related to the physiological changes that occur during the maturation process. In female fish, considerable reorganization of lipids and proteins occur as they are metabolized in the liver to produce the vitellogenin used in oocyte formation (Henderson & Tocher 1987; Jonsson et al. 1991; Martin et al. 1993; Jobling et al. 1998; Tocher 2003). Therefore, the maturation process could lead to greater changes in stable isotope values for spawning females compared to males or immature fish. This could also mean that the effect of gender and reproductive status reflect relatively greater changes in the stable isotope values of the liver tissue, since more physiological changes related to maturation are occurring in the liver compared to the dorsal muscle tissue.

The effect of life history on $\delta^{13}C_{(M-L)}$ values could be partially attributed to differences in lipid storage and use patterns between anadromous and lacustrine Arctic charr. Anadromous salmonids accumulate relatively large stores of energy during their marine feeding period in order to meet the metabolic demands associated with migration, spawning and overwintering in freshwater (Dutil 1986; Boivin & Power 1990; Jørgensen et al. 1997; Jonsson & Jonsson 1998; Rikardsen et al. 2003). In contrast, the lipid stores accumulated by lacustrine fish during the same time period do not tend to be as large (Klemetsen et al. 2003; Rikardsen et al. 2003; Amundsen & Knudsen 2009). These differences in lipid accumulation may also be associated with differences in the relative allocation of lipids to muscle and liver tissue, or differences in the types of lipids stored in each type of tissue (Jobling et al. 1998; Tocher 2003), which would lead to a difference in $\delta^{13}C_{(M-L)}$ values associated with life history strategy.

The effects of diet on $\delta^{13}C_{(M-L)}$ and $\delta^{15}N_{(M-L)}$ values could be attributed to differences in lipid and protein content among prey items. Fatty acids and amino acids are typically routed directly from the diet into the tissue of a consumer (Tocher 2003; McMahon et al. 2010) and, therefore, do not undergo trophic fractionation. However, non-essential amino acids that are not obtained from prey items must be synthesized in the liver, resulting in isotopic fractionation (Gaebler et al. 1966; Macko et al. 1986; Hare et al. 1991; Tocher 2003; McMahon et al. 2010). Therefore, consumers that feed on a low quality diet in terms of lipid and protein content would have to synthesize non-essential fatty and amino acids more often than those feeding on a relatively high quality diet (Robbins et al. 2005; McMahon et al. 2010). This could affect the

difference in muscle and liver $\delta^{13}C$ and $\delta^{15}N$ values between piscivorous and non-piscivorous individuals, as more metabolism would be occurring in the livers of Arctic charr feeding on a lower-quality, invertebrate diet.

*Impact on the interpretation of diet change data*

The magnitude of the ecological factor effects on $\delta^{13}C_{(M-L)}$ and $\delta^{15}N_{(M-L)}$ values of Arctic charr were generally relatively small. Despite their statistical significance, most of the factor effects on $\delta^{15}N_{(M-L)}$ were within the limits of analytical precision for the $\delta^{15}N$ signatures (±0.3‰) (Table 5.3). The only effects on $\delta^{15}N_{(M-L)}$ greater than this had an effect of 0.32‰, which is less than 10% of the commonly used average trophic fractionation value for $\delta^{15}N$ of 3.40‰ (Minagawa & Wada 1984; Post 2002).

If temporal changes in diet were being assessed at relatively large values of $\delta^{15}N_{(M-L)}$, on the order of a change in trophic level for instance, then the factors examined here would have relatively little impact on interpretation of the isotopic data. In other words, the magnitude of the ecological factor effects are not large enough to cause one to conclude that an Arctic charr underwent a complete dietary switch to a higher or lower trophic level when, in fact, it had not. Nor are these effects large enough to mask a complete switch to feeding at a different trophic level if one had occurred. However, two of the ecological factor effects associated with $\delta^{13}C_{(M-L)}$were greater than the average trophic fractionation value for $\delta^{13}C$ reported by Post (2002) of 0.40‰, with the effect of life history strategy also being greater than the trophic fractionation value of 1.00‰ reported by DeNiro & Epstein (1978) (Table 5.2). These effects are, therefore, large enough that one could misinterpret them as changes in feeding between trophic levels.

If changes in habitat use or migration were of interest, the impact of these ecological factors would depend on how the magnitude of the factor effects compared to the anticipated $\delta^{13}C_{(M-L)}$ values associated with the change to feeding on prey from a new area. For example, the average difference in $\delta^{13}C$ signatures between pelagic and littoral organisms reported for North American lakes by France (1995) and Post (2002) was 7-8‰. Since the difference between pelagic and littoral $\delta^{13}C$ signatures is much larger than the observed factor effects on $\delta^{13}C_{(M-L)}$, it is unlikely that these ecological factors would seriously affect questions of whether

Arctic charr underwent a dietary switch from feeding on littoral to pelagic organisms (or vice versa).

Such seemingly minor ecological factor effects may, however, become important when complete changes in diet are not expected. For example, small values of $\delta^{13}C_{(M-L)}$ or $\delta^{15}N_{(M-L)}$ could be misinterpreted as small shifts in omnivory. This is of particular concern when small diet changes are expected to coincide with physiological changes. For example, lacustrine Arctic charr often supplement their diets with seasonally available prey items, such as chironomid larvae and pupae (Amundsen 1995; Amundsen et al. 2008; Eloranta et al. 2010), during the summer months when they are also undergoing the physiological changes associated with gamete production and sexual maturation. In this case, reproductive status, gender, and a shift in diet would all lead to differences in $\delta^{13}C$ and $\delta^{15}N$ values between muscle and liver, and would likely interfere with interpretation of the isotopic data by interacting to either amplify or diminish the $\delta^{13}C_{(M-L)}$ or $\delta^{15}N_{(M-L)}$ values associated with the shifts in diet. Small effects on $\delta^{13}C_{(M-L)}$ and $\delta^{15}N_{(M-L)}$ could also confound individual niche width calculations based on the stable isotope values of tissues with different isotopic turnover times (i.e., Bearhop et al. 2004; Sweeting et al. 2005).

The effects of life history, diet, gender and reproductive status on $\delta^{13}C_{(M-L)}$ could be at least partially mitigated by removing lipids from the tissue samples prior to stable isotope analysis (Post et al. 2007). Since relative lipid content explained a significant portion of the variability in $\delta^{13}C_{(M-L)}$ values, lipid removal would likely result in smaller factor effects on $\delta^{13}C_{(M-L)}$ values. After lipid removal, these factor effects would be associated mainly with differences in the amino acid composition between tissues and not differences in lipid content. However, if a chloroform-methanol solvent is used, $\delta^{15}N$ values should be determined from tissue samples obtained prior to lipid extraction, as this solvent can remove certain proteins from the tissue in addition to lipids, thus altering the $\delta^{15}N$ signature (Dobush et al. 1985; Sweeting et al. 2006; Kelly et al. 2006; Logan & Lutcavage 2008).

*Conclusions*

This study demonstrates that factors other than a change in diet can affect the difference in $\delta^{13}C$ and $\delta^{15}N$ values between muscle and liver tissue. Since ecological factors

likely affect $\delta^{13}C_{(M-L)}$ and $\delta^{15}N_{(M-L)}$ values through differences in their physiological impacts among tissues, it is important for researchers to first identify any confounding factors that may influence the protein and lipid composition of the tissues being examined. This is especially important in studies of diet change that compare tissues that differ in metabolic activity, as those with relatively higher metabolic rates are more likely to be influenced by short-term physiological changes compared to more temporally stable tissues.

Most of the ecological factor effects observed here were not large enough to cause misinterpretation when examining major changes in diet, but could confound studies concerned with changes in omnivory. Since the effects of ecological factors are likely to vary among tissues, species and even populations, individual studies examining shifts in diet using stable isotopes should determine whether potentially confounding ecological factors have biologically meaningful impacts on the data being analyzed. Although trying to establish generalized correction factors for various ecological effects is unlikely to be a viable solution to this problem, given the potential for many interactive effects among factors and physiological differences among species, there are several ways to address the significance of ecological factor effect. This could be done simply by testing for differences in $\delta^{13}C_{(M-L)}$ and $\delta^{15}N_{(M-L)}$ among groups within a data set, as we did here, and comparing the factor effects with the level of biological significance associated with a particular study. It could also be accomplished experimentally, by assessing the effect of specific factors, or combinations of factors, under controlled conditions. Potentially confounding factors could then be included as sources of variation in statistical analyses, or studies could be purposefully designed to avoid those factor effects.

## TABLES & FIGURES

**Table 5.1:** Sampling sites and summary information for the Arctic charr data used in this study. Life history types include anadromous (A) or lacustrine (L), and the size range of fish captured for each life history type at each location is given as fork length values. Asterisks (*) indicate sites that have been given arbitrary names, as they do not have officially recognized names.

| Site | Latitude | Longitude | Life History | *n* | Size Range (mm) |
|---|---|---|---|---|---|
| Clements Markham Lake | 82° 37' N | 68° 59’ W | L | 27 | 92-456 |
| Lake C* | 82° 05' N | 68° 24’ W | L | 32 | 132-641 |
| Lake G* | 81° 50' N | 69° 14’ W | L | 29 | 218-645 |
| Lake Hazen | 81° 49' N | 71° 10’ W | L | 111 | 89-602 |
| Lac Duquet | 62° 03' N | 74° 32’ W | A | 29 | 314-572 |
| Upper Nakvak Lake* | 58° 39' N | 62° 19’ W | L | 48 | 82-474 |
| North Arm, Saglek Fjord | 58° 32' N | 63° 28’ W | A | 32 | 236-532 |
| Hebron Lake #3* | 58° 12' N | 62° 42’ W | A | 6 | 236-388 |
| | | | L | 31 | 85-434 |
| Hebron Lake #2* | 57° 58' N | 64° 01’ W | L | 34 | 77-456 |
| Okak Bay | 57° 33' N | 62° 06’ W | A | 31 | 143-494 |
| Esker Lake | 57° 09' N | 62° 53’ W | L | 35 | 84-483 |
| Tikkoatokak Bay | 56° 45' N | 62° 30’ W | A | 47 | 255-507 |
| Black Island | 56° 44' N | 61° 22’ W | A | 30 | 247-530 |
| Tasialuk Lake | 56° 44' N | 62° 42’ W | L | 34 | 80-434 |
| Tom's Pond* | 56° 41' N | 61° 38’ W | A | 15 | 316-452 |
| | | | L | 14 | 106-420 |
| Coady's Pond #2* | 56° 38' N | 63° 37’ W | L | 33 | 79-452 |
| Tasisuak Lake (Fraser River) | 56° 37' N | 62° 31’ W | A | 23 | 95-442 |
| | | | L | 43 | 65-191 |
| Anaktalak Bay | 56° 27' N | 62° 13’ W | A | 30 | 232-468 |

**Table 5.2:** The *F*-statistics, associated degrees of freedom, *P*-values, and effect sizes (± SE) associated with the terms included in the simplified models of ecological effects on $\delta^{13}C_{(M-L)}$ for anadromous and lacustrine Arctic charr when the random effect of site is considered.

| | $\delta^{13}C_{(M-L)}$ | | |
|---|---|---|---|
| | *F* | *P*-value | Effect |
| Life History | $116.48_{(1,212)}$ | < 0.001 | 1.67 ± 0.15 |
| <u>*Lacustrine*</u> | | | |
| Gender | $0.09_{(1.457)}$ | 0.760 | |
| Reproductive Status | $2.89_{(1,462)}$ | 0.090 | |
| Diet | $19.76_{(1,460)}$ | < 0.001 | 0.59 ± 0.13 |
| Gender x Reproductive Status | $3.89_{(1,457)}$ | 0.049 | |

**Table 5.3:** The *F*-statistics, associated degrees of freedom, *P*-values, and effect sizes (± SE) associated with the terms included in the simplified models of ecological effects on $\delta^{15}N_{(M-L)}$ for anadromous and lacustrine Arctic charr when the random effect of site is considered.

| | $\delta^{15}N_{(M-L)}$ | | |
|---|---|---|---|
| | *F* | *P*-value | Effect |
| <u>*Anadromous*</u> | | | |
| Gender | $4.15_{(1,234)}$ | 0.043 | 0.13 ± 0.06 |
| <u>*Lacustrine*</u> | | | |
| Gender | $22.98_{(1,459)}$ | < 0.001 | 0.27 ± 0.06 |
| Diet | $4.55_{(1.462)}$ | 0.033 | 0.18 ± 0.09 |

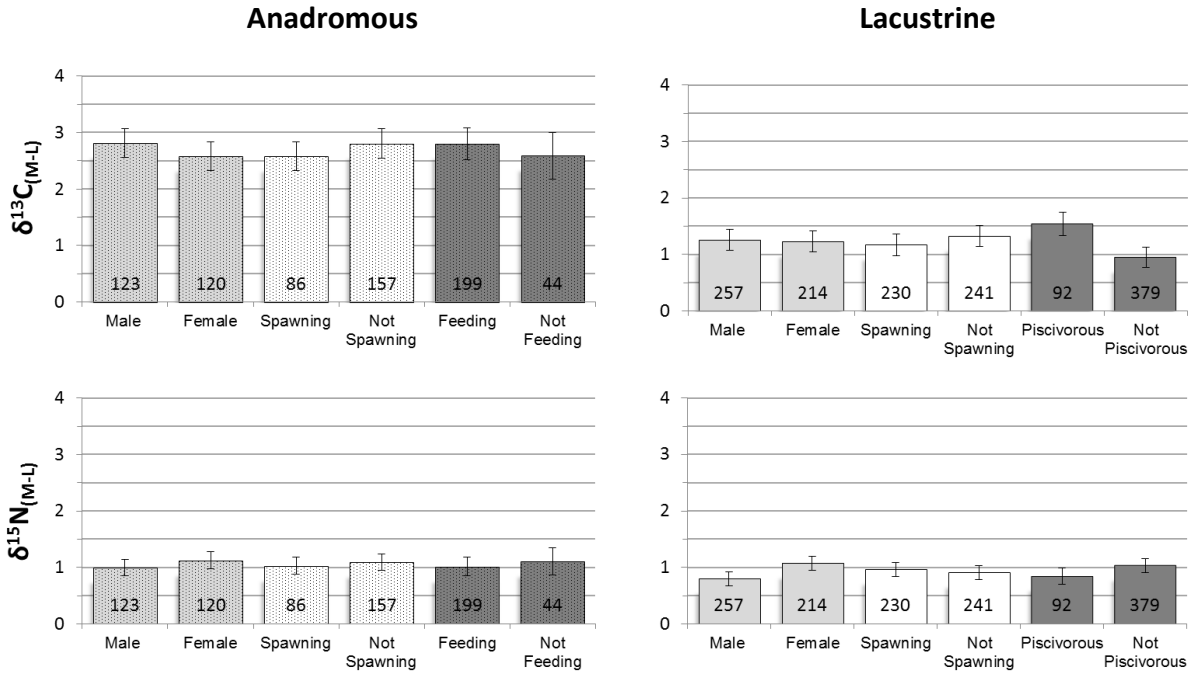


**Figure 5.1:** The estimated marginal means (± SE) of $\delta^{13}C_{(M-L)}$ and $\delta^{15}N_{(M-L)}$ values calculated for subgroups of Arctic charr when random effects related to site are controlled. Group sample sizes are indicated at the base of the bars.

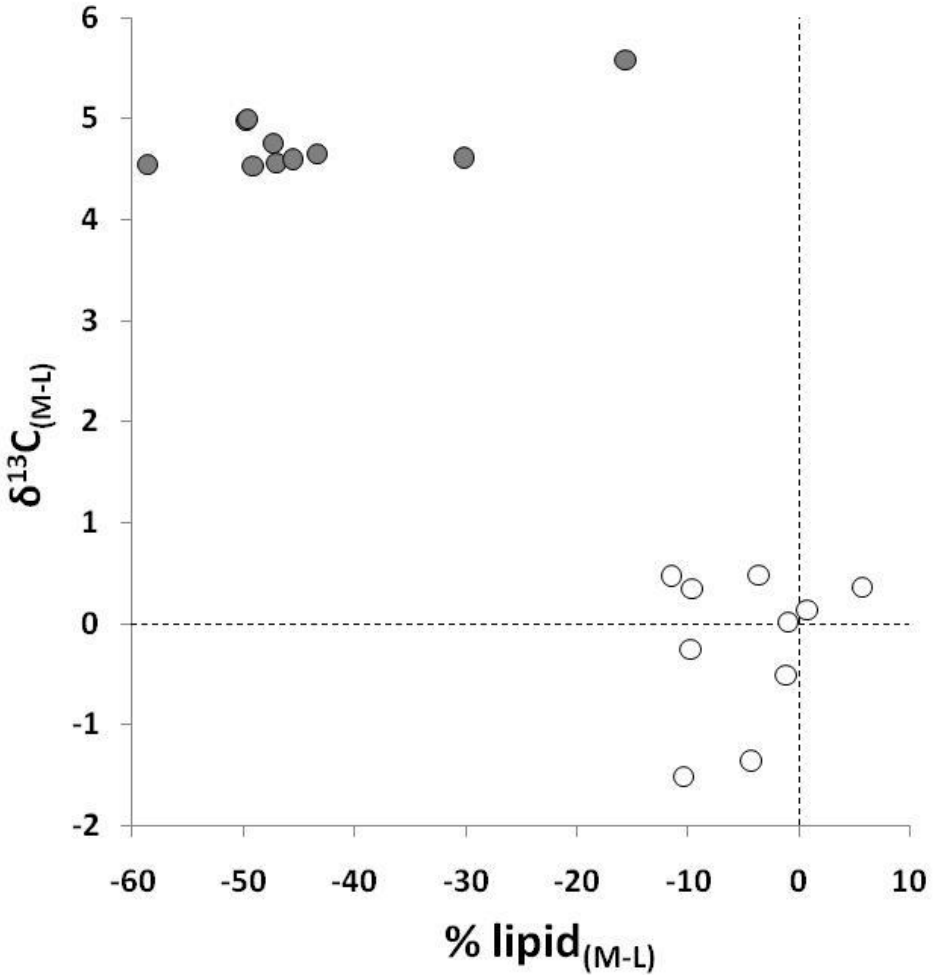


**Figure 5.2:** Percent lipid difference and $\delta^{13}C_{(M-L)}$ values for a subset of 20 individuals for which muscle and liver lipid extractions were performed. Grey circles (●) indicate individuals in the high $\delta^{13}C_{(M-L)}$ group and white circles (○) are those in the low $\delta^{13}C_{(M-L)}$ group. Dashed lines denote lipid and stable isotope tissue differences of zero.

# Chapter 6 - Summary & Conclusions

## SYNOPSIS

Arctic charr exhibit some of the most extensive phenotypic and ecological diversity that has been documented in a vertebrate species (Klemetsen 2010; 2013). Many factors play a role in the creation and maintenance of the diversity observed within this species, but the work presented here focused on ecologically-based mechanisms of diversification in Arctic charr. Specifically, the work presented in this thesis examined the relationship between environmental variability and phenotypic diversity within populations of Arctic charr.

The studies included in this thesis addressed several gaps in our understanding of how intraspecific diversity in Arctic charr is affected by temporal and spatial changes in environmental conditions. First, the data collected here demonstrated that detectable shifts in life-history traits can occur within a population of Arctic charr in response to short-term environmental fluctuations. On a spatial scale, diversification within populations of Arctic charr was also associated with differences in environmental factors within and among lakes, including differences in species community structure, climate, and lake geomorphology. However, the consistency of these patterns needs to be tested using data from populations of Arctic charr collected over larger spatial and temporal scales.

On a broader scale, this thesis work also revealed some unusual patterns of diversity in Arctic charr. The polymorphic population of Arctic charr in Lake Hazen is unusual in that it contains two morphs that appear to specialize on the same food resource but differ in their life history characteristics. This highlights the potential for diversification beyond the pelagic-littoral habitat axis that is more commonly observed in northern fish species. The differences observed between the two benthivorous morphs in Lake Hazen also stress the role that life-history traits can play in ecological diversification, where morphological traits are more often the focus. Differences in growth and maturity patterns among the three morphs in this population also suggest that cannibalism can be an important diversifying mechanism, having an effect similar to interspecific predation observed elsewhere but with the added dynamic of being an entirely intraspecific mechanism.

The data collected here also documented the differences in the relationship between ecological and morphological diversity among populations of Arctic charr. Although it may not be unexpected that among-individual ecological diversity was not tied to morphological diversity in many of these populations, these observations raised the question of what factors influence the relationship between phenotypic and ecological diversity in Arctic charr. Specifically, this highlights a gap in our understanding of how phenotypic traits other than morphology influence diet diversity within and among populations of Arctic charr, and other northern fish species. Examining the effect of ecology on stable isotope values also drew attention to the physiological differences that underlie the ecological differences more commonly observed within and among populations of northern fish species. Variability in physiological traits and their relationship to ecological diversity is another significant gap in our knowledge of diversity in Arctic charr and other northern fish species.

The following sections briefly explain the specific contributions made in each chapter to our greater understanding of diversity in Arctic charr and other species, and the role that environmental variation plays in the diversification process.

Chapter 2: *Changes in growth patterns of wild Arctic charr (*Salvelinus alpinus *(L.)) in response to fluctuating environmental conditions.*

The goal of this chapter was to test the prediction that shifts in the growth patterns of wild Arctic charr would coincide with temperature fluctuations over a contemporary time period. Consistent with this prediction, a significant relationship between annual fluctuations in sea-surface temperatures and lifetime growth patterns of Arctic charr cohorts was observed. Temperature had mixed effects on growth within a single growing season, but the cumulative lifetime temperatures experienced by these Arctic charr explained a significant portion of the variation in size-at-age among cohorts. In addition, these changes in growth patterns were better explained by coincident changes in regional prey availability, fish behavior, exploitation rates, and climate shifts than by inter-annual temperature changes alone.

These results represent two significant contributions to our understanding of the growth dynamics of fish in the wild. The changes in cohort growth patterns with temperature

demonstrated that environmental fluctuations can have cumulative effects on growth dynamics over an individual's lifetime. These changes in growth were better explained by changes in the ecosystem rather than temperature alone, which revealed that shifts in temperature have direct and indirect effects on the growth dynamics of wild fish populations.

The changes in growth observed here compliment observations from laboratory experiments, showing the effects of temperature change can be extended to fish in the wild. Laboratory experiments have shown that the potential for growth in Arctic charr and other salmonid species increases with temperature up to an optimum value, but that increased growth is only realized when individuals are able to obtain enough prey (ration) to meet the increase in metabolic demands that comes with higher temperatures (Jobling 1983; Elliot 1994; Larsson & Berglund 1998; Larsson et al. 2005). Due to the constraints associated with maintaining a controlled experimental system, the effects of temperature on growth in the studies cited above were only examined in juvenile fish over relatively short periods of time (weeks to months). Similarly, because of the logistical difficulties of obtaining long-term growth data for fish in the wild, there have been few studies of the growth dynamics of long-lived fish species like the Arctic charr. In the study presented in chapter 2, we were able to fill these gaps in our understanding of growth in wild Arctic charr by examining cohort growth patterns in a rare, long-term biological dataset that was complimented by long-term environmental data. Because of this opportunity, we were able to examine the cumulative effects of environmental factors on growth in wild fish, rather than the more common approach of examining effects during shorter, incremental periods.

In terms of our understanding of the impacts of environmental change on intraspecific diversification, this study indicates there is a connection between short-term environmental changes and changes in the size-at-age of individuals over their lifetime. This shift in body size could have implications for longer-term intraspecific diversification. For example, if assortative mating occurs with regards to body size, changes in the growth dynamics of the population could lead to evolutionary shifts in life-history characteristics. The indirect effects of temperature changes on prey availability could also affect diversification by shifting the selection optima of foraging-related traits. Alternatively, environmental fluctuations such as

these could promote adaptive phenotypic plasticity in Arctic charr (i.e. Robinson & Parsons 2002), rather than directional or disruptive selection on a quantitative trait.

This study is also particularly relevant to our understanding of the potential effects of climate change on ecosystem dynamics. Most importantly, these results highlight the importance of the indirect effects of temperature change on growth and species community structure. This observation indicates that inclusion of the indirect effects of changes in temperature and other environmental factors, such as precipitation, will improve predictive climate change models. The relationships observed here suggest the influence of past environmental conditions on growth history may have a more important impact on stock characteristics than temperatures experienced in one growing season alone. Therefore, models of growth dynamics in longer-lived species, like Arctic charr, will be improved by the inclusion of cumulative effects as they are likely to experience significant fluctuations over their lifetimes rather than directional shifts in environmental factors.

Although statistically significant relationships between temperature and growth were observed in this study, temperature explained a relatively small amount of the variation in size-at-age among these anadromous Arctic charr. This indicates there are other factors influencing the lifetime growth patterns of these wild fish, which likely includes among-population genetic differences and ecological experience. Further investigation into the population structure of this stock complex could, for example, reveal the influence of differential ecological experience as juveniles on lifetime growth patterns.

Chapter 3: *Evidence of a third morph of Arctic charr (*Salvelinus alpinus*) in Lake Hazen, Nunavut, Canada*

The study presented in Chapter 3 was undertaken in order to re-examine the question of how many morphs of Arctic charr are present in Lake Hazen using a larger dataset of morphological, life-history, and diet data that had been available previously. Two distinct morphs, one cannibalistic and one benthivorous, were identified in earlier studies that examined variation among Arctic charr from Lake Hazen. This study expanded upon these previous analyses, using an unbiased, Bayesian analysis that indicated the larger dataset of size-

at-age data was best described by three discrete clusters of individuals. Two of these clusters were consistent with the previous descriptions of a cannibalistic and a benthivorous morph. The third cluster represented an early-maturing group that attained adult body sizes smaller than those reported for the previously identified forms. Although there are alternative explanations for the occurrence of distinct size-at-age groups within a fish population, polymorphism is the most consistent explanation for the combination of differences observed in life history (growth and age-at-maturity), trophic ecology (diet), and morphology among the three groups of Arctic charr identified here.

Although this study focuses on a single population, the unusual patterns of diversification observed here help to fill some of the holes in our broader understanding of the mechanisms of diversification in Arctic charr and other species. For example, niche differentiation in polymorphic populations of northern fish species typically occurs between the benthic and limnetic habitats (Robinson & Wilson 1994; Skúlason & Smith 1995; Robinson & Parsons 2002). Ecological diversification among the three Arctic charr morphs in Lake Hazen, however, appears to occur along an axis defined by vertebrate and invertebrate prey in a benthically-rooted food web. The occurrence of a cannibalistic and invertebrate-feeding morph within a polymorphic population of Arctic charr has been observed elsewhere (Jonsson & Jonsson 2001; Finstad et al. 2006; Berg et al. 2010), but the occurrence of two morphs that feed on the same type of prey has rarely been observed in a polymorphic population (e.g. Snorrason et al. 1994). This also deviates from the pattern of differential prey use generally observed in polymorphic populations of other species (Skúlason & Smith 1995; Smith & Skúlason 1996).

The mechanisms underlying diversification of Arctic charr in Lake Hazen are also unusual among polymorphic populations because of the environmental characteristics of this lake system. Natural selection on traits associated with resource use and competition is central to most models of ecologically-based diversification and speciation (Mayr 1942; Schluter 1996). For most populations, this includes competition for resources with other species. However, because Arctic charr are the only fish species in Lake Hazen, interspecific competition does not play a role in the maintenance of diversity within this population. Most examples of ecological diversification, particularly among fish in northern lake systems, have been attributed to

selection on morphological and behavioral traits associated with foraging and prey capture (Robinson & Wilson 1994; Skúlason & Smith 1995; Robinson & Parsons 2002). However, differences in size-at-age, and age-at-maturity are the primary distinguishing characteristics among the three morphs of Arctic charr in Lake Hazen. This suggests that selection on life history traits plays a key role in diversification within this population, especially between the morphologically and ecologically similar benthivorous morphs. Further, the observations made here indicate the differences in size- and age-at-maturity between the benthivorous morphs could be maintained by size-selective predation pressure from the cannibalistic morph. Intraspecies mechanisms associated with diversification are typically limited to resource competition, predominantly competition for prey (Skúlason & Smith 1995; Smith & Skúlason 1996). Therefore, the polymorphic population of Arctic charr in Lake Hazen could be the only documented case in which predation pressure from one morph provides a mechanism for maintaining diversification between two other morphs within a single population.

The unusual patterns of diversification observed here contribute to our greater understanding of ecological speciation by highlighting the need to look beyond models of diversification that focus on differential habitat use. Specifically, the differences observed among the morphs of Arctic charr in Lake Hazen call attention to the role of cannibalism and selection on life-history traits in creating and maintaining diversity in polymorphic populations. These mechanisms will likely be more important to diversification in populations of Arctic charr in low-productivity, high-Arctic lakes, where they do not interact with other fish species. Further investigation is needed to determine how common these patterns of diversification are among species that occur in other recently deglaciated ecosystems and areas of low biodiversity, such as polymorphic fish populations found at high latitudes (e.g. Ruzzante et al. 2003).

Historically, polymorphic populations of Arctic charr and other northern fish species were identified based on the phenotypic differences observed among individuals captured in the same ecosystem (e.g Walker et al. 1988; Snorrason et al. 1994; Reist et al. 1995). The use of an unbiased, Bayesian approach to identifying morphs is a relatively new approach (Woods et al. 2012a). This is a potentially powerful tool for distinguishing among morphs that share a

common diet or have a similar appearance, as demonstrated here with the two benthivorous morphs. However, the differences observed among the morphs in Lake Hazen also emphasize the need to examine multiple traits when identifying polymorphisms. Groupings identified using one trait, such as size-at-age, could be explained by plasticity rather than adaptive divergence into distinct morphs.

Chapter 4: *Does ecological opportunity and the absence of interspecific competitors promote intrapopulation diversity in Arctic charr?*

This chapter tested the prediction that intraspecific competition and ecological opportunity promote phenotypic diversity within populations (Van Valen 1965; Roughgarden 1972; Bolnick 2001; Araujo et al. 2011). The patterns of intrapopulation morphological variation observed in the eleven populations of Arctic charr examined here did not support this prediction. However, the broader conclusions that can be drawn from this study are limited by the relatively small number of locations and samples sizes that were available for analysis. Of the studies included in this thesis, this is most directly related to the overall theme of examining the role of environmental variability in Arctic charr diversity. However, due to the limited number of samples available, this chapter is best viewed as a pilot project to developing a larger study to test the predictions that intrapopulation morphological and diet variation increases with ecological opportunity.

The study presented in chapter 4 exploits the exceptionally large latitudinal range and high potential for diversity observed in Arctic charr to expand on previous tests of the niche variation hypothesis (NVH). Because the range of Arctic charr extends further north than any other fish species, they are found in lake systems in which they do not experience interspecific competition with other fish species. Arctic charr, therefore, provide a novel opportunity to test the prediction that populations will exhibit greater among-individual variation in resource use in the absence of interspecific competition, rather than a reduction in interspecific competition. The study presented here also expands on previous tests of ecological divergence in fish to include the influence of access to the marine environment on the ecological opportunity experienced by a population observed in a lake system. Previous tests of the effects of

ecological opportunity on the diversity of fish populations have focused only on habitat heterogeneity within lake systems (Nosil & Reimchen 2005; Classen et al. 2008; Siwertsson et al. 2010; Woods et al. 2012b). However, in salmonids and other diadromous species that have the potential to utilize resources in both the freshwater and marine habitats, the ecological opportunity available in a lake system should be greatly increased by reliable access to the marine habitat.

Despite the limitations of this study, this work presented in this chapter resulted in two important contributions to our understanding of intrapopulation diversity in Arctic charr. First, testing the effects of interspecific competition and ecological opportunity revealed a suite of potentially interactive effects on the extent of ecological and phenotypic variation within populations of Arctic charr. Although the wide latitudinal range of Arctic charr offers novel opportunities for testing the NVH, it also poses some challenges due to the environmental effects associated with climatic differences at the distant northern and southern extents of the range. These include differences in relative nutrient availability, primary productivity, and biodiversity associated with contemporary differences in temperature, precipitation, and ice cover duration, as well as differences in the time since deglaciation associated with historical climatic patterns. These environmental differences have potentially interactive effects on the opportunities and limitations for diversification in Arctic charr, but the same patterns can be extended to other populations found across a range of environmental conditions. Recognizing these potential interactive effects on intrapopulation diversity draws attention to some of the assumptions made in the original version of the NVH. For example, one key assumption of many tests of the NVH is that resource diversity and availability is similar for populations in different systems. These interactive effects also emphasize the need for greater within-region sampling to control for some of these factors in future studies of Arctic charr diversity.

The second important contribution of this work was to examine the effect of environmental factors on the relationship between diet and morphological variation within populations. Historically, tests of the NVH focused on the effects of interspecific competition on morphological variation within populations. These tests produced mixed support for the hypothesis, whereas more recent quantitative studies of inter-individual diet variation typically

find positive support for the NVH (Bolnick et al. 2007; Araujo et al. 2011). This pattern has been attributed to a discrepancy between morphological and diet variation, which has been supported by logical arguments. However, there have been few attempts to actually test this prediction by quantifying the relationship between diet and morphological variation within populations (e.g. Bolnick et al. 2007; Snowberg et al. 2015). The differences in the diet-morphology relationship observed among populations of Arctic charr (Woods et al. 2013a; Knudsen et al. 2014), and other fish species (Bolnick et al. 2007; Svanback & Bolnick 2005; Svanbäck & Bolnick 2007; Bolnick & Paull 2009; Snowberg et al. 2015), raised the question of why these relationships differ among populations. This led us to predict that decreased interspecific competition and increased ecological opportunity would also lead to greater covariation between diet and morphology within populations of Arctic charr. This is a slightly different angle on more traditional tests of the NVH, which looks not only at the effects of ecological opportunity and interspecific competition on inter-individual resource use diversity, but on the phenotypic traits that underlie that diversity. Examining these hypotheses, therefore, also presents an opportunity to learn more about the contributions of different aspects of phenotypic variation, including behavior and life history traits, on the diet diversity observed within and among populations of Arctic charr.

One of the main effects of climate change expected in Arctic is the invasion of fish species into new ecosystems as they extend their range northward. This will result in significant changes to the trophic dynamics within Arctic ecosystems, and will likely result in increased interspecific competition for many Arctic charr populations. Therefore, a better understanding of the impacts interspecific competition has on intrapopulation diversity could also provide a reference for detecting and mitigating the effects of species invasions in Arctic ecosystems. It may, for example, help in identifying populations that are more vulnerable to the negative effects of invading species, and identifying population and ecosystem characteristics that affect the adaptability of Arctic charr to changes in interspecific competition.

Chapter 5: *Ecological influences on the differences in $\delta^{15}N$ and $\delta^{13}C$ values between fish tissues: implications for studies of temporal diet variation.*

We undertook the study presented in chapter 5 in order to further explore the limitations and implications of using stable isotopes to quantify ecological variation in Arctic charr & other fish species. This was a methods-based study meant to compliment the use of stable isotopes as a tool for quantifying diet diversity within and among the populations of Arctic charr examined elsewhere in this thesis (Chapters 3 & 4). The primary goal of chapter 5 was to determine whether the effects of ecological factors on the differences in $\delta^{13}C$ and $\delta^{15}N$ values between muscle and liver tissues were large enough to affect the biological interpretation of isotope data when reconstructing temporal diet patterns. Our results demonstrated that factors other than a change in diet can affect the difference in $\delta^{13}C$ and $\delta^{15}N$ values between muscle and liver tissues. These factors appear to affect $\delta^{13}C_{(M-L)}$ and $\delta^{15}N_{(M-L)}$ values indirectly, through their physiological impacts on metabolism and tissue composition. Most of the ecological factor effects observed here were not large enough to cause misinterpretation when examining major changes in diet. However, these factors could confound studies concerned with more subtle changes in omnivory, such as the among-individual diet differences we originally sought to quantify in chapter 4. The results of this study stress the need for researchers to identify potentially confounding factors that influence the protein and lipid composition of tissues whose $\delta^{13}C$ and $\delta^{15}N$ values are being compared, especially if those tissues differ in metabolic activity. The discussion presented here also emphasizes that the results of stable isotope analyses should be interpreted based on their biological significance rather than just their statistical significance.

The primary contribution of this chapter to our understanding of diversity in Arctic charr was the further development of using stable isotopes as a tool for quantifying ecological diversity. Although diversity in Arctic charr and other fish species is most often characterised by morphological and ecological differences within and among populations, differences in these traits are frequently associated with differences in diet. Stable isotopes are increasingly being used as a tool to quantify diet differences among groups, and more studies are needed to prevent over- or misinterpretation of this information.

Although not the focus of this study, the patterns observed here also drew attention to the physiological differences that underlie the ecological and life history diversity more

commonly observed in Arctic charr. Physiological diversity is an aspect of diversification and speciation not commonly studied in Arctic charr or other northern fish species. However, there has been significant work on the ecological and evolutionary impacts of physiology in other species. For example, microhabitat specialization in *Anolis* lizards has been linked with morphological adaptations to the structural aspects of their niches, as well as physiological adaptations to the climatic differences among niches (Williams 1972; Hertz et al. 2013). It is likely that variation and plasticity in physiological traits also play a key role in the diversification and speciation of Arctic charr, especially considering the different selection pressures imposed by the wide range of environmental factors, like climate and salinity, experienced by different populations of Arctic charr. As explained in chapter 2, temperature is directly tied to energy acquisition and use in this species through the physiological impact of temperature on metabolism. Therefore, it would be interesting to investigate the link between physiological adaptations to thermal habitat use and the morphological and behavioral adaptations associated with obtaining food in those habitats.

## IDEAS FOR FUTURE RESEARCH

The following are just some of the questions that arose during the process of putting together this thesis. These are general ideas for expanding on the work presented here to fill some of the gaps that still remain in our knowledge of intraspecies diversity in Arctic charr and the effects of environmental change on this species. Because stable isotopes were used as an analytical tool in most of this work, I have also included some ideas for furthering the development of stable isotopes for use in ecological research.

### *Intrapopulation diversity*

We are just beginning to collect data on the extent of inter-individual diversity in populations of Arctic charr, and few studies have examined how environmental factors may affect the extent and type of phenotypic variation found within populations. Other studies have suggested that intrapopulation diversity could affect population resiliency to environmental change (Johnson 2000; Waples et al. 2009; Hoffman & Sgrò 2011; Reed et al. 2011). A better understanding of the factors that affect intrapopulation diversity in Arctic charr could,

therefore, aid in management and conservation efforts. Important questions include: which aspects of phenotypic variation are more important to population resiliency to environmental change? How can we change management practices to maintain this type of variation? I would also like to investigate whether there is a difference in the amount of morphological and diet variation among the different ecological forms of Arctic charr. For example, do piscivorous forms, which consume prey from multiple trophic levels, also exhibit greater morphological variation than planktivorous forms, which specialize on a narrower range of prey types? Are anadromous populations less variable than lacustrine populations?

*Morphological and Ecological Diversity*

The variability in the covariance values observed here also raises questions of why the relationship between diet and morphology differs among populations of Arctic charr. Under what conditions is morphology more important to diet variability among individuals, and what environmental factors influence this relationship? Investigating this could provide more information on the role of environmental variation and different phenotypic traits in the process of ecological diversification in Arctic charr and other species. Other studies have found positive relationships between ecological opportunity and intrapopulation variation using the number of morphs in polymorphic populations as a measure of morphological and diet variation (Classen et al. 2008; Siwertsson et al. 2010; Woods et al. 2012). Here, we used the magnitude of among-individual variation in morphology to quantify intrapopulation variation. However, it is interesting to note that of the populations examined here, those known to be polymorphic (Lake Hazen, Ch X), or contained a mix of anadromous and lacustrine individuals were not necessarily more morphologically variable than the other populations. This raises the question of whether this was a product of the way morphological variation was quantified, or if it is reflective of the proportion of individuals from each morph included in the sample. Alternatively, the differences between morphs within these populations may be based on other phenotypic traits, like behavior or life history, and not necessarily reflected in morphological differences. There is evidence that individual differences in behavior may play a larger role than morphology in mitigating intraspecific competition, especially in response to short-term changes in prey availability (Swanson et al. 2003; Svanbäck & Bolnick 2007; Bolnick & Paull

2009). Traits associated with high plasticity, such as behavior and growth, are likely to be particularly important to maintaining a diet when faced with rapid environmental changes. For example, data collected from Arctic charr in Lake Windermere showed significant changes in diet over a 60+ year period, but no observable coincident changes in morphology at the population level (Corrigan et al. 2011a). It would be interesting to see how these patterns apply to temporal environmental fluctuations over different time scales.

*Physiology and Stable Isotopes*

With regards to the development of stable isotopes as a tool in ecological studies, I would like to investigate the effects of physiology on the interpretation of $\delta^{13}C$ and $\delta^{15}N$ data. I would like to know how the routing and metabolism of different amino acids and fatty acids affects stable isotope values of different tissues. Specifically, I want to test whether $\delta^{13}C$ and $\delta^{15}N$ values differ in response to seasonal changes in physiology. For fish in the northern hemisphere it is generally thought that metabolism and feeding slow greatly during the winter months. How does this affect stable isotope values? Is there a difference in how nutrients are routed through the body during the summer and winter? One would expect any nutrients consumed in winter to be used directly for metabolic purposes, while in the summer more would be processed for storage in the liver and muscle tissue. Is there a difference in which amino acids and fatty acids are routed towards storage in the summer and those used for metabolic needs in the winter? Does this have anything to do with differences in breaking down and assembling different proteins and lipids?

## CLOSING REMARKS

I first became interested in Arctic charr the day Fred Kircheis introduced me to his cozy little library. It was filled with books and papers about Arctic charr he had collected throughout his years of working with the species, many of which I have never seen anywhere else. As I read through these and listened to his stories, (over a cup of tea and cookies, of course), I became fascinated by the amazing amount of diversity that could be found within this one species. The historical and scientific accounts of the different forms of Arctic charr and the different places they were found raised many questions about speciation and what leads to the extraordinary

amount of diversity in the natural world. For Arctic charr, in particular, it led me to wonder how did this one species diversify into so many different forms? Why has this not occurred in other species? How does this influence our efforts to conserve and protect Arctic charr over the long-term?

Since that day, I have had the opportunity to see and study many different forms of Arctic charr, and meet *Salvelinus* enthusiasts from around the world. I learned a lot about the extent of diversity in Arctic charr, and a little about the environmental factors that influence it. But what I took away from all of these experiences is that this is not a species for which lines can be drawn or generalizations made. Our brains want to categorize and generalize information according to patterns, but Arctic charr are uncooperative in this. Still, those early questions still linger, and if I have learned anything about this species, it is that there is still a whole heck of a lot left to learn.

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