EVOLUTIONARY OUTCOMES OF INTERACTIONS AMONG PHENOTYPES IN POST-GLACIAL LAKES

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Abstract

Competitive interactions among sympatric phenotypes shape forms of natural selection and evolution of phenotype distributions. Populations of fish in post-glacial lakes are a model system for studying the evolutionary outcomes of competition. Fish in these lakes tend to exhibit phenotypic variability corresponding to a nearshore (benthic/littoral) to open water (limnetic/pelagic) resource use axis. In several lakes, populations have repeatedly diversified into sets of sympatric ecotypes that are phenotypically distinct from each other. Competition is predicted to drive frequency-dependent selection within and among populations of post-glacial fish. We tested whether this form of selection acts within the range of phenotypes found in generalist, single-species populations of threespine stickleback. To do this, we used experimental mesocosms with differing frequency distributions of phenotypes. We found evidence for only weak frequency-dependent survival but not frequency-dependent growth. To evaluate evidence for a possible evolutionary outcome of competitive interactions, we tested the hypothesis that levels of ecological sexual dimorphism should be higher in populations that do not co-occur with competitor species. We found similar levels of sexual dimorphism in allopatric threespine stickleback populations compared to populations sympatric with another stickleback species or prickly sculpin (an intraguild predator). Instead, we found some evidence for an alternative hypothesis that sexual dimorphism might decline with divergence from the ancestral marine stickleback phenotype. Competitive interactions affect partitioning of resources among different ecotypes and might lead to repeatability in resource use and phenotype distributions across different ecotype assemblages. Two Salmonid genera – *Salvelinus* and *Coregonus* – frequently diversify into assemblages of two to seven sympatric ecotypes in post-glacial lakes. We
evaluated evidence for repeatability of niche occupation, phenotype distributions, and niche by phenotype relationships across assemblages in these genera. We found evidence for repeatable niche occupation and niche by phenotype relationships in Coregonus, but only repeatable niche by phenotype relationships in Salvelinus. While these studies do provide evidence for a role of competitive interactions in shaping natural selection and evolution, they also suggest that predictions from theory for evolutionary consequences of competition may not always be met in fish populations in post-glacial lakes.
Lay Summary

Competition for resources, such as the food an animal eats or the habitat it occupies, shapes evolution within populations and determines which populations can co-occur in the same environment. Populations of fish in post-glacial lakes are useful for studying the effects of competitive interactions. We used an experiment to test whether natural selection within threespine stickleback fish populations stems from competition between fish with similar traits. We found weak evidence for this type of selection. Differences between males and females in traits used for acquiring resources can evolve through competition. Comparing among stickleback populations, we did not find a pattern that supported this explanation for male/female differences. Using two different groups of fish – charrs and whitefish – we compared patterns of diet and habitat use across lakes. We tested whether these patterns were highly similar across lakes, an expected result of competition. We found high similarity in whitefish but not charrs.
Preface

A version of Chapter 2 has been published as “Blain, S.A., Chavarie, L., Kinney, M.H., Schluter, D. 2022. A test of frequency-dependent selection in the evolution of a generalist phenotype. Ecology and Evolution, 12:e8831.” D. Schluter and I conceived the project. L. Chavarie, M.K. Kinney and I carried out the experiment and collected data. I analysed the data with input from D. Schluter and wrote the manuscript. D. Schluter, L. Chavarie, and M.H. Kinney contributed to editing the manuscript.

For Chapter 3, D. Schluter and I conceived the project. M. Roesti and I collected the wild-caught specimens. K. Thompson made crosses and raised the common garden specimens. I collected data from specimens and performed data analysis. I wrote the manuscript and D. Schluter contributed to editing the manuscript.

For Chapter 4, L. Chavarie, D. Schluter, and I conceived the project, with input from C. Adams, R. Knudsen, and P. Amundsen. I performed the systematic literature review and collated the data. I analysed the data with input from L. Chavarie and D. Schluter. I wrote the manuscript and L. Chavarie and D. Schluter contributed to editing the manuscript.

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List of Abbreviations

CxB – Fish from Cranby Lake by Benthic cross
CxC – Fish from Cranby Lake by Cranby lake cross
CxL – Fish from Cranby Lake by Limnetic cross
Int₀ – Mesocosms with no experimental treatment fish population
Intₐ – Mesocosms with an experimental treatment fish population with a benthic-skewed, intermediate phenotype distribution
Intₕ – Mesocosms with an experimental treatment fish population with a limnetic-skewed, intermediate phenotype distribution
LDA – Linear discriminant analysis
NMDS – Non-metric multidimensional scaling
PCA – Principal components analysis
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Chapter 1: Introduction

1.1 Ecological interactions and phenotypic diversity

Competition can drive the evolution and maintenance of phenotypic diversity both within and among populations (Slatkin 1980; Schluter 2000). When individuals or populations compete for resources, the strength of competition is expected to be highest between individuals with the most similar phenotypes (Taper and Case 1992). Competition among similar phenotypes is widespread in nature, affecting the evolution of beak shapes in *Geospiza* finches (Grant and Grant 2006), toepads in *Anolis* lizards (Stuart et al. 2014), and flowering times in *Mimulus* monkeyflowers (Kooyers et al. 2017).

There are a few possible evolutionary outcomes of competition among co-occurring phenotypes (Rueffler et al. 2006). When closely related competitor species co-occur, selection acting across the range of phenotypes in both species can drive character displacement between them (Taper and Case 1985, 1992). Within populations with a range of phenotypes related to resource acquisition, competition-driven negative frequency-dependent selection has the potential to maintain continuous phenotypic diversity (Wilson and Turelli 1986). It may also lead to the maintenance of discrete resource polymorphisms, as in crossbill populations where left-crossing and right-crossing bills are maintained (Benkman 1996). Ecological sexual dimorphism can also be an outcome of intraspecific competition. If the sexes are different in phenotypes related to resource acquisition, competition-driven frequency-dependence might cause further divergence between them (Bolnick and Doebeli 2003; De Lisle and Rowe 2015).

The effect of competition on phenotypic evolution can be shaped by other forms of interactions among individuals and species. The presence of predator species can alter
competitive interactions by creating competition for predator-free habitat space (Abrams and Chen 2002). For example, the presence of northern pike has been hypothesized to affect competitive interactions and resulting patterns of divergence in sympatric ecotypes of European whitefish (Öhlund et al. 2020b). Alternatively, intraguild predators alter competition and can drive phenotypic evolution by acting both as a competitor and a predator (Holt and Polis 1997). An example of this is prickly sculpin, which has undergone character displacement with threespine stickleback in lakes where they co-occur (Ingram et al. 2012; Miller et al. 2015; Roesti et al. unpublished). Interactions among sexes that are unrelated to resource acquisition can also impact competitive interactions within populations (Shine 1989). This might occur if sexually dimorphic traits that have evolved in response to reproductive or sexual selection impact resource acquisition. For example, sexual size dimorphism is quite common, and may evolve in response to sexual selection but also impact which resources an individual can access most easily (Stephens and Wiens 2009).

1.2 Phenotypic diversity in post-glacial lakes

Populations of fish in post-glacial lakes are a model system for studying competition among phenotypes. As the glaciers retreated following the most recent glacial maximum, numerous new lakes formed at high latitudes. These lakes were species depauperate, meaning that colonizing lineages of fish likely experience high ecological opportunity in the absence of competitors (Schluter 1996). Likely as a result of this ecological opportunity, there are many lineages of fish that have independently evolved to occupy the benthic and limnetic habitats within post-glacial lakes (Taylor 1999). Benthic habitats, sometimes referred to as littoral, are nearshore regions of the lake that tend to have vegetation and macroinvertebrate communities.
Limnetic habitats, also termed pelagic, are in open water regions of lakes and tend to contain a variety of phytoplankton and zooplankton. Threespine stickleback (*Gasterosteus aculeatus*) have evolved into sympatric benthic and limnetic species in a series of lakes along the coast of British Columbia (Schluter and McPhail 1992; Hatfield and Schluter 1999). There also are sets of sympatric ecotypes in post-glacial lakes across the northern hemisphere from multiple genera in the family Salmonidae (Kahilainen et al. 2011; Recknagel et al. 2017; Olson et al. 2019). Ecotypes in Salmonidae can occupy multiple possible habitats; benthic and pelagic habitats are particularly common (Schluter and McPhail 1993; Öhlund et al. 2020). Similar sets of benthic and limnetic ecotype pairs have been observed in the fish families Osmeridae and Centrarchidae (Robinson et al. 1996; Saint-Laurent et al. 2003).

How such benthic and pelagic assemblages form is variable. In threespine stickleback, evolution of the species pairs was facilitated by two independent invasions of the marine threespine stickleback (Taylor and McPhail 2000). The first invading population is thought to have initially adapted to resemble the intermediate generalist form found in contemporary lakes with a single species of stickleback (Schluter 2003). Following the second invasion, the marine and freshwater-adapted forms evolved in sympatry via character displacement, so that the first colonizing lineage became the benthic species and the second colonizing lineage evolved into the limnetic species (Schluter and McPhail 1992; Rundle and Schluter 1998). Other assemblages may have evolved through alternative processes. Adaptive divergence from a single colonizing lineage could occur, facilitated by either genetic changes or phenotypic plasticity (Dieckmann and Doebeli 1999; Snorrason and Skulason 2004). Alternatively, it is possible that lineages did not always adapt to fill a niche in the lake they currently occupy. Ecotypes that adapted to a
benthic or pelagic niche in one lake could have dispersed to others, allowing them to fill open niches without undergoing significant adaptation after colonization.

The effects of competition on the distribution of phenotypes within and among populations have been investigated previously within ecotype assemblages in post-glacial lakes. Observational studies in several lineages have implicated a role for competition in resource partitioning and associated phenotypic divergence among ecotypes. Experiments in threespine stickleback have demonstrated that competition can drive phenotypic divergence between sympatric ecotypes (Rundle et al. 2003; Schluter 2003). Intensity of competition also alters which resources threespine stickleback individuals access as well as the niche breadth of populations (Svanbäck and Bolnick 2007). Competitive interactions are clearly important in this system. Nonetheless, open questions remain regarding the ecological and evolutionary outcomes of these interactions under different circumstances and the degree to which these outcomes might be repeatable across environments.

1.3 Investigating competition among phenotypes in post-glacial lakes

To investigate how competitive interactions shape natural selection and evolution in populations of fish in post-glacial lakes, we used a combination of experimental, comparative, and data synthesis approaches.

For the second chapter of this dissertation, we carried out an experiment to test for negative frequency-dependent selection within the range of phenotypes present in generalist populations of threespine stickleback. According to the theory of competition-driven character displacement, the same form of selection that acts between sympatric populations to drive phenotypic divergence should act within solitary populations (Taper and Case 1992; Dieckmann
and Doebeli 1999). Specifically, with a resource gradient that is sufficiently variable relative to the phenotype distribution, higher competition for resources among more phenotypically similar individuals should lead to an advantage for individuals whose phenotypes are at a low frequency in the population (Wilson and Turelli 1986; Dieckmann and Doebeli 1999). We tested this prediction for solitary populations of threespine stickleback using a mesocosm experiment. We used treatment populations that were more limnetic-like and more benthic-like, so that one set of mesocosms contained a higher frequency of limnetic-like phenotypes while the other set contained a higher frequency of benthic-like phenotypes.

Evolution in response to competition-driven selection might depend on a population’s ecological context, including whether it is sympatric with competitor species. One possible evolutionary outcome of competitive interactions within populations is ecological sexual dimorphism (Bolnick and Doebeli 2003). In the third chapter of this dissertation, we used a comparative approach to evaluate evidence for two hypotheses related to the evolution of sexual dimorphism in the threespine stickleback. Higher levels of ecological sexual dimorphism are expected to evolve in environments where populations co-occur with few closely related competitors (Li and Kokko 2021; Stuart et al. 2021). To test this, we compared levels of sexual dimorphism in threespine stickleback populations that have evolved in sympathy with a competitor species to those that have evolved in allopatry. Another prediction from theory for ecological sexual dimorphism evolution is that it may be reduced while populations are adapting to new environmental conditions (Connallon et al. 2018). In threespine stickleback specifically, it has been suggested that this may explain higher levels of dimorphism observed in marine than benthic populations (Albert et al. 2008). We compared levels of sexual dimorphism among
ancestral marine populations and derived freshwater populations that exhibit varying degrees of phenotypic and genetic divergence.

Another possible outcome of interactions among sympatric populations of competitors is that populations might partition resources and evolve associated phenotypes in a repeatable way. In the fourth chapter of this dissertation, we used a systematic review and meta-analysis to test for repeatability in multi-ecotype assemblages of salmonids in the genera Coregonus and Salvelinus. Sets of sympatric ecotypes sometimes exhibit surprising levels of repeatability across locations where they occur (Gillespie 2004; Losos 2009). Fish ecotypes in post-glacial lakes have been described as an example of such repeatability, given that there are many examples of co-occurring benthic and pelagic forms (Schluter and McPhail 1993). It is less clear, however, if repeatability extends to assemblages containing three or more ecotypes, even though these commonly evolve in Salvelinus and Coregonus (Mcphee et al. 2012; Muir et al. 2016). We used a systematic literature review to identify assemblages containing two or more ecotypes in Salvelinus and Coregonus, and recorded data on the habitat or diet categories occupied by each ecotype as well as mean phenotypes. We assessed the similarity across assemblages in the distribution of ecological (habitat and diet) categories occupied, distributions of mean phenotypes, and relationships between ecological categories and mean phenotypes. Taken together, these studies have the potential to enrich our understanding of how competition shapes selection and phenotype distributions within populations of fish in post-glacial lakes.
Chapter 2: A test of frequency-dependent selection in the evolution of a generalist phenotype

2.1 Introduction

Populations occurring without close competitors often evolve an intermediate generalist phenotype, in contrast to the divergent specialized phenotypes that evolve via interspecific competition when species are sympatric (Brown and Wilson 1956; Slatkin 1980). This pattern, thought to be caused by ecological character displacement, has been observed in numerous traits and taxa (Schluter 2000b; Stuart and Losos 2013). Examples include intermediate body size in solitary species of *Anolis* lizards in the Lesser Antilles (Losos 1990a), beak depth in the medium beaked ground finch, *Geospiza fortis*, on Daphne Major island in the Galápagos (Schluter et al. 1985; Grant and Grant 2014), trophic traits in spadefoot toad tadpoles of both *Spea bombifrons* and *S. multiplicata* when each occurs alone in southwestern United States ponds (Pfennig et al. 2006), and gill raker length and body shape in solitary lake populations of threespine stickleback (*Gasterosteus aculeatus*) in coastal British Columbia (Schluter and McPhail 1992).

The form of selection that maintains an intermediate mean phenotype in wild solitary populations has been little investigated experimentally. In theories of character displacement and of competitive speciation, an intermediate mean phenotype in solitary populations is maintained via negative frequency dependent selection even though an intermediate phenotype is not directly favoured by selection (Wilson and Turelli 1986; Taper and Case 1992; Dieckmann and Doebeli 1999). Under this view, those resources consumed by individuals having the most common phenotypes will become depleted most quickly. This will favour individuals having
rarer phenotypes that exploit less depleted, alternative resources. If the population is randomly mating and the resource distribution is approximately symmetric, then negative frequency dependent selection will result in the maintenance of an intermediate phenotype distribution across generations (Wilson and Turelli 1986; Abrams et al. 1993; Kokko and López-Sepulcre 2007). Therefore, under the hypothesis of negative frequency dependent selection, an intermediate phenotype distribution is expected to evolve via an eco-evolutionary feedback.

While several examples of negative frequency dependent selection maintaining discrete ecologically relevant phenotypes are known (Pfennig 1992; Hori 1993; Benkman 1996; Schluter 2003; Mappes et al. 2008; Martin 2016; Bolnick and Stutz 2017), less evidence is available that this form of selection can result in the evolution of intermediate distributions of quantitative traits (but see Kusche et al. 2012).

An alternative hypothesis is that intermediate phenotypes in solitary populations are favoured regardless of the frequency distribution of phenotypes, perhaps because it allows them to access the broadest possible range of abundant resources. For example, in North American lakes, resource productivity peaks in the littoral zone in spring, and in the pelagic zone in summer (Mittelbach 1984). An intermediate phenotype would allow a fish population to exploit seasonal resource peaks in turn. Additionally, although disruptive selection would be an unstable equilibrium without negative frequency dependent selection, a bimodal resource distribution could favour alternate extreme phenotypes (Wilson and Turelli 1986; Rueffler et al. 2006). In this case, in a randomly mating population, the mean phenotype would remain intermediate. Testing for negative frequency dependent selection is therefore the first step in distinguishing the two hypotheses. Furthermore, unlike the hypothesis of negative frequency-dependent selection, the alternative hypothesis requires no feedback. A test of negative frequency dependent selection
is therefore a test of a theorized eco-evolutionary feedback in nature, evaluated against an alternative process that involves no feedback.

The present study tested for negative frequency dependent selection on a phenotypically variable, intermediate experimental population of threespine stickleback (*Gasterosteus aculeatus*). Sympatric species of threespine stickleback have diverged phenotypically via ecological character displacement along a littoral-pelagic gradient, whereas solitary populations in otherwise similar lakes are phenotypically and ecologically intermediate (Schluter and McPhail 1992). Sympatric species pairs are composed of one benthic and one limnetic species which are reproductively isolated from each other, while lakes with solitary populations have just one stickleback species (Hatfield and Schluter 1999; Rundle et al. 2000). Within solitary populations, measures of phenotypes such as body shape and gill rakers are variable and fall between those observed in the benthic and limnetic species, resulting in an intermediate distribution of phenotypes (Svanbäck and Schluter 2012). Lakes containing sympatric species pairs and those containing solitary populations of threespine stickleback are similar in their food web characteristics, including resource availability and presence of other fish species, as well as abiotic factors, such as depth and latitude (Vamosi 2003; Ormond et al. 2011). These populations are all thought to have been founded by marine threespine stickleback between 10,000 and 12,000 years ago as the lakes formed (Taylor and McPhail 2000). Previous experiments show that negative frequency dependent selection between sympatric stickleback species arises via competition for resources (Schluter 1994, 2003; Rundle et al. 2003). Further, disruptive selection has been observed within some solitary, phenotypically intermediate populations, which is consistent with frequency dependence but does not directly test for it (Bolnick 2004; Bolnick and
Lau 2008). Whether selection is frequency-dependent within the range of phenotypes present in solitary, phenotypically intermediate populations is unknown.

We tested the prediction of negative frequency dependent selection according to an eco-evolutionary feedback within intermediate phenotype distributions. To do so, we manipulated the phenotype distribution of stickleback populations in mesocosms, creating one treatment population that was more limnetic-like and one that was more benthic-like (Fig. 2.1). We then measured the effect of the two phenotype distribution treatments on the growth and survival of a phenotypically variable intermediate target population. Zooplankton and benthos, which are common threespine stickleback prey, were additionally measured to test the expectation that the two phenotype distribution treatments would differentially deplete resources. This would cause changes in invertebrate community composition that would be expected to have phenotype-dependent impacts on target population growth and survival (Matthews et al. 2016; Best et al. 2017). If selection was frequency dependent, then altering the frequency of phenotypes was predicted to affect individuals with similar phenotypes most negatively in the experimental target population (Fig. 2.1). If selection was not frequency dependent, then the performance of different phenotypes in the experimental target population may or may not be affected by the presence of treatment fish, but not their distribution of phenotypes.

2.2 Methods

2.2.1 Experimental Design

The experiment was performed in mesocosms with two distinct stages, a treatment stage and a response stage, following Matthews et al. (2016; Fig. 2.1). Although the phenotype distributions of natural solitary stickleback populations are generally unimodal and intermediate
to the extreme benthic and limnetic phenotypes of the species pairs, their mean phenotypes are variable among lakes. Due to differences in lake size and community composition, some populations exhibit more benthic-like characteristics, such as few gill rakers, and others showing more limnetic-like characteristics, such as a streamlined body shape (Miller et al. 2015; Bolnick and Ballare 2020). We exploited this variation to generate contrasting experimental treatments with more benthic-like (“IntB treatment”) or more limnetic-like (“IntL treatment”) phenotype distributions (Fig. 2.2). We chose to generate IntB and IntL treatments using solitary populations with more benthic- or limnetic-like means rather than using the more phenotypically distinct benthic and limnetic species in order to include phenotypes within the range expected in an intermediate generalist population. In the treatment stage, which began in September 2017 and lasted one month, four adult stickleback from either an IntB or IntL treatment were added to a total of 40 mesocosms. Ten mesocosms had no fish added during the treatment stage (“Int0” treatment). The phenotype frequency distributions were therefore manipulated in the treatment phase (the first month of the experiment). After a month, we removed the treatment fish and sampled zooplankton and benthic invertebrates to test for the impact of treatment on resource communities in the two main habitats. If frequency dependent selection occurred, mediated by an eco-evolutionary feedback, then the resource communities present after the treatment phase was predicted to depend on the phenotypes of treatment population fish.

In the second stage of the experiment, replicate phenotypically variable experimental target populations of 24 juvenile fish were tagged using elastomers then added to each mesocosm in October 2017. Growth rate and survival were measured in these juveniles as proxies for fitness, after their removal in December 2017. Growth rate is linked to feeding performance and fecundity in sticklebacks (Schluter 1995; Bolnick and Lau 2008; Arnegard et al. 2014). The
experimental setup therefore mimics a scenario in which adults of one generation impact juveniles of the next generation. The prediction under frequency dependence was that performance of a given target population phenotype would depend on the phenotype distribution present in the treatment phase.

2.2.2 Study Populations

Treatment and target population fish came from four types of lake stickleback populations: (1) solitary with an intermediate phenotype distribution, (2) solitary with a more limnetic-like phenotype distribution, (3) solitary with a more benthic-like phenotype distribution, (4) sympatric benthic and limnetic species pairs. We use the term “species” to refer to sympatric pairs of reproductively isolated and ecologically distinct benthic and limnetic species, and the term “populations” to refer to separate populations that would potentially interbreed if they came into contact with each other. Accurately assessing the position of phenotypically intermediate stickleback along a benthic to limnetic phenotypic axis is challenging to do accurately while individuals are still alive. We therefore relied on known differences in mean phenotypes of stickleback from different solitary populations to generate IntB and IntL treatments. There is a relatively high level of variability within these solitary populations, which lead to variation that we could not control in the degree to which treatment phenotypes were more or less limnetic-like or benthic-like (Fig. 2.2). Adult treatment fish were collected from the wild between April and June 2017 and held in aquaria in the University of British Columbia aquatics facility until their introduction into the mesocosms in September 2017.

Individuals for the IntL treatment populations were caught by minnow trap and dip net in Ambrose, North, and Garden Bay lakes. Due to a shortage of individuals resulting from mortality
in the lab prior to the start of the experiment, Intₐ treatment populations were supplemented with individuals from the limnetic species from Little Quarry and Priest lakes, in six out of the 20 limnetic-like treatment mesocosms. These individuals were also wild caught in April and May 2017 and held in aquaria until September 2017. Resource depletion did not differ among Intₐ mesocosms with different source populations. The sampled invertebrate biomass (see “Benthic Invertebrate and Zooplankton Sampling and Analysis”) was similar between mesocosms that contained limnetics and those that contained limnetic-like intermediates for both zooplankton (F₁, 18 < 0.01, p = 0.98) and benthos (F₁, 18 = 1.23, p = 0.28). Fish for the Intₐ populations were caught by minnow trap in Hoggan and Bullock lakes. Four Intₐ individuals were added to each of 20 mesocosms and four Intₐ individuals were added to another 20 mesocosms. This number of individuals was chosen because populations of four individuals were sufficient to differentially deplete resources in mesocosms in past experiments (Harmon et al. 2009; Rudman and Schluter 2016).

After the experiment, we used body shape, which varies in a repeatable way between benthic and limnetic stickleback and correlates to resource acquisition (Schluter 1995; Gow et al. 2008), to verify that Intₐ and Intₐ treatment population stickleback used and retrieved from the experiment were indeed either more benthic-like or more limnetic-like. Each recovered fish was stained with alizarin red and photographed. An additional set of wild caught stickleback of the sympatric benthic and limnetic species from Priest and Paxton Lakes were stained and photographed for comparison. 22 landmarks were used on each fish using the program tpsDig2 v 2.31 (Rohlf 2018), following the landmarks used in Ingram et al. (2012). A Procrustes analysis on the x and y coordinates of each landmark was performed using the ‘geomorph’ package in R v 4.0.3 (Adams and Otárola-Castillo 2013; R Core Team 2020). A linear discriminant analysis
was performed on the scaled and aligned coordinates corresponding to the benthic and limnetic fish using the ‘MASS’ package (Venables et al. 2019). Linear discriminant axis one therefore represented a benthic to limnetic phenotypic axis. Treatment fish were then projected onto this axis (Fig. 2.2).

We exploited among-population variation along a benthic-limnetic phenotypic axis to construct an experimental target population with high phenotypic variance. The target fish population of 24 individuals per mesocosm was a mixture of eight individuals from each of three cross types: (1) Cranby Lake females crossed to Paxton Lake limnetic males (CxL juveniles), (2) Cranby Lake females crossed to Paxton Lake benthic males (CxB juveniles), and (3) Cranby Lake females crossed to Cranby Lake males (CxC juveniles) (see “Target Juvenile Stickleback Population” below for more details on the crossed juveniles). Cranby Lake is located near Paxton Lake and contains a solitary population that is phenotypically intermediate between the benthic and limnetic species. This crossing scheme allowed us to generate an intermediate population with a wide phenotype distribution (Fig. 2.2). We chose to use a target population with inflated phenotypic variation to increase the sensitivity with which we could measure selection (Schluter 1994). A larger sample size was used for the target population than for the treatment population to account for the smaller biomass of juveniles and to allow for competition among individuals even with some mortality.

2.2.3 Mesocosm Construction and Treatment

Experimental mesocosms were constructed outdoors in 50 cattle tanks. The mesocosms had a volume of 1136 L, a depth of 64 cm, and a width of 175 cm. In May 2017, we added 12.5 kg dry weight of sand to the bottom of each mesocosm and filled them with water. Each
mesocosm was seeded with zooplankton from adjacent experimental ponds and with mud containing benthic invertebrates from a nearby reservoir pond. The mesocosms were left unmanipulated from June to August 2017, giving insects with an aquatic larval stage an opportunity to lay eggs in the tanks. To provide nutrients to stimulate phytoplankton growth, we added 0.976 g KNO3 and 0.067 g KH2PO4 to each mesocosm in August 2017.

During the experiment, mesocosms were surveyed daily for mortalities, which were removed and replaced with a fish from the same population type (IntB or IntL) to maintain a density of four fish per mesocosm. After the month-long treatment phase, treatment fish were removed by minnow trap and dip net over a two-week period. All treatment population individuals were recovered in twenty-five of the forty treatment mesocosms, and between zero and three individuals were recovered in the remaining fifteen mesocosms. The decision was made nonetheless to proceed with adding the target fish as we assumed that these individuals had died in the substrate at the bottom of the tank or were eaten by predatory birds or insects and were not recoverable without creating undue disruption to the mesocosms. The timing of these assumed deaths during the experiments is unknown. Results with all mesocosms included are presented in the main text, and results from only tanks where all four fish were recovered are included in supplementary materials. The direction of results is consistent between both datasets, with some differences in statistical significance given differences in sample size (see Results, Table A.1, Table A.2).

2.2.4 Benthic Invertebrate and Zooplankton Sampling and Analysis

Between the first and second stages of the experiment, four zooplankton samples were taken through the water column in each cattle tank using a 5.08 cm diameter PVC pipe with a
tennis ball attached to a rope that could be pulled in to act as a stopper. Samples were stained and preserved in iodine. They were later identified to a taxonomic level ranging from family to subclass and the length was measured using an ocular micrometer in a dissecting microscope. We used data on Daphniidae as well as calanoid and cyclopoid copepods to represent pelagic resource availability (Schluter and McPhail 1992). Length measurements of Daphniidae and Copepoda specimens were used to estimate biomass, using length-weight regressions from Dumont et al. (1975). Biomass estimates were not normally distributed, so they were ln-transformed.

Two 120 cm$^2$ samples of benthic substrate were taken using a dipnet from standardized locations in each mesocosm – one near the mesocosm edge, and one near the centre. The full depth of substrate was sampled at each location. Samples were searched by hand for benthic invertebrates for up to 20 mins, immediately after collections. Benthic invertebrates were preserved in ethanol, and later identified and measured using an ocular micrometer in a dissecting microscope. Identification ranged from a family to a class level and length measurements were converted to biomass using published length-weight regressions (Benke et al. 1999; Baumgärtner and Rothhaupt 2003; McKinney et al. 2004; Miyasaka et al. 2008).

The benthos and zooplankton biomass estimates were each divided by the surface area of the sample taken, so that all estimates were in μg/cm$^2$. We calculated the total biomass (μg/cm$^2$) as the sum from each mesocosm. We then log-transformed each biomass estimate after adding the constant to 0.1 to allow zero values to be included in the dataset. The data were not normally distributed (Shapiro-Wilk normality test: $W = 0.96$, $p = 0.002$), so we used a two-group Mann-Whitney U test to determine whether invertebrate biomass in each mesocosm depended on fish presence/absence treatment ($Int_0$ vs. $Int_L/Int_B$).
We predicted that Int$_B$ and Int$_L$ fish would more efficiently deplete benthos and zooplankton, respectively. To test this, we first converted sample type to a numeric value (benthos = 0, zooplankton = 1) and calculated the slope of log-transformed biomass against sample type for each mesocosm. We then used a two-group Mann-Whitney U test on the slopes between treatments under the alternative hypothesis that the slope between sample type and biomass was greater in Int$_B$ than Int$_L$ mesocosms.

To test for shifts in community composition in invertebrate communities, we first divided counts of individuals per taxonomic category by the surface area of the sample taken, then calculated Bray-Curtis distances between tanks using the ‘vegan’ package in R (Oksanen et al. 2020). We then evaluated the effect of treatment fish presence/absence (Int$_0$ vs. Int$_L$/Int$_B$) and treatment fish phenotype (Int$_L$ vs. Int$_B$) on those distances using the function ‘adonis()’ which conducts a multivariate analysis of variance using distance matrices (Anderson 2001; Oksanen et al. 2020). To visualize these distances, we used non-metric multidimensional scaling (NMDS) with four dimensions. We then used linear models to test whether there was a difference among treatments along any of those four axes.

### 2.2.5 Target Juvenile Stickleback Population

CxL, CxC, and CxB crosses were performed throughout May 2017 in the field and then transported to the UBC aquatics facility to be hatched and raised in aquaria. Crosses were performed by mixing eggs from one gravid Cranby Lake female with one crushed teste from a Paxton limnetic, Paxton benthic, or Cranby male. They were held in aquaria until transportation to the mesocosms. For 10 Int$_L$, 10 Int$_B$, and 5 Int$_0$ mesocosms, fish were individually marked with elastomer tags to identify their cross type and allow measurement of individual growth
rates. Due to logistical constraints, in the other 25 mesocosms CxC juveniles were batch marked with elastomer tags by giving the same type of elastomer tag to each fish. Mesocosms were assigned randomly to contain individually or batch-marked populations. CxL juveniles and CxB juveniles were the most morphologically distinct cross types, so these fish were left unmarked. The individually marked and batch marked fish required different methods of analysis. For mesocosms with individually marked fish, the fish is the sampling unit (nested within mesocosm). Including batch marked fish required using the mesocosm as the sampling unit, with an average growth change calculated for each cross type in each mesocosm.

At the end of the experiment, CxL juveniles and CxB juveniles retrieved were identified by a discriminant function analysis of their overall body shape, using the same landmarks used for treatment population fish. We performed a linear discriminant analysis on the scaled and aligned coordinates for individually marked fish of known cross type. The results of this analysis were used to classify remaining individuals. Individuals not assigned to a cross type with posterior probability higher than 95% were removed from later analyses.

2.2.6 Growth and Survival Estimates

Standard lengths were measured from photographs of target population fish taken before introduction to and after removal from mesocosms, using the program ImageJ (Schneider et al. 2012). The photographs were taken of the left side of each fish with a ruler in the frame of the photo. Each fish was also weighed at both time points by placing the fish in a tupperware container with water on a zeroed scale. Growth was calculated for all individually marked fish as the natural log of measured length and weight at the end of the experiment minus the natural log of the same measurements at the beginning of the experiment. For all mesocosms, we calculated
average change in length for each cross type. This was calculated as the mean length of fish of a cross type in a mesocosm at the end of the experiment minus the mean length of fish of a cross type in a mesocosm at the beginning. Whether or not individuals survived could be determined for the individually marked fish only. There were, therefore, four different response variables: (1) length change of individually marked fish, (2) weight change of individually marked fish, (3) survival of individually marked fish, and (4) length change of batch marked and individually marked fish.

2.2.7 Treatment Fish Presence/Absence Effects

To evaluate the predicted effect of treatment fish presence/absence in each of the three response variables, we tested for a difference in each mean growth and proportion survived between Int₀ mesocosms, where treatment fish were absent, and mesocosms where treatment fish were present (Int_L and Int_B). We used a Welch’s two sample t-test with the alternative hypothesis that growth in Int₀ mesocosms was greater than in Int_L and Int_B mesocosms. We estimated standardized effect sizes with Cohen’s D. Cohen’s D values near 0.2 and 0.5 are generally considered to be small and moderate, respectively, while an effect size of 1.2 is considered very large (Sawilowsky 2009).

We additionally tested whether the presence of treatment fish affected the slope of the relationship between target fish phenotype and outcome (specifically weight change, length change, and proportion survival). To do this, we followed the methods outlined below for comparisons between slopes in Int_L and Int_B mesocosms (see “Tests of Selection”) but instead compared mesocosms where treatment fish were absent (Int₀) and present (Int_L and Int_B). Because this did not address any of our predictions for the experiment, these results are included
in the Supplement (Table A.3). Slopes of regressions of survival on body shape along the benthic-limnetic axis tended to be larger in treatment fish absence mesocosms than in treatment fish presence mesocosms (Table A.3). In several comparisons, the slopes of regressions of growth (weight and length) on benthic-limnetic body shape were smaller in treatment fish absence mesocosms than in treatment fish presence mesocosms (Table A.3).

2.2.8 Tests of selection

For mesocosms with individually marked fish, we estimated the slope of the relationship between LD1 (which corresponded to an axis of body shape from benthic-like to limnetic-like) and each length and weight change. These slopes were expected to be non-zero due to intrinsic differences in growth rates among stickleback phenotypes (Fig. 2.1; Hatfield and Schluter 1999). We then tested whether slopes from IntL mesocosms differed from those from IntB mesocosms using a Welch’s two-sample t-test. If selection were negative frequency dependent, we would expect fish with more limnetic-like phenotypes (i.e., CxL fish) to exhibit higher growth in IntB relative to IntL mesocosms (Fig. 2.1). This would correspond to a more negative slope between growth and body shape in IntL than IntB mesocosms. We then repeated this test with a cross type converted to numeric values (CxB = -1, CxC = 0, CxL = 1) as the predictor instead of LD1.

For fish from all mesocosms (individually marked and batch marked), we calculated the mean length and mean LD1 for the three cross types from each mesocosm then calculated a slope between those variables for each mesocosm. We used a Welch’s two-sample t-test to evaluate whether slopes from IntL mesocosms were less than slopes from IntB mesocosms. This test was repeated with cross type converted to numeric values as the predictor for each slope.
For survival, we first calculated the mean LD1 and proportion survived for the three cross types from each Int\(L\) and Int\(B\) mesocosm. We calculated a slope for each mesocosm using these three points, then evaluated whether the slopes in Int\(L\) mesocosms were less than those in Int\(B\) mesocosms using a one-sided Welch’s two-sample t-test. We then repeated this test with cross type converted to numeric values as the predictor.

### 2.3 Results

#### 2.3.1 Invertebrate biomass response

Invertebrate community biomass, sampled after treatment fish were removed and before the addition of the experimental target population, was greater overall in control (Int\(0\)) than fish addition (Int\(B\) and Int\(L\)) mesocosms (Fig. 2.3A; \(W = 301, p < 0.01\)), confirming food resource depletion in the presence of fish. The slope of regressions of biomass on invertebrate sample type differed slightly in the predicted direction between Int\(B\) and Int\(L\) mesocosms and not significantly (Fig. 2.3B; \(W = 218, p = 0.44\)).

Invertebrate community composition differed between the control (Int\(0\)) and fish addition treatment (Int\(B\) and Int\(L\)) mesocosms (multivariate ANOVA: \(F_{1, 47} = 2.64, p < 0.01\)), indicating an effect of resource depletion in the presence of fish. In contrast to the first prediction from the frequency dependence hypothesis, we did not detect a difference in community composition between Int\(B\) and Int\(L\) mesocosms (multivariate ANOVA: \(F_{1, 37} = 0.95, p = 0.47\)). Int\(0\) was differentiated from Int\(L\) and Int\(B\) along the third NMDS axis (Fig. A.1; \(F_{2, 46} = 16.76, p < 0.01\)), but treatment groups did not vary along the first \(F_{2, 46} = 0.08, p = 0.93\), second \(F_{2, 46} = 0.18, p = 0.83\) or fourth axes \(F_{2, 36} = 2.39, p = 0.10\).
2.3.2 Survival among experimental target fish

Mean survival of experimental target fish was similar between mesocosms in which treatment fish had been present and absent (Fig. 2.4; $t_{4.53} = -0.9$, $p = 0.42$, Cohen’s D = -0.54). As predicted under negative frequency dependent selection, the slope of regressions of survival on cross type differed between treatments (Fig. 2.4; $t_{14.69} = 2.34$, $p = 0.03$, Cohen’s D = 1.05). The limnetic-like treatment (Int_L) reduced survival of the most limnetic-like experimental fish relative to the most benthic-like experimental fish. Conversely, the benthic-like treatment (Int_B) reduced survival of the most benthic-like target fish relative to the most limnetic-like target fish. For slopes of regressions relating survival to body shape of experimental fish, the difference was in the same direction but only marginally significant (Fig. A.2; $t_{16.89} = 1.96$, $p = 0.07$, Cohen’s D = 0.89).

2.3.3 Growth rates among experimental fish

Food depletion by treatment population fish impacted experimental target fish growth. Mean growth of individually-marked fish was highest in Int_0 mesocosms (treatment fish absent) when measured by weight change (Fig. 2.5A; $t_{823} = 8.89$, $p < 0.01$, Cohen’s D = 3.01) and length change (Fig. 2.5B; $t_{9.05} = 4.99$, $p < 0.01$, Cohen’s D = 2.19). The result was the same for length change of batch-marked fish (Fig. A.3; $t_{16.62} = 2.8$, $p = 0.01$).

Slopes of regressions of growth rate on cross type did not significantly differ between frequency treatments (Int_B and Int_L) for weight change in individually-marked fish (Fig. 2.5A; $t_{16.93} = 1.25$, $p = 0.23$, Cohen’s D = 0.56) and length change in batch-marked fish (Fig. A.3A; $t_{35.87} = 0.78$, $p = 0.44$, Cohen’s D = 0.25). For length change in individually-marked fish,
differences in slopes of the relationship between growth and cross type were not significant and not in the predicted direction (Fig. 2.5B; \( t_{14.32} = -0.04, p = 0.97, \text{Cohen’s D} = -0.02 \)).

Slopes of regressions of growth rate on body shape did not differ between frequency treatments (IntB and IntL) when measured in individually-marked fish by weight change (Fig. A.4A; \( t_{16.95} = -0.84, p = 0.41, \text{Cohen’s D} = -0.38 \)) and length change (Fig. A.4B; \( t_{16.91} = -0.33, p = 0.74, \text{Cohen’s D} = -0.15 \)). Slopes of regressions of growth rate on body shape were not significantly different when measured by length change in batch-marked fish (Fig. A.3B; \( t_{32.15} = 1.09, p = 0.28, \text{Cohen’s D} = 0.36 \)).

### 2.4 Discussion

When a randomly mating population evolves on a symmetric resource gradient, resource competition is predicted to result in frequency dependent selection leading to the evolution of an intermediate phenotype (Taper and Case 1992; Dieckmann and Doebeli 1999). Alternatively, selection might directly favour an intermediate phenotype without frequency dependent selection. We carried out an experimental test of frequency dependent selection via an eco-evolutionary feedback using intermediate populations of threespine stickleback and detected only weak effects. Survival selection was weakly frequency dependent. The direction of estimates was variable when growth was used as a fitness metric and point estimates were small and uncertain. Resource depletion occurred with detectable effects on growth, suggesting that competition for food was nevertheless present. We conclude that frequency dependent selection is likely to be present, but if so, it is not strong.

Aspects of the experimental conditions warrant caution in drawing conclusions about the role of frequency dependent selection on stickleback populations. Performing the experiment in
mesocosms might have restricted the width of the resource gradient, such as by representing a primarily benthic environment. Character displacement theory shows that a narrow resource gradient weakens frequency dependent selection (Taper and Case 1985; Dieckmann and Doebeli 1999). Furthermore, this experiment was run on a short time frame. It is possible that a longer period of resource depletion would be required to generate a noticeable impact of the different phenotypes on the environment. This also means that only one part of the target population’s life cycle was measured, so stronger effects may have emerged if there was more time for juvenile growth or if effects were measured over multiple generations. Additionally, adult sticklebacks were used as a treatment population whereas juvenile sticklebacks were used as a target population. Given that adult and juvenile stickleback have differences in morphology and gape width, it is possible that they would consume resources differently. As a result, it is possible that frequency dependence would only be observed among individuals of the same age class. Despite the caveats, we have shown that frequency dependent selection, if present within this range of phenotypes, is not always strong and easily detectable. Although this is not the final word on frequency dependence in this system, we nevertheless suggest that the results have interesting implications for our understanding of the evolutionary processes acting in intermediate populations.

Our results are somewhat surprising because they seem at odds with theory for trait evolution along a resource gradient in the presence of competition (Roughgarden 1976; Taper and Case 1985). They are additionally puzzling because frequency dependent selection has been detected between sympatric species of threespine stickleback differing in mean phenotype (Schluter 1994, 2003; Rundle et al. 2003). However, under existing theory, frequency dependent directional selection is expected to weaken with greater similarity of competing individuals.
(Schluter 2000a). Therefore, differences between sympatric and solitary populations might prevent similar intensities of selection from occurring in both contexts. At the start of the character displacement process in stickleback, the phenotype distribution in lakes containing two sympatric species is thought to have been broader overall than that in single-species, solitary populations (Taylor and McPhail 2000; Svanbäck and Schluter 2012). Phenotypes within intermediate populations might always overlap significantly in resource use, or the overlap between limnetic-like and benthic-like phenotypes might be higher when each occurs in the absence of alternative phenotypes. Variation in resource use within and among intermediate populations may therefore not be large enough to exert detectably different ecological impacts or to generate an eco-evolutionary feedback, and therefore frequency dependent selection. A broader phenotype distribution than that found within populations may be necessary to generate strong frequency dependence in stickleback.

Another possible explanation for our finding of weak selection is that the resource distribution was too narrow in mesocosms relative to the breadth of resources utilized by consumers. For strong frequency dependence driven by an eco-evolutionary feedback to emerge, resource distributions must wide enough for individuals with uncommon phenotypes to have undepleted resources to access (Rainey and Travisano 1998; Dieckmann and Doebeli 1999). For a population of individuals exploiting most of the resources in an environment with a narrow resource distribution, this may not be the case. Stickleback populations are most phenotypically variable and most commonly experience disruptive selection (another possible outcome of frequency dependent selection – see below) in intermediate-sized lakes with relatively equal ratios of benthic to limnetic habitat (Bolnick and Lau 2008; Bolnick and Ballare 2020). These may therefore be the habitats in which frequency dependence within intermediate populations is
strongest and most likely to be detected. Nonetheless, previous experiments have shown that phenotypically divergent stickleback cause divergent ecosystem effects in mesocosms, and that these ecosystem effects can generate eco-evolutionary feedbacks (Harmon et al. 2009; Des Roches et al. 2013; Matthews et al. 2016; Rudman and Schluter 2016). Those experiments, however, used a wider distribution of phenotypes with greater differences between phenotype treatments. Weak or absent frequency dependent selection could instead be a consequence of the way in which phenotypes deplete resources, and the degree of overlap between them. If individuals within intermediate stickleback populations consume a broader or more plastic range of resources, then individuals with different phenotypes may exhibit more overlap in resource use. This would mean that increasing the frequency of one phenotype would impact other phenotypes more or less equally, leading to a lack of strong frequency dependence.

A prediction of the same theory, which we did not test here, is that selection on intermediate populations should be disruptive (Wilson and Turelli 1986). Surveys and field experiments have found that selection is variable and sometimes disruptive in single-species populations of threespine stickleback, depending on lake characteristics, and that the strength of disruptive selection is density-dependent (Bolnick 2004; Bolnick and Lau 2008). However, in those lakes where disruptive selection does occur it also tends to be quite weak (Bolnick and Lau 2008). Disruptive selection has also been detected in an experimental pond population of F₂ hybrids between sympatric benthic and limnetic species (Arnegard et al. 2014). In both cases, disruptive selection could have been generated by either frequency dependence or a bimodal resource distribution (Wilson and Turelli 1986; Rueffler et al. 2006). In phenotypically intermediate populations of S. multiplicata spadefoot toads, which are another set of solitary populations from a character displacement series, disruptive selection is present and generated by
competition between phenotypically similar individuals, as predicted by character displacement theory (Martin and Pfennig 2009). The present experiment demonstrated that frequency dependence is hard to detect even with the inflated variance of our target experimental populations. We thus suggest that frequency dependent selection may be present, but weak within the limited range of phenotypes in solitary populations.

Our findings are broadly consistent with a particularly well-studied intermediate natural population, the medium ground finch *G. fortis* on Daphne Major Island in the Galápagos. Mean beak size in this population is intermediate between the means of the small and medium ground finch species that occur in sympatry on most other islands (Schluter et al. 1985). Decades of field study have shown that on Daphne Major, selection on *G. fortis* is typically directional and varies in direction and strength from year to year. The net effect is to maintain the population at an intermediate phenotype (Schluter et al. 1985; Grant and Grant 2014). The fluctuating selection and resulting evolution are closely tied to annual variation in environmental factors, particularly rainfall (Grant and Grant 2014; Nosil et al. 2018). This suggests that frequency dependent selection within the range of phenotypes in the population might not be the main cause of an intermediate phenotype in the solitary *G. fortis* population, although this has not been tested experimentally. Given the results of the present experiment along with weak and spatially varying disruptive selection in solitary populations (Bolnick 2004; Bolnick and Lau 2008), the same might be true in stickleback.
Figure 2.1 Expectations for growth rate under frequency dependent (A) and frequency independent selection (B). The lines in the two panels illustrate the expected relationship between phenotype and growth in each mesocosm type - Int_B (benthic-like treatment), Int_L (limnetic-like treatment), and Int_0 (no fish control). Under frequency dependent selection (A), the growth of alternate extreme phenotypes is depressed under contrasting Int_L and Int_B treatments (shown as lines with different slopes). In the absence of frequency dependent selection (B), the relationship between phenotype and growth does not depend on treatment phenotype. Mean growth in both treatments is depressed compared with the Int_0 treatment, in which no fish were
added prior to introduction of target fish. (C) Experimental design. There were three main time points in the experiment. At time point 1, four adult treatment fish with benthic-like (Int_B) or limnetic-like (Int_L) phenotypes were added to each of 40 mesocosms, with 10 left as no fish controls (Int_0). They were removed at time point 2, and we sampled zooplankton and benthic invertebrates. At time point 3, identical phenotypically variable target populations of 24 juvenile hybrids were added to each mesocosm. We measured the growth and survival of these experimental target fish.
Figure 2.2 Position of different experimental fish phenotypes along a linear discriminant axis. Each point represents one individual. Benthic and limnetic individuals are from the species pair populations in Priest and Paxton lakes (squares), the Int_B and Int_L individuals were the fish used in the treatment phase of the experiment (circles), and the CxB, CxC, and CxL individuals were the experimental target population (triangles). All target population individuals are from the individually-marked dataset. Body shapes were quantified after the experiment, so individuals included in this figure were only those that survived the experiment.
Figure 2.3 (A) Total invertebrate biomass. Circles represent the total biomass (μg/cm²) of invertebrates sampled from a mesocosm. Diamonds represent medians, while error bars represent one standard deviation. On the y-axis, biomass is given on a natural log scale. (B) Invertebrate biomass by habitat. Points represent the total biomass (μg/cm²) of invertebrates sampled from a mesocosm on a log scale, with lines joining biomass estimates from the same mesocosm. Diamonds represent medians for each sample type from each IntB and IntL mesocosms.
Figure 2.4 Relationship between survival and cross type in contrasting treatments. Cross was converted to a numeric value, with CxB = -1, CxC = 0, and CxL = 1. Each thin line represents the relationship between growth and cross type in one mesocosm while bold lines represent the mean slopes for mesocosms with each treatment.
Figure 2.5 Relationship between growth, measured by weight (A) and length (B), and cross type in contrasting treatments. Cross was converted to a numeric value, with CxB = -1, CxC = 0, and CxL = 1. Each thin line represents the relationship between growth and cross type in one mesocosm while bold lines represent the mean slopes for mesocosms with each treatment.
Chapter 3: Sexual dimorphism in the threespine stickleback across ecological contexts

3.1 Introduction

Ecological sexual dimorphism, whereby the sexes differ in ecologically-relevant traits, is common in nature (Temeles et al. 2000; Butler et al. 2007; De Lisle and Rowe 2015). However, what causes it to evolve in some populations but not others is not well understood, and there are multiple hypotheses from theory. Sexual dimorphism in ecologically-relevant traits can evolve as a by-product of selection for traits involved in mate choice or reproduction (Shine 1989). Alternatively, sexual dimorphism can result from intraspecific resource competition. If the resources easily accessed by the most common phenotypes in a population are depleted most quickly, disruptive selection can favour lower-frequency phenotypes that access underused resources (Wilson and Turelli 1986). As long as the traits have some difference between the sexes in their underlying genetics, greater differences between the sexes are expected to evolve (Bolnick and Doebeli 2003; Cooper et al. 2011). In populations with low levels of assortative mating among phenotypes, sexual dimorphism might evolve as an alternative to ecological speciation in sympatry (Bolnick and Doebeli 2003). Levels of ecological sexual dimorphism may also depend on a population’s history of adaptation. Sex-specific selection is expected to be reduced when a population is adapting to a new environment because both sexes will experience the same direction of selection if both are maladapted (Connallon et al. 2018). A comparative approach using closely related populations or species that vary in both their degree of ecological
sexual dimorphism and their ecological context may provide a way to distinguish among these alternative hypotheses (Stephens and Wiens 2009; Stuart et al. 2021).

Comparative approaches have been used to demonstrate that dimorphism in ecologically relevant traits evolves via selection based on mate choice and reproductive roles (Shine 1991; Nylin et al. 1993; Ronco et al. 2019). There have also been several empirical tests of the hypothesis that sexual dimorphism evolves in response to intraspecific competition, but they have provided partial answers. Experimental manipulations of both population density and the relative frequency of each sex using Notophthalmus viridescens salamanders demonstrated that competition between individuals of the same sex can lead to disruptive selection on sexually dimorphic feeding morphology as well as negative frequency-dependence (De Lisle and Rowe 2015). Therefore, despite the different underlying genetics, ecological sexual dimorphism can evolve through the same form of selection that has been shown to drive character displacement between closely related sympatric species (Pfennig and Murphy 2002; Schluter 2003).

A prediction of the competition hypothesis is that ecological sexual dimorphism should be greater in the absence of closely related competitor species than in their presence (Li and Kokko 2021; Stuart et al. 2021). This hypothesis requires that there be a wide enough resource gradient that there are resources underused by the most common phenotypes and that the resource gradient is similar between locations where competitor species are present and absent (Bolnick and Doebeli 2003; Li and Kokko 2021). It is expected that this resource gradient can be subdivided among competitor species or between the sexes, but not both (Bolnick and Doebeli 2003). Consistent with this prediction, sexual size dimorphism declines with species richness in assemblages of Liolaemus lizards (Pincheira-Donoso et al. 2018). However, similar levels of dimorphism in the presence and absence of competitor species were observed for traits affecting
habitat occupation in *Anolis caroliensis* lizard populations, body size in emydid turtles, and body size in island-dwelling mammal and lizard communities (Stephens and Wiens 2009; Meiri et al. 2014; Stuart et al. 2021). Alternatively to this prediction, if sexually dimorphic traits are different from those evolving in response to competition, then the evolution of sexual dimorphism may not be constrained by co-occurrence with competitors (Cooper et al. 2011).

Threespine stickleback populations in small coastal lakes of British Columbia vary in the number of competing species with which they co-occur (Taylor and McPhail 2000; Vamosi 2003). Threespine stickleback occur as solitary populations in most lakes, but also occur as a pair of phenotypically and ecologically divergent species in a few lakes (Schluter and McPhail 1992; Nagel and Schluter 1998). The sympatric species differ in several traits associated with resource acquisition and habitat use, including gill raker morphology, overall body shape, jaw morphology, spines and lateral plates (Schluter and McPhail 1992; Vamosi and Schluter 2004; McGee et al. 2013). This divergence was at least partly the outcome of resource competition (Rundle et al. 2003; Schluter 2003). Populations of stickleback also vary in their co-occurrence with prickly sculpin, a competitor and possible intraguild predator (Vamosi 2003). Stickleback that are sympatric with sculpin exhibit more gill rakers and an elongated body shape, both of which are adaptations associated with foraging in a more limnetic environment, as well as enhanced defensive traits (Ingram et al. 2012; Miller et al. 2015). Populations of stickleback occurring without a second stickleback species and without sculpin exhibit other characteristics expected of a population facing reduced interspecific competition, such as increased phenotypic variation and greater phenotypic plasticity than sympatric stickleback populations (Svanbäck and Schluter 2012).
Marine stickleback colonized these coastal lakes following deglaciation, roughly 12,000 years ago, and adapted in parallel to freshwater lake environments (Hohenlohe et al. 2010; Morris et al. 2018; Magalhaes et al. 2021). Despite having a similar time of divergence from marine stickleback, freshwater threespine stickleback populations form a continuum of phenotypic and genomic divergence from the ancestral marine form. Benthics are more diverged than limnetics while there is more variability among solitary and sculpin-sympatric populations in their degree of divergence from marines (Chhina et al. 2022). Threespine stickleback populations also exhibit varying degrees of sexual dimorphism in ecologically relevant traits (Reimchen and Nosil 2004; Bolnick and Lau 2008), which allows a test of whether sexual dimorphism varies with the number of competing species. The competition hypothesis predicts that ecological sexual dimorphism should be greater in the absence of a second stickleback species or prickly sculpin than in their presence.

We also used the same populations to test the alternative hypothesis that sexual dimorphism evolves as a by-product of ecological selection on populations locally adapting to a new environment. This hypothesis predicts that sex-specific selection should be reduced in populations adapting to new environments because selection is in the same direction, rather than predicting directly how sexual dimorphism should evolve with mean phenotype (Connallon et al. 2018; De Lisle et al. 2018). To evaluate this prediction, we tested whether sexual dimorphism is correlated to the degree of divergence from marine populations. The logic of this test is based on previous observations that the benthic species from Paxton Lake exhibits less dimorphism than marine stickleback (Albert et al. 2008) and that more limnetic freshwater populations, which are more similar to the marine form phenotypically, have higher levels of body shape dimorphism (Spoljaric and Reimchen 2008). Repeated divergence to a varying extent from the marine form
allowed us to test whether sexual dimorphism changed consistently the further populations diverged from marines and the more selection both sexes experienced in the same direction.

In sum, we compared the degree of sexual dimorphism in ecologically-relevant traits in populations occurring alone in lakes against sexual dimorphism when populations co-occur either with a second stickleback species or with prickly sculpin. First, we expected stickleback populations without sympatric competitors to exhibit greater sexual dimorphism than threespine stickleback populations occurring in the presence of a competing fish species. This greater sexual dimorphism is predicted to evolve specifically along a limnetic-benthic gradient, which is the same gradient partitioned by stickleback when sympatric with competing fish species. Alternatively, we predicted that sexual dimorphism might be reduced with greater divergence from marine stickleback. This means that along a phenotypic axis of benthic-marine divergence, we expected marine stickleback to exhibit more dimorphism than benthic stickleback, with limnetic, solitary, and sculpin-sympatric stickleback exhibiting intermediate levels of dimorphism. Additionally, the competition hypothesis predicts that, to some extent, sexual dimorphism in solitary populations should occur along the phenotypic axis of interspecific character displacement, while the divergence hypothesis predicts that sexual dimorphism in marine stickleback should occur along the phenotypic axis of marine – benthic population divergence.

3.2 Methods

3.2.1 Specimen sampling

We caught stickleback specimens to use for phenotyping from wild populations between April and July in 2016 – 2018, using minnow traps and dip nets. Populations of both the benthic
and limnetic species (which we refer to as “Stickleback-sympatric”) were sampled from Little Quarry, Paxton, and Priest Lakes (Fig. 3.1A). Sculpin-sympatric stickleback populations were sampled from McNair, Roselle, Paq, Morton, Ambrose, Blackwater, Ormond, Merrill, North and Pachena Lakes (Fig. 3.1A). Solitary populations of stickleback occurring neither with another stickleback species nor with sculpin were sampled from Weston, Bullock, Black, Kirk, Cranby, Maxwell, Mike, Trout, Klein, and Hoggan Lakes (Fig. 3.1A). All lakes occur in different drainage basins and almost all contain cutthroat trout (Vamosi 2003). We sampled marine fish from Cowichan River, Oyster Lagoon, and Little Campbell River. All samples were euthanized in the field with buffered MS-222 and preserved in 95% ethanol.

3.2.2 Common garden experiment

We raised stickleback from a subset of populations to quantify sexual dimorphism in a common garden and estimate the degree to which it is genetically based. We performed intra-population crosses using wild adult stickleback from several lakes between April and June 2017. Solitary population crosses were performed from Bullock and Klein Lakes, sculpin-sympatric crosses were from North and Paq Lakes, and benthic and limnetic crosses were from Priest Lake. The offspring from each cross were raised in replicate aquaria and fed brine shrimp as juveniles and bloodworms as adults following (Chhina et al. 2022). All samples were euthanized in April and May 2018 after reaching adulthood and preserved in 40% formalin.

3.2.3 Phenotyping

Wild-caught samples were gradually transitioned from 95% ethanol to water before being fixed in 10% formalin. Both wild-caught and lab-grown samples were then stained with alizarin
red, following standard procedures (Peichel et al. 2001). All samples were categorized as “male” or “female” based on the presence of testes or ovaries. Samples were photographed on the right side, except where that side of the fish was visibly damaged. We then obtained coordinates of 25 landmarks from each specimen photograph using tpsDig (Rohlf 2018). We used the same landmarks as in Albert et al. (2008), but removed the two landmarks indicating the pelvic girdle location because this trait was absent in some benthic-adapted populations. We performed a Procrustes analysis to scale, coordinate, and align landmarks across specimens using “gpagen()” in Geomorph (Adams et al. 2018). We took linear measurements using calipers (Fig. S1) and counted lateral plates on the right side of each stained specimen. We counted gill rakers on the lower and upper sides of the first gill arch and measured the lengths of the three posterior-most gill rakers on the upper first gill arch using a dissecting microscope. We size-corrected linear trait measurements to account for variation among individuals related to age and growth rates. To do this, we first regressed log-transformed values of each trait on log-transformed standard length. We combined all fish in one regression, thereby assuming equal slopes across populations. We then calculated the trait size predicted for each fish at the grand mean standard length (Vamosi 2002).

3.2.4 Phenotype analysis – wild caught fish

To test the prediction that sexual dimorphism should be greater in solitary than stickleback-sympatric populations, we performed principal components analyses (PCA) on the covariance matrix of body shape landmark coordinates using all solitary, benthic, and limnetic populations. The covariance matrix was used because landmarks were already scaled and therefore had similar variances. Hereafter, the major axis (PC1) from this analysis is referred to
as the ‘benthic – limnetic axis’. To test the prediction that sexual dimorphism should be greater in solitary than sculpin-sympatric populations, we performed principal components analysis on the covariance matrix of body shape landmark coordinates using solitary and sculpin-sympatric specimens. Hereafter, PC1 from this analysis is referred to as the ‘solitary – sculpin-sympatric axis’. To test the prediction that sexual dimorphism should vary with phenotypic divergence from the marine ancestral form, we performed principal components analyses on the covariance matrix of body shape landmark coordinates using both marine and freshwater populations, including marine, solitary, stickleback-sympatric, and sculpin-sympatric populations. Hereafter, PC1 from this analysis is referred to as the ‘marine – benthic axis’ since the benthic populations are the most divergent in shape from the marine ancestor. We used separate PCA’s for each of these population subsets to generate the phenotypic axes along which sexual dimorphism should occur under each prediction. We estimated the magnitude of sexual dimorphism along each of these axes within each population as the difference between female and male mean values. We then took the absolute value of these magnitudes to estimate patterns in amount of dimorphism rather than its direction. To quantify the phenotypic divergence of each population from the marine ancestor, we calculated the mean value for each population along the marine – benthic axis.

Because sexual dimorphism in body shape may not align to a particular axis of population divergence, we also estimated ‘total’ sexual dimorphism in body shape as the length of a divergence vector between the multivariate means for males and females of each population, following (Stuart et al. 2017). To evaluate whether the phenotypic axis of sexual dimorphism aligns to axes associated with population divergence, we calculated the angle between the direction of male – female divergence in the populations predicted to exhibit elevated
dimorphism along a particular axis and the major axes of population divergence: benthic – limnetic, solitary – stickleback-sympatric, and marine – benthic. We calculated a single divergence vector between all benthic and all limnetic individuals. We estimated the angle between this benthic – limnetic divergence vector and the sexual dimorphism divergence vector from each solitary population. We then calculated a divergence vector between all solitary and all sculpin-sympatric individuals and estimated the angle between this divergence vector and the total sexual dimorphism vector in each solitary population. To evaluate the alignment between sexual dimorphism and a marine – benthic axis of divergence, we calculated a divergence vector between all marine and all benthic individuals, then calculated the angle between this vector and the total sexual dimorphism vector for each marine population. Because direction of alignment between the vectors is arbitrary, we standardized all angles to fall between 0 and 90° by calculating its difference from 90°.

We also calculated and compared sexual dimorphism in size, armor and in gill raker traits between populations. Body size was measured as centroid size. Armor was measured using the first principal component from the correlation matrix for number of lateral plates, size-corrected length of pelvic girdle, and size-corrected length of first dorsal, second dorsal, and pelvic spines. Gill raker variation was measured using the first principal component from the correlation matrix of two gill raker counts and three size-corrected gill raker lengths. We quantified sexual dimorphism for gill rakers, armour, and body size as the absolute value of the difference between mean values of females and males in each population.

To evaluate support for the competition hypothesis, we used two-sample t-tests of the difference in sexual dimorphism along a benthic – limnetic axis, total body shape dimorphism,
and sexual dimorphism in body size, gill rakers, and armour between solitary and stickleback-sympatric populations. We then used two-sample t-tests to compare levels of sexual dimorphism along the solitary – sculpin-sympatric axis, as well as sexual dimorphism in total body shape, body size, gill rakers, and armour, between solitary and sculpin-sympatric populations. To evaluate support for the phenotypic divergence hypothesis, we first regressed dimorphism along a marine – benthic axis on mean population body shape along the marine – benthic axis. We then regressed dimorphism in total body shape, body shape, gill rakers, and armour on mean population body shape along the marine – benthic axis.

3.2.5 Phenotype analysis – common garden experiment

To evaluate whether sexual dimorphism in each trait has a genetic basis, we compared sexual dimorphism in wild and lab-raised stickleback. We used a subset of populations spanning the competitive contexts included in the study: Priest Lake Benthic and Limnetic (stickleback-sympatric), Bullock and Klein lakes (solitary), and North and Paq lakes (sculpin-sympatric). We estimated total sexual dimorphism in body shape in each population as described above, separately for wild and lab-raised individuals. We also estimated dimorphism in body size, as described above for lab-raised and wild-caught samples separately. We estimated sexual dimorphism in gill raker traits using the first principal component from the correlation matrix of five gill raker traits based on wild and lab-raised individuals from the subset of populations. We similarly estimated sexual dimorphism in armour traits using the first principal component from the correlation matrix of five armor traits. We then calculated a t-test of the difference between females and males from a population, separately for lab and wild samples.
We then determined whether sexual dimorphism measured in wild-caught fish is repeated in common garden samples. For each population we had a set of repeated measures for each trait: one wild-caught and one lab-raised. We then evaluated repeatability, defined as high similarity of repeated measures within populations relative to variation among populations, by fitting a linear mixed model with sexual dimorphism as the response variable and population as a random effect. Our repeatability metric was the variance among populations in sexual dimorphism divided by the sum of the variance within and variance among populations in sexual dimorphism (Lessells and Boag 1987).

3.3 Results

3.3.1 Sexual dimorphism and character displacement

None of the predictions of the competition hypothesis were upheld. Levels of dimorphism along a benthic – limnetic body shape axis were similar between stickleback-sympatric (each benthic and limnetic population) and solitary populations ($t_{11.5} = 1.54, p = 0.15$; Fig. 3.2A). In contrast to the prediction of the competition hypothesis, total body shape dimorphism was higher in stickleback-sympatric than solitary populations ($t_{12.7} = -3.03, p = 0.01$; Fig. B.1A). Levels of sexual dimorphism were similar between solitary and stickleback-sympatric populations in body size ($t_{6.46} = 0.98, p = 0.36$; Fig. B.4), gill rakers ($t_{8.1} = -0.57, p = 0.58$; Fig. B.2B), and armour ($t_{13.19} = 0.84, p = 0.42$; Fig. B.3A).

Similarly, sculpin-sympatric and solitary populations did not differ in dimorphism along a sculpin-sympatric – solitary body shape axis ($t_{14.1} = 0.62, p = 0.54$; Fig. 3.2C). Total body size dimorphism was similar in solitary and sculpin-sympatric populations ($t_{15.2} = 0.09, p = 0.93$; Fig. B.1B). Solitary and sculpin-sympatric populations were also similar in sexual size dimorphism.
3.3.2 Sexual dimorphism and divergence from ancestral form

In contrast, there was stronger support for the hypothesis that decreased sexual dimorphism evolved with degree of divergence from the marine form. There was a negative relationship between dimorphism in body shape along the marine – benthic body shape axis and position along that axis (slope = -0.32, 95% C.I. = [-0.50, -0.14]; Fig. 3.3A). Marine populations tended to be most dimorphic and more benthic-like populations tended to be least dimorphic. When marine populations were removed from the analysis, the relationship was still negative but not significantly different from no relationship (slope = -0.13, 95% C.I. = [-0.39, 0.13]). Total sexual dimorphism in body shape was also negatively related to position along the marine – benthic body shape axis (slope = -153.91, 95% C.I. = [-246.22, -61.59]; Fig. 3.3B).

There was no relationship between sexual dimorphism in gill rakers and population mean position along the marine – benthic body shape axis (slope = 1.25, 95% C.I. = [-8.88, 11.38]; Fig. B.3A). The same was true of armour (slope = 0.06, 95% C. I. = [-2.69, 2.8]; Fig. B.3B) and body size (slope = -1.41, 95% C. I. = [-7.70, 4.87]; Fig. B.4).

3.3.3 Alignment to phenotypic axis of population divergence

The angles between a vector of total dimorphism in body shape and a vector of benthic – limnetic divergence in body shape indicated that dimorphism did not align closely to a benthic – limnetic axis in solitary populations (mean $\theta = 72.90$, range = [55.51, 89.88]; Fig. B.5). The phenotypic axis of total dimorphism was different from that of solitary – sculpin-sympatric
divergence in solitary populations (mean $\theta = 81.30$, range = [62.99, 87.97]; Fig. B.5). The angle between a marine – benthic divergence vector and a female – male divergence vector indicated some, but not particularly close, alignment between the two axes of divergence in marine populations (mean $\theta = 61.61$, range = [52.50, 67.12]; Fig. B.5).

### 3.3.4 Repeatability of lab-grown and wild-caught dimorphism estimates

Lab-grown populations did exhibit sexual dimorphism, but the repeatability of dimorphism across lab-grown and wild-caught samples was trait-dependent (Fig. B.6). There were somewhat similar levels of total body shape dimorphism in lab-grown and wild-caught samples, as there was more variation among populations than between sample types (repeatability = 0.63). However, there was more variation between sample types within populations than among populations when dimorphism was measured by body size (repeatability = 0.15), gill rakers (repeatability = 0.37), and armour (repeatability = 0.20).

### 3.4 Discussion

Sexual dimorphism in ecologically relevant traits has been predicted to be elevated in populations that do not co-occur with closely related competitor species in a process analogous to ecological character displacement between species (Bolnick and Doebeli 2003; De Lisle and Rowe 2015; Li and Kokko 2021). We tested this prediction in the threespine stickleback that occur with and without a sympatric stickleback competitor, and also in stickleback that occur with and without the intraguild predator prickly sculpin. The prediction was not upheld. Levels of sexual dimorphism in body shape, gill rakers, and armour trait were similar between freshwater solitary populations and those sympatric with either stickleback or sculpin. Instead,
sexual dimorphism in body shape declined as populations exhibited greater divergence from the ancestral marine stickleback. This aligns with the alternative prediction that dimorphism decreases with more divergent local adaptation because maladapted populations experience more selection in the same direction and hence reduced sex-specific selection (Connallon et al. 2018; De Lisle et al. 2018). Results were the same for sexual dimorphism along a marine-freshwater divergence axis in body shape and total sexual dimorphism in body shape. This effect, however, was noisy and not statistically significant when estimated only among freshwater populations. Additionally, it was trait dependent and was not observed in gill rakers, armour traits, or body size. Using a common garden experiment, we found moderate to low repeatability of sexual dimorphism between wild-caught and lab-raised populations, suggesting that phenotypic plasticity likely contributes to dimorphism.

Our finding that sexual dimorphism does not depend on competitive environment contrasts with theory but is consistent with results from other study systems. Similar levels of ecological sexual dimorphism have been observed across communities of emydid turtles that vary in the number of competitor species present (Stephens and Wiens 2009). *Anolis carolinensis* populations that co-occur with a closely related competitor species, *Anolis sagrei*, are similar in dimorphism to solitary populations (Stuart et al. 2021). This is somewhat surprising, given that sexual dimorphism can theoretically evolve via a process analogous to ecological character displacement between species (De Lisle and Rowe 2015). Sympatric *A. carolinensis* and *A. sagrei* have diverged via character displacement between the species on short time scales (Stuart et al. 2014). In contrast to ecological character displacement between the sexes, ecological character displacement between species evolves readily (Schluter 2000a; Pfennig and Pfennig 2010).
One explanation for the lack of character displacement between the sexes is that the strength of competition-driven divergent selection between the sexes is generally weak or inconsistent enough in nature that it does not lead to repeated evolution across populations in different competitive contexts. It is possible that the direction of selection on body shape is different during the breeding season relative to other times of the year. Additionally, although the sexes are somewhat phenotypically different, they are generally less differentiated than species.

Divergent selection is expected to be highest at an intermediate level of phenotypic similarity between competing populations (Schluter 2000b), and sexes may be similar enough that divergent selection is instead relatively weak. A second explanation is that solitary threespine stickleback populations may have evolved alternative outcomes to intraspecific resource competition. Solitary populations exhibit wider niche breadths and greater plasticity than stickleback-sympatric populations, both of which would be expected to evolve under disruptive selection generated by resource competition (Svanbäck and Schluter 2012). Therefore, solitary populations may access a greater diversity of resources than sympatric populations to alleviate intraspecific competition, but not in a way that is partitioned between the sexes.

A third explanation is that solitary populations may not meet the conditions in which dimorphism is most likely to evolve. Ecological sexual dimorphism is theoretically less likely to evolve in populations with habitat heterogeneity and a polygynous mating system, both of which are features of all threespine stickleback populations (Li and Kokko 2021). Additionally, dimorphism is most likely to arise through intersexual resource competition if there is high individual specialization (Bolnick and Doebeli 2003; Cooper et al. 2011). Stickleback from solitary, stickleback-sympatric, and sculpin-sympatric populations all exhibit individual specialization in habitat and diet (Matthews et al. 2010; Bolnick and Ballare 2020). Experimental
studies of stickleback populations in enclosures show that they exhibit greater individual niche
widths when not co-occurring with sculpin compared to experimental populations that did co-
occur with sculpin (Bolnick et al. 2010), while experimental populations exhibited greater
individual specialization at high relative to low densities (Svanbäck and Bolnick 2007). These
results suggest that individual specialization may not be higher in solitary relative to competitor-
sympatric populations, which may have some effect on the evolution of ecological sexual
dimorphism.

It is still possible that ecological sexual dimorphism is shaped by competition in
stickleback, but that propensity for evolution of sexual dimorphism is not greater in solitary than
competitor-sympatric populations. De Lisle (2019) suggests that threespine stickleback
constitutes a compelling case for the evolution of sexual dimorphism by ecological intersexual
character displacement. As evidence, he pointed to the fact that dimorphism in ecologically
relevant traits has been observed repeatedly in stickleback, across both marine and freshwater
populations (Kitano et al. 2007; Aguirre and Akinpelu 2010; Cooper et al. 2011; McGee and
Wainwright 2013). Across a set of wild stickleback populations co-occurring with variable fish
communities, higher levels of dimorphism were observed in populations with reduced disruptive
selection (Bolnick and Lau 2008). This would be consistent with dimorphism evolving in
response to disruptive selection and ultimately reducing the strength of that form of selection. If
populations vary in their propensity for sexual dimorphism due to differences in genetic variation
for sexual dimorphism, then this could lead to variation among populations that does not
correspond to competitive environment (Bolnick and Doebeli 2003). Although containing
solitary and competitor-sympatric populations are generally ecologically similar, another
possibility is that some aspect of the environment restricted both co-occurrence of sympatric
stickleback species and the evolution of ecological sexual dimorphism (Vamosi 2003; Ormond et al. 2011; Miller et al. 2019). Therefore, the assumption that resource gradients are the same across lakes where competitor species are present and absent might not be met (Bolnick and Doebeli 2003; Li and Kokko 2021).

In contrast to the prediction from the competition hypothesis, sexual dimorphism in body shape declined in freshwater populations with divergence from the ancestral marine form. This aligns with Albert et al.’s (2008) observations that a marine stickleback population exhibited more dimorphism than a benthic population. Spoljaric and Reimchen (2008) also observed a relationship between ecological sexual dimorphism and ecological context in freshwater stickleback populations, finding that more limnetic populations exhibit greater body shape dimorphism than more benthic populations. It has been previously hypothesized that ecological sexual dimorphism is a source of standing phenotypic variation that may have facilitated the rapid adaptation of ancestral marine stickleback to freshwater environments (Aguirre et al. 2008). Theory suggests that sexual dimorphism may be reduced as populations locally adapt (Connallon et al. 2018). If a population evolves towards a new phenotypic optimum, directional selection may favour alleles that affect both sexes similarly, in contrast with the divergent sex-specific selection on differentially expressed alleles that causes dimorphism to evolve. In this case, even if one sex in the marine species is more similar to an optimal freshwater phenotype, both likely experienced selection favouring traits that tend to be observed in freshwater populations, such as shorter spines and larger relative head size. This might have led to the observed pattern of particularly reduced dimorphism along a marine – benthic phenotypic axis in freshwater populations that are most phenotypically differentiated from the marine species.
For sexual dimorphism to evolve in response to sex-specific selection, it must have a genetic basis. Our common garden experiment indicated that dimorphism is likely plastic to some extent. There is substantial evidence that some of these traits, including gill raker number and lengths, spine lengths, and number of plates, exhibit genetically-based differences between populations (Glazer et al. 2015; Miller et al. 2015; Thompson et al. 2018). However, given that phenotypic differences between the sexes are relatively small in magnitude, there could still be some plasticity in these traits on a scale that matters for the magnitude of sexual dimorphism. Further, while body shape along a benthic – limnetic phenotypic axis is not highly plastic in benthic, limnetic, or marine populations, there is substantial plasticity within solitary populations (Svanbäck and Schluter 2012). If sexual dimorphism is phenotypically plastic, then levels of sexual dimorphism likely respond to present environmental conditions, including resource availability.

Sex-specific selection can arise through the competition-driven mechanisms described above, but it can also result from sexual selection or differences in reproductive roles (Hedrick and Temeles 1989). If the source of selection is related to reproduction, the resulting evolution may still have ecological implications (Shine 1989). This may be expected particularly where traits that are involved in reproductive roles are also involved in resource acquisition, as in mouth-brooding cichlids (Ronco et al. 2019). In the breeding season, male sticklebacks build nests, court females, and care for eggs by fanning to supply oxygen and removing dead or unfertilized eggs to prevent fungal growth (Foster et al. 2008). Female sticklebacks continue foraging, often in social groups, while producing eggs (Shaw et al. 2007). It is possible that ecological sexual dimorphism is an incidental by-product of adaptation to different reproductive roles (Shine 1989). Limnetic males, for example, tend to have more benthic-like body shapes.
This may be because, while breeding, they occupy nearshore areas where benthics commonly forage, but it could cause them to access more benthic invertebrate prey at other times of the year. This would mean there is reduced sex-specific selection in more benthic habitats, which could contribute to the relationship we observed between dimorphism and divergence from marine phenotypes, if there is a closer match in more benthic populations in optimal phenotype based on ecological selection and optimal body shape for reproductive roles. Additionally, it is possible that reproductive, sexual, and ecological selection all interact in some way to produce the observed sexual dimorphism in sticklebacks. To address this, it will likely be necessary to experimentally manipulate degree of maladaptation and quantify sex-specific selection. It would additionally be useful to estimate magnitudes of selection on sexually dimorphic, ecologically relevant traits both during and outside the breeding season.
Figure 3.1 (A) Map of populations included in the study. Two sets of populations – stickleback-sympatric and sculpin-sympatric – co-occur with a closely related competitor species. All freshwater populations were established when lakes were colonized by an ancestral marine population post-glaciation. (B) Predicted differences among populations in levels of sexual dimorphism. Under the competition hypothesis, dimorphism was predicted to be higher in solitary populations than in stickleback-sympatric or sculpin-sympatric populations. Under the divergence from marines hypothesis, dimorphism was predicted to be highest in marine stickleback and lowest in benthic stickleback, with intermediate levels of dimorphism in limnetic, solitary, and sculpin-sympatric stickleback populations.
Figure 3.2 Sexual dimorphism in solitary stickleback and in stickleback sympatric with either another stickleback (A and B) or with sculpin (C and D). Each point represents sexual dimorphism in one population, quantified as the absolute value of the difference in means along a principal component between females and males within a population. (A) and (C) show dimorphism in body shape, with PCA’s performed separately for the sets of populations involved
in each comparison, while (B) and (D) show dimorphism in gill rakers, with a PCA performed on all populations together (solitary, stickleback-sympatric, sculpin-sympatric, marine). Light pink indicates limnetic stickleback-sympatric populations while dark pink indicates benthic stickleback-sympatric populations.

Figure 3.3 Sexual dimorphism in body shape along a marine to benthic phenotypic axis. In all panels, each point represents one population, showing the relationship between position along a marine to benthic PC1 for body shape and a metric for dimorphism in body shape. (A) Dimorphism was quantified as the difference in means (female – male) along a principal component within a population. (B) Dimorphism was quantified as the total difference in shape between males and females.
Chapter 4: Patterns and repeatability of multi-ecotype assemblages of sympatric salmonids

4.1 Introduction

Geographically distinct assemblages of coexisting ecotypes can be strikingly similar to one another, regardless of whether they are intraspecific morphs or species (Mahler et al. 2013; Gillespie et al. 2018; Knotek et al. 2020). This suggests that the process giving rise to ecological diversity may be, to some degree, repeatable (Schluter and McPhail 1993; Losos et al. 1998; Blount et al. 2018; Jamie and Meier 2020). This process can involve divergent evolution between sympatric ecotypes, nonrandom coexistence of previously evolved ecotypes, or some combination thereof (Rundell and Price 2009). Repeated evolution can result from parallel natural selection favouring similar phenotypes, or it can result from similar developmental and genetic constraints on the direction of evolution in independent ecotype assemblages (Yoder et al. 2010; Brakefield 2011; McGlothlin et al. 2018). Additionally, for entire assemblages to resemble each other, ecological processes driving community assembly must also be repeated in different locations (Schluter 1990; Gillespie 2004).

It is unclear whether repeatability of ecotype assemblages changes with the number of ecotypes present in an assemblage. There are many examples of repeated ecotype pairs, but relatively few examples of repeatable multi-ecotype assemblages. This could simply be because assemblages of two ecotypes resembling each other is statistically more likely than resemblance across assemblages of three or four ecotypes. If there is are environmental differences between two and multi-ecotype assemblages, this could also lead to a breakdown in repeatability (Losos
and Schluter 2000; Wagner et al. 2014; Recknagel et al. 2017). Even if one resource axis is repeated across environments, additional resource axes may not be, making differentiation along multiple axes more variable (Harmon et al. 2005). Alternatively, greater diversity within an assemblage may instead lead to higher repeatability across multi-ecotype assemblages in which niches are filled and how ecotypes are phenotypically differentiated. If there are limited niches within an environment, then with more ecotypes present it could become difficult for new ecotypes to evolve or invade, leading to assemblages with more similarity in composition (MacArthur 1969; Kinzig et al. 1999). Certain ecotypes may also be more likely to be added to an assemblages only when others are already present, which could lead to repeated patterns across multi-ecotype assemblages (Broderson et al. 2018).

There are a few examples in which repeatability does appear to extend to assemblages with multiple ecotypes. Anolis ecomorph assemblages in the Greater Antilles are highly repeatable among islands and also exhibit nestedness in habitat occupation: perching habitats occupied in low diversity assemblages form a subset of those in larger assemblages (Losos 2009). Assemblages of two to four sympatric ecomorphs of Tetragnatha spiders in Hawaii show high similarity and also exhibit nestedness in habitat and diet niche occupation (Gillespie 2004). The nestedness in niche use and similarity across assemblages in these systems suggests that their ecological context and phenotypic variation interact in a way that produces repeatable outcomes. There seems to be little indication that repeatability declines with diversity, but there are too few examples available to know whether this is generally true.

Ecotype assemblages might exhibit elements of both repeatability and idiosyncrasy. Even in systems where there is evidence for repeated assembly of the same sets of ecotypes, variability in the strength and direction of ecological selection as well as standing (epi)genomic variation can
lead to variable phenotypes (Oke et al. 2017). Additionally, the degree to which evolution and phenotypic plasticity appear to produce repeatable outcomes may depend on the kind of traits and how precisely they are measured (Blount et al. 2018). It is possible that repeatability in broad habitat and diet categories (“ecological niche”) may exist but repeatability in underlying specific morphological traits might be absent. Although there are many examples of repeated adaptive trait evolution for a given niche (Losos 2009; Elmer et al. 2014; Riesch et al. 2014; Fernandez-Mazuecos et al. 2020), two populations that occupy similar habitats may instead exhibit dissimilar morphology (Oke et al. 2017; Bolnick et al. 2018). While a few examples of assemblage-wide convergence in phenotype distributions have been observed (Marchinko et al. 2004; Cardoso and Price 2010; Mahler et al. 2013), it remains quite rare (Losos 2010). Repeatability would also only be expected where resource gradients are highly similar between locations, which can be difficult to verify.

Fish in post-glacial lakes are a classic example of repeated assemblages of co-occurring ecotypes (Schluter and McPhail 1993; Mcphee et al. 2012). In many previously glaciated regions of the northern hemisphere, a number of fish species that colonized new lakes at the end of the ice age quickly gave rise to pairs of ecotypes whose members tend to divide resources in the same way and have consistent sets of morphological and life history differences associated with this split (Smith and Skúlason 1996; Hendry 2009; Oke et al. 2017). Most of these pairs include a pelagic (sometimes termed “limnetic”) ecotype that consumes zooplankton in open water regions of the lake and a benthic ecotype that occupies nearshore areas and eats primarily macroinvertebrates (Matthews et al. 2010). The repeated occurrence of this set of ecotypes across several families of fish suggests both that there is a fundamental benthic-to-
pelagic ecological gradient in post-glacial lakes and that this is a gradient along which it is consistently possible for freshwater fish to adapt (Schluter 2000b).

It is possible that there are multiple resource axes within a lake, with the number of axes likely dependent on lake features such as size and shape, so that additional ecotypes are not constrained to a benthic to pelagic resource gradient (Recknagel et al. 2014, 2017; Wagner et al. 2014). Among the post-glacial fish in which sympatric ecotypes have evolved, two salmonid genera – *Salvelinus* and *Coregonus* – are unique in exhibiting multiple instances of assemblages of three or more ecotypes in addition to pairs (Schluter and McPhail 1993; Smith and Skúlason 1996). Multiple *Salvelinus* species have been described as exhibiting an unusually high level of phenotypic and ecological intraspecific variability (Klemetsen 2013; Muir et al. 2016). This ecological variability spans lacustrine, riverine, and anadromous life histories, as well as diets that range from benthic invertebrates to pelagic zooplankton to other fish (Klemetsen 2010; Mcphee et al. 2012; Muir et al. 2016). *Coregonus* species have high levels of variability in habitat occupation within and across lake environments, as well as dietary variation ranging from zooplankton to larger freshwater invertebrates (Bernatchez et al. 2010). If the niches occupied are repeatable with three or more co-occurring ecotypes in *Salvelinus* and *Coregonus*, this will imply that phenotypic variation and ecological gradients are structured in a way that allows repeated evolution and assembly of multi-ecotype communities.

Different instances of ecotype evolution and assembly vary in the mechanisms underlying differentiation, whether based on genetics or phenotypic plasticity, as well as their degree of reproductive isolation (Hendry 2009). They are also variable in their degree of phylogenetic independence, and in many cases phylogenetic relationships are unknown. Population genetic analyses of both *Salvelinus* and *Coregonus* assemblages suggest that most evolved independently.
in situ, even among lakes in the same drainage (Pigeon et al. 1997; Østbye et al. 2006; Jacobs et al. 2020). However, in a few cases there is evidence that ecotypes may have evolved in one lake then dispersed to another, based on high genetic similarity between *Salvelinus* planktivore ecotypes in two Scottish lakes, *Salvelinus* piscivore ecotypes in two Russian lakes, and *Coregonus* assemblages in adjacent lakes in Switzerland (Hudson et al. 2011; Jacobs et al. 2020). Nonetheless, these assemblages have been evolving largely independently of each other since their formation in most cases. In the absence of more detailed phylogenetic information, we have treated assemblages as though they are statistically independent replicates.

We assessed the repeatability of assemblages containing up to seven taxa or populations and up to five ecotypes in two salmonid genera, *Salvelinus* and *Coregonus*. We defined repeatability here as high similarity across assemblages in niche occupation, phenotype distributions, and niche by phenotype associations, with assemblage defined as a set of closely related ecotypes in one lake. We used published data on morphological, life history, diet, and habitat use to assess patterns of repeatability and trait divergence in ecotypes of *Coregonus* and *Salvelinus* that are sympatric in freshwater lakes through a meta-analysis. We aimed to address two overarching questions. First, does repeatability occur and does it depend on the number of sympatric ecotypes? We measured degree of repeatability in two-ecotype lakes and then tested whether it extended beyond those to multi-ecotype assemblages. Assemblages were regarded as repeatable if they exhibited greater similarity in the frequencies of ecotypes and of phenotypic means than expected by chance, or if they form nonrandom patterns such as a nested sequence of ecotypes. Assemblages were considered to lack repeatability if frequency distributions and occurrence patterns of ecotypes resembled expected patterns if populations were distributed randomly among ecotype categories. Second, do sympatric ecotypes exhibit repeatable patterns of trait
divergence? We evaluated this for assemblages ranging from two to five ecotypes by using differences between ecotypes in trait means.

4.2 Methods

4.2.1 Systematic literature review

We performed a systematic literature review to identify papers with data on wild caught fish from lakes with two or more co-occurring populations, closely related species, or taxa (hereafter referred to as “populations”) forming separate ecological and morphological clusters, from the genus *Salvelinus* or *Coregonus*, following standard systematic review guidelines (Moher et al. 2015; Mikolajewicz and Komarova 2019). We used searches in Web of Science, Scopus, and bioRxiv to identify papers that potentially reported primary research on lineages at an early stage of divergence in these two genera (see Supplemental Methods and Fig. C.1 for more details). We included studies that reported sympatric populations that were considered by the authors to be intraspecific or part of a species complex of recently diverged lineages. For example, described species from the *C. artedi* and *C. laveretus* species complexes were included (Østbye et al. 2005; Crête-Lafrenière et al. 2012). Studies that provided information on sympatric *Salvelinus* or *Coregonus* ecotypes within lakes were identified by a scan of the abstract followed by full text review. We retained 127 studies with data from 104 assemblages following the full text review and included in the meta-analysis. For 85 lakes, all ecotypes were identified as filling a diet or lake habitat niche. We extracted usable trait data for 93 assemblages.

For each assemblage, we recorded the number of phenotypically distinct populations described by the authors (Table C.1). If the sample size was less than five individuals for a population of given ecotype, that population was excluded from our analysis due to a lack of
information about those populations. In most cases, this resulted in the assemblage being excluded from the analysis because one or no populations were left in the dataset. Assemblages from two lakes, Ellasjoen and Kalarskii-Davatchan, were retained in the analysis following the removal of data for one population with insufficient sample size. Mean, sample size, and a measurement of error were recorded for gill raker count, body length, age, and stable isotopes indicative of dietary resources ($\delta^{13}$C and $\delta^{15}$N). These variables were selected because of their relationship to resource use and because they are commonly measured (Sánchez-Hernández et al. 2019). When necessary, metaDigitise was used to extract data from figures and convert standard error and 95% confidence interval estimates to standard deviation to measure variability (Pick et al. 2019). We also recorded commonly reported lake characteristics, including latitude, longitude, surface area, and maximum depth (Table C.2). All analyses were run in R version 4.0.3 (R Core Team 2020). All statistical tests were performed separately for Coregonus and Salvelinus.

4.2.2 Ecotypes

We produced four assemblage datasets: one for each Coregonus and Salvelinus, and with ecotypes defined by either diet consumed or the lake habitat occupied. We classified populations into ecotypes based on descriptions provided in each paper. The habitat ecotype categories for both genera were “littoral/benthic”, “pelagic”, “profundal”, “generalist”, and “shallow”. The diet categories were “planktivore”, “benthivore”, and “generalist invertivore” for Coregonus and “planktivore”, “benthivore”, “generalist invertivore”, “piscivore”, and “omnivore” for Salvelinus. In some cases, the descriptions that we initially recorded were later recategorized to facilitate comparisons across lakes. For habitat ecotype IDs, ecotypes originally documented as “all”, “pelagic & littoral”, “pelagic & littoral & profundal”, “pelagic & profundal”, “benthic &
pelagic”, and “deep & shallow” were classified as “generalist”. “Limnetic” ecotypes were
categorized as “pelagic,” whereas “deep”, “deepwater”, and “abyssal” were defined as
“profundal”; and “shallow littoral & pelagic” was reclassified as “shallow”. For diet ecotype IDs,
planktivorous and zooplanktivore were reclassified as “planktivore”; “piscivore & invertivore”
and “generalist” were reclassified as “omnivore”; and “gammaridivore” was reclassified as
“benthivore”.

4.2.3 Repeatability of ecotypes across lakes

Repeatability can be defined as higher similarity between assemblages than expected by
chance in the frequency distribution of ecotypes. In this case we would expect to see matching
among assemblages in the occurrence of ecotypes, nonrandom patterns in the frequency
distribution of ecotypes, and a repeatable pattern in which ecotypes additionally occur when
assemblages contain more than two ecotypes. Therefore, we quantified similarity in three ways:
as ecotype-for-ecotype matching, as associations in the occurrence of ecotypes within
assemblages, and as a pattern of nestedness among assemblages differing in the number of
ecotypes.

We evaluated similarity of population assemblages using ecotype-for-ecotype matching
(often referred to as “species-for-species matching”) (Schluter 1990). Ecotype-for-ecotype
matching occurs when the frequency distribution of ecotypes exhibited by populations is more
similar across sites compared to a null distribution where populations are assigned randomly to
ecotypes, while keeping the number of populations per lake and per ecotype constant. We tested
for this separately in two-population lakes, three-population, and with all populations included
(i.e., two to seven per lake). To test for ecotype-for-ecotype matching, we constructed
contingency tables with ecotypes as columns (either diet or habitat) and lakes as rows, counting the number of populations in each ecotype (Schluter 1990). We calculated the $\chi^2$ statistic using the R function “chisq.test()”. To generate a null distribution, we simulated contingency tables using the R function “r2dtable()”, which generates tables with an algorithm that shuffles the frequencies while maintaining the observed row and column totals (Patefield 1981). We then compared the observed $\chi^2$ to the null distribution with a two-tailed test. To quantify whether assemblages were more similar or dissimilar than expected by chance, we calculated the ratio of the $\chi^2$ statistics to the degrees of freedom (df). For $\chi^2$/df, a value less than one would indicate similarity and a value greater than one would indicate dissimilarity (because df is the mean of the distribution).

As a second measure of repeatability we tested for associations between population ecotypes within assemblages using the V-ratio (Schluter 1984). V-ratios estimate whether ecotypes are more likely to occur together (or not occur together) than expected by chance. An association would arise, for example, if two ecotypes were more likely to occur in the same assemblage (positive association) or less likely to occur together (negative association) than expected by chance (Schluter 1984). If pelagic and littoral/benthic ecotypes tend to occur together, this would be a positive association, but if planktivore and piscivore ecotypes tend to only occur in separate assemblages, this would be a negative association. A pattern of positive association would indicate similarity among assemblages in which ecotypes occur, while a pattern of negative association would imply dissimilarity among assemblages. To estimate V-ratios, we used a presence-absence matrix with ecotypes and lakes as variables, where “1” indicated that a lake contained an ecotype in a diet or habitat category and “0” indicated that it did not. These were calculated using “V.ratio()” from the package “bipartite()” (Dormann et al.
To evaluate significance, we used a two-sided test compared to a null distribution generated from simulations carried out via the function “oecosimu()” (Oksanen et al. 2020). We used the algorithm “r1” with 10,000 simulated matrices. With the r1 algorithm, row (lake) sums were fixed while column (ecological category) sums were not. Instead, the probability of occurrence in each ecological category depended on the marginal frequency of that column.

We then evaluated evidence for a pattern of nestedness of ecotype occurrence across assemblages relative to a null distribution where ecotypes are randomly distributed across lakes, with the number of ecotypes per lake held constant. This pattern would emerge if the ecotypes in lakes with few ecotypes form a subset of the categories occupied in lakes where more ecotypes are present. If ecotypes in 2-ecotype assemblages are not consistently observed in 3- or 4-ecotype assemblages, this would be consistent with a lack of nestedness. The “NODF” score from the function “nestednodf()” in vegan version 2.5-7 was used to evaluate nestedness (Almeida-Neto et al. 2008; Oksanen et al. 2020). NODF estimates the percentage of overlap between lakes with fewer ecotypes relative to those with more, with higher NODF values indicating greater evidence for nestedness. We evaluated the null hypothesis of no nestedness against the one-sided alternative hypothesis that nestedness is greater than expected by chance. To do this, we again simulated a null distribution of 10,000 matrices with the algorithm “r1”.

4.2.4 Repeatability of trait frequency distributions in ecotype assemblages

If there is repeated assemblage-wide convergence in phenotypes, then the frequency distribution of population mean trait values in assemblages would be expected to be highly similar across lakes. To test for repeatability of trait distributions for gill raker counts and total body length among populations and across lakes, we evaluated whether the sets of trait values in
each lake were more similar than if they were randomly drawn from the same distribution of possible values (Schluter 1990). Alternatively, a lack of repeatability would be implicated by evidence that sets of trait values were not drawn from the same distribution or by a lack of evidence for either similarity or dissimilarity. This is a form of ecotype-for-ecotype matching, testing for matching of frequency distributions of continuous traits rather than discrete categories.

We used two-tailed k-sample Anderson-Darling tests to evaluate whether mean trait distributions were unusually similar or divergent among assemblages (Scholz and Stephens 1987). This is a rank order test that evaluates whether samples are drawn from the same unspecified distribution. We used the function “ad.test()” from the R package “kSamples”, with significance evaluated by comparing the observed AD statistic to a distribution of 10,000 simulated statistics (Scholz and Zhu 2019). We first converted fork and standard length to total body length using conversion parameters from FishBase (Boettiger et al. 2012; Binohlan et al. 2021). For lakes with multiple and independent published estimates of mean trait values for each of the co-occurring ecotypes, a weighted mean of estimates for a particular trait value was used. Because the most common habitat categories in both Coregonus and Salvelinus were littoral/benthic, profundal, and pelagic, we repeated the tests retaining only those ecotypes.

### 4.2.5 Trait differences between ecological niche categories

Continuous traits might exhibit repeated differences associated with ecotype. As another test of repeatability of continuous traits, we estimated similarity among lakes in the difference in trait means between ecotypes (habitat or diet). Assemblages would be considered similar if the difference in trait values between sympatric ecotypes is large relative to the differences among
lakes in trait values. A lack of repeatability would emerge if trait differences between sympatric
ecotypes are small or variable in direction among lakes.

To estimate this form of repeatability, we fit a linear model with trait value (population
means) for each ecological category as the response variable and lake and niche as fixed effects,
weighted by the inverse of the sampling variance for each trait mean. We restricted this analysis
to lakes with two or more populations in different ecotypes. We also limited the ecotypes
included to those most commonly observed: littoral/benthic, pelagic, and profundal for habitat
and planktivore, benthivore, and piscivore for diet. We then estimated the variance explained by
ecological differences among lakes (sum of squares (SS) of the interaction between ecological
category and lake) relative to all variation among ecological categories. This was calculated as:
\[
D = \frac{SS_{\text{Niche} \times \text{Lake}}}{SS_{\text{Niche}} + SS_{\text{Niche} \times \text{Lake}}},
\]
where SS is calculated sequentially (type 1 sums of
squares) with “Niche” entered in the model before “Lake”. Possible values for D vary between 0
and 1. A value close to 0 would indicate low among-lake variability in the relationship between
ecotype and traits relative to total variation among ecotypes, and therefore high similarity among
lakes.

4.3 Results

4.3.1 Repeatability of niche occupation across lakes

Frequency distributions of populations of habitat ecotypes were more similar among 2-
population Coregonus lakes than expected by chance, indicating evidence for ecotype-for-
ecotype matching (\(\chi^2/df < 0.01, p < 0.001\); Fig. 4.2B). The numbers of Coregonus ecotypes in
diet groups across 2-population lakes showed a similar pattern (\(\chi^2/df = 0.23, p = 0.051\); Fig.
4.3B). In 3-population Coregonus lakes, numbers of populations in habitat ecotypes were similar
across lakes ($\chi^2/df = 0, p < 0.001$; Fig. 4.2B), as were the numbers in each diet ecotype ($\chi^2/df = 0, p = 0.004$; Fig. 4.3B). Thus, *Coregonus* exhibits matching in ecotypes. This pattern held when all *Coregonus* lakes having two to four co-occurring populations are analyzed at once (habitat: $\chi^2/df = 0.84, p = 0.050$; Fig. 4.2B; diet: $\chi^2/df = 0.41, p < 0.001$; Fig. 4.3B). In most lakes, with ecotype defined by diet, population pairs tended to include one benthivore and one planktivore ecotype, while trios contained two benthivore and one planktivore ecotype. Four-population lakes contained two benthivore ecotypes, one planktivore ecotype, and a generalist invertivore ecotype.

Results for *Salvelinus* population pairs were quite different from those for *Coregonus*. In 2-population *Salvelinus* lakes, the frequency distributions of populations of different ecotypes showed no ecotype-for-ecotype matching (habitat: $\chi^2/df = 1.11, p = 0.79$; Fig. 4.2A; diet: $\chi^2/df = 0.90, p = 0.11$; Fig. 4.3A). Significant ecotype-for-ecotype matching emerged in *Salvelinus* trios. The number of populations of each diet ecotype was overly similar ($\chi^2/df = 0.69, p = 0.010$; Fig. 4.3A) but the same was not true of habitat ecotypes ($\chi^2 = 1.11, p = 0.68$; Fig. 4.2A). Across *Salvelinus* lakes with two to seven co-occurring populations, there was no ecotype-for-ecotype matching of habitat ($\chi^2 = 1.12, p = 0.82$; Fig. 4.2A) or diet ecotypes ($\chi^2 = 0.91, p = 0.13$; Fig. 4.3A). Ecotype assemblages were highly variable for *Salvelinus*. Assemblages containing two to seven populations commonly included a combination of benthivore, planktivore, and/or piscivore ecotypes. Generalist invertivore and omnivore ecotypes also occurred in assemblages with varying numbers of populations but were observed less frequently. In 3-population lakes, which did exhibit diet ecotype matching, assemblages tended to contain either benthivore, planktivore, and piscivore ecotypes or benthivore, piscivore, and omnivore ecotypes.
For Coregonus, ecotypes were positively associated across lakes. Some ecotypes were more likely to co-occur in the same assemblages than expected by chance (diet: $V$ [95% CI] = 17.06 [2.99, 14.39], $p = 0.050$; Fig. C.4; habitat: $V$ [95% CI] = 20.30 [9.25, 18.20], $p = 0.005$; Fig. C.5). Planktivore, benthivore, and generalist invertivore ecotype tended to co-occur, as did pelagic, littoral/benthic, and profundal ecotypes. Conversely, for Salvelinus, ecotypes within an assemblage showed no evidence for associations across lakes. This indicates that ecotypes were neither unusually likely nor unlikely to co-occur in the same assemblage, suggesting a random distribution of ecological category occupation across assemblages when defined by diet ($V$ [95% CI] = 7.92 [2.73, 10.93], $p = 0.458$; Fig. C.4) or habitat ($V$ [95% CI] = 5.44 [2.08, 8.86], $p = 0.830$; Fig. C.5).

Coregonus ecotypes exhibited a pattern of nestedness when ecotypes were defined by diet, with the ecotype assemblages in lakes with few ecotypes tending to form a subset of those with more ecotypes occupied (NODF [95%] = 34.74 [34.63], $p = 0.027$; Fig. C.4). This was a consequence of the same pattern that led to ecotype-for-ecotype matching, where lakes with two ecotypes contained benthivore and planktivore ecotypes and lakes with a third ecotype additionally contained a generalist invertivore. However, with ecotypes defined by habitat, Coregonus assemblages did not exhibit a pattern of nestedness (NODF [95%] = 47.15 [48.05], $p = 0.111$; Fig. C.5). While littoral/benthic and pelagic ecotypes are commonly present when two categories are filled, and a profundal ecotype is often added when a third category is filled, the presence of generalist and shallow ecotypes in lakes with few ecotypes disrupts a pattern of nestedness. The variability in Salvelinus assemblages in ecotype assemblages led to an absence of nestedness when ecotypes were defined by diet (NODF [95%] = 39.33 [41.53], $p = 0.205$; Fig. C.4) and by habitat (NODF [95%] = 22.82 [27.63], $p = 0.576$; Fig. C.5).
4.3.2 Repeatability of trait values across lakes

In contrast to population frequencies in ecotypes, frequency distributions of gill raker counts were divergent across lakes rather than overly similar for both *Coregonus* (AD = 56.47, \(p < 0.001\); Fig. 4.5) and *Salvelinus* (AD = 13.15, \(p < 0.001\); Fig. 4.5). Similarly, distributions of total lengths were divergent and not similar across lakes for *Coregonus* (AD = 24.5, \(p < 0.001\); Fig. 4.5) and *Salvelinus* (AD = 47.0, \(p < 0.001\); Fig. 4.5). Distributions of total lengths of pelagic, littoral/benthic, and profundal ecotypes were also not similar for both *Coregonus* (AD = 17.8, \(p = 0.021\)) and *Salvelinus* (AD = 9.5, \(p = 0.073\)). In *Coregonus*, total length distributions of planktivore and benthivore ecotypes were divergent (AD = 14.22, \(p = 0.006\)), but distributions of planktivore, benthivore, and piscivore ecotypes were neither more divergent nor more similar than expected by chance in *Salvelinus* (AD = 14.65, \(p = 0.707\)). Overall, there was no support for ecotype-for-ecotype matching in continuous traits for either *Coregonus* or *Salvelinus* assemblages.

4.3.3 Trait differences among ecotypes

The index of similarity in trait-ecotype association, \(D\), can range from 0 to 1, with values close to 0 indicating small differences (high similarity) among lakes. High similarity occurs when ecotypes differ markedly in mean trait values and these trait differences are parallel between lakes. In *Coregonus*, the level of similarity was trait-dependent. For gill raker counts in *Coregonus*, similarity among lakes in trait-ecotype association was relatively high (diet: \(D = 0.115\); habitat: \(D = 0.077\); Fig. 4.5; Fig. C.6). Benthivore ecotypes tended to have fewer gill rakers than planktivore ecotypes. Pelagic ecotypes tended to have more gill rakers than either
littoral/benthic or profundal ecotypes. In contrast, similarity was low for total length (diet: $D = 0.518$; habitat: $D = 0.439$; Fig. 4.4; Fig. C.6). Similarity among lakes for stable isotopes depended on which was measured. Similarity was high for $\delta^{13}$C (diet: $D = 0.091$; habitat: $D = 0.095$; Fig. C.6), with littoral/benthic ecotypes tending to have higher values than pelagic or profundal ecotypes and benthivores tending to have higher values than planktivores. Similarity was moderate to low for $\delta^{15}$N (diet: $D = 0.838$; habitat: $D = 0.321$; Fig. C.6).

In *Salvelinus*, similarity among lakes depended on which trait was measured and whether ecotypes were classified by habitat or diet. For total length, similarity among lakes was high with ecotypes defined by diet ($D = 0.139$; Fig. 4.4), with piscivores having longer bodies than other ecotypes, but moderate with ecotypes defined by habitat: $D = 0.460$; Fig. 4.4; Fig. C.7). There was a moderate to low level of similarity for $\delta^{13}$C (diet: $D = 0.290$; habitat: $D = 0.565$; Fig. C.7) but high similarity for $\delta^{15}$N (diet: $0.030$; habitat: $0.084$; Fig. C.7). Profundal ecotypes tended to have higher $\delta^{15}$N values than pelagic or littoral/benthic ecotypes while piscivore ecotypes tended to have higher $\delta^{15}$N values than planktivore or benthivore ecotypes. For fish age, there was high similarity with ecotypes defined by diet ($D = 0.093$; Fig. C.7), with piscivores having higher ages than planktivore or benthivore ecotypes, but low similarity with ecotypes defined by habitat ($D = 0.961$; Fig. C.7). Both *Salvelinus* and *Coregonus*, therefore, exhibited some evidence for among-lake similarity in the relationships between trait values and ecotypes.

### 4.4 Discussion

We found strong evidence for repeatability of ecotype assemblages in *Coregonus* and weaker evidence in *Salvelinus*. *Coregonus* assemblages showed evidence of ecotype-for-ecotype matching, positive associations in the occurrence of ecotypes, and nestedness among
assemblages differing in the number of ecotypes. In contrast, *Salvelinus* ecotype assemblages were highly variable. Neither genus exhibited matching in the frequency distribution of mean trait values of assemblages, but trait-ecotype associations were highly similar among lakes. The high repeatability observed in one genus is consistent with some degree of consistency in ecological selection and standing (epi)genomic variation among populations in that group. However, low repeatability in the other genus indicates that ecological context and lineage-specific factors are clearly also important in the evolution of ecotypes.

We assessed whether repeatability was present within two-ecotype assemblages, and whether there is evidence that it extends beyond that. Within *Coregonus*, repeatability across multi-ecotype assemblages was evident, suggesting that where repeatability is present in two-ecotype assemblages it does not necessarily break down with the addition of a third or fourth ecotype. However, in *Salvelinus*, neither two-ecotype nor multi-ecotype assemblages exhibited clear patterns of repeatability. The pattern in two-ecotype *Coregonus* assemblages was consistent with two-ecotype assemblages of other fish genera in post-glacial lakes, in which a littoral/benthic and a pelagic ecotype commonly co-occur (Schluter and McPhail 1993). Across both *Coregonus* and *Salvelinus*, littoral/benthic, pelagic, and profundal were all commonly filled habitat categories while planktivore and benthivore were commonly filled diet categories. This lends support for the idea that the profundal niche may represent a third ecological axis along which post-glacial fish can easily evolve (Præbel et al. 2013). *Salvelinus* assemblages additionally often contained a piscivorous ecotype that co-occurred with planktivore and benthivore ecotypes at similar frequencies. The nested patterns of diet category occupation in *Coregonus* suggests that evolution is ordered to some extent, with some ecotypes occurring only when others are also present. When a benthivore and a planktivore ecotype are already present in
a *Coregonus*, it seems that a second benthivore ecotype may be the most likely to evolve as a third ecotype and a generalist invertivore is the most likely fourth ecotype. This contrasts with the non-nested pattern in *Salvelinus*, where each possible ecotype occurs in two-ecotype assemblages. Nestedness in assemblages may also be indicative that the processes driving divergent adaptation among ecotypes are, to some extent, deterministic (Losos et al. 1998; Mahler et al. 2013). Presence/absence of ecological categories may be also be nested among lakes if lakes with certain abiotic features or biotic communities tend to drive the evolution and assembly of a particular set and number of ecotypes (Losos and Schluter 2000; Wagner et al. 2014).

Why *Coregonus* and *Salvelinus* differ in their degree of repeatability is unclear. It is possible that higher repeatability in *Coregonus* that *Salvelinus* is a consequence of greater evolutionary constraint, as *Salvelinus* may exhibit more intraspecific variability in development, physiology, and ecology (Klemetsen 2013; Muir et al. 2016). Ecotypes in *Salvelinus* are commonly piscivorous and omnivorous, which are two dietary niches that are never filled by *Coregonus* ecotypes. This greater ecological flexibility could allow *Salvelinus* ecotypes to evolve to exploit the best available resources, rather than being constrained to the same directions of divergence. The small body sizes of *Coregonus* species relative to some *Salvelinus* ecotypes could be a constraint that precludes the development of piscivorous or omnivorous *Coregonus* ecotypes. Alternatively, *Salvelinus* ecotypes could occupy different or more variable lake habitats than *Coregonus*, with less consistency in the selective landscapes they experience. Repeatability is most likely to be seen where resource distributions are similar between lakes, and this may be truer of lakes inhabited by *Coregonus* than by *Salvelinus*. Despite the similarities between the two genera, such as the overlapping ecotypes in both, there are environmental and/or
intrinsic differences that lead to more variability in patterns of divergence in *Salvelinus* than *Coregonus*.

Repeatability depended on whether it was assessed using broad ecotype categories or continuous trait values, with high variability in distributions of mean phenotypes for both genera. This was unsurprising in *Salvelinus*, given that assemblages contain variable sets of ecotypes. However, in *Coregonus*, the similar distributions of ecotypes across lakes could have been expected to be matched by a similar distribution of mean phenotypes. Therefore, even if lakes contain sets of ecotypes with repeatable niche occupation, this does not equate to their containing similar phenotype distributions. Mean trait values could depend on the genetic variation and propensity for phenotypic plasticity present in founding populations as well as the specifics of the ecological context each population occupies (Ghalambor et al. 2007; Landry et al. 2007; Marques et al. 2019). The existence of a pelagic niche, for example, may be repeated across many lakes, but the specific environments different pelagic fish experience can be quite variable. Perhaps limited repeatability in phenotype distributions in *Salvelinus* and *Coregonus* is unsurprising, given the large spatial and taxonomic scale over which sympatric ecotypes in the genera have evolved. Littoral/benthic and pelagic stickleback ecotypes, which exhibit highly repeatable differences in body size, body shape, gill raker number and length, and trophic position (Schluter and McPhail 1992; Gow et al. 2008; Matthews et al. 2010), evolved from the same ancestral species of marine stickleback and occur in a limited geographic region (Taylor and McPhail 2000; Vamosi 2003). In contrast, the ecotype assemblages in the present study occur across the northern hemisphere and are descended from ancestral populations that are quite divergent between many lakes (Crête-Lafrenière et al. 2012).
Sympatric ecotypes in both *Salvelinus* and *Coregonus* did exhibit trait differences that were characteristic of habitat or diet categories. There were some clear patterns that aligned with expectations, such as more gill rakers in planktivore than benthivore ecotypes in *Coregonus* and larger body sizes in piscivore ecotypes than others in *Salvelinus*. For *Coregonus*, repeatability of trait by ecotype relationships emerged with ecotypes defined by both habitat and diet, but for *Salvelinus* there was more similarity among assemblages with ecotypes defined by diet. This may result from *Salvelinus* ecotypes that occupy similar habitats but use different diets. Pelagic *Salvelinus alpinus* ecotypes, for example, may be either planktivorous or piscivorous (Snorrason et al. 1994). Associations between ecologically relevant traits and niche occupation matches observations from other multi-ecotype assemblages that exhibit elements of repeatability. *Anolis* lizards in the Greater Antilles adapt repeatably to the same niches with similar differences in fore- and hindlimb length, body size, and tail length (Losos 1990b) and *Tetragnathus* spiders in Hawaii exhibit repeatable differences in body size and colour (Gillespie 2004). Several traits not included in our study, including fin morphology, jaw morphology, and overall body shape, have been associated with each habitat and diet in each *Coregonus* and *Salvelinus* (Smith and Skúlason 1996; Taylor 1999; Mcphee et al. 2012). Therefore, it is possible that a more extensive suite of traits may characterize among-ecotype niche differences in these genera, similar to what has been observed in *Anolis*.

In *Salvelinus*, but also to some extent in *Coregonus*, there was a lack of repeatability across assemblages by some metrics. One factor underlying this could be the young age of *Salvelinus* and *Coregonus* ecotypes. The *Anolis* adaptive radiation is comparatively very old (46.3–64.4 million years, Poe et al. 2017), which means that species have likely had sufficient time to evolve towards their evolutionary optimum (McGlothlin et al. 2018). Evolution in
younger ecotypes, however, is highly constrained by genetic variation present in the founding population as there has not been sufficient time for new beneficial mutations to arise (Barrett and Schluter 2008). Furthermore, phenotypic variation in several Salvelinus and Coregonus ecotype assemblages is highly plastic, which has the potential to lead to higher variability among specific ecological contexts. Even within Salvelinus and Coregonus, whether genetic or plastic changes underlie divergence may impact which traits diverge and to what extent. Assemblages of ecotypes in these genera fall along a speciation continuum (Hendry 2009). This continuum ranges from genetic differentiation and evidence of reproductive isolation to ecological differentiation generated almost entirely by plasticity (Parsons et al. 2011). Ecotype differentiation may involve similar trait differences whether it has a genetic or plastic basis. For example, developmental trajectories are altered in some cases to produce phenotypes suited to access different resources, and this alteration has a largely genetic basis in some systems and a more plastic one in others (Skúlason et al. 2019). However, some traits may not have much plasticity, or a plastic response may depend on the genetic variation present in the population.

To understand what drives repeatability or its absence in this system, it will be necessary to know more about both the mechanisms driving divergence among sympatric ecotypes and the genetic basis that underlies them. Differences in mean trait values can evolve via divergent selection for alternative phenotypes (Rundle et al. 2003; Doebeli et al. 2007). This form of selection can arise via selection favouring phenotypes able to effectively access distinct resources or if intraspecific competition generates selection for phenotypes that are at a low frequency within the population (Rueffler et al. 2006; Abrams et al. 2008). Alternatively, divergent selection can arise through competition for predator-free space. Comparative studies in Coregonus have suggested that predation may be the driving ecological interaction (Öhlund et al.
2020α), while others have implicated competition (Landry et al. 2007). What combination of these mechanisms drives divergent selection may have implications for which traits are expected to diverge repeatedly in response.
Figure 4.1 (A) Map of lakes containing two or more co-occurring ecotypes of *Salvelinus* or *Coregonus*. (B) Pelagic, littoral/benthic, and profundal *Coregonus laveretus* ecotypes from Lake Skrukkebukta (Præbel et al. 2013). (C) Piscivore and planktivore *Salvelinus alpinus* ecotypes from Loch Erich (Maitland and Adams 2018). (D) Three generalist and one pelagic *Salvelinus namaycush* ecotypes from Great Bear Lake (Chavarie et al. 2013). (E) Dwarf and normal *Coregonus clupeaformis* ecotypes from Cliff Lake (Bernatchez et al. 2010).
Figure 4.2 Numbers of sympatric populations grouped by habitat ecotypes across lakes. Each row represents one lake, with the colour of the tiles in each row indicating the number of populations in a habitat category found within a lake. (A) *Salvelinus* ecotypes, (B) *Coregonus* ecotypes.
Figure 4.3 Numbers of sympatric populations grouped by diet ecotypes in lakes. Each row represents one lake, with the colour of the tiles in each row indicating the number of populations in a diet category found within a lake. (A) *Salvelinus* ecotypes, (B) *Coregonus* ecotypes.
Figure 4.4 (A) Body length distributions in assemblages. Each dot represents the mean total body length of a population, with colour and shape indicating genus. (B – E) Differences in body lengths between paired habitat or diet ecotypes, for (B – D) \textit{Salvelinus} and (E) \textit{Coregonus}. Each point represents the mean of all populations in a lake from a diet or habitat category and lines connect ecotypes from the same lake.
Figure 4.5 (A) Gill raker count distributions in assemblages. Each dot represents the mean gill raker count for a population, with colour and shape indicating genus. (B – D) Differences in gill raker counts between paired habitat ecotypes. Each point represents the mean of all populations in a lake from a diet or habitat category and lines connect ecotypes from the same lake. All populations are *Coregonus* because there was an insufficient sample size for comparisons in *Salvelinus*.
Chapter 5: Conclusion

Evolution in response to competition is common in nature (Schluter 2000b). Competition drives divergence between sympatric species that overlap in resource use and leads to changes in phenotypic distributions within populations (Benkman 1996; Pfennig and Murphy 2002). In this dissertation we addressed a few possible outcomes of competition among phenotypes. Specifically, we tested for competition-driven natural selection within experimental populations, we compared ecological sexual dimorphism among populations in different competitive contexts, and we assessed repeatability across assemblages of ecotypes that differ in resource use.

5.1 Competition and natural selection within populations

In chapter two, we tested for competition-driven frequency-dependent selection within experimental populations. Phenotypic variation within intermediate generalist populations can theoretically be maintained by negative frequency-dependent selection (Taper and Case 1992). We tested for frequency-dependent selection by manipulating the relative frequency of phenotypes within experimental populations in mesocosms. We found that frequency-dependence was weak to absent within the range of phenotypes included in the experiment. This indicated that selection within intermediate generalist populations of threespine stickleback likely differs from sympatric specialist species, which evolved in response to competition-driven frequency-dependent selection (Rundle et al. 2003; Schluter 2003). Instead, it is possible that an intermediate phenotype distribution is maintained because selection is stabilizing or because the direction of selection fluctuates through time (Grant and Grant 2014). These forms of selection
could still be shaped by competition within intermediate threespine stickleback populations. Intensity of competition affects which resources individuals access within experimental intermediate populations of threespine stickleback, as well as the strength of selection on those populations (Bolnick 2004; Svanbäck and Bolnick 2007; Araújo et al. 2008). The results from chapter three of this dissertation suggest that the same phenotypes may be favoured in a particular competitive environment, regardless of the frequency distribution of phenotypes in the population.

5.2 Evolution within populations in different ecological contexts

The evolution of greater ecological sexual dimorphism is a possible outcome of competition within populations (De Lisle and Rowe 2015, 2017). If the evolution of sexual dimorphism is competition-driven, then higher levels of dimorphism are expected to be observed in the absence of competitor species (Stuart et al. 2017; Li and Kokko 2021). We found no evidence to support this hypothesis, instead finding similar levels of ecological sexual dimorphism between threespine stickleback populations that are sympatric with closely related competitor species (either another stickleback species or prickly sculpin) and populations that are not. Instead, we found that sexual dimorphism in body shape declines with divergence from the marine stickleback phenotype, which was ancestral to the freshwater populations. An alternative hypothesis consistent with this pattern is that sexual dimorphism declines as populations adapt to new environments because reduced sex-specific selection is expected within maladapted populations (Connallon et al. 2018).

If this alternative hypothesis explains variation in sexual dimorphism among populations of threespine stickleback, then competitive interactions were likely to have affect the evolution
of sexual dimorphism indirectly rather than directly. Limnetic threespine stickleback species are more phenotypically and genetically similar to marine populations than benthic species are (Jones et al. 2012; Chhina et al. 2022). The phenotypic differences between limnetic and benthic species are a result of competition-driven selection that caused the two species to evolve to specialize on different resources (Schluter 1995, 2003; Rundle et al. 2003). Similarly, competition with prickly sculpin, an intraguild predator, has shaped distributions of phenotypes that vary along a marine to benthic phenotypic axis in several threespine stickleback populations (Ingram et al. 2012; Miller et al. 2015). Therefore, competitive interactions affect population phenotype distributions and the divergence of those phenotypes from the marine ancestor. If divergence from marine phenotypes determines the degree of sexual dimorphism in populations, this implicates a possible indirect effect of competitive interactions on levels of sexual dimorphism.

5.3 **Repeatability of evolution and assembly among populations**

Assemblages of ecotypes sometimes display a surprising level of similarity, with sets of ecotypes with similar phenotypes and filling the same ecological niches occurring repeatedly (Losos 2009; Gillespie et al. 2018). Competition is thought to have shaped evolution and species assembly in several of these repeated assemblages. For example, Caribbean *Anolis* lizards compete for perching locations, leading to the evolution of ecomorphs that subdivide arboreal and terrestrial habitats based on the substrate that best matches their toe and limb phenotypes (Losos 1994; Mahler et al. 2013). Assemblages of freshwater fish ecotypes in post-glacial lakes commonly fill benthic and pelagic habitats, suggesting repeatable differentiation driven by competition for lake habitat and dietary resources (Schluter and McPhail 1993; Skulason and
Smith 1995). In chapter four of this dissertation, we assessed repeatability of ecotypes assemblages within two Salmonid genera – *Coregonus* and *Salvelinus* – in which sets of two to seven sympatric ecotypes are common. Within *Coregonus*, there was high similarity across assemblages in which ecotypes, defined by diet or habitat, co-occur. Ecotype assemblages in *Salvelinus* were highly variable, indicating a lack of repeatability. In both genera, distributions of mean phenotypes were dissimilar across assemblages. However, there were repeated ecotype by phenotype associations in both *Coregonus* and *Salvelinus* assemblages.

The results of this chapter do not directly demonstrate a role for competition in structuring ecotype assemblages in *Coregonus* and *Salvelinus*, but they are consistent with one. The patterns underlying repeatability in *Coregonus* ecotype assemblages – unusually high similarity in ecological niche use, nestedness among assemblages with different numbers of ecotypes, positive associations in ecotype co-occurrence – are all expected in communities structured by competition (Schluter 1984, 1990; Nicholas J. Gotelli 2000; Ulrich and Gotelli 2007). Even in *Salvelinus*, which exhibited a lack of similarity across assemblages, sympatric populations tended to fall into different ecotypes, suggesting some partitioning of resources. This could arise through processes of community assembly, as ecologically distinct populations may be more likely to occur together due to reduced interpopulation competition (Chesson 2000). Alternatively, phenotypic and ecological differences between ecotypes could have evolved in sympatry through ecological character displacement (Taper and Case 1985).

### 5.4 Comparing across divergent assemblages in post-glacial lakes

Threespine stickleback are tractable as an experimental and genetic system. Because of this, numerous selection and evolution experiments, as well as common garden and crossing
experiments, have been used to answer questions about how the benthic and limnetic species assemblages evolved (Albert et al. 2008; Conte et al. 2015; Miller et al. 2015; Rennison et al. 2019; Schluter et al. 2021). It would be difficult to perform many of these experiments with *Coregonus* and *Salvelinus* species, which generally have much larger body sizes and much longer lifespans than threespine stickleback. There are many similarities between threespine stickleback species pairs and Salmonid ecotype assemblages, including the tendency to evolve along a benthic-pelagic environmental gradient within species depauperate post-glacial lakes (Schluter and McPhail 1993; Skulason and Smith 1995). However, it is unclear whether some of the results from threespine stickleback would translate to ecotype assemblages in *Salvelinus* and *Coregonus*.

Our finding in chapter two of this dissertation indicated that the strength of frequency-dependent selection depends on the range of phenotypes present in threespine stickleback populations because we found much weaker frequency dependence than has previously been observed with a broader range of phenotypes (Schluter 2003). In chapter four, we found that phenotype distributions for body size and gill raker counts were highly variable across assemblages in *Coregonus* and *Salvelinus*. It is possible that variation among systems in their phenotype distributions could impact the strength of frequency-dependent selection driving evolution within those systems. Therefore, it is unclear to what degree the form of selection that drove divergence between threespine stickleback species pairs is similar to selection driving divergence among sympatric *Coregonus* and *Salvelinus* ecotypes.

In threespine stickleback, phenotypic shifts in response to closely related sympatric competitors have a well characterized genetic basis (Arnegard et al. 2014; Miller et al. 2019). Threespine stickleback in lakes do exhibit phenotypic plasticity that is shaped by the resources
they access, but this plasticity does not form the basis of phenotypic differences between benthic and limnetic species (Svanbäck and Schluter 2012). In Coregonus and Salvelinus assemblages, meanwhile, the degree to which phenotypic shifts are genetically based is much more variable and much less clear (Skúlason et al. 2019). This could have implications for the low repeatability in phenotype distributions observed among Coregonus and Salvelinus ecotype assemblages. Threespine stickleback assemblages, in contrast, exhibit repeated phenotype distributions in several traits (Gow et al. 2008). There may also be implications of this for the process by which Coregonus and Salvelinus ecotypes evolve in response to competition.

5.5 Future directions

There are several open questions relating to the evolutionary consequences of competitive interactions among phenotypes in post-glacial lakes. In particular, there are still gaps in our knowledge about the interplay between natural selection and evolution in these populations. In chapter two, we tested for frequency-dependent selection over a one-month period. This allowed us to estimate effects of phenotype frequencies in a population on survival and growth of juvenile fish during that time period but did not give us insight into how frequency-dependence might affect fish over the course of their life cycle. Importantly, we do not know whether there would have been frequency-dependent effects on relative reproductive success of phenotypes, as well as what effect this might have had on the phenotypic composition of a next generation. Additionally, if selection is instead fluctuating or stabilizing, it would be interesting to know which phenotypes are favoured within populations, how this changes through time, and how the form of selection on intermediate populations affects the distribution of phenotypes.
In chapter three of this dissertation, we demonstrated a pattern consistent with a reduction in sexual dimorphism through adaptation to a new environment. While this pattern is suggestive, we did not test this hypothesis directly. If sexual dimorphism declines through the process of adaptation to a new environment, then it should be possible to observe reduced sex-specific selection in maladapted threespine stickleback populations. Maladapted populations have been shown to commonly experience reduced sex-specific selection (De Lisle et al. 2018). It would be interesting to know whether this is the case in threespine stickleback, as well as the genetic changes involved in the evolution of reduced dimorphism. Alternatively, if benthic-adapted populations exhibit reduced sexual dimorphism because benthic males and females experience more similar environments throughout their lifespan than limnetic females and males do, then reduced sex-specific selection should be observed in populations that primarily occupy a benthic but not pelagic environment.

The links between selection, evolution, and resulting phenotypes are unclear across many Coregonus and Salvelinus ecotype assemblages. Assemblages in these genera vary in the degree to which phenotypic differences among ecotypes are based in genetics versus phenotypic plasticity (Parsons et al. 2011; Lundsgaard-Hansen et al. 2013). They also vary in the degree of reproductive isolation among ecotypes and the ecological and evolutionary processes by which they were assembled (Hendry 2009; Crête-Lafrenière et al. 2012; Præbel et al. 2013). It would be exciting to address how phenotypes varying in their degree of plasticity might respond to competition-driven selection, and how this might affect the maintenance and repeatability of ecotype assemblages. Additionally, in this system disparate processes seemingly lead to some level of similarity across assemblages. These assemblages may, therefore, represent a useful
system for addressing what ecological and phenotypic outcomes might be shared or distinct across different evolutionary and ecological processes of community assembly.
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Appendices

Appendix A  Supplementary materials for Chapter 2

Figure A.1. NMDS of invertebrate community composition. Each point represents one mesocosm, with colour indicating treatment (Int₀ = yellow circle, Int₇ = green triangle, Int₉ = red square). Ellipses represent a 95% confidence interval for each treatment. Labels indicate the ordination of invertebrate taxa.
Figure A.2. Target population survival in contrasting frequency distributions of phenotypes of individually-marked fish. Each line represents one mesocosm. More negative LD1 values indicate a more benthic-like body shape while fish with more positive LD1 values have a more limnetic-like body shape.
Figure A.3. Target population growth in contrasting frequency distributions of phenotypes of both individually- and batch-marked fish. Each line represents one mesocosm. In (A) cross was converted to a numeric value, with CxB = -1, CxC = 0, and CxL = 1. In (B), more negative LD1 values indicate a more benthic-like body shape while fish with more positive LD1 values have a more limnetic-like body shape.
Figure A.4. Target population growth in contrasting frequency distributions of phenotypes of individually-marked fish. Each line represents one mesocosm. More negative LD1 values indicate a more benthic-like body shape while fish with more positive LD1 values have a more limnetic-like body shape.
Table A.1. Tests of the difference between treatment fish presence (Int\(_L\) and Int\(_B\)) and absence (Int\(_0\)). For dataset = 1, all mesocosms were included. For dataset = 2, we included only mesocosms where all four treatment stage fish were recovered.

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<th>df</th>
<th>p-value</th>
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Table A.2. Tests of frequency-dependent selection. For tests where slope predictor is "cross type", CxB individuals were coded as -1, CxC individuals were coded as 0, and CxL individuals were coded as 1. For dataset = 1, all mesocosms were included. For dataset = 2, we included only mesocosms where all four treatment stage fish were recovered.

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<td>16.95</td>
<td>0.41</td>
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<td>0.97</td>
<td>-0.02</td>
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<td>16.93</td>
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<td>0.56</td>
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<tr>
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<td>1.45</td>
<td>8.92</td>
<td>0.18</td>
<td>0.79</td>
</tr>
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</table>
Table A.3. Tests of differences in slope between fish presence and absences. For tests where slope predictor is "cross type", CxB individuals were coded as -1, CxC individuals were coded as 0, and CxL individuals were coded as 1. For dataset = 1, all mesocosms were included. For dataset = 2, we included only mesocosms where all four treatment stage fish were recovered.

<table>
<thead>
<tr>
<th>slope predictor</th>
<th>slope response</th>
<th>fish</th>
<th>dataset</th>
<th>mesocosms</th>
<th>t-stat</th>
<th>df</th>
<th>p-value</th>
<th>Cohen's D</th>
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</thead>
<tbody>
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<td>body shape (LD1)</td>
<td>log(final length / initial length)</td>
<td>batch-marked and individually-marked</td>
<td>1</td>
<td>47</td>
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<td>13.50</td>
<td>0.03</td>
<td>-0.87</td>
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<tr>
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<td>batch-marked and individually-marked</td>
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<td>33</td>
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<td>batch-marked and individually-marked</td>
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<td>0.02</td>
<td>-0.90</td>
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<td>log(final length / initial length)</td>
<td>batch-marked and individually-marked</td>
<td>2</td>
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<td>20.13</td>
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<td>7.93</td>
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<td>9.66</td>
<td>0.03</td>
<td>-1.16</td>
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<td>-0.61</td>
<td>7.22</td>
<td>0.56</td>
<td>-0.32</td>
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<td>1</td>
<td>25</td>
<td>1.06</td>
<td>12.60</td>
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<td>0.43</td>
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<td>1.12</td>
<td>15.20</td>
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<td>0.49</td>
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<tr>
<td>mean LD1 for each cross type</td>
<td>proportion survived</td>
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<td>24</td>
<td>2.25</td>
<td>5.85</td>
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<td>proportion survived</td>
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<td>18</td>
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<td>6.06</td>
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<td>2.18</td>
<td>9.40</td>
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Table A.4. Population history and number of treatment fish recovered between treatment and target population phases.

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<th>Lake</th>
<th>Recovered</th>
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<td>IntB</td>
<td>Bullocks</td>
<td>4</td>
</tr>
<tr>
<td>T3-6</td>
<td>IntB</td>
<td>Bullocks</td>
<td>4</td>
</tr>
<tr>
<td>T3-8</td>
<td>IntB</td>
<td>Bullocks</td>
<td>4</td>
</tr>
<tr>
<td>T4-7</td>
<td>IntB</td>
<td>Bullocks</td>
<td>4</td>
</tr>
<tr>
<td>T5-1</td>
<td>IntB</td>
<td>Bullocks</td>
<td>4</td>
</tr>
<tr>
<td>T6-3</td>
<td>IntB</td>
<td>Bullocks</td>
<td>4</td>
</tr>
<tr>
<td>T1-7</td>
<td>IntB</td>
<td>Bullocks</td>
<td>3</td>
</tr>
<tr>
<td>T2-8</td>
<td>IntB</td>
<td>Bullocks</td>
<td>3</td>
</tr>
<tr>
<td>T4-8</td>
<td>IntB</td>
<td>Bullocks</td>
<td>2</td>
</tr>
<tr>
<td>T4-5</td>
<td>IntB</td>
<td>Bullocks</td>
<td>1</td>
</tr>
<tr>
<td>T1-2</td>
<td>IntB</td>
<td>Hoggan</td>
<td>4</td>
</tr>
<tr>
<td>T4-3</td>
<td>IntB</td>
<td>Hoggan</td>
<td>4</td>
</tr>
<tr>
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<td>IntB</td>
<td>Hoggan</td>
<td>4</td>
</tr>
<tr>
<td>T4-9</td>
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</tr>
<tr>
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<td>IntB</td>
<td>Hoggan</td>
<td>4</td>
</tr>
<tr>
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<td>Hoggan</td>
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</tr>
<tr>
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<td>IntB</td>
<td>Hoggan</td>
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</tr>
<tr>
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<td>IntL</td>
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</tr>
<tr>
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<td>Little Quarry</td>
<td>4</td>
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<tr>
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<td>Little Quarry</td>
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<tr>
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<td>Little Quarry</td>
<td>3</td>
</tr>
<tr>
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<td>IntL</td>
<td>North</td>
<td>4</td>
</tr>
<tr>
<td>T3-4</td>
<td>IntL</td>
<td>North</td>
<td>4</td>
</tr>
<tr>
<td>T5-8</td>
<td>IntL</td>
<td>North</td>
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</tr>
<tr>
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<td>IntL</td>
<td>North</td>
<td>3</td>
</tr>
<tr>
<td>T2-9</td>
<td>IntL</td>
<td>North</td>
<td>3</td>
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<tr>
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<td>IntL</td>
<td>Paq</td>
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<td>IntL</td>
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<td>T1-6</td>
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<td>Paq</td>
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<tr>
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<td>IntL</td>
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<tr>
<td>T3-7</td>
<td>IntL</td>
<td>Priest</td>
<td>4</td>
</tr>
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Figure B.1. Dimorphism in total body shape, quantified as the length of a vector of divergence between females and males in each population, compared between solitary populations and (A) stickleback-sympatric populations or (B) sculpin-sympatric populations. Each point represents one population and colour indicates population type. In (A) light pink indicates limnetic stickleback-sympatric populations and dark pink indicates benthic stickleback-sympatric populations.
Figure B.2. Sexual dimorphism in solitary and competitor-sympatric populations. Each point represents sexual dimorphism in one population, quantified as the difference between females and males within a population in means along the first principal from a PCA performed on armour traits with all populations together (solitary, stickleback-sympatric, sculpin-sympatric, marine). Light pink indicates limnetic stickleback-sympatric populations while dark pink indicates benthic stickleback-sympatric populations.
Figure B.3. Sexual dimorphism in body shape along a marine to benthic phenotypic axis. In both panels, each point represents one population, showing the relationship between position along a marine to benthic PC1 for body shape and dimorphism in (A) gill rakers and (B) armour traits. Dimorphism was quantified as the t-statistic of the difference in means along a principal component between females and males within a population.
Figure B.4. Sexual dimorphism in body size, quantified as the centroid size of body shape landmarks. Each point indicates the average centroid size for one sex in one population while error bars indicate standard deviation.
Figure B.5. Alignment of sexual dimorphism to axes of population divergence. Each point represents the angle between a vector quantifying divergence between males and females in each population and (A) a vector quantifying body shape divergence between limnetic and benthic populations, (B) a vector quantifying body shape divergence between sculpin-sympatric and solitary populations, (C) a vector quantifying body shape divergence between marine and benthic populations.
Figure B.6. Comparison between lab-raised and wild-caught fish in estimated levels of sexual dimorphism. Dimorphism was quantified as (A) the length of a vector of body shape differences between males and females, (B) differences between males and females in centroid size, (C) differences between males and females along a PC 1 of gill raker measurements, and (D) differences between males and females along a PC 1 of armour measurements.
Appendix C Supplementary materials for Chapter 4

C.1 Supporting methods – systematic literature review details

We searched Web of Science on 20-Sep-2019 using the search terms [TS= (ecotype OR morph OR polymorphism OR polyphenism) AND TS=(morphology OR diet OR phenotype) AND TS=(charr OR Dolly Varden OR Lake Trout OR Brook Trout OR whitefish OR Cisco OR Brown Trout OR salvelinus OR coregonus OR coregonids OR salmonid*]. This search returned 331 results from 1989-2019. Following a visual scan of each abstract to determine whether the paper included information about co-occurring Salvelinus or Coregonus ecotypes within lakes, 166 papers were eliminated. The full text was assessed for 165 papers. We also downloaded titles and abstracts of all papers that have cited Skulason and Smith 1995 from Scopus on 31-Oct-2019. This paper was chosen as a foundational review of intraspecific polymorphisms that is commonly cited by researchers studying co-occurring salmonid ecotypes. This returned 474 unique citations. 320 eliminated in the abstract scan for not fitting topic requirements. 70 papers were retained after excluding duplicates with the Web of Science results. We performed a Scopus search on 12-Nov-2019 using the search terms (TITLE-ABS-KEY (ecotype OR morph OR polymorphism OR polyphenism OR radiation) AND TITLE-ABS-KEY (charr OR trout OR whitefish OR cisco OR Salvelinus OR Coregonus OR coregonid* OR salmonid*) AND TITLE-ABS-KEY ( morpholog* OR diet OR phenotyp*)). This search returned 577 unique results. 430 were retained after excluding duplicates with previous searches. 333 papers were eliminated in the abstract scan, and the full text was assessed for 97 papers. We performed a bioRxiv search on 05-Dec-2019 with “full text or abstract or title "*morph* salmonid*" (match whole all)”. We scanned the full text of all 27 results from this search.
In the abstract scan, studies were included if they reported on lakes containing two or more co-occurring *Salvelinus* or *Coregonus* ecotypes. In the full text scan, a study was retained if it unambiguously included morphology, trophic ecology, or life history information about co-occuring *Salvelinus* or *Coregonus* ecotypes within lake. We required that this information was collected from wild caught fish, not fish raised in captivity. Additionally, all ecotypes included in this study were lacustrine, rather than riverine or migratory.
Figure C.1. PRISMA diagram showing the stages of the systematic literature review used to select studies for inclusion in the meta-analysis.
Figure C.2. Frequency distribution of diets and habitats across all lakes. This plot includes ecotype counts from lakes with two to seven co-occurring ecotypes where all ecotypes were identified as belonging to a diet or habitat category.

Figure C.3. Frequency distribution of diets and habitats across lakes. This plot includes counts from lakes with two to seven co-occurring ecotypes in which all ecotypes are identified by habitat or diet. Abbreviations in (A): lit = littoral/benthic, pel = pelagic, pro = profundal, gen = generalist, sha = shallow. Abbreviation in (B): ben = benthivore, pla = planktivore, pis = piscivore, gen = generalist, on = omnivore.
Figure C.4. Presence/absence matrices of ecotypes grouped by diet across lakes. For all plots, each row represents one lake, with the coloured tiles in each row indicating the presence of an ecotype and white tiles represented the absence of an ecotype in that lake. (A) includes lakes containing two or more *Salvelinus* ecotypes while (B) includes lakes containing two or more *Coregonus* ecotypes.
Figure C.5. Presence/absence matrices of ecotypes grouped by habitat across lakes. For all plots, each row represents one lake, with the coloured tiles in each row indicating the presence of an ecotype and white tiles represented the absence of an ecotype in that lake. (A) includes lakes containing two or more *Salvelinus* ecotypes while (B) includes lakes containing two or more *Coregonus* ecotypes.
Figure C.6. Differences in trait values between sympatric ecotypes in *Coregonus*. Each point represents the mean of all populations in a lake from a diet or habitat category and lines connect ecotypes from the same lake.
Figure C.7. Differences in trait values between sympatric ecotypes in *Salvelinus*. Each point represents the mean of all populations in a lake from a diet or habitat category and lines connect ecotypes from the same lake.
Table C.1 Number of assemblages with different levels of diversity

<table>
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<tr>
<th></th>
<th>2-ecotype</th>
<th>3-ecotype</th>
<th>4-ecotype</th>
<th>5-ecotype</th>
<th>6-ecotype</th>
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<td>12</td>
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<td>1</td>
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<tr>
<td><em>Coregonus</em></td>
<td>30</td>
<td>11</td>
<td>6</td>
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<td>1</td>
<td>1</td>
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</table>

Table C.2 Summary of lake characteristics

<table>
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<tr>
<th></th>
<th>Latitude</th>
<th>Longitude</th>
<th>Maximum depth (m)</th>
<th>Surface area (km²)</th>
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</thead>
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<tr>
<td><em>Salvelinus</em></td>
<td>42.5 – 81.8°</td>
<td>-155 – 160°</td>
<td>5.5 – 614</td>
<td>0.12 – 31790</td>
</tr>
<tr>
<td><em>Coregonus</em></td>
<td>45.9 – 70°</td>
<td>-137 – 33.2°</td>
<td>15 – 614</td>
<td>2 – 28000</td>
</tr>
</tbody>
</table>

a Numbers are the range of values observed for lakes with assemblages in each genus.

b Note that not all lakes had available data for these variables.