Habitat Use, Movement Patterns, and Spatial Population Structure of Polyphenic Sunfish

by

Will M.C. Jarvis

A Thesis
presented to
The University of Guelph

In partial fulfilment of requirements
for the degree of
Master of Science
in
Integrative Biology

Guelph, Ontario, Canada

© Will M.C. Jarvis, August, 2018
ABSTRACT

HABITAT USE, MOVEMENT PATTERNS, AND SPATIAL POPULATION STRUCTURE OF POLYPHENIC SUNFISH

Will M.C. Jarvis
University of Guelph, 2018

Advisor: Dr. Beren Robinson

Populations are often spatially structured such that phenotype distributions reflect adaptive phenotype-habitat associations. I investigated two questions about how environmental heterogeneity contributes to spatially structured phenotypic variation in a polyphenic population of sunfish. 1) How does sunfish habitat use in exposed lake shorelines influence spatial population structure? I found that sunfish from exposed shoreline habitat varied in diet and phenotype among sites but were more similar in body form to sunfish from shallow littoral habitat than to sunfish from open water pelagic habitat. 2) How do patterns of connectivity between habitat patches likely influence spatial population structure? Using a between year mark-recapture study, I found rates of movement between habitats sufficient for gene flow to homogenize any genetic differences. Understanding patterns of habitat use and connectivity in polyphenic sunfish populations will generate hypotheses about how spatial population structure is generated and maintained in the initial stages of adaptive diversification.
ACKNOWLEDGEMENTS

I want to first thank my advisor Beren Robinson for his continual support, guidance, and inspiration over the 4 years that I have worked with him. I also would like to thank past and present members of the Robinson and McLaughlin labs for all their encouragement and help, and for making my grad school experience an enjoyable one; particularly Caleb Axelrod, Karalea Cantera, Nick Sakitch, Jade Dawson, David Gislason, Rachel Holub, Rob McLaughlin, and Emelia Myles-Gonzalez. I want to additionally thank Kathryn Peiman and Colby Nolan for their significant contributions to the field and lab work that went into this thesis.

Thank you to my committee members, Karl Cottenie and Kevin McCann, for pushing me to think ecologically and for all their feedback on my thesis.

I also want to acknowledge the funding that made this work possible; a Natural Sciences and Engineering Research Council of Canada grant to Beren Robinson, The Norman James Aquatic Animal Ecology Award, and a Graduate Tuition Scholarship from the College of Biological Sciences at the University of Guelph.

Thank you to the Ashby Lake Protective Association and the Ontario Ministry of Natural Resources for allowing us to work on Ashby Lake, and to Bob and Christine Gautier for accommodating our stay.

Thank you to my mom, dad, Mac, and Goose.

And finally, I want to thank Sarah Dolson for always understanding and making me laugh.
## TABLE OF CONTENTS

ABSTRACT ......................................................................................................................... ii

ACKNOWLEDGEMENTS ................................................................................................. iii

TABLE OF CONTENTS ................................................................................................. iv

LIST OF TABLES .............................................................................................................. v

LIST OF FIGURES ......................................................................................................... vi

General Introduction ...................................................................................................... 1

Chapter 1: Habitat Use and Consequences for Spatial Population Structure .............. 7
  1.1 Introduction ................................................................................................................. 7
  1.2 Methods ....................................................................................................................... 11
  1.3 Results ......................................................................................................................... 14
  1.4 Discussion .................................................................................................................... 18
  1.5 Tables ........................................................................................................................ 23
  1.6 Figures ......................................................................................................................... 24

Chapter 2: Connectivity and Spatial Population Structure ........................................... 37
  2.1 Introduction .................................................................................................................. 37
  2.2 Methods ....................................................................................................................... 42
  2.3 Results ......................................................................................................................... 46
  2.4 Discussion .................................................................................................................... 47
  2.5 Figures ........................................................................................................................ 52

General Conclusion ....................................................................................................... 60

REFERENCES ............................................................................................................... 62
LIST OF TABLES

Table 1.1 Summary of sunfish sample sizes for each collection site across the three collection trips.-----------------------------------------------23

Table 1.2 Summary of the effect of site on sunfish traits and the least squared means or actual means (body size only) for each trait among collection sites (where ES refers to exposed shoreline site 1 and 2). Different letters indicate significantly different contrasts between sites from Student’s t-tests of least squared means (jaw size, PC2, PC3, parasite load) or actual means (body size)..................................................................23

Table 1.3 The canonical loadings (correlation) on the first linear discriminant axes for each predictor variable used in the linear discriminant analysis. The linear discriminant function was trained with phenotype data from littoral and pelagic sunfish, and then used to classify exposed shoreline sunfish as littoral or pelagic..................................................................23
LIST OF FIGURES

Figure 1.1 Map of Ashby Lake, ON indicating collection sites; littoral (Lit) and pelagic (Pel) reference sites, exposed shoreline site 1 (ES1), and exposed shoreline site 2 (ES2)……………24

Figure 1.2 The locations of 17 homologous landmarks used for geometric morphometric analysis…………………………………………………………………………………………...25

Figure 1.3 Deformations grids visualizing whole body shape variation along principle component axes (head is facing to the right). Shown here are the individuals with the maximum and minimum principle component score for each axis. PC2 expressed variation in body depth, head length, and the orientation of the premaxilla jaw bone relative to the rest of the head. PC3 expressed variation in relative head size and the length of the premaxilla jaw bone relative to total head size…………………………………………………………………………………….26

Figure 1.4 Relationships between oral jaw width and body size by collection site. Sunfish collected from littoral and pelagic habitat had the largest and smallest jaw width respectively, after accounting for body size. Sunfish from exposed shorelines were intermediate between littoral and pelagic reference sunfish (ES2) or not different from littoral reference sunfish (ES1). The slope of the relationship between oral jaw width and body size did not differ between sites………………………………………………………………………………………………27

Figure 1.5 Relationships between shape principle component 2 and body size by collection site. Sunfish collected from pelagic habitat had relatively deeper bodies, shorter heads, and more terminal mouths than sunfish collected from littoral or exposed shoreline (ES) habitat after accounting for body size. There was a significant negative relationship between PC2 and body size; increasing body size was related to deeper bodies, shorter heads, and more terminal mouths. Letters indicate significantly different contrasts from pairwise Student’s t-tests of least squared means…………………………………………………………………………………………….28

Figure 1.6 Relationships between shape principle component 3 and body size by collection site. Sunfish collected from littoral habitat and exposed shoreline 2 (ES2) had larger heads and longer premaxilla jaw bones than those from pelagic or exposed shoreline 1 (ES1) after accounting for body size. There was a weak relationship between PC3 and body size. Letters indicate significantly different contrasts from pairwise Student’s t-tests of least squared means…………………………………………………………………………………………………………29

Figure 1.7 Boxplots of sunfish body size by collection site. Boxes show the mean value, and the upper (75th) and lower (25th) quartiles. Sunfish from pelagic and exposed shoreline 2 sites were larger than those from exposed shoreline 1 and littoral. Letters indicate significantly different contrasts from pairwise Student’s t-tests of means…………………………………………………………………………………………………………30

Figure 1.8 The effect of body size and collection habitat on the number of external blackspot parasites. Sunfish from littoral and exposed shoreline 2 tended to have higher parasite loads than exposed shoreline 1 and pelagic sunfish. There was a positive effect of body size on parasite load
across all collection sites. Letters indicate significantly different contrasts from pairwise Student’s t-tests of least squared means………………………………………………………… 31

Figure 1.9 Classifications of sunfish from exposed shoreline sites as either littoral or pelagic ecotypes based on the linear discriminant function. The linear discriminant function was trained with phenotype data from littoral and pelagic sunfish, and then used to classify exposed shoreline sunfish as littoral or pelagic. The linear discriminant function predicted collection site with adjusted jaw size, body size, PC2, PC3, and parasite load………………………………………32

Figure 1.10 Boxplots of the linear discriminant axis scores by collection site. The linear discriminant function was trained with phenotype data from littoral and pelagic sunfish, and then used to classify exposed shoreline sunfish as littoral or pelagic. The linear discriminant function predicted collection site with adjusted jaw size, body size, PC2, PC3, and parasite load. Letters indicate significantly different contrasts from pairwise t-tests…………………………………..33

Figure 1.11 The proportion of sunfish with insects present in their stomach across collection sites. More littoral sunfish had insects in their stomachs than did pelagic sunfish, while an intermediate number of exposed shoreline sunfish had insects in their stomachs……………….34

Figure 1.12 Boxplots of the number of *Daphnia magna* found in each sunfish stomach across collection sites. Sunfish from the pelagic site and exposed shoreline site 2 had more *Daphnia magna* in their stomachs on average than sunfish from the littoral site or exposed shoreline site 1…………………………………………………………………………………………………..35

Figure 1.13 Relationships between body condition and body size by collection site. There is no difference in body condition between collection sites, and no relationship with body size…… 36

Figure 2.1 The effect of body size of jaw size by initial collection habitat. Sunfish from littoral habitat had wider jaws on average than those from pelagic habitat……………………………………52

Figure 2.2 The effect of body size and initial collection habitat on the number of external blackspot parasites. Sunfish from littoral habitat had higher parasite loads than those from pelagic habitat…………………………………………………………………………………………………………………………………………………………………..53

Figure 2.3 Boxplots of sunfish body size by initial marking habitat type. Boxes show the mean value, and the upper (75th) and lower (25th) quartiles. Tails indicate the extent of the data. Sunfish from the pelagic habitat had a larger mean body size than those from the littoral habitat……..54

Figure 2.4 Map of the north basin of Ashby Lake showing recorded sunfish movements between habitat patches between 2016 and 2017. Circle symbols indicate mark and recapture sites (pelagic = blue, littoral = red). Arrows indicate the direction of an individual’s movement……55

Figure 2.5 Counts of individuals originally marked in 2016 in each habitat type. Colours indicate where those individuals were recaptured in 2017: in the alternate habitat type, in a different site of the same habitat, or the same site. There is no difference in the proportion of individuals
captured in the alternate habitat, but the proportion of individuals that moved between patches was greater in the pelagic habitat.

*Figure 2.6* The effect of body size on the predicted probability of moving among pelagic habitat patches. There is a significant negative effect of body size on the predicted probability of moving between patches.

*Figure 2.7* The lack of effect of adjusted jaw size on the predicted probability of moving between habitat patches within the pelagic habitat.

*Figure 2.8* The lack of effect of body condition on the predicted probability of moving between habitat patches within the pelagic habitat.
General Introduction

Populations of individuals are often spatially structured such that phenotypes are non-randomly distributed, instead reflecting adaptive phenotype-habitat associations (Manel et al., 2003). The spatial distribution of phenotypes can influence demographic (e.g. population growth rate) and evolutionary parameters (e.g. gene flow) that are important determinants of population dynamics (Manel et al., 2003) and adaptive evolutionary responses (Kinnison & Hairston, 2007). For example, the population dynamics of the Granville fritillary butterfly in Finland are governed by a balance between stochastic local extinctions and recolonization through a network of populations, or a metapopulation (Hanski et al., 1995). For this species, local recolonization and extinction risk are both highly dependent on the spatial arrangement of populations (Saccheri et al., 1998). Spatial population structure is similarly important for evolutionary processes like adaptive divergence, for example when the spatial distribution of phenotypes determines opportunities for assortative mating and competitive interactions (Rice, 1987; Caillaud & Via, 2000). Because of its importance for both ecology and evolution, it is critical to understand how spatial population structure is generated and maintained under various scenarios of environmental heterogeneity. Here, I investigated the spatial population structure of ecologically and phenotypically diverse pumpkinseed sunfish (Centrarchidae: *Lepomis gibbosus*) within a single lake, specifically extending our characterization of phenotype-habitat associations to an understudied habitat type, and quantifying connectivity between habitat patches over one year.

Adaptive phenotype-habitat associations can occur across a range of scales from microhabitat use within populations to adaptive divergence among populations. I consider a population to be a group of conspecific individuals that is spatially distinct from other groups of individuals where members of the group can potentially interbreed (Wells & Richmond, 1995). A metapopulation is a set of populations with some amount of migration between breeding seasons (Wells & Richmond, 1995). Ecologists have a long history of studying the spatial structure of metapopulations and the effects of migration on local population dynamics and persistence, but evolutionary biologists also acknowledge these different scales when they distinguish processes regulating adaptive divergence in sympathy (within populations) and allopatry (among populations).
Geographic scale often informs our view of population structure. Here, I focus on fine scale spatial population structure within a single lake population of fish because of its relevance to competitive and reproductive interactions. In this thesis, I studied the spatial population structure of sunfish within a single lake roughly 2.59 km$^2$ in area. I consider a whole lake to be an environment containing a set of distinct habitat patches. Habitat patches are identified by spatially discrete clusters of reproductive activity that are separated by some geographic barrier where no reproduction occurs, for example deep open water, or a rocky point separating shallow bays.

Spatial population structure is the product of environmental heterogeneity, the variation in abiotic (e.g. temperature) and biotic (e.g. predator assemblage) conditions between habitat patches, and the dispersal capacities of individuals (Hanski, 2012). The environment occupied by a population is rarely homogenous but is instead made up of multiple habitat patches of varying type and quality. Differences in habitat conditions can result in different locally optimal phenotypes, such as when ecological performance involves strong trade-offs that preclude ecological generalists (Futuyma & Moreno, 1988). With sufficient genetic variation, diversifying selection for alternate optimal phenotypes can lead to local adaptation (Rainey & Travisano, 1998). A useful example can be found in populations of *Timema cristinae* walking stick insects that occupy different habitat patches characterized by a single shrub type and the patch size (Sandoval, 1994). Each shrub type has a different colour background and so has an optimal colour phenotype that allows camouflage from predators, while the size of the patch determines the local carrying capacity (Sandoval, 1994; Nosil, Crespi & Sandoval, 2003). A predictable spatial distribution of phenotypes across habitat patches emerges in this system that is generated by the pattern of environmental heterogeneity the population experiences (Sandoval, 1994; Nosil, Crespi & Sandoval, 2003). However, the effect of environmental heterogeneity is dependent upon its spatial scale relative to the dispersal capacities of the individuals, or the ‘connectivity’ of a population.
Spatial population structure can be homogenized or maintained by the patterns of connectivity between habitat patches (Garcia-ramos & Kirkpatrick, 1997; Nosil & Crespi, 2004; Hanski, 2012). Connectivity is a combination of the physical properties of paths between patches and the dispersal capacity of individuals. It links habitat patches demographically and genetically through the movement of individuals and their genes (Hendry & Taylor, 2004; Hanski, 2012). Connectivity has important population consequences when differences in quality between habitat patches results in local populations with positive growth rates that are net exporters of migrants (sources), or with negative growth rates where they can only be maintained by receiving migrants (sinks) (Pulliam, 1988; Dias, 1996). From an evolutionary perspective, source populations that produce migrants can create a migration load in sink populations, as immigrants bring non-adaptive genes, preventing local adaptation (Bolnick & Nosil, 2007; Bolnick, Caldera & Matthews, 2008). Populations of blue tit (*Parus caeruleus*) in southern France provide an informative case of source-sink dynamics, where birds occupy habitat patches with either deciduous or sclerophyllous forest types (Dias & Blondel, 1996). Sclerophyllous habitat patches are sinks because birds there have mismatched breeding phenology that prevents exploitation of peak caterpillar abundance. Conversely, deciduous habitat patches are sources as birds there are well matched to prey population dynamics. Local adaptation in sclerophyllous sink habitat patches is prevented because they receive a high migration load from the deciduous source habitat patches which spatially dominate the environment (Dias & Blondel, 1996). Despite the variation in conditions between deciduous and sclerophyllous habitat patches, there is a more uniform spatial distribution of phenotypes. In this case, connectivity between habitat patches degrades spatial structuring of blue tit populations.

Models examining different scenarios of environmental heterogeneity and connectivity find that three basic patterns of spatial phenotype distributions can emerge; locally adapted specialists in each habitat patch, a single habitat generalist across all habitat patches, or a single dominant habitat specialist across all habitat patches (Hanski & Mononen, 2011). Generally, higher habitat heterogeneity (i.e. larger ecological differences between habitat patches) promotes local adaptation while higher connectivity breaks down spatial population structure. An exception to this rule is when connectivity between habitat patches is additionally influenced by matching
habitat choice (Edelaar, Siepielski & Clobert, 2008; Scheiner, 2016). When individuals can match themselves to the habitat type where they are best adapted, then connectivity can allow a positive feedback between local adaptation and reduced migration that promotes spatial population structure and adaptive divergence (Ravigne, Dieckmann & Olivieri, 2009; Scheiner, 2016)

Beyond being a determining factor in population persistence, spatial population structure also can influence community level processes (McCann, Rasmussen & Umbanhowar, 2005). The amount of connectivity between habitat patches and local adaptation within habitat patches will influence how interacting populations move energy through food webs (Bolnick et al., 2003; McCann, Rasmussen & Umbanhowar, 2005). For instance, energy flow between habitat patches will tend to be limited in a population structured into distinct clusters of locally adapted phenotypes (Quevedo, Svanbäck & Eklov, 2009; Svanback et al., 2015). Alternatively, if a population has high connectivity between habitat patches or less local adaptation, energy flow between habitat patches will be enhanced (Vander Zanden & Vadeboncoeur, 2002; McCann, Rasmussen & Umbanhowar, 2005). Ultimately, some level of connectivity can affect whole food web stability and productivity by stabilizing species interactions, although too much connectivity can be destabilizing (Rooney & McCann, 2012).

Despite the importance of understanding the origins and maintenance of spatial population structure, and a solid theoretical foundation, there are few case studies of how spatial population structure arises in natural settings (Hanski, 2012). Here, I investigated several questions about how spatial population structure is generated and maintained in an ecologically and phenotypically diverse (‘polyphenic’) population of pumpkinseed sunfish (Centrarchidae: Lepomis gibbosus). Here, I use polyphenic to refer to a population where phenotypic diversity has a significant plastic component as opposed to a polymorphic population where phenotypic diversity is primarily the result of genetic diversity. Pumpkinseed sunfish are a widespread freshwater fish found throughout eastern North America, with the most northerly distribution of the Lepomis sunfishes. Typically, they exploit the littoral (near shore, benthic) habitat, but in low productivity lakes with limited littoral habitat and no competitors for zooplankton prey (e.g.
bluegill sunfish, *L. machrochirus*), pumpkinseed will also exploit the pelagic (open water, limnetic) habitat (Robinson, Wilson & Margosian, 2000). These lake habitats differ in abiotic conditions such as physical complexity and water clarity, and biotic conditions such as prey availability and predator risk. For example, littoral habitat has a greater abundance and diversity of benthic macroinvertebrates like snails and insect larvae, while pelagic habitat has a greater abundance and diversity of zooplankton (Robinson et al., 1993; Jastrebski & Robinson, 2004). These polyphenic sunfish present an ideal opportunity to address questions about how spatial population structure is generated and maintained.

There are predictable associations between phenotypes and habitat type within polyphenic populations of pumpkinseed sunfish, characteristic of spatially structured populations. There are differences in functional foraging traits between sunfish from littoral and pelagic habitats, including body shape, pharyngeal jaw size, and gill raker density (Robinson et al., 1993; Gillespie & Fox, 2003; Colborne et al., 2016). These morphological differences characterize different phenotypes within the population that are associated with a specific ecology, or ‘ecotype’. Sunfish likely face diversifying selection for these alternate phenotypes as morphological differences are related to foraging performance on habitat-specific prey types (Parsons & Robinson, 2007) and components of fitness like growth and body condition (Robinson & Wilson, 1996; Jastrebski, 2001; Colborne et al., 2016). Genetic variation underlies some of these phenotypic differences between ecotypes, but these traits also have strong plastic developmental responses to local conditions that likely contribute to phenotype-habitat associations (Robinson & Wilson, 1996; Parsons & Robinson, 2007). This general pattern of phenotype-habitat association is repeated in multiple sunfish populations in post-glacial lakes in northeastern North America (Weese, Ferguson & Robinson, 2012).

We do not fully understand how connectivity between habitat patches shapes population structure within these populations. Within a summer growing season, sunfish ecotypes exhibit strong patch fidelity, segregating between littoral and pelagic habitat patches for foraging and reproduction (McCairns & Fox, 2004). A mark-recapture study of sunfish during the summer found 97% and 98% probability of site fidelity for pelagic and littoral individuals, respectively
Additionally, among sunfish transplanted between habitat patches, there was a 74% and 93% probability of returning to their site of capture for pelagic and littoral individuals, respectively (McCairns & Fox, 2004). However, sunfish live up to 10 years, and they leave summer habitats patches in the fall to overwinter in deeper waters and return to summer season sites in the spring (personal observation). It is unclear how much they may move between habitat patches between years. Genetic differentiation at neutral loci is low or non-existent in all polyphenic sunfish populations, which suggests substantial gene flow between ecotypes (Weese, Ferguson & Robinson, 2012; Colborne et al., 2016). A major goal of this thesis is to improve our understanding of connectivity in these populations and how this may influence population structure.

In chapter 1, I explored spatial population structure in a polyphenic population of pumpkinseed sunfish, specifically quantifying how a previously unstudied habitat, exposed lake shorelines, influences spatial population structure. This builds on our current understanding of spatial structuring across the littoral-pelagic habitat divergence to include other frequently used habitat types in post-glacial lakes. In chapter 2, I used a mark-recapture study to estimate between-year rates of adult sunfish movement and to understand how connectivity between habitat patches influences spatial population structure. This specifically extends our understanding of connectivity in these populations to a scale that has not been previously studied. The findings of this thesis increase our understanding of how environmental heterogeneity and connectivity interact to generate spatial population structure in populations of sunfish. The perspectives gained will further the long-term goal of identifying the factors that regulate adaptive divergence in postglacial lake fishes (Robinson & Wilson, 1994; Robinson, Wilson & Margosian, 2000).
Chapter 1: Habitat Use and Consequences for Spatial Population Structure

1.1 Introduction

Individuals in a population are often geographically structured such that the distribution of phenotypes across space follows predictable patterns of phenotype-habitat association (Manel et al., 2003). This ‘spatial population structure’ is thought to result from diversifying selection generated by strong trade-offs in ecological performance between patchily distributed habitats that vary in local conditions and quality (Rainey & Travisano, 1998; Rundle & Nosil, 2005; Hanski, 2012). Spatial population structure can influence important demographic (e.g. population growth rate) and evolutionary (e.g. gene flow) dynamics (Manel et al., 2003), therefore it is critical to understand how specific patterns of spatial population structure arise and are maintained. To support this goal, we need more empirical studies of spatial population structure under various scenarios of environmental heterogeneity (Hanski, 2012). In this chapter, I explore spatial population structure in an ecologically and phenotypically diverse (‘polyphenic’) population of pumpkinseed sunfish (Centrarchidae: Lepomis gibbosus), specifically in a previously unstudied habitat, the exposed shoreline of oligotrophic lakes.

The key determinant of spatial population structure is variation in abiotic (e.g. temperature) and biotic (e.g. competitor and predator assemblage) conditions between habitat patches, and the performance trade-offs that these differences can produce. Performance trade-offs are one of the most generally accepted patterns in biology, often identified by improved performance at one task resulting in reduced performance at another (Kneitel & Chase, 2004). In a heterogeneous environment made up of multiple habitat patches that differ in local conditions, performance trade-offs can contribute to diversifying selection that favours local adaption, where new, habitat-specific phenotypes evolve from a single ancestral type (Rainey & Travisano, 1998; Rundle & Nosil, 2005; Hereford, 2009). So, by contributing to strong diversifying selection, performance trade-offs can lead to the formation of spatial population structure.

Typically, models evaluating the factors that regulate within-population spatial structure are simplified to consist of discrete habitat types (i.e. course-grained habitat heterogeneity), whereas
environmental variation in nature can also include ‘fine-grained’ variation in the form of ecological gradients. The spatial population structure generated under continuous environmental gradients where some individuals experience intermediate or mixed environmental conditions is not as well understood (Rundle & Nosil, 2005; DeWitt, 2016). Environmental gradients may lead to a spatial pattern of corresponding phenotypic gradients (Doebeli & Dieckmann, 2002; Mullen & Hoekstra, 2008), however, intermediate conditions might also select for unique phenotypes (DeWitt, 2016). For example, in an intermediate habitat with a mix of resource types, a generalist phenotype that exploits multiple resource types may be more fit than a specialist phenotype that exploits one resource type well. Therefore, empirical studies of spatial population structure should consider any intermediate habitat types and their effects on spatial population structure in order to better understand how environmental heterogeneity contributes to spatial population structure.

Intermediate habitats can also sometimes be of lower quality or ‘marginal’ in terms of energy availability or predation risk. Populations in high quality habitat patches will tend to have positive growth rates and to produce migrants (sources) while those in marginal habitat patches will have negative growth rates, and only persist through immigration from high quality habitats (sinks) (Pulliam, 1988; Dias & Blondel, 1996). Local adaptation to marginal habitat patches will be difficult as the local population suffers a migration load as immigrants bring maladaptive genes from source populations (Bolnick & Nosil, 2007; Bolnick, Caldera & Matthews, 2008). This migration load degrades spatial population structure by homogenizing genetic differences between populations. The strength of migration load will depend on the connectivity between habitat patches, a topic I address in chapter two.

In this chapter I investigate how the exposed shoreline habitat may differ in local conditions and perhaps quality, and so contribute to spatial population structure in an ecologically and phenotypically diverse population of pumpkinseed sunfish. Clear phenotype-habitat associations exist between inshore shallow water (littoral) and offshore deep water (pelagic) habitats across replicate sunfish populations in low productivity post-glacial lakes (Robinson, Wilson & Margosian, 2000; Jastrebski & Robinson, 2004; Weese, Ferguson & Robinson, 2012). Typical
littoral habitat is shallow, sheltered, has soft sediments with considerable aquatic vegetation and high shoreline inputs of organic materials debris (Eklöv, 1997). It typically supports a high abundance of macroinvertebrate prey and a large microbial decomposer community (Eklöv, 1997). The pelagic habitat is deep open water where high productivity phytoplankton populations support abundant zooplankton, a prey resource for many pelagic fishes. Typically, the pelagic habitat has very little physical structure, but in some lakes, it can be interspersed with submerged rocky shoals that function similarly to reefs, providing shelter and access to pelagic resources for smaller fish species. In polyphenic populations of sunfish, ecological performance trade-offs appear to exist between ecotypes that specialize on prey from pelagic versus littoral habitats; zooplankton and benthic macroinvertebrates respectively (Robinson & Wilson, 1996; Colborne et al., 2016). There is continuous phenotypic variation in functional morphological traits with respect to body shape, gill raker density, and pharyngeal jaw size, but individuals at the extremes of this distribution are only found in their respective habitats (Robinson & Wilson, 1996; Gillespie & Fox, 2003; Colborne et al., 2016). These traits are functionally related to feeding performance, where individuals with more intermediate phenotypes (hereafter ‘intermediates’) perform worse than either specialist at feeding on their respective prey type in the lab (Parsons & Robinson, 2007), and contribute to reduced body condition in the field (Robinson et al. 1996). However, we know little about the ecology of intermediates in the wild, such as how much they may move among habitats, or whether the exposed shoreline is a marginal habitat that might contribute to within-population spatial structure in polyphenic sunfish.

In lakes with polyphenic sunfish populations, in addition to typical littoral and pelagic habitats sunfish are often present in a third habitat that we refer to as ‘exposed shorelines’. Exposed shoreline is nearshore habitat which may be lower quality because there is minimal shelter or vegetation, and it has a steep, rocky bottom that quickly drops off into deep pelagic habitat leaving only a thin band of rocky shoreline (Jastrebski & Robinson, 2004). In oligotrophic lakes, this habitat type spatially dominates the inshore littoral zone of the lake relative to sheltered littoral bays that are more sheltered and contain soft muddy substrates that support macrophyte primary productivity. Thus, productivity along exposed shorelines may be
significantly lower than in either pelagic or littoral habitats. Despite its proximity to the pelagic habitat, plankton sampling reveals that zooplankton are less abundant compared to further offshore in the pelagic habitat (unpublished data). Nevertheless, both littoral and pelagic prey may be available to fish in exposed shoreline habitat because of the spatial proximity to both shoreline and deep pelagic waters. If this is the case, sunfish that occupy exposed shorelines may experience a mix of littoral and pelagic conditions and prey resources. In addition, we do not know how a mix of environmental conditions may influence the local phenotypic variation and its contribution to spatial population structure at the scale of the whole lake.

In this chapter I addressed two general questions; 1) What phenotypes are associated with the exposed shoreline habitat in polyphenic populations of sunfish? 2) What is the condition of exposed shoreline sunfish relative to littoral and pelagic sunfish? To address my first question, I first tested the assumption that sunfish in exposed shoreline experience a mix of littoral and pelagic environmental conditions by quantifying sunfish diet relative to pelagic and littoral habitats using stomach content analysis. I then quantified phenotypic diversity within and among three lake habitats (littoral, exposed shoreline, and pelagic). I hypothesized that local prey availability structures phenotype distributions through performance trade-offs as in other studies comparing fish from littoral and pelagic habitats (Gillespie & Fox, 2003; Riopel, Robinson & Parsons, 2008; Colborne et al., 2016). If performance trade-offs are structuring phenotype distributions, then I predicted that sunfish occupying exposed shoreline habitat would have phenotypes intermediate between littoral and pelagic sunfish. To address my second question, I quantified body condition, a proxy for performance, of sunfish from exposed shoreline relative to pelagic and littoral habitats. By improving our understanding of how sunfish use exposed shoreline habitat relative to littoral and pelagic lake habitats, this study will lead to new hypotheses about how habitat heterogeneity contributes to spatial population structure and adaptive divergence in polyphenic populations of pumpkinseed sunfish.
1.2 Methods

1.2.1 Study Location

Ashby Lake, ON (45°05'N, 77° 21’W), is an oligotrophic Canadian Shield lake, with a surface area of 2.59 km$^2$ and a maximum depth of 36.6 m. It primarily consists of deep, open water pelagic habitat punctuated by islands and submerged rocky shoals. A narrow band of shallow shoreline habitat (‘exposed shoreline’; < 2 m depth, < 3 m from shore) encompasses 53% of the shallow lake area (<3m depth). Exposed shoreline typically has sand, gravel, or rocky substrate, is regularly exposed to wind-driven wave action that removes soft substrates and so supports minimal macrophyte vegetation. More productive littoral habitat with soft muddy substrate and more vegetation is restricted to sheltered bays (Jastrebski & Robinson, 2004). Submerged rocky shoals in the pelagic habitat are functionally similar to coral reefs, providing access to zooplankton prey, physical shelter from predators, and suitable sites for reproduction (Jastrebski & Robinson, 2004; Colborne et al., 2016). Pumpkinseed sunfish are found in all three habitats, and the population exhibits a littoral-pelagic polyphenism with divergent diets, morphology, and male reproductive behaviour between sunfish occupying littoral and pelagic habitats that is strongly influenced by plastic developmental responses (Jastrebski & Robinson, 2004; Berchtold et al., 2015; Colborne et al., 2016). However, the ecology of sunfish that occupy the extensive exposed shoreline habitat is unknown.

1.2.2 Sample Collection and Processing

I sampled adult sunfish by angling from 4 sites in summer 2017 over three collection trips (1$^{st}$: June 26$^{th}$ – 28$^{th}$; 2$^{nd}$: July 11$^{th}$; and 3$^{rd}$: August 23$^{rd}$ - 24$^{th}$). I sampled from one each of pelagic and littoral reference sites and two exposed shoreline sites ($n_{total} = 206$; Table 1.1; Fig. 1.1) because the littoral and pelagic biology is already well characterized (Robinson, Wilson & Margosian, 2000; Colborne et al., 2016). In the analyses below, I test for effects of site to evaluate variation among exposed shorelines relative to the reference littoral and pelagic sites. I euthanized sunfish and measured their wet weight (g), external oral jaw width (mm), and standard length (mm). Larger oral jaw size after adjusting for body size is related to greater littoral resource use (i.e. more benthic invertebrates), and so can distinguish habitat ecotypes (Jarvis et al., 2017) as well as potentially microhabitat use within habitats (Axelrod unpublished
results). I then took a photograph of the left-side of each individual for subsequent geometric morphometric analysis of body shape in relation to habitat use (Weese, Ferguson & Robinson, 2012). I then fixed sunfish in 10% buffered formalin for 24 hours before identifying sex and removing the stomach, which was stored in 70% ethanol for later diet analysis.

1.2.3 Geometric Morphometric Analysis

I only used sunfish collected post-reproduction in August for phenotypic analysis to reduce any effects of reproduction on traits. I digitized 17 homologous landmarks on the lateral images of sunfish using ImageJ (Fig. 1.2). I extracted centroid size from the landmark coordinates; a multidimensional measure of body size accounting for size variation in both \( x \) and \( y \) directions (R package ‘geomorph’, Adams & Otárola-Castillo, 2013). I then applied a generalized Procrustes analysis to removes the effects of scale, isometry, rotation, and position on shape from the landmark coordinates. I used Principle Component Analysis to visualize and identify major axes of shape variation for all individuals and sites combined. The first principle component primarily accounted for artificial variation in caudal position due to the freezing process and so was excluded from subsequent analyses. Principle components two and three each accounted for more than 10% of additional shape variation and so were included in subsequent analyses (PC2 = 16.7%; PC3 = 14.4%).

1.2.4 Sunfish Habitat Use, Diet, and Performance

Prey diversity and abundance differs between littoral and pelagic habitats, with large benthic invertebrates more common in inshore littoral habitat and zooplankton more common in the pelagic habitat (Robinson et al., 1993; Colborne et al., 2016). However, little is known about the diet of sunfish that occupy exposed shorelines. Exposed shoreline has limited soft sediments and vegetation ideal for large benthic invertebrates (Werner, Mittelbach & Hall, 1981), but nearby deep water may provide access to pelagic zooplankton (Keast & Welsh, 1968). I compared the diets of sunfish from exposed shoreline relative to littoral and pelagic habitats by evaluating the stomach contents among sunfish collected from all sites during the first two collection trips (June, July; Table 1.1). I identified the contents of each individual’s stomach under a dissection microscope and assessed the presence and abundance of large benthic
invertebrates (snails, adult insects, larval insects) and zooplankton (daphnia, copepods) relative to reference littoral and pelagic sites (Colborne et al., 2016). Parasites can indicate long-term habitat use when they have a specialized life cycle limited to hosts found predominantly in a particular habitat. I counted the number of trematode ‘blackspot’ parasites (*Neascus* spp.) encysted in the skin and fins of each individual (both sides) because these parasites primarily infest hosts with littoral-type diets (Robinson, Wilson & Margosian, 2000; Gillespie & Fox, 2003).

Habitats expose resident individuals to conditions that can consistently influence individual performance such that some habitats can be thought of as high or low quality. To assess the quality of exposed shoreline relative to reference littoral and pelagic habitat, I calculated individual body condition using the Scaled Mass Index, which has been shown to perform better than either mass to length ratios or residuals from an OLS regression (Peig & Green, 2010). Body condition is used as a proxy for individual fat reserves, and is thought to reflect relative performance in fishes in northern temperate lakes where fat reserves are critical for over-winter survival (Peig & Green, 2010).

1.2.5 Statistical Analysis

To assess the phenotype of sunfish from the exposed shoreline habitat, I compared mean jaw size and body shape (PC2 and PC3) among collection sites using ANCOVA accounting for covariation in body size (standard length, mm), and initially including an interaction between site and body size to allow for different allometric relationships between sites. There were no significant interactions between body size and site in any of these models (see results), so these interaction effects were removed from the final models. The effects of site and body size on parasite load were evaluated with a general linear model with a Poisson distribution and log link function for the count data (O’Hara & Kotze, 2010). I then used pairwise Student’s t-tests to compare trait least-squared means between sites after statistically accounting for any effect of body size. Mean body size differences between sites were tested with ANOVA. Exposed shoreline sunfish phenotypes were then characterized using a linear discriminant function using traits that differed between the littoral and pelagic reference sites (adjusted jaw size, body size,
parasite load, PC2, and PC3) to classify exposed shoreline sunfish as more littoral or more pelagic. The linear discriminant function was trained using trait data from the reference littoral and pelagic sites, and then applied to exposed shoreline sunfish. I then used exact binomial tests of the proportions classified as pelagic or littoral for each exposed shoreline site. Lastly, I compared mean linear discriminant axis 1 scores using ANOVA.

To assess sunfish diets in exposed shoreline in June and July, I analyzed the contribution of littoral-benthic prey and pelagic prey separately. To assess the contribution of littoral-benthic prey to sunfish diets, I used pairwise Barnard’s Tests to compare the presence/absences of insects in sunfish stomachs among collection sites. To assess the contribution of pelagic prey to sunfish diets, I used a general linear model with a Poisson distribution and log link function to compare the number of *Daphnia magna* in sunfish stomachs among collection sites (O’Hara & Kotze, 2010).

To assess sunfish performance in exposed shoreline habitat relative to littoral and pelagic habitats, I tested for differences in body condition among littoral, pelagic, and exposed shoreline sites using ANCOVA while accounting for sex and body size. Lastly, to determine if any traits that differed between reference littoral and pelagic sites were related to performance, I used a general linear model to evaluate whether body condition was related to oral jaw size, body size, PC2, PC3, sex, parasite load, site, and 2-way interaction effects of each parameter with site. PC2, parasite load, and all 2-way interactions were not significant and so were dropped from the final model. All analyses were performed in R 3.5.0. or JMP Pro 13 (R Development Core Team, 2018; SAS, 2018).

1.3 Results

1.3.1 Patterns of body shape and size variation

Shape principle components 2 and 3 where included in subsequent analysis because they each accounted for more than 10% of total shape variation (PC2 = 16.7%; PC3 = 14.4%). PC2 expressed variation in body depth (deep vs. elongated), head length, and the orientation of the premaxilla jaw bone (landmarks 1-11; Fig. 1.2) relative to the rest of the head (Fig. 1.3). The
orientation of the premaxilla jaw bone reflects how the mouth opens and is typically related to foraging strategy; for example, a more terminal mouth opens directly forward, while a more superior mouth opens facing upward and a subterminal mouth opens facing downwards (Keast & Webb, 1966). Here, lower scores on the PC2 axis indicate individuals with relatively deeper bodies, shorter heads, and a more terminal mouth position (Fig. 1.3). PC3 expressed variation in the length of the premaxilla jaw bone (landmarks 1-11; Fig. 1.2) relative to body size (Fig. 1.3), which may be related to gape width (Keast & Webb, 1966). Here, lower scores on the PC3 axis indicate individuals with relatively larger heads and longer premaxilla (larger mouths) (Fig. 1.3).

To determine if standard length was a suitable measure of body size, I assessed the correlation between centroid size and standard length (mm) with a Pearson’s correlation test. They were highly correlated ($r = 0.99$, $t_{129} = 96.12$), so I used standard length as my measure of body size in all further analyses for simplicity. There was a positive effect of body size on oral jaw width ($F_{1, 126} = 755.88$, $p < 0.0001$; Fig. 1.4) and a negative effect on PC2 scores ($F_{1, 126} = 193.54$, $p < 0.0001$; Fig. 1.5). There was also weak evidence of a negative effect of body size on PC3 scores ($F_{1, 126} = 3.81$, $p = 0.053$; Fig. 1.6). There was no evidence of differences in allometry between collection sites for any of these traits (all site x standard length interactions: $p > 0.17$).

1.3.2 Sunfish Phenotype and Habitat Use

Sunfish compared between the littoral and pelagic reference sites had greater phenotypic differences than any other possible site contrast (Table 1.2) and differed in a pattern consistent with prior studies of polyphenic sunfish populations (Robinson, Wilson & Margosian, 2000; Riopel, Robinson & Parsons, 2008; Berchtold et al., 2015). Pelagic sunfish were on average larger, had narrower oral jaws, lower PC2 shape scores, higher PC3 shape scores, and had lower parasite loads than sunfish sampled from the littoral habitat (Table 1.2). Sunfish sampled from exposed shorelines were not consistently intermediate between the littoral and pelagic reference samples (Table 1.2). For example, sunfish mean standard length differed between the two exposed shoreline samples; exposed shoreline site 2 sunfish were the same size as pelagic sunfish ($t_{127} = -0.22$, $p = 0.82$; Fig. 1.6) while exposed shoreline site 1 sunfish were the same size as littoral sunfish ($t_{127} = -0.37$, $p = 0.71$; Fig 1.7).
External oral jaw width was larger (after adjusting for body size) for sunfish collected from the reference littoral site compared to the reference pelagic site ($t_{126} = 6.81, p < 0.0001$; Fig. 1.4). Sunfish jaw width also differed between exposed shorelines (Table 1.2); exposed shoreline 2 sunfish had narrower jaws than the littoral reference samples ($t_{126} = -3.28, p = 0.0014$; Fig. 1.4) and wider jaws than the pelagic reference samples ($t_{126} = 3.59, p = 0.0005$; Fig. 1.4), while exposed shoreline 1 sunfish were not different from the littoral sample ($t_{126} = -1.66, p = 0.099$; Fig. 1.4).

Body shape traits also differed between collection sites (Table 1.2). After accounting for body size, sunfish sampled from the pelagic habitat had lower principle component 2 scores (deeper bodies, shorter heads, and more terminal mouths) than the reference littoral sample and both samples of exposed shoreline sunfish ($t_{126} = -3.41, p < 0.0001$; Fig. 1.5). Sunfish sampled from the pelagic habitat had higher principle component 3 scores (smaller head size and premaxilla jaw bone) than littoral sunfish ($t_{126} = 4.27, p < 0.0001$; Fig. 1.6). PC3 scores also differed between the exposed shoreline samples. Sunfish from exposed shoreline 1 were not different from the reference pelagic sample ($t_{126} = 1.20, p = 0.23$; Fig. 1.6) whereas fish from exposed shoreline site 2 were not different from the reference littoral site ($t_{126} = -0.57, p = 0.57$; Fig. 1.6). Oral jaw width and PC3 were significantly correlated with each other (Pearson’s $R = -0.30, t_{129} = -3.63, p = 0.0004$). Sunfish with larger oral jaw widths tended to have longer premaxilla and larger heads.

Parasite load increased with body size and differed among collection sites ($\chi^2_{(3, 126)} = 515.32, p = < 0.0001$; Fig. 1.8). There was a significant interaction effect between body size and collection site ($\chi^2_{(3, 126)} = 595.41, p = < 0.0001$). Sunfish from the reference littoral site had higher parasite loads than sunfish from the reference pelagic site ($t_{126} = 556.6, p < 0.0001$; Fig. 1.8) as found previously (Robinson, Wilson & Margosian, 2000). Sunfish sampled from the two exposed shoreline sites also differed in parasite load. Sunfish sampled from exposed shoreline 2 site had high parasite loads similar to those from the littoral reference sample, whereas sunfish
from exposed shoreline 1 had low parasite loads similar to fish sampled from the pelagic reference sample (Fig. 1.8).

The linear discriminant function classified more exposed shoreline 1 sunfish as littoral than pelagic (prop. = 0.70, \(p = 0.035\); Fig. 1.9), while it classified exposed shoreline 2 sunfish as littoral or pelagic equally (prop. = 0.56, \(p = 0.60\); Fig. 1.9). Sunfish from exposed shoreline 1 were not significantly different from littoral sunfish in LD1 scores (\(t_{127} = -1.46, p = 0.15\); Fig. 1.10), whereas sunfish from exposed shoreline 2 were intermediate in LD1 scores (higher than the reference littoral site: \(t_{127} = 3.67, p = 0.0004\), and lower than the reference pelagic site: \(t_{127} = -5.29, p < 0.0001\); Fig. 1.10). The predictor variables in order of strongest to weakest loading on LD1 were adjusted jaw size, PC2, PC3, body size, parasite load (Table 1.3), although all traits other than parasite load were highly correlated with LD1.

1.3.3 Sunfish Diet

Sunfish from the reference littoral site had a slightly higher proportion of insects in their stomach than did pelagic sunfish (\(z = -1.91, p = 0.07\); Fig. 1.11). An intermediate proportion of exposed shoreline sunfish had insects in their stomachs as both sites were not different from littoral (\(p > 0.1\); Fig. 1.11) or pelagic sites (\(p > 0.1\); Fig 1.11). Primarily, I identified dragonfly and damselfly larvae (Order: Odonata), chironomid larvae (Order: Diptera), adult beetles (Order: Coleoptera), and adult ants (Order: Hymenoptera). I found that pelagic sunfish had higher numbers of *Daphnia magna* in their stomachs than littoral sunfish (\(t_{19} = 3.82, p = 0.0003\); Fig. 1.12). Sunfish from exposed shoreline site 2 also had high numbers of *Daphnia magna* in their stomachs like pelagic sunfish (\(t_{19} = 1.49, p = 0.14\); Fig. 1.12), while sunfish from exposed shoreline site 1 had low numbers of *Daphnia magna* in their stomachs like littoral sunfish (\(t_{15} = -0.06, p = 0.95\); Fig. 1.12).

1.3.4 Sunfish Performance

Body condition did not vary significantly with standard length, nor did it differ among sites (\(F_{3, 126} = 1.73, p = 0.17\); Fig. 1.13), suggesting that sunfish in the different habitats had equivalent energy reserves in August. Combining sunfish from all four sites, however, revealed
that higher body condition was related to oral jaw width and body shape variation on PC3. Individuals with higher body condition tended to have larger oral jaws ($t_{127} = 2.85, p = 0.0051$), and larger heads and premaxilla bones (PC3: $t_{127} = -3.76, p = 0.00026$).

1.4 Discussion

There was considerable phenotypic variation among sunfish from different lake habitats suggesting that phenotypic variation in this population is spatially structured by habitat type, as found in previous studies of polyphenic sunfish (Robinson, Wilson & Margosian, 2000; Berchtold et al., 2015; Colborne et al., 2016). Littoral and pelagic sunfish were phenotypically and ecologically differentiated in a pattern consistent with previous studies of polyphenic sunfish; pelagic sunfish had deeper bodies, smaller jaws (narrower oral jaw width and shorter premaxilla), and a more terminal mouth than littoral sunfish, as well as having more insects and less zooplankton in their diet. This study expands on those prior findings by considering the effect of exposed shoreline habitat on the pattern of spatially structured phenotypic variation. Sunfish from exposed shorelines had oral jaw widths and shape PC2 scores more similar to sunfish from the reference littoral than from the reference pelagic site. Whereas, variation in body size and shape PC3 scores indicate phenotypic differences between sunfish from different exposed shoreline sites. Sunfish habitat use was similarly divergent; exposed shoreline 1 sunfish had low parasite loads like pelagic fish while exposed shoreline 2 sunfish had high parasite loads like littoral fish. Exposed shoreline sunfish diets were intermediate between littoral and pelagic sunfish with respect to macroinvertebrate consumption, but sunfish from exposed shoreline sites differed in their consumption of zooplankton. There was no evidence that patterns of phenotype-habitat association or parasite load translated into differences in body condition, at least in August. This suggests either that exposed shorelines are not a low-quality habitat with respect to foraging or that sunfish there are temporary residents that acquire energy elsewhere. However, if body size is related to fitness in sunfish, then more pelagic sites may be superior habitats in Ashby lake.

When assessing these traits in combination with discriminant function analysis, one exposed shoreline site studied here (ES1) had sunfish with phenotypes that were classified as
more littoral, while sunfish from the other exposed shoreline site (ES2) were slightly more intermediate between littoral and pelagic phenotypes. ES1 sunfish did not differ from littoral sunfish for any of these traits except for head size and premaxilla length (PC3 scores), where they were more like pelagic sunfish. ES2 sunfish were like littoral sunfish with respect to body shape but were intermediate between littoral and pelagic sunfish with respect to mean jaw size, and more like pelagic sunfish in mean body size. So, it appears that exposed shoreline sunfish are similar to littoral sunfish for most traits but that there is substantial phenotypic variation among exposed shoreline sites so that at some sites, sunfish are intermediate between littoral and pelagic ecotypes.

Sunfish among exposed shoreline sites also differed in their long-term habitat use as they had different parasite loads. Exposed shoreline 1 sunfish had lower parasites loads than littoral sunfish, while exposed shoreline 2 sunfish had similar parasite loads to littoral sunfish. In these populations trematode ‘blackspot’ parasites (*Neascus* spp.) can indicate long-term habitat use as they have a specialized life cycle limited to snail hosts found predominantly in the littoral habitat (Robinson, Wilson & Margosian, 2000). Consistent with this, littoral sunfish had higher parasite loads than pelagic sunfish here. The difference in parasite load suggests greater dietary variation among sunfish from exposed shoreline habitat.

There was dietary variation among sunfish from exposed shoreline habitat in terms of zooplankton consumption, but not macroinvertebrate consumption. Sunfish from the pelagic reference site and exposed shoreline site 2 had more *Daphnia magna* in their stomachs on average than sunfish from the littoral site or exposed shoreline site 1. More littoral sunfish had insects in their stomachs than did pelagic sunfish, while an intermediate number of exposed shoreline sunfish from both sites had insects in their stomachs. The variation in diet among exposed shoreline sites may explain the variation in phenotype. Sunfish from exposed shoreline site 2 had more pelagic phenotypes than sunfish from exposed shoreline site 1, which is consistent with their diet consisting of more zooplankton. A goal moving forward will be to test for relationships between individual diet and phenotype.
There was no difference in body condition among habitat types which suggests exposed shoreline is not lower in energetic quality than littoral or pelagic habitats. However, it may be the case that exposed shoreline supports lower densities of sunfish than either littoral or pelagic habitat, and the sunfish that do occupy exposed shoreline have equal relative performance because they face less competition for resources. If exposed shoreline habitat supports lower densities of sunfish, this will have implications for local adaptation as the sunfish there may be subject to a migration load (Bolnick & Nosil, 2007; Bolnick, Caldera & Matthews, 2008). Migration loads degrade spatial population structure by homogenizing genetic differences between populations as immigrants introduce maladaptive genes. It is important to note that I only looked at sunfish body condition in August, which is post-spawning. Reproduction has a high energetic cost for both male and female sunfish (Danylchuk & Fox, 1994; Rios-Cardenas & Webster, 2005), which might put all sunfish at low body condition regardless of habitat. However, body size has been linked to reproductive success for the related bluegill sunfish (*Lepomis macrochirus*) (Cargnelli & Neff, 2006) and so may be a better indicator of long term fitness. If this is the case, then more pelagic habitats may represent higher quality habitat for sunfish.

Jaw size (oral jaw width and premaxilla length) and relative head size were related to body condition across all habitats, which suggests that these traits functionally enhance sunfish performance across all habitat types. Jaw size limits the upper size limit of accessible prey, so larger jaws should increase the prey resources available to an individual (Wainwright, 1991). Larger head size may be related to performance in a variety of ways. For example, it may reflect an expanded internal pharyngeal apparatus; either larger pharyngeal jaws which allow crushing hard prey items (Wainwright, 1991) or more robust and/or more numerous gill rakers which allow feeding on zooplankton (Sanderson, Cech & Patterson, 1991; Schluter, 1993).

The phenotypic variation between exposed shoreline sites may result from several processes. Sunfish in these populations are highly plastic (Robinson & Wilson, 1996; Parsons & Robinson, 2006) and exposed shoreline habitat likely has some mix of littoral and pelagic environmental cues. If sunfish in exposed shoreline have high site fidelity (McCairns & Fox, 2007).
2004), then variation in the strength of these cues among exposed shoreline sites could generate variation in plastic responses (DeWitt, 2016). It would be useful to differentiate which environmental cues trigger each phenotypic trait examined here, and then compare cues across sites to test if variation in cues corresponds to phenotype-habitat patterns found here. Phenotypic variation between exposed shoreline sites could also reflect different rates of immigration from littoral and pelagic habitats, but we have evidence that movement to and between shoreline habitat patches is very low compared to among pelagic patches for example (see chapter 2). Lastly, phenotypic variation between exposed shoreline sites could reflect evolutionary responses to selection. Despite high plasticity in these populations, there is also evidence of genetic differences in body shape and in plastic responses (Robinson & Wilson, 1996; Parsons & Robinson, 2006). However, it remains unclear what environmental features would generate diversifying selection between exposed shoreline sites. Regardless, the high phenotypic variation between exposed shoreline sites makes the spatial pattern of population structure more complex than previously understood because this habitat covers roughly 50% of the shallow littoral habitat in this lake.

Across species, there is considerable variation in the extent of phenotypic and genetic divergence within adaptively diverging populations, ranging from continuous phenotypic variation in panmictic populations to discrete, reproductively isolated species pairs (Hendry, 2009; Nosil, Harmon & Seehausen, 2009). The capacity for adaptive genetic divergence is thought to reflect the balance between gene flow and diversifying selection (Lenormand, 2002; Hendry & Taylor, 2004; Nosil & Crespi, 2004). However, the role of intermediate or marginal habitats in facilitating or constraining adaptive divergence is frequently overlooked. Here we have shown that considering intermediate habitats as a homogenous group can be oversimplifying patterns of spatial population structure, as there may be substantial variation in phenotype distributions between sites. Considering intermediate habitats as a spectrum of environmental conditions between two high quality habitats will reveal more about how environmental heterogeneity generates spatial population structure in a given system.
The next steps are to clarify the proximate mechanisms leading to phenotypic variation among exposed shoreline habitat, determine whether exposed shoreline actually supports lower densities of sunfish, and determine whether exposed shoreline provides opportunities for movement between littoral and pelagic habitat (see chapter 2). We can begin to address the first uncertainty by assessing sunfish diets; specifically, whether sunfish maintain consistently generalist or specialist diets across habitat types. A more generalist diet is expected to generate more intermediate plastic responses (Parsons & Robinson, 2007; DeWitt, 2016) and may account for the phenotypic variation between exposed shoreline sites seen here. To address the second uncertainty, we need to do conduct a mark-recapture study focused on estimating relative sunfish densities between habitats. If exposed shoreline does support lower sunfish densities, this could result in a migration load limiting local adaptation. In chapter 2 I investigate sunfish movement patterns within and among habitats, and how this influences spatial population structure.

Sunfish from exposed shoreline habitat are phenotypically more similar to littoral sunfish than pelagic, but there is high phenotypic variation among exposed shoreline sites. Sunfish also differed among exposed shoreline sites in their habitat use and diet as they had different parasite loads and reliance on zooplankton prey, however there was no evidence that exposed shoreline sunfish have lower performance than those inhabiting the littoral and pelagic habitats, at least with respect to August body condition. Key uncertainties remain about the proximate mechanisms leading to within habitat phenotypic variation, whether exposed shoreline supports lower sunfish densities, and whether exposed shoreline provides opportunities for sunfish movement. This study expands our understanding of how habitat heterogeneity contributes to spatial population structure in polyphenic sunfish populations, as exposed shoreline habitat appears to increase the complexity of spatial structuring over littoral and pelagic habitat alone. Considering intermediate habitats as having a spectrum of environmental conditions between higher quality habitats will reveal more about patterns of spatial population structure than considering intermediate habitats as a homogenous group.
1.5 Tables

<table>
<thead>
<tr>
<th>Site</th>
<th>Date</th>
<th>June 26th – 28th</th>
<th>July 11th</th>
<th>August 23rd – 24th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Littoral</td>
<td>10</td>
<td>10</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Exposed Shoreline 1</td>
<td>6</td>
<td>10</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Exposed Shoreline 2</td>
<td>10</td>
<td>10</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Pelagic</td>
<td>10</td>
<td>10</td>
<td>34</td>
<td></td>
</tr>
</tbody>
</table>

*Table 1.1* Summary of sunfish sample sizes for each collection site across the three collection trips.

<table>
<thead>
<tr>
<th>Trait</th>
<th>$\chi^2$</th>
<th>F</th>
<th>df</th>
<th>p</th>
<th>Littoral</th>
<th>ES1</th>
<th>ES2</th>
<th>Pelagic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jaw Size</td>
<td>n/a</td>
<td>16.61</td>
<td>3, 126</td>
<td>&lt; 0.0001</td>
<td>10.79$^A$</td>
<td>10.54$^A$</td>
<td>10.26$^B$</td>
<td>9.76$^C$</td>
</tr>
<tr>
<td>PC2</td>
<td>n/a</td>
<td>6.45</td>
<td>3, 126</td>
<td>0.0004</td>
<td>0.0017$^A$</td>
<td>0.0017$^A$</td>
<td>0.0023$^A$</td>
<td>0.0053$^B$</td>
</tr>
<tr>
<td>PC3</td>
<td>n/a</td>
<td>11.84</td>
<td>3, 126</td>
<td>&lt; 0.0001</td>
<td>-0.0049$^A$</td>
<td>0.0034$^B$</td>
<td>-0.0065$^A$</td>
<td>0.0067$^B$</td>
</tr>
<tr>
<td>Body Size</td>
<td>n/a</td>
<td>7.44</td>
<td>3, 127</td>
<td>0.00013</td>
<td>89.17$^A$</td>
<td>88.00$^A$</td>
<td>99.22$^B$</td>
<td>98.53$^B$</td>
</tr>
<tr>
<td>Parasite Load</td>
<td>515.32</td>
<td>n/a</td>
<td>3, 126</td>
<td>&lt; 0.0001</td>
<td>0.51$^A$</td>
<td>-0.42$^B$</td>
<td>0.44$^A$</td>
<td>-0.69$^B$</td>
</tr>
</tbody>
</table>

*Table 1.2* Summary of the effect of site on sunfish traits and the least squared means or actual means (body size only) for each trait among collection sites (where ES refers to exposed shoreline site 1 and 2). Different letters indicate significantly different contrasts between sites from Student’s t-tests of least squared means (jaw size, PC2, PC3, parasite load) or actual means (body size).

<table>
<thead>
<tr>
<th>Trait</th>
<th>LD1 Loading</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasite Load</td>
<td>0.100</td>
</tr>
<tr>
<td>Adjusted Jaw Size</td>
<td>0.775</td>
</tr>
<tr>
<td>PC2</td>
<td>0.683</td>
</tr>
<tr>
<td>PC3</td>
<td>-0.582</td>
</tr>
<tr>
<td>Body Size</td>
<td>-0.520</td>
</tr>
</tbody>
</table>

*Table 1.3* The canonical loadings (correlation) on the first linear discriminant axes for each predictor variable used in the linear discriminant analysis. The linear discriminant function was trained with phenotype data from littoral and pelagic sunfish, and then was used to classify exposed shoreline sunfish as littoral or pelagic ecotypes.
1.6 Figures

Figure 1.1 Map of Ashby Lake, ON indicating collection sites; littoral (Lit) and pelagic (Pel) reference sites, exposed shoreline site 1 (ES1), and exposed shoreline site 2 (ES2).
Figure 1.2 The locations of 17 homologous landmarks used for geometric morphometric analysis.
Figure 1.3 Deformations grids visualizing whole body shape variation along principle component axes (head is facing to the right). Shown here are the individuals with the maximum and minimum principle component score for each axis. PC2 expressed variation in body depth, head length, and the orientation of the premaxilla jaw bone relative to the rest of the head. PC3 expressed variation in relative head size and the length of the premaxilla jaw bone relative to total head size.
Figure 1.4 Relationships between oral jaw width and body size by collection site. Sunfish collected from littoral and pelagic habitats had the largest and smallest jaw width respectively, after accounting for body size. Sunfish from exposed shorelines were intermediate between littoral and pelagic reference sunfish (ES2) or not different from littoral reference sunfish (ES1). The slope of the relationship between oral jaw width and body size did not differ between sites.
Figure 1.5 Relationships between shape principle component 2 and body size by collection site. Sunfish collected from pelagic habitat had relatively deeper bodies, shorter heads, and more terminal mouths than sunfish collected from littoral or exposed shoreline (ES) habitat after accounting for body size. There was a significant negative relationship between PC2 and body size; increasing body size was related to deeper bodies, shorter heads, and more terminal mouths. Letters indicate significantly different contrasts from pairwise Student’s t-tests of least squared means.
Figure 1.6 Relationships between shape principle component 3 and body size by collection site. Sunfish collected from littoral habitat and exposed shoreline 2 (ES2) had larger heads and longer premaxilla jaw bones than those from pelagic or exposed shoreline 1 (ES1) after accounting for body size. There was a weak relationship between PC3 and body size. Letters indicate significantly different contrasts from pairwise Student’s t-tests of least squared means.
Figure 1.7 Boxplots of sunfish body size by collection site. Boxes show the mean value, and the upper (75th) and lower (25th) quartiles. Sunfish from pelagic and exposed shoreline 2 sites were larger than those from exposed shoreline 1 and littoral. Letters indicate significantly different contrasts from pairwise Student’s t-tests of means.
Figure 1.8 The effect of body size and collection habitat on the number of external blackspot parasites. Sunfish from littoral and exposed shoreline 2 tended to have higher parasite loads than exposed shoreline 1 and pelagic sunfish. There was a positive effect of body size on parasite load across all collection sites. Letters indicate significantly different contrasts from pairwise Student’s t-tests of least squared means.
Figure 1.9 Classifications of sunfish from exposed shoreline sites as either littoral or pelagic ecotypes based on the linear discriminant function. The linear discriminant function was trained with phenotype data from littoral and pelagic sunfish, and then used to classify exposed shoreline sunfish as littoral or pelagic ecotypes. The linear discriminant function predicted collection site using a linear weighted combination of adjusted jaw size, body size, PC2, PC3, and parasite load (see Table 1.3).
Figure 1.10 Boxplots of the linear discriminant axis scores by collection site. The linear discriminant function was trained with phenotype data from littoral and pelagic sunfish, and then used to classify exposed shoreline sunfish as littoral or pelagic. The linear discriminant function predicted collection site using a linear weighted combination of adjusted jaw size, body size, PC2, PC3, and parasite load (Table 1.3). The discriminant function correctly classified 89% of the littoral and pelagic sunfish to their collection habitat. Letters indicate significantly different contrasts from pairwise t-tests.
Figure 1.11 The proportion of sunfish with insects present in their stomach across collection sites. More littoral sunfish had insects in their stomachs than did pelagic sunfish, while an intermediate number of exposed shoreline sunfish had insects in their stomachs.
Figure 1.12 Boxplots of the number of *Daphnia magna* found in each sunfish stomach across collection sites. Sunfish from the pelagic site and exposed shoreline site 2 had more *Daphnia magna* in their stomachs on average than sunfish from the littoral site or exposed shoreline site 1.
Figure 1.13 Relationships between body condition and body size by collection site. There is no difference in body condition between collection sites, and no relationship with body size.
Chapter 2: Connectivity and Spatial Population Structure

2.1 Introduction

Individual movement has profound effects on both population demography and evolutionary dynamics, and so it is critical to understand when and why individuals move between habitat patches. Connectivity, a function of the physical properties of paths between habitat patches and the dispersal capacity of individuals, links patches demographically and genetically through the movement of individuals and their genes (Hanski, 2012). In turn, these links influence the vital rates of populations like reproduction and survival (Hanski et al., 1995; Gonzalez et al., 1998; Gilbert, Gonzalez & Evans-Freke, 1998). By facilitating gene flow, connectivity can also influence several evolutionary processes. Depending on the level of gene flow, connectivity can limit local adaptation (Lenormand, 2002; Hendry & Taylor, 2004; Nosil & Crespi, 2004), reduce inbreeding depression (Saccheri et al., 1998), or intensify the opportunity for selection (Kirkpatrick, 2000; Nosil, Crespi & Sandoval, 2003). Despite its significance for population and evolutionary biology, there is a lot we still do not know about how connectivity operates and influences spatial population structure in natural settings under various scenarios of environmental heterogeneity (Harrison, 1999; Hanski, 2012). In this chapter, I investigated how connectivity operates and might influence spatial population structure in an ecologically and phenotypically diverse (‘polyphenic’) population of pumpkinseed sunfish (Centrarchidae: Lepomis gibbosus).

Connectivity strongly influences spatial population structure, the geographic distribution of phenotype-habitat associations found both within and among natural populations. In heterogeneous environments, habitat is often patchily distributed with some number of functional pathways between patches that facilitate connectivity (Cowie & Krebs, 1979; McNamara, 1982). The physical characteristics of pathways (e.g. distance, permeability) and the dispersal capacities of individuals determines the level of connectivity, a measure of the likelihood of exchange of individuals between habitat patches (Hanski, 2001). Pathways can be thought of as habitat where individuals can survive in transit, but incur some cost (energy expenditure, predation risk) that make them unsuitable for long term survival and reproduction (Gilbert, Gonzalez & Evans-
Freke, 1998). Pathways connecting habitat patches with different locally adaptive phenotypes allow individuals to move between patches, altering the spatial distribution of phenotypes (Hanski, 2012). There are many reasons why an individual might leave a habitat patch, including avoiding local competition, poor local habitat quality, phenotype-habitat mismatch (Edelaar, Siepielski & Clobert, 2008; Bolnick et al., 2009), or due to a passive dispersal life-history strategy (Bowler & Benton, 2004; Edelaar, Siepielski & Clobert, 2008). The mechanism underlying individual movement will determine how connectivity influences spatial population structure.

Local adaptation to different habitat patches reflects the balance between two contrasting processes: diversifying selection and gene flow (Lenormand, 2002; Hendry & Taylor, 2004; Nosil & Crespi, 2004). Diversifying selection can lead to the accumulation of genetic differences between groups in different habitats, while gene flow homogenizes genetic differences by exchanging genes between groups. Gene flow consists of two main events: the successful movement of organisms between populations (i.e. connectivity), and the successful establishment of new genes into the population through survival and sexual reproduction after movement is complete. Since connectivity facilitates the movement of genes between populations, it plays a key role in local adaptation.

Connectivity can have a particularly strong influence on spatial population structure when large differences in quality exist between habitat patches, resulting in source-sink population dynamics in extreme cases. High quality habitat patches with larger carrying capacities become net exporters of migrants (sources) while low quality or ‘marginal’ habitat patches become importers of migrants (sinks) (Pulliam, 1988; Dias, 1996). Populations in sink habitats cannot evolve local adaptation to these patches because they often suffer a migration load where immigrants from source populations introduce non-adaptive alleles (Bolnick & Nosil, 2007; Bolnick, Caldera & Matthews, 2008). For example, local adaptation by *Timema cristinae* walking stick insects to habitat patches with different colour backgrounds is prevented by large neighboring habitat patches that produce many migrants (Sandoval, 1994; Nosil & Crespi, 2004).
In this case, high connectivity impedes local adaptation and the associated development of spatial population structure.

Nevertheless, local adaptation to habitat patches can take place under high connectivity scenarios when individuals can choose their habitat based on ‘phenotype match’, the adaptive fit between a phenotype and the local environment (Kawecki, 1995; Edelaar, Siepielski & Clobert, 2008). Theory indicates that this is because matching habitat choice generates a positive feedback between local adaptation and reduced migration, eventually leading to genetic divergence between populations in different habitat patches (Kawecki, 1995; Ravigne, Dieckmann & Olivieri, 2009; Scheiner, 2016). Traits that facilitate matching habitat choice can be favoured by natural selection when mismatches between phenotypes and the local habitat incurs fitness costs, and individuals can assess multiple habitat types (Edelaar, Siepielski & Clobert, 2008). It is therefore important to test hypotheses about why individuals choose to remain or leave a local patch when studying connectivity, as this will determine the effect of connectivity on spatial population structure.

Despite connectivity being critical to the formation of spatial population structure, there are few case studies seeking to understand the factors that govern connectivity in natural settings (Hanski, 2012). Here, I investigated connectivity and reasons for individual movement in a polyphenic population of pumpkinseed sunfish. In lakes with polyphenic sunfish populations, there is substantial spatial environmental variation between habitat patches, both in the physical characteristics and available prey (Jastrebski & Robinson, 2004). Sunfish in these populations primarily exploit littoral (shallow, near-shore) or pelagic (open-water) habitat types; littoral habitat is typically shallow, sheltered, densely vegetated and host to a wide array of benthic macroinvertebrate prey, while pelagic habitat is a deep, open water environment punctuated by islands and submerged rocky shoals where zooplankton are the primary prey available. Sunfish also occupy exposed shoreline habitat, but we now have evidence that exposed shoreline sunfish are more similar to littoral sunfish than pelagic with respect to habitat use and phenotype (see chapter 1). Phenotypic mismatch with habitat type likely incurs a fitness cost through reduced foraging performance (Parsons & Robinson, 2007), which is indicated by the reduced body
condition (a proxy for fat reserves) of individuals with mismatched phenotypes in littoral and pelagic habitats (Robinson & Wilson, 1996; Colborne et al., 2016). A key trait that associated with littoral and pelagic habitat types is jaw width; larger jaw widths are linked to increased foraging performance on benthic macroinvertebrates like snails (Wainwright, 1991; Robinson et al., 1993). While we can see clear patterns of spatial population structure in these populations, we know little about the patterns of connectivity between habitat patches and how this might influence spatial population structure.

Adult sunfish have strong habitat patch fidelity during the annual summer growing season (McCairns & Fox, 2004) but it is unknown if this extends between years as sunfish migrate between their over-wintering habitat in deeper waters and summer growing season habitats. For example, a mark-recapture study of sunfish movement during the summer growing season found 97% and 98% probability of site fidelity for pelagic and littoral sunfish, respectively (McCairns & Fox, 2004). Additionally, among sunfish that were experimentally transferred between habitat patches, there was respectively a 74% and 93% probability of returning to their site of capture for pelagic and littoral individuals, indicating greater habitat fidelity by littoral origin sunfish compared to pelagic origin sunfish (McCairns & Fox, 2004). However, movement can also occur over longer annual time scales as this species can live up to 10 years in the wild. Pumpkinseed sunfish leave summer habitat patches during the fall, presumably to overwinter in deeper waters, and return to them in the spring (personal observation), but the probability of returning to the same or to a different habitat patch between years is unknown. If adult sunfish change habitat or patches between years, then one possible explanation is that they do so to minimize costs of phenotypic mismatch. Alternatively, movement among habitat patches between years may not be governed by matching habitat choice but instead be a response to density-dependent processes such as resource competition (Bowler & Benton, 2004).

Within season patch fidelity will reduce gene flow between populations in littoral and pelagic habitats to some extent, and additionally there is some assortative mating in these populations (Colborne et al., 2016; Jarvis et al., 2017). Despite these barriers to gene flow, measurable genetic differentiation at neutral loci is low or non-existent in polyphenic populations
of pumpkinseed sunfish, which indicates substantial gene flow with respect to neutral alleles (Weese, Ferguson & Robinson, 2012; Colborne et al., 2016). This would suggest that there is a) movement between habitats, b) survival of immigrants, and c) reproduction with immigrants occurring. Genetic divergence between ecotypes at loci for traits with strong fitness consequences is possible, but this has not yet been explored.

In this chapter, I asked two general questions; 1) What is the connectivity between littoral and pelagic habitat patches, and among patches within habitat types, between years? 2) Is individual movement predicted by phenotype, condition, or local patch population density? I addressed the first question by assessing adult movement between years using a mark-recapture study. Based on the high level of gene flow indicated by measurements of genetic divergence at neutral markers, I predicted high rates of movement both between patches within habitat types and between littoral and pelagic habitats. I addressed the second question by testing whether phenotypic traits measured prior to tagging or local population density better predicted the probability of movement between years. I hypothesized that individuals with a mismatched phenotype for their current habitat patch are more likely to switch patch to increase their phenotypic match and limit the performance costs of mismatch. If this is true, I predicted that individuals with low residual jaw width (mismatched) and low body condition in littoral habitat would be more likely to switch to pelagic habitat, and vice versa. If movement is to avoid competitive interactions, I predicted that individuals facing higher local population density would be more likely to move between habitat patches to mitigate the costs of competition. This work will increase our understanding of how connectivity might contribute to spatial population structure in polyphenic sunfish, as well as provide an empirical test of the effect of connectivity in a natural setting.
2.2 Methods

2.2.1 Study Location

See chapter 1

2.2.2 Marking

To determine how much movement occurs between and within littoral and pelagic habitat patches between years, I employed a mark-recapture study of sunfish from summer 2016 to summer 2017. Between June 24\textsuperscript{th} and July 19\textsuperscript{th}, 2016, we collected 517 sunfish from littoral (L) and 468 from pelagic (P) habitats in Ashby Lake through a combination of angling (L: \(n = 235\); P: \(n = 209\)) and trapping (L: \(n = 281\); P: \(n = 260\)) to minimize behavioural bias. We sampled 12 pelagic and 17 littoral sites from across the northern basin of Ashby Lake (approximately \(\frac{1}{4}\) of the lake surface area). Pelagic sites were defined as the shallow area (< 3m) of a single rocky shoal, which had an average area of 1530 m\(^2\). Littoral sites were defined as the shallow area (< 3m) along a 100 m stretch of shoreline, roughly corresponding to the home range size of sunfish (~3000 m\(^2\)) (Fish & Savitz, 1983). Of the 17 littoral sites, 7 were exposed shoreline sites as opposed to more typical littoral sites, but since we have evidence that exposed shoreline sunfish are more similar to littoral sunfish than pelagic with respect to habitat use and phenotype (see chapter 1), we classified all of these as littoral sites to improve our statistical power at the expense of heterogeneity detail. We measured individual wet weight (g), external oral jaw width (mm), and standard length (mm). We then marked each fish with an individually coded wire tag (CWT: 1.1 mm Length x 0.25mm Dia., Northwest Marine Technologies, Inc.) inserted into dermal tissue at the anterior base of the anal fin. We also clipped 1/2 the length of the 4\textsuperscript{th} dorsal spine and the anterior portion of the dorsal fin on each individual to facilitate visual identification of marked fish. Following marking and phenotype measurements, all fish were released at their site of capture.

2.2.3 Recapture

In summer 2017, I returned to recapture sunfish through a combination of angling and trapping. I limited my recapture effort to the northern basin of Ashby lake where marking took place because this provided the best opportunity to recapture marked individuals. I attempted to
evenly distribute recapture effort between angling and trapping but angling proved substantially more efficient at collecting sunfish (76.1% angling vs. 23.9% trapping). I sampled at all locations within the basin where sunfish can be found between June 6th and August 24th, 2017. I captured 4874 sunfish (L: n = 1956; P: n = 2918) and identified marked individuals visually by checking for a clipped dorsal fin spine, and with a magnetic tag detector (Northwest Marine Technologies, Inc.). I found 74 sunfish with clipped dorsal fins and spines, but only 46 of these had tags, indicating 37.8% tag loss. Tag loss was not related to body size (Z73 = -0.22; p = 0.83). All sunfish with clipped spines were euthanized, CWTs were extracted by dissection and the fish preserved in 10% buffered formalin.

2.2.4 Sunfish phenotype, performance, and habitat use

In the lab, I identified individuals from their tags and determined sex. To assess phenotype, I used oral jaw size, a trait that is related to greater littoral resource use (i.e. more benthic invertebrates), and distinguishes between habitat ecotypes (Jarvis et al., 2017). I calculated jaw size as the residuals from an OLS regression of external oral jaw width (mm) against standard length (mm). To assess performance, I calculated a body condition factor using the Scaled Mass Index, which has been shown to perform better than either mass to length ratios or residuals from an OLS regression (Peig & Green, 2010). Body condition is used as a proxy for individual fat reserves, and is thought to reflect relative performance in fishes where fat reserves are critical for over-winter survival (Peig & Green, 2010). I excluded individuals with standard length < 75 mm as sunfish below this size have different growth curves and so are outliers from the adult mass ~ length relationship (Osenberg et al., 1988). For both trait calculations, I used a larger data set combining the marked recaptured sunfish with 30 additional randomly selected marked (not-recaptured) sunfish from each habitat type (n_total = 106). This should increase the accuracy of individual trait estimates for this population. Lastly, to assess habitat use I counted the number of trematode ‘blackspot’ parasites (Neascus spp.) encysted in the skin and fins of each individual (both sides) because these parasites primarily infest sunfish via a benthic invertebrate intermediate host (Robinson, Wilson & Margosian, 2000).
To determine if morphology, performance, or habitat use differed between sunfish collected from littoral and pelagic habitats during the marking phase, I assessed jaw size, body condition, and parasite load of the marked recaptures respectively. I first used ANCOVA with the trait of interest as the dependent variable, and body size (standard length, mm), initial habitat type, and their interaction as independent variables. Parasite counts were modeled using a general linear model with a Poisson distribution and log link function to deal with count data (O’Hara & Kotze, 2010). I used ANOVA to test whether mean body size (standard length, mm) differed between habitat types.

2.2.5 Mark-Recapture Analysis

I first assessed whether the rates of movement between habitat sites and between habitat types differed between sunfish marked in littoral and pelagic habitats using Fisher’s exact test. Only 3 individuals switched between the littoral and pelagic habitats, which was insufficient to test predictive factors of habitat switching. However, a moderate number of individuals moved between sites, especially within the pelagic habitat (see results). To determine more generally whether traits predicted individual movement between sites, I used a general linear model (family = binomial, link = logit) predicting the odds of moving using jaw size, body size, initial habitat type, jaw size*initial habitat, body size*initial habitat, initial site density, body condition, sex, and parasite load. I used reproductive activity as a proxy for the relative density of sunfish within sites (see Sunfish Density below). Only body size*initial habitat had a significant effect on the odds of moving, so I ran general linear models (family = binomial, link = logit) predicting the odds of moving using body size, initial site density, body condition, sex, and parasite load for each initial habitat group independently. All analyses were done in R 3.5.0. (R Development Core Team, 2018).

2.2.6 Population Size

I estimated total population sizes of sunfish in each of the littoral and pelagic habitats within the study area using the modified Lincoln-Peterson estimator suggested by (Seber, 1982):

\[
N = \frac{M (C + 1)}{(R + 1)}
\]
Where N is the size of the population at time of marking, M is the number of individuals marked in the first sample, C is the total number of individuals captured in the second sample, and R is the number of individuals in second sample that are marked. I included recaptured individuals with missing tags in this analysis as they reflect real recaptured marks despite lacking individual identity. This estimator is nearly unbiased with 7 or more recaptures (Seber, 1982), as is the case here. However, the assumption that the population is closed (i.e. no births, deaths, immigration, emigration) is likely violated because the year between marking and recapture would allow for these effects to alter the relative number of marks in the population. Additionally, relative to the marked sample, my recapture sample was biased towards individuals collected by angling (76% of marked vs. 45% of recaptured), and towards larger individuals (mean = 39.5 g vs. mean = 28.5 g). However, these recapture biases were not different between recapture samples from littoral and pelagic habitats (p > 0.05). Lastly, during the recapture phase, some individuals were likely captured multiple times as unmarked sunfish were released at the end of each collection trip. These assumption violations are all expected to lead to overestimation of population size. However, my estimate does not include the number of juvenile sunfish in the population, as they are too small to be marked (< 60mm). I calculated confidence intervals for population size estimates using the Poisson distribution as there was less than 50 marked recaptures, and R/C > 0.10 (Seber, 1982).

2.2.7 Sunfish Density

To estimate the population density in each habitat type, I first measured the surface area of each habitat type within the study area using ArcGIS. Littoral habitat was measured as the area between the shoreline and the 3m depth contour. Pelagic habitat was measured as the total area shallower than 3m around each rock shoal. Total littoral habitat area was 253 500 m$^2$, while total pelagic habitat area was 37 240 m$^2$. I then divided the estimated population size for each habitat by the measured surface area. I did not have measures of my relative effort for each site, so I could not estimate individual site population density. However, I distributed my recapture effort approximately equally in each habitat type.
As a proxy for relative site population density, I used reproductive survey data collected over two summer breeding seasons in 2015 and 2017. Sites were surveyed every 2-3 days by snorkelling, and the number of active nests were counted. The highest number of active nests observed within a season was taken as the estimate of reproductive activity at that site in that year. I then took the mean of the two years.

2.3 Results

2.3.1 Sunfish phenotype, performance, and habitat use

Among marked recaptures, sunfish collected from littoral habitat sites had larger jaws than those collected from pelagic habitat sites ($t_{42} = -2.50, p = 0.017$; Fig. 2.1), and higher parasite loads than those collected from pelagic habitat sites ($z = -7.39, p > 0.0001$; Fig. 2.2). On average, marked recaptures collected from pelagic habitat sites were larger than those from littoral habitat sites ($t_{43} = 2.86, p = 0.0065$; Fig. 2.3). There was no difference in mean body condition of fish between habitats ($t_{38} = 0.082, p = 0.94$).

2.3.2 Movement Analysis

Out of the marked sunfish that retained their tag (n = 46), 17 individuals moved between habitat sites (37.0%; CI: 23.2%, 52.5%; Fig. 2.4, 2.5). Of those, 3 individuals switched between littoral and pelagic habitats (6.5%; CI: 1.4%, 17.9%; Fig. 2.4, 2.5), which was not enough to test whether phenotypic match predicted habitat switching. There was more movement between habitat sites within the pelagic habitat, 50.0% (12/24), than between habitat sites within littoral habitat, 10.5% (2/19) ($p = 0.0038$). No individual factor predicted the odds of movement among habitat sites in the whole recapture sample ($p > 0.1$), but an interaction between body size and initial habitat was weakly significant ($z = 1.73, p = 0.084$). Within pelagic habitat, the probability of movement was predicted by body size, with smaller individuals more likely to have moved between years than larger individuals ($z = 2.04, p = 0.042$; Fig. 2.6). Jaw size (Fig. 2.7), body condition (Fig. 2.8), initial site density, sex, and parasite load did not significantly predict whether individuals moved between sites (all $p > 0.1$). No factor predicted the odds of movement among littoral habitat sites ($p > 0.1$), although with only 2 individuals moving, this test had little power.
2.3.3 Population Size, Density, and Immigration Rate

Within the north basin of Ashby L., I estimated a population size of 29,760 (CI: 21,520, 42,580) sunfish in the littoral habitat, at a density of 0.12 individuals/m², and 31,770 (CI: 23,970, 43,140) sunfish in the pelagic habitat, at a density of 0.85 individuals/m². So, pelagic habitat appears to have around a 7.1x higher population density than the littoral habitat. The general rate of 6.5% movement between habitat types translates to an estimated per year immigration rate ($m$) of 0.077 (2/26) into pelagic habitat, and 0.050 (1/20) into littoral habitat. We have no data on what proportion of the population reproduces, but the effective population size ($N_e$) would be a fraction of this size as nest locations are limiting.

2.4 Discussion

Here, I present evidence consistent with gene flow between sunfish in littoral and pelagic lake habitats. Only 6.5% of recaptured sunfish switched between littoral and pelagic habitats from 2016 to 2017, but this rate of movement is likely high enough to allow sufficient gene flow to homogenize neutral genetic differences. When $N_em >> 1$, neutral genetic population structure ($F_{st}$) approaches 0 (Mallet, 2001). If only 1% of the sunfish population in Ashby L. reproduces each year, then $N_em$ would still be much greater than 1 (25.15 for the pelagic population and 14.32 for the littoral population). Both habitat types have relatively large population sizes of around 30,000 individuals each, so genetic drift probably has a small effect here. Thus, for neutral genetic differences to accumulate, either the migration rate must be much lower, immigrants must have poor survival after arriving in the new habitat, or there must be strong assortative mating against immigrants. There is some evidence of assortative mating based on phenotype in these populations, but it is weak and varies between years (Jarvis et al., 2017). So, this result is consistent with previous findings that there is no spatial population structure with respect to neutral allelic variation in polyphenic sunfish populations (Weese, Ferguson & Robinson, 2012; Colborne et al., 2016).

We did not have a large enough sample of individuals that switched between habitats to test whether switching is predicted by matching habitat choice. Within the pelagic habitat,
movement between different pelagic habitat patches (shoals) was not predicted by jaw size which is what we would expect as these patches are all highly similar with respect to local conditions. Movement between patches was also not predicted by body condition, a common proxy for individual performance. This suggests that individual sunfish do not decide to return to or leave their previous patch based on how well they performed in that habitat. However, we did find evidence that body size predicted movement among patches within the pelagic habitat. Smaller individuals were more likely to have moved between patches than larger individuals, which suggests that smaller fish may face greater intraspecific competition than larger individuals. So, there is now some evidence that movement between habitat patches is non-random in polyphenic sunfish populations, at least for pelagic sunfish.

There was more movement between patches within the pelagic habitat than within the littoral habitat. This may be the result of the higher densities in the pelagic habitat if individuals move to mitigate the effects of conspecific competition. However, there was no evidence that competition for reproductive opportunities drives movement since patch nesting density did not have an effect on movement. Despite high movement within the pelagic habitat, there was no movement from pelagic patches to geographically accessible littoral patches (Fig. 2.4). This suggests that sunfish are expressing some form of habitat choice as otherwise we would expect there to be similar rates of movement among patches within the pelagic habitat, and between pelagic patches and geographically accessible littoral patches. It is not clear how or why sunfish are choosing to remain within the pelagic habitat, but it may be because it is more productive than the littoral habitat in Ashby lake, at least with respect to zooplankton abundances (unpublished data). Alternatively, it may be because sunfish imprint to their habitat type early in life (Stamps & Swaisgood, 2007). If sunfish do undergo habitat imprinting, then this could strongly facilitate habitat selection and further adaptive divergence (Berner & Thibert-Plante, 2015). Lastly, higher movement within the pelagic habitat may be the result of more homogenous environmental conditions between pelagic patches than between littoral patches. Here, littoral habitat represents all shallow shoreline habitat including typical high quality littoral habitat and lower quality exposed shoreline habitat. There is some phenotypic and habitat use variation between exposed shoreline and littoral sites (see Chapter 1). Differences in
environmental conditions between littoral and exposed shoreline sites could limit opportunities for movement between littoral habitat patches.

The sunfish population density in the pelagic habitat was substantially higher than in the littoral habitat, indicating that the pelagic habitat here represents an ecological opportunity for pumpkinseed sunfish (Yoder et al., 2010). Typically, models of how species colonize novel habitats find that initial establishment is difficult as the new populations have high extirpation rates. That does not seem to be the case here, as the pelagic habitat must be highly productive to support such high densities of sunfish. Ecological opportunities are typically expected to impose strong directional selection early during colonization (Barrett, Rogers & Schluter, 2008; Rees, 2009). This may help explain the phenotypic spatial population structure in polyphenic sunfish, despite the moderate gene flow between ecotypes.

The estimates of total population size provided here are coarse approximations as several assumptions were violated (see results). Most importantly, these estimates are based off two observations of each individual: initial capture and recapture. More accurate population size estimators make use of repeated observations of each individual to include estimates of survival, but this was not possible with the coded wire tag method which required lethal sampling. As addressed in the results section, all assumptions violated here are expected to lead to a moderate overestimation of population size (Seber, 1982). The confidence intervals I used are conservative to address this issue (Seber, 1982). Nevertheless, if the lower confidence limit for each population is used as our size estimate, our inferences about gene flow and population density do not change significantly.

It is important to recognize that connectivity does not exclude the possibility that adaptive genes are segregated between populations in each habitat type. Despite our evidence supporting connectivity between littoral and pelagic sunfish populations, we find consistent patterns of spatial population structure with respect to functional morphological traits, as shown in chapter 1 as well as several prior studies (Robinson, Wilson & Margosian, 2000; Weese, Ferguson & Robinson, 2012; Berchtold et al., 2015). We also have some evidence of genetic
divergence for functional foraging traits and plastic responses (Robinson & Wilson, 1996; Parsons & Robinson, 2006), so it is possible that there is genetic population structure with respect to adaptive alleles here. It can be difficult to detect signatures of genetic population structure in populations that have been adaptively diverging over relatively short time-scales (<10,000 years), also known as the “grey zone” (Bailleul et al., 2017). A key uncertainty is whether the genotypes and phenotypes of individuals that switch between habitat types constitute random or directed gene flow in this population. Directed gene flow, for example through matching habitat choice, is expected to facilitate adaptive divergence by sorting adaptive genes into their respective habitats (Ravigne, Dieckmann & Olivieri, 2009; Scheiner, 2016). Ultimately, the characteristics of individuals that do move between habitats will determine the effect of connectivity on spatial population structure.

Despite a good theoretical basis for why matching habitat choice should occur, there are limited examples in nature (Edelaar, Siepielski & Clobert, 2008; Edelaar & Bolnick, 2012), and for those examples, the results are mixed (positive: Karpestam et al. 2012; negative: Camacho et al. 2015). Studies that demonstrate evidence consistent with matching habitat choice typically fail to satisfy all criteria necessary for a strong inference of matching habitat choice (Edelaar, Siepielski & Clobert, 2008). For example, a key expectation is that immigrants have higher expected fitness following successful habitat choice (Edelaar, Siepielski & Clobert, 2008), but this has rarely been sufficiently tested. The lack of empirical examples likely reflect the logistical challenges of a) phenotyping a large enough sample of individuals, b) who then switch between habitat types, and c) subsequently can be recaptured for assessing local performance and survival (Camacho, Canal & Potti, 2015). Individuals who switch between habitats may be hard to find in large numbers as matching habitat choice is expected to lead to lower migration rates over time (Ravigne, Dieckmann & Olivieri, 2009; Scheiner, 2016). If matching habitat choice has been expressed by polyphenic sunfish populations for a long enough period, then migration rates may have decreased over time

The obvious next step for this research is to continue the mark recapture study, both to increase our sample of habitat switching individuals and to determine the consistency of
movement rates over time. Patterns of movement may vary from year-to-year for a variety of reasons, such as changes in local population density, predation risk, or resource availability. Another important unknown is whether immigrants have inferior performance, survival, and reproductive success relative to non-migrants. If immigrants have low survival and/or low reproductive success, then gene flow between littoral and pelagic ecotypes will be further restricted despite immigration. An artificial transplant of sunfish between habitat types could demonstrate whether transplanted sunfish perform poorly compared to resident sunfish. At least over short time scales transplanted sunfish appear to have high survival (McCairns & Fox, 2004) but this needs to be examined over longer time scales before we can draw strong conclusions.

Here, I presented evidence consistent with sufficiently high connectivity between littoral and pelagic habitats to allow high gene flow between sunfish occupying these two lake habitats. However, this does not exclude the possibility that there is adaptive differentiation between populations in each habitat type. We did not have a large enough sample of individuals that switched between habitat types to test whether switching is predicted by matching habitat choice. However, we did find evidence that size predicts movement among pelagic patches, suggesting that competition may play a role in movement, as expected under most models of adaptive divergence (Schluter, 2000). Ultimately, whether sunfish express habitat matching behaviour will determine the effect of connectivity on adaptive divergence. Although we were unable to test this hypothesis, this work increased our understanding of connectivity in this population, specifically that there appears to be some habitat matching, at least by pelagic sunfish, and that rates of movement between patches is much higher in the pelagic habitat and may be the result of size-based competition.
2.5 Figures

*Figure 2.1* The effect of body size of jaw size by initial collection habitat. Sunfish from littoral habitat had wider jaws on average than those from pelagic habitat.
Figure 2.2 The effect of body size and initial collection habitat on the number of external blackspot parasites. Sunfish from littoral habitat had higher parasite loads than those from pelagic habitat.
Figure 2.3 Boxplots of sunfish body size by initial marking habitat type. Boxes show the mean value, and the upper (75th) and lower (25th) quartiles. Tails indicate the extent of the data. Sunfish from the pelagic habitat had a larger mean body size than those from the littoral habitat.
Figure 2.4 Map of the north basin of Ashby Lake showing recorded sunfish movements between habitat patches between 2016 and 2017. Circle symbols indicate mark and recapture sites (pelagic = blue, littoral = red). Arrows indicate the direction of an individual’s movement.
Figure 2.5 Counts of individuals originally marked in 2016 in each habitat type. Colours indicate where those individuals were recaptured in 2017: in the alternate habitat type, in a different site of the same habitat, or the same site. There is no difference in the proportion of individuals captured in the alternate habitat, but the proportion of individuals that moved between patches was greater in the pelagic habitat.
Figure 2.6 The effect of body size on the predicted probability of moving among pelagic habitat patches. There is a significant negative effect of body size on the predicted probability of moving between patches.
Figure 2.7 The lack of effect of adjusted jaw size on the predicted probability of moving between habitat patches within the pelagic habitat.
Figure 2.8 The lack of effect of body condition on the predicted probability of moving between habitat patches within the pelagic habitat.
General Conclusion

In this thesis, I investigated the habitat use and movement patterns of polyphenic sunfish in a heterogenous environment. In chapter 1 I found that sunfish from exposed shoreline habitat varied in phenotype and diet among sites but were generally more similar in body form and habitat use to sunfish from shallow littoral habitat than to sunfish from open water pelagic habitat. This expands our current understanding of spatial population structuring along the littoral-pelagic habitat divergence to include another frequently used habitat in post-glacial lakes. The next steps are to identify the proximate mechanisms generating within habitat phenotypic variation and determine whether exposed shoreline supports lower sunfish densities. These findings demonstrate that exposed shoreline habitat appears to increase the complexity of spatial structuring over littoral and pelagic habitat alone, suggesting that considering intermediate habitats as a gradient of environmental conditions between higher quality habitats should reveal more about patterns of spatial population structure than considering them as a homogenous group.

In chapter 2 I found that sunfish move between habitats at moderate rates that could result in sufficient gene flow to homogenize genetic differences. However, the proximate causes of between habitat movement remains unclear. Additionally, I found that annual rates of movement among patches is much higher in the pelagic habitat and that because movement was predicted by smaller body size, that this may reflect avoidance of size-based competition. This specifically expands our understanding of connectivity to a longer time scale between successive years in polyphenic sunfish populations. We did not have a large enough sample of individuals that switched between habitat types to test whether sunfish express matching habitat choice. To determine the effect of connectivity on gene flow and adaptive divergence, we need to determine if switching is predicted by matching habitat choice and whether immigrants have inferior performance, survival, and reproductive success relative to non-migrants.

The spatial distribution of phenotypes in a population can influence both demographic and evolutionary parameters that are key components of population dynamics (Manel et al., 2003)
and adaptive evolutionary responses (Kinnison & Hairston, 2007). Understanding patterns of
habitat use and connectivity in polyphenic sunfish populations will help generate hypotheses
about how spatial population structure is generated and maintained during the preliminary stages
of adaptive diversification. This perspective will help advance the long-term goal of identifying
the factors that regulate adaptive divergence in postglacial lake fishes (Robinson & Wilson,
REFERENCES


Harrison S & EB. 1999. Habitat fragmentation and large-scale conservation: what do we know


R Development Core Team. 2018. R: A language and environment for statistical computing.


SAS. 2018. JMP.


