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Genetic And Demographic Consequences Of Lake And River Habitat Fragmentation On Fishes In Vermont

Peter T. Euclide
University of Vermont

GENETIC AND DEMOGRAPHIC CONSEQUENCES OF LAKE AND RIVER HABITAT FRAGMENTATION ON FISHES IN VERMONT

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Peter T. Euclide

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Dissertation Examination Committee:

J. Ellen Marsden, Ph.D., Advisor
Matthew J. Wargo, Ph.D., Chairperson
Jason D. Stockwell, Ph.D.
C. William Kilpatrick, Ph.D.
Cynthia J. Forehand, Ph.D., Dean of the Graduate College

ABSTRACT

Globally, habitat fragmentation has had a major impact on the conservation and management of many species and is one of the primary causes of species extinction. Habitat fragmentation is loosely defined as a process in which a continuous habitat is reduced to smaller, disconnected patches as the result of habitat loss, restriction of migration or the construction of barriers to movement. Aquatic systems are particularly vulnerable to habitat fragmentation, and today an estimated 48% of rivers are fragmented worldwide. My dissertation evaluates how habitat fragmentation has influenced the populations of four different species of fish in the Lake Champlain basin. In chapter 1 I summarize the current state of habitat fragmentation research, I broadly describe habitat fragmentation, review how habitat fragmentation pertains to population genetics, and describe the legacy of habitat fragmentation in the Lake Champlain basin. In chapters 2, 3 and 4 I evaluate and discuss the impact of nine lake causeways on the population structure of slimy sculpin (*Cottus cognatus*), rainbow smelt (*Osmerus mordax*), and lake whitefish (*Coregonus clupeaformis*). The genetic effects of causeways are limited. However, causeways appear to have had a significant influence on rainbow smelt demographics, and the genetic structure observed in lake whitefish may be a product of reduced effective population size resulted from commercial harvest in the late 1800s. In chapter 5 I evaluate how the basin-wide population of tessellated darters (*Etheostoma olmstedi*) is naturally structured throughout Lake Champlain and three different major tributaries and evaluates the effect that different types of habitat fragmentation (dams, causeways, and natural fall lines) have on tessellated darter populations. Tessellated darters appear to be highly structured by river drainage but not by dams, causeways or fall lines. My dissertation highlights how comparative population genetic studies can be used to identify patterns of isolation within large populations. My results stress the value of reporting both the presence and absence of barrier induced population sub-structuring.

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1.1. Habitat fragmentation as a global issue

How species' and population diversity is distributed across landscapes has been a key question in ecology for more than a century, and has led to research that describes the effect of both natural and man-made barriers on species distributions and genetic structure (Forman, 1995). As human populations continue to increase, so does habitat fragmentation, degradation, and loss (With & Crist, 1995; Ewers & Didham, 2006). Fragmentation impairs ecosystems by changing ecosystem services, promoting dispersal of exotic species, and damaging core habitat (Trombulak & Frissell, 2000; Broadbent *et al.*, 2008). Additionally, fragmented populations are often subject to reduced gene flow among sub-populations, which can weaken species' ability to react to changes in their environment (Macarthur & Wilson, 1967; Templeton *et al.*, 1990). As a result, species in fragmented landscapes are often at a higher risk of extinction than species in contiguous landscapes (Fahrig, 2002). For these and other reasons, fragmentation is considered one of the root causes of increasing rates of species extinctions worldwide (Fahrig, 1997; Henle *et al.*, 2004). Therefore, an important step in both conservation of endangered species and management of natural resources is to understand how different forms of habitat fragmentation influence species at the population level.

To evaluate how habitat fragmentation has influenced species assemblages and populations, researchers have developed and utilized a variety of observational, experimental, and modeling techniques (Haddad *et al.*, 2015; Williams *et al.*, 2016; Yeager *et al.*, 2016). Experimental manipulations of patch size can identify how

fragmentation influences community richness and abundance (e.g., Kareiva, 1987). However, meta-analyses of these experiments show a lack of consistency in results, emphasizing the variation in species- and landscape-specific responses to fragmentation (Debinski & Holt, 2000). Simulations of habitat fragmentation have often been used to construct null models to compare to observed data and predict how systems might be impacted by future fragmentation (e.g., Sisk, Haddad & Ehrlich, 2013). Two types of models common in habitat fragmentation research are extinction-colonization (EC) and birth-immigration-death-emigration (BIDE) models, and whereas they differ in their approach, both find that extinction rates increase with fragmentation (Fahrig, 2002). More recently, landscape models that combine geographic data with genetic and species natural history data have been used to identify barriers and potential corridors within landscapes (Rees *et al.*, 2008; Elliot *et al.*, 2014). Finally, the design, interpretation, and parameterization of fragmentation experiments and models would not be possible without observational, field-based fragmentation research that describes how habitat fragmentation has impacted hundreds of different species, from plants to insects, large mammals, and fish (e.g., Gerlach & Musolf, 2000; Ramalho *et al.* 2014; Hansen *et al.*, 2014; Couchoux, Seppä & van Nouhuys, 2016).

In terrestrial systems, habitat fragmentation exists in many different forms, including urbanization, deforestation, and road construction. Fragmentation by roads and deforestation negatively impacts animal movements and seed and pollen dispersal (Gerlach & Musolf, 2000; Ramalho *et al.*, 2014). Additionally, species richness and community composition often differ between fragmented and un-fragmented habitats

(Quinn & Harrison, 1988). However, the size and direction of this effect often differs. While some studies find decreased species richness is associated with fragmentation, others find the exact opposite (Debinski & Holt, 2000; Haddad *et al.*, 2015). One fairly consistent trend, however, is that increased fragmentation leads to decreased population size and increased rates of local extinction (Saccheri *et al.*, 1998; Fahrig, 2002). Another consistent finding is that habitat fragmentation often has indirect, negative effects on species, such as changes in soil temperature and salinity near roads affecting nearby plant growth, and increased active and passive harassment of wildlife (Trombulak & Frissell, 2000).

In aquatic systems, fragmentation is largely a consequence of dams and their impact on fish movement, habitat connectivity, and habitat loss due to changes in hydrology and sediment transport (Ligon, Dietrich & Trush,1995; Bessert & Orti, 2008; Wang *et al.*, 2010). In the U.S. alone there are an estimated 75,000 dams (Graf, 1999) and many of them pose significant barriers to a range of fish species, obstructing movement and limiting access to suitable habitat. Worldwide, the number of dams continues to rise and as of 2015 an estimated 48% of global rivers are at least moderately impacted by fragmentation and flow regulation (Grill *et al.*, 2015). While many species are impacted, dams and other instream barriers have the most impact on highly migratory fishes such as salmonids, sturgeon, and lamprey that have upstream spawning habitat (Hall, Jordaan & Frisk, 2011). Dams are still one of the largest threats to anadromous Pacific salmon stocks and central to anadromous Atlantic salmon (*Salmo salar*) recovery efforts (Roni *et al.*, 2002; Brown *et al.*, 2013). While commercially harvested species such as salmon are

most often cited when discussing instream barriers, habitat fragmentation has contributed to diminished populations of almost all anadromous species from forage fish such as alewife (*Alosa pseudoharengus*), blueback herring (*Alosa aestivalis*) and American shad (*Alosa sapidissima*) to game fish such as striped bass (*Morone saxatilis;* Beasley & Hightower, 2000; Kocovsky, Ross & Dropkin, 2009). For most of these species, however, dams do not fragment populations, but instead decrease the available spawning and nursery habitat by preventing upstream and downstream migration and damaging existing spawning habitat through sedimentation and altered flow (Ligon, Dietrich & Trush,1995; Sheer & Steel, 2011).

Migratory fish species may be most directly affected by instream barriers but non-migratory species are also affected. For many stream residents, barriers can damage habitat and limit gene flow isolating once-contiguous dendritic populations (Clemento *et al.*, 2009). One common impact of new barriers is a decrease in species diversity both above and below the barrier due to a loss in habitat complexity (Ligon, Dietrich & Trush,1995; Wang *et al.*, 2010). When a new barrier is built, the area above the barrier often transitions to a more lentic state, leading to the extirpation of many lotic species while areas below the barrier are also affected by flow regulation affecting seasonal flood cycles crucial to many species' life histories (Agostinho, Pelicice & Gomes, 2008). However, of importance is that the impact of the barriers themselves on community diversity is often small in comparison to other environmental factors, such as river size, flow, and land use (Cumming, 2004; Wang *et al.*, 2010). Additionally, as in terrestrial environments, the effect of habitat fragmentation in aquatic systems is species-specific,

which makes the prediction of a species' sensitivity to habitat fragmentation difficult (Ewers & Didham, 2006).

What to do about aquatic habitat fragmentation is complicated by the conservation benefits of dams and other barriers. One of the best examples of conflict between negative and positive impacts are dams in the Laurentian Great Lakes watershed. At least 12,000 dams exist in the Great Lakes watershed including many small, out-of-use dams that could be removed (Januchowski-Hartley *et al.*, 2013). Even small, out-of-use barriers limit up-stream movement of many species of fish, resulting in diminished species richness above barriers (Dodd *et al.*, 2003). Additionally, many endangered or threatened species use Great Lakes tributaries for reproduction (e.g., adfluvial lake sturgeon, *Acipenser fulvescens*) or as their primary habitat (e.g., northern madtom, *Noturus stigmosus;* Auer, 1996; Lane, Portt & Minns, 1996). However, in the mid-1970s managers began using low-head barriers as a method to prevent spawning by invasive sea lamprey (*Petromyzon marinus*; Hunn and Youngs, 1980). Using barriers to limit sea lamprey access to spawning habitat has been a successful form of control and additional lamprey-control barriers have been added to some streams (Lavis *et al.*, 2003). Therefore, making management decisions about aquatic habitat fragmentation requires information about how multiple species and preferably the entire community will be impacted by the addition or removal of fragmentation.

Relative to terrestrial and riverine systems, lakes are generally not subject to fragmentation. Fragmented lakes provide a novel system to draw parallels between aquatic and terrestrial system in meta-analyses. Unlike lotic systems which are 1-

dimensional and movement is largely limited to upstream or downstream, lentic systems, like terrestrial environments, are more 2-dimensional whereby fish are free to choose multiple routes to the same destination. One human impact in lake systems akin to terrestrial fragmentation is causeways. Most causeways connect islands to the mainland across marine ecosystems (e.g., connecting Venice, Singapore, and Bahrain to the mainland), or are used to reclaim land or protect land from tidal flooding (e.g., the system of polder dykes and Zuiderzee Works in the Netherlands), but are uncommon in freshwater lakes. When present, causeways divide lentic environments and could limit the movement of aquatic species (Fechhelm, 1999; Fechhelm *et al.*, 1999). Movement across most causeways is generally still possible through one or more openings built into the causeway to allow some water flow or boat passage. Therefore, causeways may limit gene flow similar to roads, deforestation, or other landscape-altering practices where some passage between patches is still possible. However, no studies have evaluated if causeways limit gene flow, which makes lake causeways a novel area of research.

As human populations increase, so does habitat fragmentation and degradation (With & Crist, 1995; Ewers & Didham, 2006). While many aspects of the impact of habitat fragmentation are still debated, the negative effects on natural communities are well established, and the idea that habitat fragmentation leads to increased rates of extinction is widely accepted (Wilcox & Murphy, 1985; Fahrig, 2003). Therefore, a better understanding of the consequences of watershed-wide habitat fragmentation is needed to inform management and conservation decisions about barrier creation and removal in watersheds throughout the world.

1.2. Genetic consequences of habitat fragmentation

Genetic diversity is required for evolution of species, and is positively correlated with population and individual fitness (Reed & Frankham, 2003). Loss of genetic diversity through inbreeding generally leads to decreased fitness and increased inbreeding depression (Saccheri *et al.*, 1998; Vrijenhoek, 1998; Perrin & Mazalov, 2000). Because habitat fragmentation often reduces population size and increases spatial isolation, fragmentation is generally hypothesized to erode genetic variation and lead to increased rates of genetic drift and population sub-structuring. However, the influence of habitat fragmentation in population genetics is varied, and often species-specific (Henle *et al.*, 2004). Nonetheless, maintenance of genetic diversity has been recognized by the International Union for Conservation of Nature (IUCN) as a conservation priority (McNeely *et al.*, 1990), and understanding how human activities, such as those that lead to habitat fragmentation, affect genetic structure and diversity is important to protect and conserve native species.

In terrestrial environments, habitat fragmentation has had inconsistent effects on population genetics. Genetic diversity of plant populations is often reduced with increased fragmentation and reduced population size; however, the effects can be small, and gene flow among sub-populations is often still common (Young, Boyle & Brown, 1996). Studies of terrestrial animals have also found variable effects of habitat fragmentation on population structure and diversity. Whereas most studies still find a relationship between genetic diversity and population size, many species appear to be robust against the hypothesized impact of habitat fragmentation on increasing inbreeding

and decreasing genetic diversity (Mitrovski *et al.*, 2007). When fragmentation limits access to dispersal pathways, however, populations do generally show signs of increased genetic sub-structuring (Gerlach & Musolf, 2000; Barr *et al.*, 2015). Additionally, erosion of diversity and sub-structuring is often higher in specialists, and populations that were small prior to fragmentation (Harrison & Bruna, 1999; Holderegger & Di Giulio, 2010).

The effects of fragmentation on the population genetics of aquatic species are also variable (Blanchet *et al.*, 2010). However, barriers to gene flow are easier to identify in aquatic systems, making causative studies more feasible than in terrestrial systems. Freshwater environments are naturally very fragmented (e.g., dendritic rivers systems, isolated small ponds and lakes), and populations living in these systems are often isolated from one another with only a single possible dispersal route (Campbell Grant, Lowe & Fagan, 2007). This natural fragmentation is thought to be partially responsible for the disproportionate level of species diversity present in freshwater versus marine habitats (Dias *et al.*, 2013). Populations in river systems are especially vulnerable to habitat fragmentation, and can be subject to high levels of local extinction, especially when migration is unidirectional or if the system is small (Fagan, 2002). Therefore, construction of new barriers magnifies the effects of existing patterns of isolation and restricted dispersal present in most freshwater fish populations.

As in terrestrial environments, increased fragmentation in aquatic systems is predicted to lead to decreased genetic diversity and increased population genetic sub-structuring. Several studies have shown that noticeable changes in population structure and genetic diversity of fish species separated by dams can occur within less than 100 years (e.g.,

Neraas & Spruell, 2001; Wofford, Gresswell & Banks, 2005). For example, as a result of several dams built in the Sense river basin of Switzerland, bullhead (*Cottus gobio*) had diminished genetic diversity in headwater regions consistent with a lack of upstream dispersal (Junker *et al.*, 2012). Similarly, European chub (*Squalius cephalus*) showed higher genetic structure in streams with large in-stream barriers than in an adjacent un-fragmented stream (Gouskov & Vorburger, 2016). While a degree of population sub-structuring in freshwater systems is natural, further decreased population connectivity is an additional stressor to many populations already negatively affected by habitat degradation, overfishing, and other anthropogenic impacts and is therefore a conservation and management concern (Coleman *et al.*, 2018).

Evaluating the genetic diversity and structure of populations continues to be an important tool in conservation and management of fish populations (Vrijenhoek, 1998; Schwartz, Luikart & Waples, 2007). For example, following the collapse of lake trout populations in the Great Lakes in the mid-1900s, the genetic diversity of the remnant populations in Lake Superior has decreased and shows signs of genetic bottlenecks; therefore, conserving genetic diversity is central to lake trout (*Salvelinus namaycush*) recovery efforts (Guinand *et al.*, 2003). Genetic data have been used to define or redefine management units for commercial fishing (VanDeHey *et al.*, 2009). In Lake Michigan, genetic assessment of commercially fished lake whitefish (*Coregonus clupeaformis*) showed that lake whitefish landed in each management unit were comprised of multiple genetic stocks suggesting that all spawning stocks need to be considered when setting catch limits (Andvik *et al.*, 2016). For endangered or threated species, quantifying genetic

structure can help maximize time and resources by identifying populations of concern for conservation (Aben *et al.*, 2016; Li *et al.*, 2016). In the Missouri River, dams caused increased isolation by distance and decreased genetic diversity in endangered blue sucker (*Cycleptus elongates*) populations (Bessert & Orti, 2008). As information of genetic diversity and structure becomes increasingly efficient and affordable to acquire, population genetic analysis has become an essential step in the development of management and conservation plans (Begg & Waldman, 1999; Mace, 2004).

More recently, genetic research has focused on understanding how landscapes influence the connectivity of populations (Manel *et al.*, 2003; Storfer *et al.*, 2007; Balkenhold & Landguth, 2011). Landscape genetic research often attempts to evaluate multiple landscape pressures concurrently through the use of models and simulations (Hand *et al.*, 2014). The predictive capability of models has been used to identify what the effects of barriers may be in the future (Landguth *et al.*, 2014). Though powerful, these modern techniques have drawbacks, often sacrificing field research for laboratory and computational work (Richardson *et al.*, 2016). This has led to a recent call for more field-based research that combines null model techniques with traditional genetic sampling across a range of taxa and landscapes (Richardson *et al.*, 2016).

Universally, the small and isolated populations created by habitat fragmentation are at an increased risk of diminished genetic diversity, increased population sub-structuring, and increased risk of inbreeding depression. While not all species have the same levels of sensitivity to these effects, the ability to predict which species are sensitive is an important part of conservation and natural resources management (Henle *et al.*, 2004;

Ewers & Didham, 2006). As modern molecular techniques make collecting and analyzing population genetic data more efficient and affordable, understanding the genetic structure of populations has become central to species conservation and management (Schwartz, Luikart & Waples, 2007).

1.3. Habitat fragmentation in the Lake Champlain basin

Lake Champlain has a long history of fragmentation. Geologically, the Champlain Valley has experienced extensive change over the last 20,000 years. During this time, Vermont experienced glaciation, reversals in lake outflow direction, large fluctuations in lake size, and changes in salinity when, for a 1,500 to 2,000-year period, the region was connected to the Atlantic Ocean (Cronin *et al.*, 2008; Marsden & Langdon, 2012). Following European colonization in the 1700s, many dams and weirs were built in the Vermont tributaries of Lake Champlain, and causeways were constructed in the lake by the mid-1800s. The causeways divide the lake into four distinct basins and may be partially responsible for large differences in productivity and water quality among basins (Myer & Gruendling, 1979; LCBP, 2015).

The Lake Champlain drainage basin has a distinct fall line that runs north to south, parallel to the lake on the Vermont side (Figure 1.1). Following the last glaciation, the area that is now considered the Lake Champlain valley was inundated, allowing for many species, such as many fishes and unionid mussels, to colonize above the fall line (Smith, 1985; Langdon, Ferguson & Cox, 2006). Following a decrease in lake level approximately 10,000 years before present, the fall line was uncovered and now act as a

natural barrier to many species of fish and shaped stream species assemblages seen today (Marsden & Langdon, 2012). Presently, the fall line is approximately 46 m in elevation and partially eroded but major waterfalls or cascades can be easily identified in most tributaries that cross the fall line.

During the 1800s, dams were built on most of the major tributaries to Lake Champlain, including the Great Chazy, Little Chazy, Salmon, Little Ausable, Ausable, Boquet, Winooski, Lamoille, and Missisquoi rivers and Otter Creek. Though many of the smaller weirs and mill dams have been removed, 463 dams remain in the Lake Champlain watershed and over 800 remain in the entire state (Bushman, 2016). Dams built on two of the largest tributaries to Lake Champlain, the Missisquoi and Winooski rivers, were built below the natural fall line and cut off many species of fish such as Atlantic salmon, walleye (*Sander vitreus*), and lake sturgeon (*Acipenser fulvescens*) from their historic spawning habitat (Marsden & Langdon, 2012). Additional dams throughout the watershed have impacted the populations of these and many fish including redhorses and other suckers (Catostomidae) and lake whitefish.

Dams in Lake Champlain are a controversial subject and have had both positive and negative effects on natural populations. Hydroelectric dams in the Winooski River, one of the largest tributaries to Lake Champlain, are known barriers to Atlantic salmon, and while most of the dams have fish passage systems, they still appear to have a negative impact on recruitment. A recent assessment suggested that only 65% of stocked salmon smolts were successful in finding downstream passage; less than half of downstream passage was through the bypass indicating mortality could be an issue (Nyqvist *et al.*,

2017). Barriers in the Richelieu River that connects Lake Champlain to the St. Laurence River and ultimately the Atlantic Ocean have also been reported to impact native species. The two dams on the Richelieu River and the lock at St. Ours, Quebec are thought to have prevented American eels (*Anguilla rostrate*) from reaching Lake Champlain where they were once abundant (Verreault, Mingelbier & Dumont, 2012). Dams in the Lake Champlain basin have also played an important role in protecting some native species. Dams provide refuge habitat for many species from exotic species such as limiting range expansions of zebra mussels thereby protecting unionid mussels (Marsden & Hauser, 2009). Additionally, dams serve as an important management tool used to limit spawning habitat for nuisance sea lamprey populations which have had a negative influence on lake trout and Atlantic salmon recovery efforts (Marsden *et al.*, 2003). Finally, many dams have historical or cultural value to communities in Vermont, making dam removal a sensitive issue to some stakeholders (Fox, Magilligan & Sneddon, 2016). Given the complex combination of negative and positive properties of dams in the Lake Champlain basin, understanding what affect they have on natural communities is important to make informed decisions about barrier removal or construction.

Since the mid-1800s, construction of nine major causeways has progressively divided Lake Champlain into relatively isolated regions (Northeast Arm, Malletts Bay, Carry Bay, The Gut, Missisquoi Bay, and the northern section of the northwest arm; Figure 1.1; Table 1.1). The causeways range from 300 m to 5.25 km long; all have narrow openings (24 to 250 m) to allow passage of boat traffic (Marsden & Langdon 2012). The openings are generally shallow (2-8 m deep) and therefore may be inaccessible to cold-water fish

species during lake stratification when surface waters are warm (Table 1.2). Causeways on either side of Carry Bay and the Gut (which separate the Northeast Arm from the Main Lake) are relatively shallow and become stagnant and heavily vegetated in the summer because of the restricted flow. These seasonal changes may exacerbate the existing barrier to fish movement by lowering the habitat suitability for fish that prefer cold, oligotrophic parts of the lake. While causeways are predicted to be only partial barriers to fish movement, little is known about which species of fish pass through openings. One of the only studies that has discussed fish movement through causeway openings was conducted on tagged sea lamprey and indicated that lamprey were able to cross through causeway openings, likely while attached to host fish (Howe, Marsden & Bouffard, 2006). Although causeways provide many services such as recreational opportunities, vehicle transit, and nursery habitat for endangered turtles, causeways have been a point of contention in Vermont. Public concern that the Missisquoi Bay causeway could be partially responsible for the high nutrient levels that cause algal blooms in Missisquoi Bay led to the widening of the Missisquoi Bay causeway opening in 2004 despite scientific research indicating a larger opening would have almost no influence on water circulation within the bay (Watzin, 2006). While common in Lake Champlain, causeways, especially those that significantly divide a lake into parts, are not a common feature in most lakes and therefore very little is known about the environmental impact of causeways on lake hydrology or fish movement.

The environmental impact of causeways has been evaluated in only a few other systems. The large causeway built for the Southern Pacific Railway that crosses Great Salt Lake in

Utah has been shown to prevent water mixing among lake basins. This division has resulted in differences in hydrology, salinity, and species assemblages on either side of the causeway (Post, 1977). Salinity was also different on either side of a 15-km long causeway across Urmia Lake in Iran (Zeinoddini, Tofighi & Vafaee, 2009). Species assemblage changes were also seen 30 years after the construction of a causeway across the Petitcodiac River estuary, in New Brunswick, zooplankton communities represented those of a disturbed environment and many of the larvae of anadromous fish previously abundant in the estuary were absent, suggesting the causeway may have blocked fish passage into the estuary (Aube, Locke & Klassen, 2005). Similar to dams, however, the impact of what on fish movement is inconsistent; for example, a mark-and-recapture study of Arctic cisco (*Coregonus sardinella*) around a causeway built near Pruhoe Bay, Alaska found that the causeways had no effect on adult Arctic cisco movement but may limit juvenile movement (Craig & Griffiths, 1981; Fechhelm *et al.*, 1999). Despite these examples, studies of fragmentation in lakes remain limited, and most focus primarily on how shoreline development impacts fish distribution (Scheuerell & Schindler, 2004), rather than the impact they have on movement and dispersal. However, all studies do suggest that causeways can have a significant environmental impact and therefore should be included in the habitat fragmentation literature.

The long history and diversity of habitat fragmentation in the Lake Champlain basin makes it an excellent location to study the effects of aquatic barriers on fishes. My dissertation uses the Lake Champlain system to fill major gaps in fragmentation literature associated with lake habitat fragmentation by assessing the population genetic structure

of multiple species across lake causeways and evaluating the how different barriers influence the population structure of a species that lives in both lentic and lotic environments. These aims were accomplished by using a combination of genetic, demographic, historic, and environmental data.

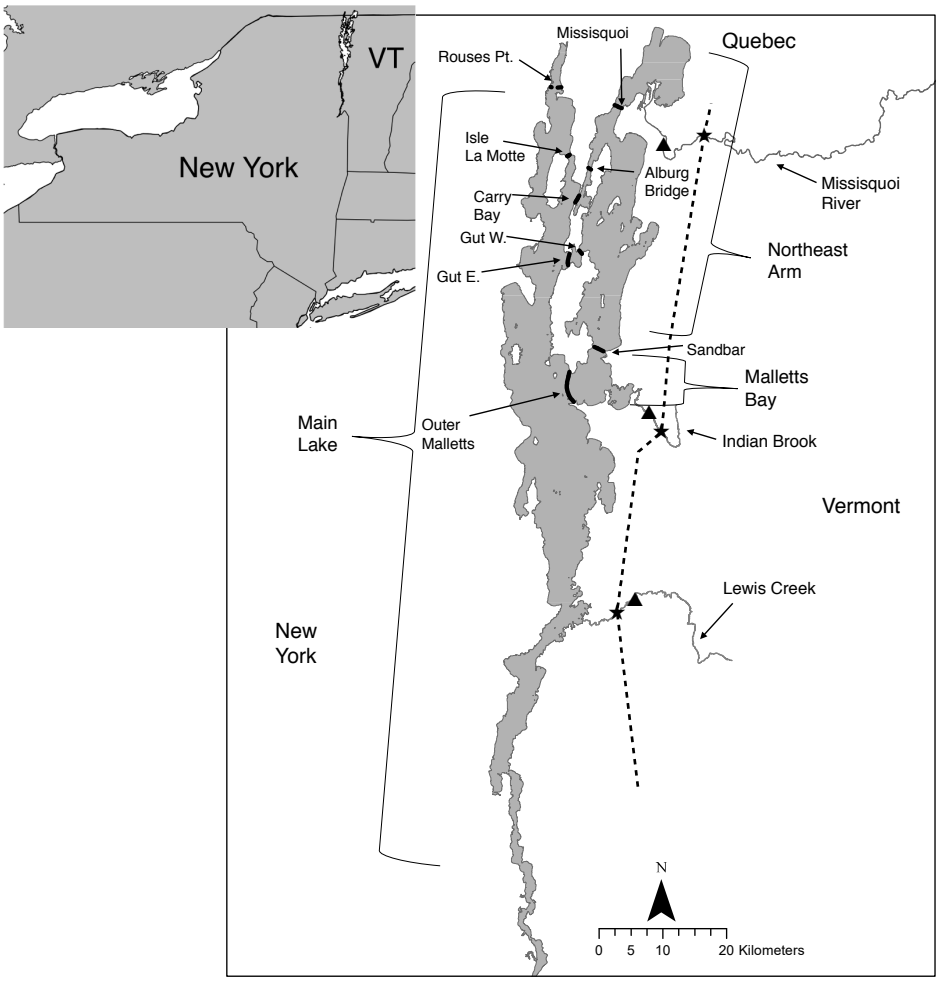

Figure 1.1: Location of Lake Champlain and major features discussed in text. Short dashed line indicates the approximate location of the natural fall line. Brackets indicate the approximate designation of the three primary basins of Lake Champlain isolated by causeways. Causeways are denoted as black lines and labeled in the map, exact locations of dams and fall lines in the three rivers sampled in Chapter 5 are denoted by triangles, and stars respectively.

Table 1.1: Descriptions of all major causeways present in Lake Champlain. Data from Marsden and Langdon 2012 and field measurements.

Causeway	Date Constructed	Number of Openings	Length (m)	Length of opening(s) (m)	Average depth (m)
Sandbar Causeway	1850	1	1281	19	1.3
Rouse's Point	1851	1	1738	965	3.5*
Isle La Motte	1882	1	520	19	3.3
Gut W. Causeway	1886	1	1984	57	4.9
Alburg Bridge	1886	1	464	277	7.7
Gut E. Causeway	1892	1	492	58	3.9
Outer Malletts Causeway	1899	2	5091	80	4.0*
Carry Bay Causeway	1899	2	1319	85	6.0
Missisquoi Bay	1938	1	1251	255	4.0*

*Average depth estimated from chart (NOAA Coast Survey Chart 1997)

Table 1.2: Mean and standard deviation (SD) of temperature data (°C) collected in nine of the 11 causeway openings in Lake Champlain. Names correspond to causeways shown in in Figure 1.1.

	month	1	2	3	4	5	6	7	8	9	10	11	12
Outer Malletts S.	Mean	0.9	0.9	1.6	4.8	11.6	17.8	23.4	23.9	21.5	13.0	7.8	3.5
	SD	0.8	0.5	1.2	2.1	3.2	3.0	1.5	1.2	2.4	3.0	1.9	2.4
Outer Malletts N.	Mean	1.1	1.0	1.6	4.6	11.2	17.5	22.6	24.1	21.4	13.5	8.3	4.2
	SD	0.9	0.4	0.9	2.1	2.9	2.8	1.5	0.9	1.9	2.7	1.9	2.2
Sandbar	Mean	0.8	0.9	1.6	4.8	11.5	17.4	23.4	23.9	20.1	12.8	7.5	2.2
	SD	0.4	0.4	1.3	2.2	3.7	3.3	2.3	2.0	2.9	3.2	2.3	2.0
Gut E.	Mean	1.3	1.1	1.6	4.2	9.5	15.8	22.3	23.9	21.6	14.6	9.1	4.5
	SD	0.8	0.5	1.0	1.9	2.6	2.6	1.6	0.8	1.7	2.8	1.5	2.1
Gut W.	Mean	1.7	1.4	2.1	5.5	11.5	17.0	21.5	22.9	22.3	17.6	12.6	5.8
	SD	0.7	0.4	1.1	2.1	3.3	2.3	1.4	1.0	2.2	5.1	6.5	5.4
Alburg Bridge	Mean	1.5	2.5	2.3	6.5	12.6	18.9	23.2	24.3	21.5	12.9	6.7	2.8
	SD	0.6	0.4	1.2	2.5	2.0	2.3	1.4	0.9	1.9	3.6	1.8	1.6
Carry S.W.	Mean	1.3	1.3	2.0	6.1	11.8	18.2	22.6	24.3	21.3	13.1	7.8	3.5
	SD	0.5	0.5	1.1	2.4	2.6	2.5	1.5	0.6	1.9	3.0	1.7	2.0
Isle La Motte	Mean	1.1	1.2	2.0	6.0	11.8	18.1	21.1	NA	18.2	10.7	7.2	3.4
	SD	0.4	0.3	1.0	2.6	3.0	2.5	1.0	NA	0.9	2.3	2.1	2.1
Missisquoi	Mean	1.7	2.8	2.3	6.6	14.0	20.4	23.9	24.7	20.8	12.1	6.3	2.5
	SD	0.6	0.5	1.1	2.8	2.6	2.4	1.2	1.0	2.2	3.2	2.0	1.7

CHAPTER 2: LACK OF POPULATION GENETIC STRUCTURE OF SLIMY SCULPIN IN A LARGE, FRAGMENTED LAKE[1]

2.1. Abstract

Most of what is known about sculpin population structure comes from research in streams; however, slimy sculpins (*Cottus cognatus*) are also a common benthic species in deep lakes. In streams, sculpins are considered to be a relatively inactive species, moving only small distances and characteristically have high levels of genetic structure. I examined population genetic structure of slimy sculpin across multiple barriers and over distances up to 227 km in Lake Champlain (USA, Canada) and Lake Ontario (USA, Canada) to determine if lake populations of sculpin are also highly structured. I predicted that slimy sculpin populations in Lake Champlain would be structured by six causeways as well as by distance, Lake Ontario populations would be structured only by distance, and differences between the lakes would be large relative to within-lake differences. I examined microsatellite variation among 200 slimy sculpins from Lake Champlain and 48 slimy sculpins from Lake Ontario to evaluate patterns of population connectivity and structure. Slimy sculpins were genetically distinct between lakes there was no evidence of population sub-structuring within either lake but. I conclude that sculpin form a single, panmictic population of in Lake Champlain and another potentially panmictic population in Lake Ontario, with no indication of genetic isolation by distance. Our results contrast

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[1] Euclide P.T., Flores N.M., Wargo M.J., Kilpatrick C.W. & Marsden J.E. (2017) Lack of genetic population structure of slimy sculpin in a large, fragmented lake. *Ecology of Freshwater Fish*, 1–11.

with data from sculpin in streams, suggesting distance and habitat fragmentation exert little influence on population connectivity of benthic fish in lakes. One possible explanation for this could be the comparatively large population size of sculpins in lakes compared to streams or a difference in dispersal strategies between lake and stream populations.

2.2. Introduction

Patterns of genetic variation across a species' range generally result from historic, extrinsic factors such as physical isolation due to glaciation or changes in climate (Hewitt, 1996; Petit *et al.*, 2003), whereas genetic structure of populations across smaller spatial scales are often the result of contemporary environmental conditions such as habitat availability or fragmentation. Among freshwater aquatic habitats, lotic waters are particularly susceptible to anthropogenic change (e.g., channelizing, siltation, dewatering) and fragmentation (e.g., construction of dams, weirs, and roads with poorly placed culverts; Templeton *et al.,* 1990; Dynesius & Nilsson, 1994; Ligon, Dietrich & Trush,1995; Graf, 1999). The combination of the naturally complex structure of lotic systems with high amounts of anthropogenic disturbance often leads to high levels of population isolation and genetic structure of species living in streams and rivers (e.g., Bessert & Orti, 2008; Gouskov & Vorburger, 2016). In contrast, large lentic systems often have less habitat complexity, especially offshore lake regions, and little habitat fragmentation. Understanding how environmental heterogeneity in lakes may influence population genetic structure is nonetheless central to understanding recent evolutionary change and species' vulnerability to anthropogenic alterations.

Determining relationships between environmental and genetic variation is particularly important for fish species that inhabit both lentic and lotic habitats, despite differences in flow, habitat complexity, connectivity, and habitat predictability (Ryder & Pesendorfer, 1989). Lentic and lotic populations of the same fish species can differ in dispersal and genetic structure, and are often genetically distinct from one another. For example, home ranges of 21 fish species in lakes were found to be 19 – 23 times larger than 25 fish species in rivers by Minns (1995), indicating movement patterns differ between lotic and lentic habitats. Additionally, patterns of genetic differentiation have been found between lentic and lotic populations of sticklebacks and cyprinids (McKinnon & Rundle, 2002; Collin & Fumagalli, 2011).

Though sculpins (Cottidae) are widely distributed in lakes and streams, little is known about their genetic structure in lentic systems. Based primarily on lotic research, sculpin are generally considered to be sedentary, and disperse only short distances. For example, mottled sculpins (*Cottus bairdi*) in a small tributary in North Carolina showed patterns of genetic isolation by distance across 5.6 km, and the estimated migration rates between sites separated by less than 300 m were small (Lamphere & Blum, 2012). Mottled sculpin sampled in tributaries of eastern Lake Michigan also showed strong patterns of genetic structure even across short distances (Homola *et al.*, 2016). Assessment of sculpin behavior and ecology also suggests that sculpin do not move long distances. Mottled sculpin implanted with PIT tags had a maximum displacement distance from the tagging location of about 511 m over one year, and more than 74% of individuals moved less than 100 m from where they were tagged during a one-year study (Breen *et al.*, 2009).

Similarly, slimy sculpins (*Cottus cognatus*) in Little River, New Brunswick, had detectable differences in stable isotope composition among sites separated by less than 10 km, suggesting slimy sculpin have small home ranges (Gray, Cunjak & Munkittrick, 2004). Otolith microchemistry of slimy sculpin also indicated that individuals generally move less than 10 km from their natal location throughout their lifetime (Clarke, Telmer & Shrimpton, 2015). Few studies, however, have examined sculpin movement or genetic structure in lentic systems. *In situ* behavioral studies of slimy sculpin in lakes are challenging because they prefer depths greater than 25 m and cold water (less than 15ºC; Otto & Rice, 1977; Brandt, 1986). Lakes generally have lower habitat complexity and have few or no barriers akin to dams to limit dispersal, thus I predict that population connectivity and genetic structure of sculpin may be different in lakes than in streams.

To better understand sculpin ecology and population connectivity in lentic systems, I examined the genetic structure of slimy sculpins in two large lakes. Lake Champlain served as our focal system. Lake Champlain is a partially fragmented lake divided into three basins by causeways that may restrict slimy sculpin dispersal, providing a lentic equivalent to a fragmented lotic system (Marsden & Langdon, 2012). I also examined two slimy sculpin populations from Lake Ontario as an outgroup to assess consistency of trends in population structure among lakes, and between lake and stream populations. The two lakes have a similar fish community and trophic status, but Lake Ontario is much larger than Lake Champlain (longest axis is 311 km relative to 193 km in Lake Champlain), lacks habitat fragmentation, and due to its size is more likely to have higher isolation by distance among fish populations. The two lakes have been isolated for

approximately 10,000 years, providing a context for genetic differences resulting from isolation. Examining sculpin in Lake Champlain and Lake Ontario allows us to assess potential genetic differences resulting from isolation between lakes, isolation by distance within lakes, and isolation by fragmentation in two systems with similar environments.

2.3. Methods

2.3.1. Study sites:

Lake Champlain is a long (193 km) and narrow (20 km at the widest point) lake spanning the border of New York and Vermont, USA and Quebec, Canada. The portion of the lake with deep water suitable for slimy sculpin is approximately 110 km long. The lake has a maximum depth of 122 m and an average depth of 19.5 m. Three large islands naturally divide the northern portion of Lake Champlain into eastern and western arms (Figure 2.1). The construction of six causeways built between 1850 and 1900 have linked the islands to the mainland and have isolated the lake further into three major basins: the Main Lake, Malletts Bay, and the Inland Sea (Figure 2.1; Marsden & Langdon 2012). All the causeways have at least one shallow (1-7 m deep) opening that allows some flow of water and passage of boats and fish; Carry Bay and the Island Line causeways each have an additional non-navigable opening. Lake Ontario is 311 km long, 85 km wide, with a average depth of 84 m and a maximum depth of 244 m; apart from a series of islands in the northeastern portion (Bay of Quinte), the lake lacks physical isolating structures.

Slimy sculpin prefer water temperatures less than 10ºC and rarely inhabit temperatures greater than 15ºC; to assess whether causeways would be expected to act as a substantial

barrier to sculpin, I measured seasonal changes in water temperature in causeway openings. HOBO® temperature probes were placed on the bottom of all causeways openings except the northwest opening to Carry Bay (Figure 2.1). Temperature was recorded at openings once per hour for 12 months. Slimy sculpins are generally only found in water greater than 25 m deep, therefore depth profiles of all but the Island Line causeway (Figure 2.1) openings were measured using a weighted line from a small boat and depth of the remaining two Island Line causeway openings was estimated using chart data (NOAA Coast Survey 1997).

2.3.2. Fish sampling and genetic analysis

Two hundred slimy sculpin were sampled during August and September 2014 and May, June and July 2015 using benthic trawls at seven sites throughout Lake Champlain (Figure 2.1). Forty-eight slimy sculpin were sampled in October 2016 from two locations approximately 230 km apart in Lake Ontario, NY, one near Fairhaven, New York (43° 29.231'N, -76° 38.053'W) and one near Hamilton, Ontario (43° 20.462'N, 79° 27.736'W). Individuals were euthanized by cooling directly on ice, measured to the nearest millimeter (total length), and caudal fins were collected following protocols outlined in LaHood *et al.* (2008) or frozen.

DNA was extracted from fin clips using standard procedures from a DNeasy Blood and Tissue Kit (Qiagen). The concentration of DNA template was verified on a NanoDrop and ranged from 6 – 100 ng/µl of DNA, though most samples contained between 30 and 50 ng/µl. Following extraction, polymerase chain reaction (PCR) amplification was conducted for 10 microsatellite loci previously identified for sculpin (Table 2.1). Markers

were multiplexed when possible in 25 µl reactions using 2X Q5 High Fidelity DNA Polymerase Master Mix (New England BioLabs Inc.), and 20 pmol of a fluorescently labeled forward primer and un-labeled reverse primer, and 6 – 100 ng of the DNA template. The general PCR program used was 98°C for 2 min, 30 cycles at 98°C for 30 s at marker-specific annealing temperature (Table 2.1), 72°C for 45 s, followed by a final extension of 72°C for 10 min. Fragment analysis of PCR products was conducted in the University of Vermont Advanced Genome Technologies Core using an Applied Biosystems 3130 Genetic Analyzer and a ROX 500 size standard and scored using GENEMAPPER software (Applied Biosystems).

2.3.3. Statistical analysis:

Conformance to Hardy-Weinberg equilibrium (HWE) expectations at each locus was estimated using Markov chain Monte-Carlo methods in ARLEQUIN (Excoffier & Lischer, 2010) with 100,000 step burn-in and 900,000 step determination. Any deviations from HWE were assessed for heterozygote excess or deficiency and significance levels were adjusted using a Bonferroni correction. All loci were assessed for the presence of null alleles with MICRO-CHECKER version 2.2.3 (Van Oosterhout *et al.*, 2004). To quantify the genetic diversity for each locus, the number of alleles per locus was determined and observed (H_O) and expected (H_E) heterozygosity calculated using GenAlEx (Peakall & Smouse, 2006, 2012). Allelic richness was calculated using rarefaction in FSTAT version 2.9.3.2 (Goudet, 1995). To test whether diversity varied between sites and lakes, mean observed heterozygosity and allelic richness were evaluated for differences between Lake Ontario and Lake Champlain and among Main

Lake sites and sites in Malletts Bay and the Inland Sea in Lake Champlain by comparing observed data to 10,000 permutations in FSTAT. As an additional estimate of diversity, effective population size of each sampled location was calculated using a linkage disequilibrium method in N_eESTIMATOR (Do *et al.*, 2014) with minimum acceptable allele frequencies of 0.05, 0.02, and 0.01. Following estimation, a minimum allele frequency of 0.02 was chosen because large changes in effective population size were found between a 0.05 and 0.02 minimum allele frequency, suggesting 0.05 may have been too stringent for our dataset.

Possible genetic structure between lakes and among sites was evaluated using pairwise comparisons of F_{ST}, and their associated levels of significance were calculated in ARLEQUIN. First, population structure was evaluated by calculating F_{ST} values between Lake Champlain and Lake Ontario. Next, F_{ST} values were calculated within each lake to determine if sculpin populations were structured within lakes. To test for a possible Wahlund effect resulting from early stage isolation, differences in H_O vs. H_E of the total Lake Champlain sculpin population was measured using a Bartlett test executed in R version 3.3.0 using the bartlett.test() function available in the stats package (R Core Team, 2015). To identify statistically significant differences in allelic variance among sites, analysis of molecular variance (AMOVA) was calculated using ARLEQUIN. AMOVAs were run hierarchically, as indicated in Table 1.2 groupings. Sample sites were first grouped by lake, and Lake Champlain slimy sculpin were compared to Lake Ontario slimy sculpin. Next, slimy sculpin from Lake Champlain were analyzed separately, comparing all sampled sites in the Main Lake to sites sampled in the Inland Sea to

determine if causeways could explain differences in allele frequencies. The site in Malletts Bay was excluded because it was the only site sampled in the basin.

To assess whether populations are isolated by distance, Lake Champlain and Lake Ontario were analyzed separately. In Lake Champlain, a pairwise F_{ST} matrix was compared against a pairwise matrix of geographic distance using a Mantel's test to determine whether differences in genetic variation among slimy sculpin sample locations correspond to geographic distance measured as the shortest possible route by water between two sites. Mantel tests were conducted in IBDWeb using 10,000 permutations (Jensen, Bohonak & Kelley, 2005). Pairwise genetic distance was estimated between the two Lake Ontario sites to evaluate whether similar levels of isolation by distance occur in Lake Ontario and Lake Champlain. Because only two sites were sampled in Lake Ontario I was unable to run a Mantel test, however I expected the F_{ST} between sites in Lake Ontario to be similar to F_{ST} between the two furthest sites in Lake Champlain if the effect of isolation by distance is similar in both lakes.

To further examine how slimy sculpin populations were structured among and within lakes, discriminate analysis of principle components (DAPC) and Bayesian STRUCTURE analysis were used to identify clusters of individuals representing populations (Pritchard *et al.*, 2000; Jombart, 2008; Jombart, Devillard & Balloux, 2010). DAPC is a multivariate analysis that maximizes genetic differentiation between groups while minimizing within-group variation. The relationship between sample sites was evaluated hierarchically; DAPC was first run using the complete dataset to visualize the relationship between all samples sites in Lake Ontario and Lake Champlain, then using

only individuals from Lake Champlain. All DAPCs were conducted in R version 3.3.0 using the ADEGENET version 2.0.1 (Jombart, 2008; R Core Team, 2015). Bayesian STRUCTURE analysis was also run hierarchically, first on the total dataset and subsequently on only Lake Champlain individuals. STRUCTURE was run 10 times for each value of k = 1 – 10 with settings of 500,000 replicates and an initial burn-in of 100,000 replicates. The most likely number of clusters (k) was then assessed using ΔK estimated in STRUCTURE HARVESTER (Evanno, Regnaut & Goudet, 2005; Earl & vonHoldt, 2012) and the most likely estimates of k were consolidated into a single best estimate using CLUMPP (Jakobsson & Rosenberg, 2007).

2.4. Results

2.4.1. Habitat suitability:

Average depth of each causeway opening at mean lake level (29.1 m above sea level) varied among causeways, ranging from less than 1.0 m at the Sandbar causeway to just over 7.0 m at the Alburg Passage causeway. However, even when adjusted to the maximum reported lake level of 31.6 m the depth of all openings was less than 10.0 m. Temperature in causeway openings ranged from near 0.0 ºC in January and February when sensors became frozen in ice to 22 – 25 ºC during July and August. For causeway openings with at least 365 days of available temperature data (N = 4), temperature was above the adult sculpin avoidance temperature of 15 ºC for 37 ± 2% of the year and above the preferred temperature of 9 ºC for 53 ± 3% of the year (Otto & Rice, 1977).

2.4.2. Genetic data

Genetic diversity differed slightly between lakes but was consistent within lakes. Locus Cco14 exhibited inconsistencies in allele scoring and was therefore removed from analysis. No loci showed signs of null alleles. All loci except locus Cott213 were polymorphic at all sites with 5 to 25 alleles per locus. All loci at all sites were in HWE following a sequential Bonferroni correction. Observed (H_O) and expected (H_E) heterozygosity was moderate for all sites (average = 0.59 and 0.58, respectively; Table 2.2). Observed heterozygosity was significantly higher ($p = 0.03$) in Lake Champlain (0.62) than in Lake Ontario (0.51) but consistent among sites within each lake. Mean allelic richness of loci was higher ($p = 0.01$) in Lake Champlain (5.9) than in Lake Ontario (5.2). Allelic richness was similar among all sites within Lake Champlain, ranging from 5.6 at Sunset Isle to 6.2 at Inland Sea North. No significant differences in allelic richness were found among Main Lake (5.8), Malletts Bay and Inland Sea populations (6.0; $p = 0.53$). Effective population size was moderate to high for all populations and the upper limit of the confidence interval always included infinity. Effective population sizes of Hamilton and Fairhaven sites in Lake Ontario were estimated to be 140.1 and 101.5 individuals. Within Lake Champlain, effective population sizes tended to be higher at Main Lake sites than Malletts Bay or the Inland Sea. Barber Point, Shelburne Bay and Sunset Isle exhibited the highest effective population sizes in the Main Lake ($Ne = \infty$), followed by Grand Isle ($Ne = 223.1$). Malletts Bay and the Inland Sea North and South sites had more moderate estimated effective population sizes ($Ne = 226.3$, 139.4, and 433.1, respectively).

2.4.3. Between-lake genetic structure:

Sculpin in Lake Ontario were genetically distinct from sculpin in Lake Champlain. Pairwise F_{ST} values between Lake Ontario and Lake Champlain populations were large (0.065 - 0.118) relative to within-lake pairwise comparisons (Table 2.3). When populations in Lake Champlain were compared to populations in Lake Ontario, 10.4% of allele frequency variation occurred between lakes (AMOVA $p < 0.001$) while 89.7% of the variation occurred within individual populations. Both DAPC and a delta k analysis of STRUCTURE indicated the presence of two clusters, offering further evidence of between-lake population structure (Figure 2.2).

2.4.4. Within-lake genetic structure:

Evidence of weak to no genetic differentiation was found among sampled populations within Lake Champlain and Lake Ontario. Pairwise estimates of F_{ST} were small (0.00 - 0.016; Table 2.3). Only two comparisons had F_{ST} values significantly greater than zero, though both corresponded to values less than 0.02. Additionally, there was no indication of a reduction of heterozygosity across loci characteristic of a Wahlund effect (Bartlett test $p = 0.91$). When populations in the Main Lake were compared to populations in the Inland Sea, less than 1% (AMOVA $p = 0.53$) of allele frequency variation occurred between basins while 99.8% of the variation occurred within individual populations. Subsequent runs of STRUCTURE and DAPC examining substructure within Lake Champlain did not reveal any further clustering, suggesting the presence of a single panmictic population (Figure 2.2).

No correlation was observed between waterway distance (the shortest distance by water between two sites) and pairwise F_{ST} in Lake Champlain ($r^2 = 0.08$; $p = 0.82$; Figure 2.3)

indicating that populations of slimy sculpin were not isolated by distance. Additionally, pairwise F_{ST} was zero between Fairhaven and Hamilton in Lake Ontario, similar to pairwise F_{ST} among sites in Lake Champlain. However, Fairhaven and Hamilton are separated by more than 220 km, about four times the maximum distance between sites in Lake Champlain, indicating a lack of isolation by distance in Lake Ontario.

2.5. Discussion

Our findings indicate that, although slimy sculpin in Lake Champlain and Lake Ontario have comparable genetic diversity to slimy and mottled sculpin in streams and rivers (Huff, Miller & Vondracek, 2010; Lamphere & Blum, 2012), they exhibit little to no within-lake genetic structure even across numerous barriers and distances up to 227 km (Breen *et al.*, 2009; Lamphere & Blum, 2012). The lack of any observed genetic structure indicates that sculpins in Lake Champlain and Lake Ontario represent single panmictic populations. The relatively large genetic differences observed between lakes Ontario and Champlain were expected, considering that the lakes have been isolated since the last glacial retreat approximately 10,000 years ago (Rayburn, Franzi & Knuepfer, 2007). Although Lake Ontario and Lake Champlain remain connected by the St. Lawrence River, this route is unlikely to provide enough connectivity to maintain a genetically homogeneous population; transit between the lakes would entail a 360-km downstream trip in the St. Lawrence River, followed by 130 km of upstream dispersal through the Richelieu River, or vice versa.

Low genetic structure is usually a feature of highly connected populations with high mobility and capacity for dispersal (Muths *et al.*, 2013; Thompson *et al.,* 2015).

However, adult slimy sculpin are not considered highly mobile. Adult sculpin in streams have patchy distributions and tend to maintain home ranges of 1 to 5 river-km (Galloway *et al.*, 2003; Gray, Cunjak & Munkittrick, 2004). However, little information exists about the movement of slimy sculpin in lakes. Nonetheless, the lack of any genetic structure among sculpin populations in Lake Champlain is particularly surprising given the fragmentation of the lake by causeways. Several of our sample sites were separated by large areas of shallow habitat not usually inhabited by slimy sculpins. For example, Malletts Bay and Sunset Island are only 3 km apart, but separated by a 5-km causeway built on top of a shallow (1–3 m deep) 1 km wide sandbar. To maintain the level of population connectivity I observed, sculpin would need to disperse across at least 1 km of unsuitable habitat. To migrate from the Inland Sea to the Main Lake, slimy sculpin must pass through at least two causeways via 2–5 km of shallow (1-10 m) water. For these deep-water fish, the depth and temperature of the causeway openings should be a substantial barrier to movement (Scott & Crossman, 1973; Otto & Rice, 1977). Causeway openings were, however, within an acceptable temperature range for slimy sculpin (< 10 ºC) during the early spring, late fall and winter (50 – 70% of the year). Thus, adult slimy sculpins might disperse through the openings during these times. Given the moderate level of differentiation between Lake Champlain and Lake Ontario populations which have been isolated for thousands of years, it is possible that within Lake Champlain insufficient time has passed to detect the effects of isolation by causeways. Though I cannot conclusively refute the hypothesis that not enough time has passed to see the effects of isolation, there was little evidence of genetic structure or a Wahlund effect

indicative of early stage isolation found in our study (Wahlund, 1928). Therefore, I suggest time since isolation is not the most important factor limiting population differentiation.

Genetic panmixia in the absence of adult movement could be the result of larval dispersal. In marine systems, larval fish commonly disperse substantial distances (100 – 1000 km) by advection (Pineda, Hare & Sponaugle, 2007). In the Great Lakes, models of yellow perch larval drift suggest individuals could drift from southern to northern Lake Michigan, a distance of 200 - 300 km, before settling to the bottom (Beletsky *et al.*, 2007). Deepwater sculpin *Myoxocephalus thompsonii* larvae are known to be pelagic (Geffen & Nash, 1992), but slimy sculpin larvae are generally assumed to be benthic, which would limit their likelihood of dispersal (e.g., Lantry *et al.*, 2007, GLFC Sculpin Workshop, 2007). Nevertheless, slimy sculpin larvae have been found in the water column during spring icthyoplankton tows in Lake Huron (Martin, Czesny & Wahl, 2011; Roseman & O'Brien, 2013) and throughout the summer in Lake Michigan, suggesting that larvae may remain pelagic long enough to disperse long distances by advection before settling to the bottom (Geffen & Nash, 1992). Summer surface current velocities in Lake Champlain and Lake Ontario are comparable to Lake Michigan (Rao & Murthy, 2001; McCormick *et al.*, 2008), so larval sculpins could disperse long distances through advection.

Larval advection could also explain why lake causeways have little to no effect on slimy sculpin populations. The flow of water through causeway openings can be substantial (34,000 – 325,000 $m^3 hr^{-1}$) and thus may facilitate larval drift among basins (Myer &

Gruendling, 1979). However, flow direction varies among openings, and can be almost entirely unidirectional; for example, water through the Carry Bay and Grand Is-North Hero causeways flows predominately west into the Main Lake, flowing in the opposite direction from the Main Lake into the Inland Sea only 15% of the time (Myer & Gruendling, 1979). Therefore, currents in causeway openings could facilitate asymmetric movement among basins.

Alternatively, lack of genetic structure in slimy sculpin in lakes could be explained by extremely large populations. The effective population size of sculpin in three of the seven sites sampled in Lake Champlain was estimated to be infinity, and the upper confidence interval from all sites included infinity. However, the lower confidence interval for effective population size for all sites was less than 450, similar to effective population sizes observed in stream populations of sculpin that showed significant levels of structure (Dennenmoser, Rogers & Vamosi, 2014). Given that population structure has been identified in species with very large population sizes (e.g., Foley *et al.*, 2013), I suggest that that large population size alone is unlikely to explain the lack of genetic structure observed in Lake Champlain and Lake Ontario.

The lack of genetic structure and isolation by distance of slimy sculpin in our study contrasts with the high genetic structure observed in stream populations collected only a few kilometers apart (Junker *et al.*, 2012; Dennenmoser, Rogers & Vamosi, 2014; Table 2.4). In 12 other microsatellite-based studies of sculpins I identified similar observed heterozygosity and allelic richness but substantially lower F_{ST} than any other study (Table 2.4). All but one of the 12 other microsatellite studies of sculpin focused on rivers or river

systems and the remaining study focused on coastal populations. Therefore, the higher population structure seen in these studies could be partially explained by the higher degree of physical fragmentation and unidirectional flow in rivers than in our lake system. However, even when compared to pairwise estimates in relatively unfragmented systems our pairwise F_{ST} estimates were often an order of magnitude smaller than the minimum pairwise F_{ST} in other studies.

My findings highlight how little is known about the life history and dispersal of sculpin in lakes and suggest that there may be significant differences in behavior and life history between lotic and lentic populations. Other studies have also indicated that the ecology and evolution of lentic and lotic fish populations can differ substantially (Swain & Holtby, 1989; Minns, 1995; Istead, Yavno & Fox, 2015). I recommend that future research should focus on determining whether low genetic structure in lakes is a general trait for the Cottidae family by expanding research to other common lentic and lotic species such as mottled sculpin. Additionally, I propose that direct assessment of adult and larval movement of sculpin in streams and in lakes would be an important next step in determining how sculpin populations remain connected. Finally, our results emphasize the importance of examining ecology and population structure in a variety of habitats to accurately characterize family- and species-wide trends.

Table 2.1: Characteristics of 10 microsatellites amplified in slimy sculpin. Shown are the GenBank marker name, repeat motif, forward and reverse primer sequence, fluorophore tail, amplified size range, annealing temperature (Ta) and citation for the source of the marker.

marker	repeat	primer (5' - 3')		size range	Ta	source
Cco02	Tri	F: TTCTTGTTCTCCGTCTTGAGC	HEX	227-254	59	Fujishin et al. 2009
		R: CCCATCTTCTCCTCCTGTCC				
Cco08	Tri	F: TTGCAAACTTCAGACAGTAAAGC	FAM	87-111	55	Fujishin et al. 2009
		R: GCTGAGAATCCAGGAAGGAG				
Cco13	Tri	F: CCTGGAATTTCACCAAGGTC	NED	221-248	55	Fujishin et al. 2009
		R: TCACAACAAAGCCAGAGGAC				
Cco17	Tri	F: TCGTCTTGGAAATGGAAAGC	HEX	69-142	55	Fujishin et al. 2009
		R: CATGTCAGCAGGATATCACGTC				
Cco11	Di	F: GCAGGAGGAACACGAAGATG	NED	198-230	60	Fujishin et al. 2009
		R: CTCAAGGAACTACACACACATGC				
Cco14	Tetra	F: CATAAAACCTGTGGCTTTGG	HEX	NA	60	Fujishin et al. 2009
		R: GACGCTCTGCTGGAGAGATG				
Cott105	Di	F: TCCTACAGGGTGCGATCGTG	FAM	322-346	60	Nolte et al. 2005
		R: TGCAGGAGTCAGGACTCTGC				
Cott128	Di	F: TCTGTGGGTGTTTGGTCGTG	HEX	314-350	60	Nolte et al. 2005
		R: TGAACTCTGCACATGACTGC				
Cott113	Di	F: AGCGCCAGAATGCAGCATCC	FAM	132-142	60	Nolte et al. 2005
		R: AGTGTGGCGAGCCCAAGATC				
Cott213	Di	F: TTGCCATGGATTTGAGGCAG	NED	331-333	60	Nolte et al. 2005
		R: AGCATTGCTATTATCAGGCTGC				

Table 2.2: Site-specific summary statistics of slimy sculpin genotypes taken from nine microsatellite loci grouped by lake, basin, and site. N = number of individuals genotyped, Na = mean number of alleles per locus, H_O = observed heterozygosity, H_E = expected heterozygosity, Ne = effective population size, nPA = number of private alleles and AR = mean allelic richness across all loci.

Site	N	Na	H_O	H_E	Ne	nPA	AR
Lake Champlain							
Main Lake							
Grand Isle	30	6.9	0.651	0.601	223.1	1	5.79
Sunset Isle	30	6.7	0.628	0.600	∞	3	5.59
Shelburne Bay	30	7.2	0.618	0.593	∞	2	5.94
Barber Pt.	30	7.2	0.609	0.612	∞	4	5.86
Inland Sea							
Inland Sea N.	31	7.4	0.640	0.631	139.4	5	6.17
Inland Sea S.	31	7.1	0.562	0.595	433.1	4	5.81
Malletts Bay							
Malletts Bay	18	6.1	0.617	0.586	226.3	1	5.92
Lake Ontario							
Fairhaven	24	6.1	0.534	0.509	101.5	3	5.40
Hamilton	24	5.8	0.486	0.480	140.1	4	5.09

Table 2.3: Pairwise F_{ST} (below the diagonal) and corresponding p-values ± standard deviation (above the diagonal) calculated in ARLEQUIN for slimy sculpin sampled from two sites in Lake Ontario (Fairhaven and Hamilton) and three major basins in Lake Champlain isolated from one another by causeways. The three basins were the Main Lake (Grand Isle, Sunset Isle, Shelburne Bay, Barber Point), the Inland Sea (north and south sites), and Malletts Bay.

	Grand Isle	Sunset Isle	Shelburne Bay	Barber Pt	Inland Sea N.	Inland Sea S.	Malletts	Fairhaven	Hamilton
Grand Isle	*	0.045 ±0.024	0.973 ±0.018	0.874 ±0.024	0.847 ±0.034	0.333 ±0.054	0.910 ±0.017	0.000 ±0.000	0.00 0±0.000
Sunset Isle	0.009	*	0.604 ±0.053	0.676 ±0.041	0.198 ±0.030	0.009 ±0.009	0.189 ±0.057	0.000 ±0.000	0.000 ±0.000
Shelburne Bay	-0.008	-0.003	*	0.829 ±0.038	0.532 ±0.042	0.153 ±0.031	0.910 ±0.029	0.000 ±0.000	0.000 ±0.000
Barber Pt	-0.007	-0.004	-0.005	*	0.964 ±0.014	0.288 ±0.057	0.955 ±0.020	0.000 ±0.000	0.000 ±0.000
Inland Sea N.	-0.006	0.003	0.000	-0.007	*	0.802 ±0.032	0.847 ±0.024	0.000 ±0.000	0.000 ±0.000
Inland Sea S.	0.001	0.016	0.005	0.002	-0.004	*	0.423 ±0.047	0.000 ±0.000	0.000 ±0.000
Malletts	-0.009	0.005	-0.009	-0.011	-0.006	0.001	*	0.000 ±0.000	0.000 ±0.000
Fairhaven	0.091	0.098	0.083	0.096	0.106	0.115	0.065	*	0.694 ±0.039
Hamilton	0.111	0.118	0.108	0.119	0.130	0.141	0.091	-0.004	*

Table 2.4: Diversity and basic environmental metrics from 12 microsatellite studies of sculpin compared to the slimy sculpin in Lake Champlain and Lake Ontario. Distance estimates are based approximately from site maps or mantel plots when no exact numbers are reported as indicated by a '~'. Data not reported in the cited study is indicated by 'NR'.

species	number of loci	region/river	H_O	allelic richness	mean/range of pairwise F_{ST}	distance range (km)	source
Cottus asper	10	American, Tuolumne, Kings rivers, California	0.311	1.38	0.238	~3-200	Baumsteiger & Aguilar, 2014
Cottus asper	14	Lower Fraser River, British Columbia, Canada	0.577	6.31	0.128	~10-500	Dennenmoser et al., 2014
Cottus asper	11	Northern California streams and rivers	0.366	3.02	0.010 - 0.501	2-1,250	Baumsteiger et al., 2016
Cottus asperrimus	9	Hat Creek Fault, California	**0.385	5.25	0.32	25-Aug	Kinziger et al., 2016
Cottus bairdi	12	Nantahala River, North Carolina	0.598	NR	0.026	0.3-5.6	Lamphere & Blum, 2012
Cottus bairdi	6	Lake Michigan tributaries, Michigan	0.32	2.7	0.235	~3-400	Homola et al., 2016
Cottus beldingi	8	Truckee River, Nevada	0.665	NR	−0.002 - 0.046	~2-78	Peacock et al., 2016
Cottus cognatus	8	Northern Mississippi River and tributaries	0.62	5.85	*0.450	~5-120	Huff et al. 2010

Cottus gobio	10	Sense River, Switzerland	0.52	4.19	0.058	0.5-40	Junker et al., 2012
Cottus gobio	7	River Rye, England	**0.528	5.04	0.268	0.2-80	Hänfling & Weetman, 2006
Cottus gulosus	10	American, Tuolumne, Kings rivers, California	0.141	1.16	0.634	~3-200	Baumsteiger & Aguilar, 2014
Cottus gulosus	6	Northern California streams and rivers	0.18	2.12	0.596	40-602	Baumsteiger et al., 2014
Cottus pitensis	6	Northern California streams and rivers	0.114	1.35	0.267	7-285	Baumsteiger et al., 2014
Trachidermus fasciatus Heckel	16	Coast of Qinhuangdao and Ariake Sea, China	0.831	9.64	0.054	70 - 1200	Li et al., 2016
Cottus cognatus	9	Lake Champlain, Vermont	0.617	5.87	***0.000	3-77	present study
Cottus cognatus	9	Lake Ontario, New York, USA/Ontario, CA	0.51	5.25	***0.000	227	present study

* Data from a recent reintroduction from three source populations; **Expected, not observed heterozygosity presented; *** Data from single, pairwise comparison.

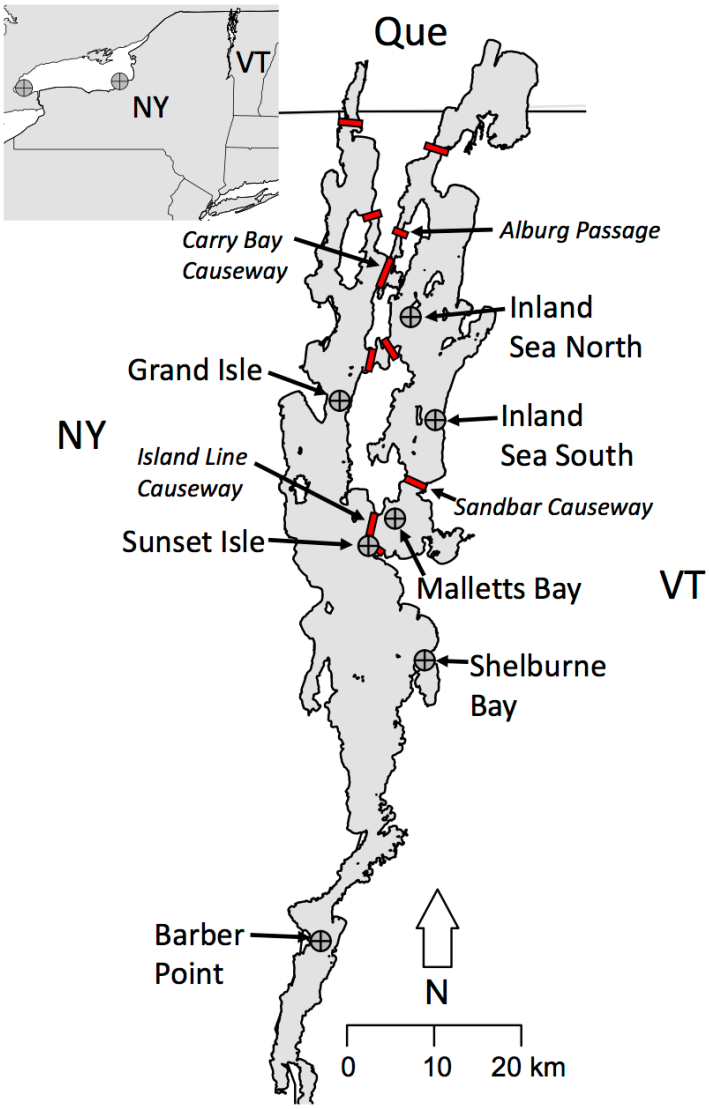

Figure 2.1: Sample sites indicated by open crossed dots for slimy sculpin in Lake Champlain and Lake Ontario (inset map), and location of nine causeways (red bars) hypothesized to pose barriers to fish movement.

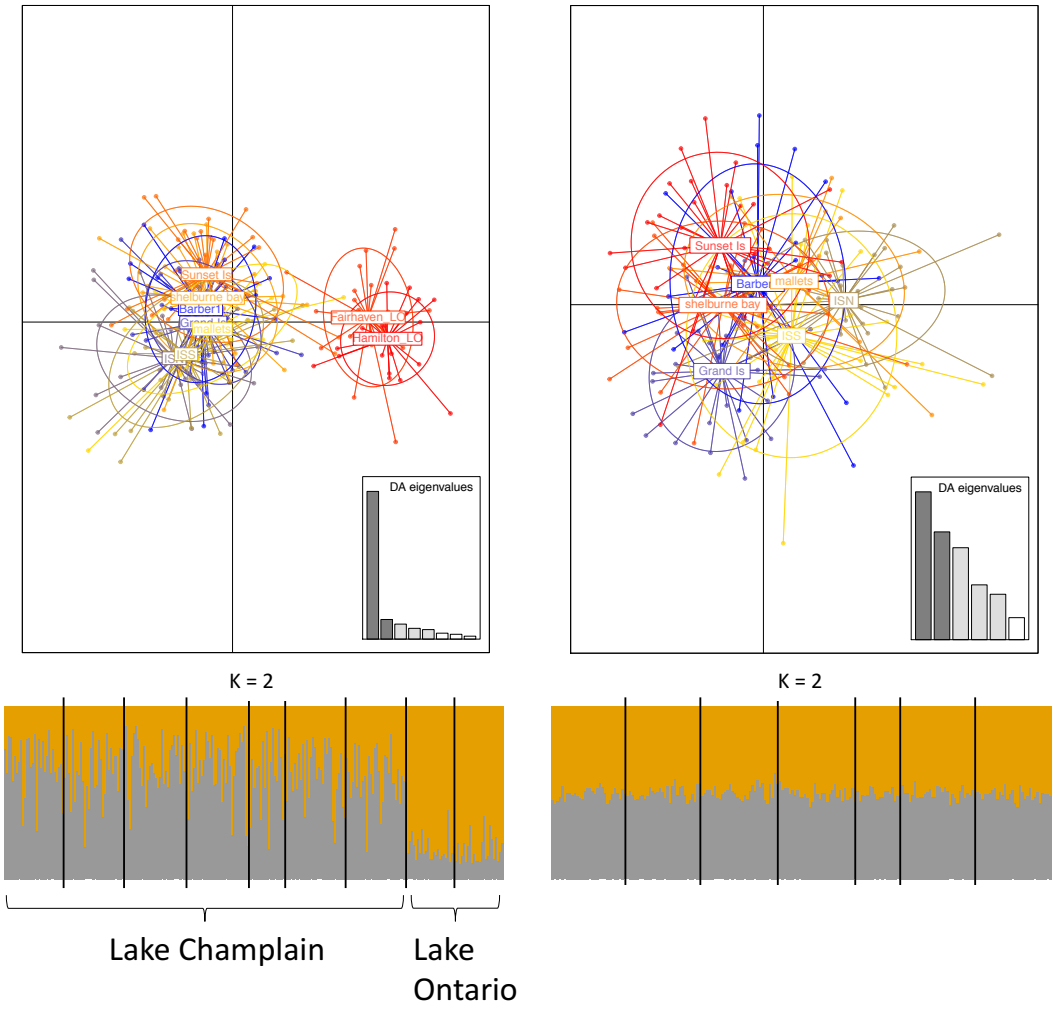

Figure 2.2: Clustering of two Lake Ontario and seven Lake Champlain slimy sculpin populations (left) based on DAPC (top) and STRUCTURE (bottom) and the same data for only Lake Champlain (right). In the scatterplot of DAPC results, individuals are represented by dots and sampled populations are coded by color and encircled with inertia ellipses. The STRUCTURE barplot is a graphical representation of individual membership coefficient to each cluster (vertical bars). Colors represent different estimated clusters of a single admixed individual. Based on results from ΔK analysis, only K = 2 are shown.

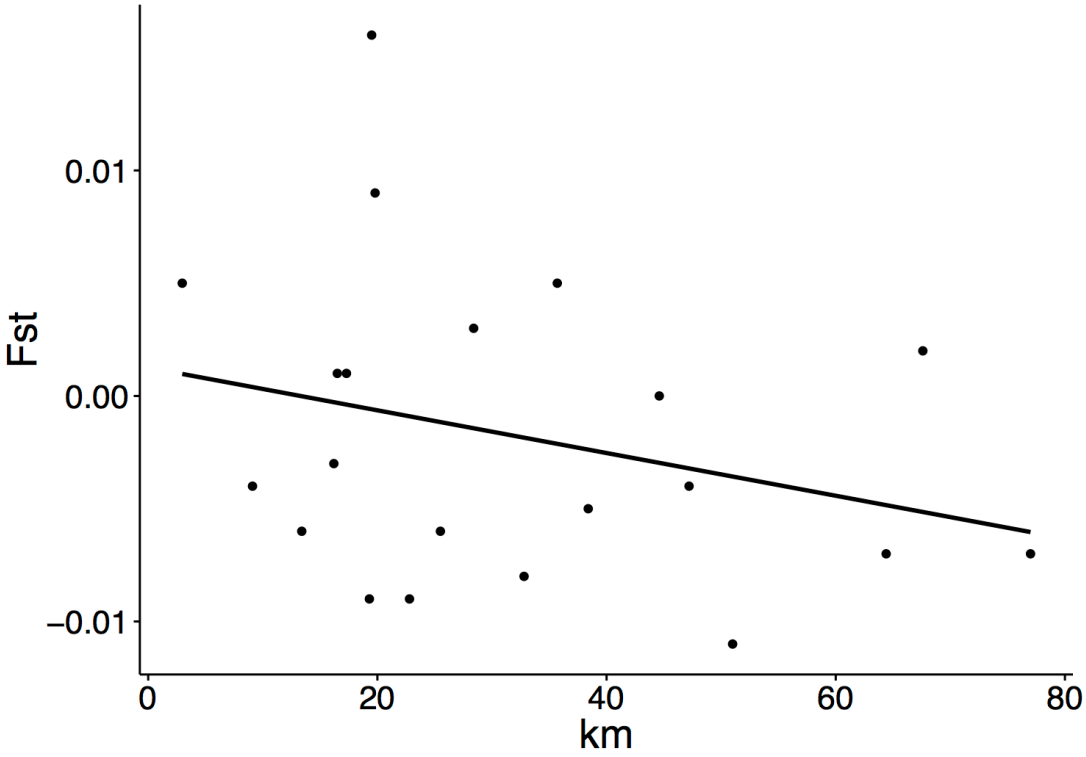

Figure 2.3: Correlations between waterway distance and all pairwise F_{ST} genetic distance estimates for slimy sculpins from seven locations in Lake Champlain.

CHAPTER 3: GENETIC VERSUS DEMOGRAPHIC STOCK STRUCTURE OF RAINBOW SMELT IN A LARGE FRAGMENTED LAKE

3.1. Abstract

Boundary delineation of fish stocks plays an important role in fisheries management but the results of stock identification often depend on the technique used and the management goal. Historically, stocks were identified by place of capture, population demography and morphology, but recently genetic stock identification has become more standard. Here I evaluate the stock structure of rainbow smelt (*Osmerus mordax*) in three fragmented basins of Lake Champlain using 26 years of population demographic data collected by the Vermont Fish and Wildlife Department and genotype data from six microsatellite loci. Length, age, and catch-per-unit-effort of smelt captured different basins suggested that the smelt from different basins in Lake Champlain are at least partially isolated from one another. However, no genetic differences among smelt were identified suggesting that there is still gene flow among basins. Therefore, rainbow smelt in Lake Champlain should be considered to consist of at least three demographic stocks, but a single genetic stock. Our results indicate that care should be taken when using only a single method of stock identification otherwise

important aspects of population structure could be missed leading to erroneous conclusions about stock recruitment and mortality.

3.2. Introduction

Stock assessment is central to successful fisheries management (Dickey-Collas *et al.*, 2010; Price *et al.*, 2017). Therefore, classifying the limits of stock identification and delineation techniques continues to be an important area of fisheries research. Stock assessment strategy generally falls into one of two categories, genetic or phenotypic (Begg, Friedland & Pearce, 1999). While genetic assessment provides direct evidence of reproductive isolation among stocks, phenotypic assessments based on geometric morphometrics or demography provide indirect evidence of prolonged post-larval isolation of stocks (Begg, Friedland & Pearce, 1999). Since their development, molecular techniques have become the gold standard for stock assessment (Begg & Waldman, 1999; Begg, Hare & Sheehan, 1999). Though the definition of 'stock' varies, the concept of stock almost always implies genetic continuity among individuals (Ihssen *et al.*, 1981). Modern molecular techniques make identification of reproductively isolated fish stocks quick and simple, and allow for detailed mixed stock analysis (Sweijd *et al.*, 2000; Ward, 2000). The increased efficiency and decreased cost of genetic sample processing has led to the broad application of genetic techniques to identify and monitor fisheries and a move to refine or create new management areas based on genetic data (Reiss *et al.*, 2009).

Prior to development of molecular techniques, stock identification centered around phenotypic differences between stocks using morphometrics, demographics, life history variation, and, more recently, otolith microchemistry (Begg, Friedland & Pearce, 1999). Though both molecular and phenotypic methods are valid for stock analysis, the two methods can contradict each other (Swain & Foote, 1999). The contradiction between methods is in part because in large populations, which are common for many species of fish, even a small amount of migration (less than 1%) is enough to eliminate genetic differentiation between groups while demographic differences may be able to persist with up to 10% migration between stocks (Hastings, 1993). Therefore, using a combination of both molecular and morphometric techniques may be the best way to identify stock structure.

In lakes, stocks are rarely physically isolated from one another, and differences between stocks are driven by spatial isolation of spawning sites or currents that affect the dispersal of early life stages (VanDeHey *et al.*, 2009; Sepulveda-Villet & Stepien, 2011). Lake Champlain, a 1127 km^2 lake between New York and Vermont and Quebec, is an example of an anthropogenically fragmented lake. Three large islands connected by six causeways divide the northern portion of Lake Champlain into three major basins: the Main Lake, Malletts Bay, and the Northeast Arm, leaving only small openings in the causeways for movement of fish and boats between basins (Figure 3.1; Marsden & Langdon, 2012). The physical fragmentation of Lake Champlain has led state agencies to focus assessment and management at the basin level, though very little research has been conducted to determine the level of connectivity among basins.

The three basins of Lake Champlain vary in size, trophic status, mean depth, and species community (Potash, Sundberg & Henson, 1969; LCBP, 2015). The Main Lake is mesotrophic (9 – 17 µg/l chlorophyll), with an average depth of 29 m and maximum depth of 120 m, and contains 82% of the total volume of the lake. The Northeast Arm is mesotrophic with two eutrophic bays (14 – 19 µg/l chlorophyll), has an average depth of 13 m and maximum depth of 49 m, and contains 13% of the total volume of the lake. Malletts Bay is oligotrophic (8 – 12 µg/l Chlorophyll), has an average depth of 13 m and maximum depth of 32 m, and contains just 3% of the total volume of the lake. The community composition of each basin varies; for example, species which prefer deep, cold water such as salmonids, sculpins (*Cottus* spp.), and *Mysis diluviana* are generally more common in the Main Lake than in either of two the smaller basins.

Despite the small openings in each causeway, the causeways may limit fish movement. The openings are shallow (< 10 m) and warm (22 – 25 ºC during July and August) and should therefore be at least a seasonal barrier to fish that live in cold and deep water. However, Euclide *et al.*, (2017) found that populations of slimy sculpin (*Cottus cognatus)* were panmictic across causeways and distance, even though adult sculpin move only short distances (Gray, Cunjak & Munkittrick, 2004; Breen *et al.*, 2009). One hypothesis that explains this phenomenon is that causeways are barriers to adult fish but not to planktonic larvae, resulting in lakewide genetic population connectivity even in the absence of adult dispersal across causeways. If this is the case, the genetic stock structure of a species could indicate a single mixed population, while growth and mortality measured in adults may be basin-specific.

Causeways in Lake Champlain may also restrict movement of rainbow smelt (*Osmerus mordax*), a key forage fish species for walleye (*Sander vitreus)* and salmonids (Marsden & Langdon, 2012). Density, growth, and diet of age-0 and age-1 rainbow smelt appears to differ among basins, suggesting that the restriction of fish movements between basins by causeways has resulted in demographically distinct stocks (Stritzel Thomson *et al.*, 2011). Similar differences in population characteristics of rainbow smelt (i.e., length-frequency distributions, fecundity, and growth) have been shown in Lake Superior among three zones along the Minnesota shoreline (Luey & Adelman, 1984). Smelt populations in these zones were also shown to be genetically distinct (Schreiner *et al.*, 1984). In Lake Champlain, rainbow smelt were the major conduit of energy from primary consumers to higher trophic levels (Kirn & LaBar, 1996). Therefore, an understanding of the stock structure and population dynamics of rainbow smelt in Lake Champlain is important for management of the recreational fishery. I hypothesized that lake causeways have led to detectable levels of genetic and demographic population structure within Lake Champlain.

3.3. Methods

3.3.1. Study species

Rainbow smelt are native to Lake Champlain and were the main pelagic planktivore until alewife (*Alosa pseudoharengus)* invaded the lake in 2004 (Marsden & Langdon, 2012). Unlike in the Great Lakes, rainbow smelt in Lake Champlain are not adfluvial, but spawn in the lake (Plosila, 1984; Marsden & Langdon, 2012). Generally, rainbow smelt spawn

shortly after ice-out when water temperatures rise above 4.4 C (Becker, 1983). However, O'Brien *et al.* (2012) found a stream-spawning cohort in May and a later, lake-spawning cohort in July in St. Martin Bay, Lake Huron, suggesting spawning time and habitat can vary. Spawning substrate of rainbow smelt is varied and includes gravel, sand, and submerged vegetation (Scott & Crossman, 1973). Therefore, rainbow smelt may be able to successfully spawn in a wide variety of locations in Lake Champlain. Rainbow smelt larvae are planktonic and can be found in the water column throughout the summer (Tin & Jude, 1983). Young-of-year (YOY) rainbow smelt remain in warm water (10–20°C) near or above the thermocline while adult rainbow smelt (age-1 and older) are found in cool (<10–12°C) deep water (Simonin *et al.*, 2012).

3.3.2. Fish sampling (genetics)

Rainbow smelt for genetic analyses were sampled from Malletts Bay, the Northeast Arm, and two sites in the Main Lake (Barber Point and Valcour Island) of Lake Champlain by the Vermont of Fish and Wildlife Department (VTFWD) during the annual forage fish survey in 2012 (Figure 3.1). Additional samples for genetic analysis were collected from Juniper Island during bottom trawls on the University of Vermont R/V Melosira during June 2015. Individuals were euthanized by cooling directly on ice, measured to the nearest millimeter (total length), and caudal fin clips were collected following protocols outlined in LaHood *et al.,* (2008) or taken from whole frozen fish.

3.3.3. Genetic analysis

DNA was extracted from 167 rainbow smelt fin clips using standard procedures from a DNeasy Blood and Tissue Kit (Qiagen). The concentration of DNA template was verified on a NanoDrop and ranged from 6 – 100 ng/µl, though most samples contained between 30 and 50 ng/µl. Samples with more than 50 ng/µl of DNA were diluted with molecular Biology Grade Water (Mediatech Inc.) to 50 ng/µl. Following extraction, polymerase chain reaction (PCR) amplification was conducted for eight previously identified microsatellite loci (Table 3.1). Markers were multiplexed when possible in 25 or 12.5 µl reactions. Loci Osmo12, Osmo16, Osmo45, and Osmo157 (Saint-Laurent, Legault & Bernatchez, 2003) were amplified using 2X Q5 High Fidelity DNA Polymerase Master Mix (New England BioLabs Inc.), and 20 pmol of a fluorescently labeled forward primer and unlabeled reverse primer, and 5 – 50 ng of the DNA template. The general PCR program used for these loci was 98°C for 2 min, 30 cycles at 98°C for 30 s at marker-specific annealing temperature (Table 3.1), 72°C for 45 s, followed by a final extension of 72°C for 10 min. Loci Omo1, Omo3, Omo5, and Omo11 (Coulson *et al.*, 2006) were amplified using 2X Taq Master Mix (New England BioLabs Inc.), and 20 pmol of a fluorescently labeled forward primer and unlabeled reverse primer, and 5 – 50 ng of the DNA template. The general PCR program used for these loci was 95°C for 2 min, 30 cycles at 95°C for 30 s, 20s at marker-specific annealing temperature, 68°C for 30 s, followed by a final extension of 68°C for 10 min. Fragment analysis of PCR products was conducted in the University of Vermont Advanced Genome Technologies Core using an Applied Biosystems 3130 Genetic Analyzer and a ROX 500 size standard and scored using GENEMAPPER software (Applied Biosystems).

All loci were assessed for the presence of null alleles with MICRO-CHECKER version 2.2.3 (Van Oosterhout *et al.*, 2004). Conformance to Hardy-Weinberg equilibrium (HWE) expectations at each locus, observed (H_O) and expected (H_E) heterozygosity, F_{IS} and allelic richness was estimated using the basicStats() function of the diveRsity package in R version 3.3.3 (Keenan *et al.*, 2013; Team, 2015). Any deviations from HWE following Bonferroni correction for multiple comparisons were assessed for heterozygote excess or deficiency in the diveRsity package for R. Effective population size of each sampled location was calculated using a linkage disequilibrium method in N_eESTIMATOR (Do *et al.,* 2014) with minimum acceptable allele frequencies of 0.02.

To evaluate whether basins supported genetically distinct stocks of rainbow smelt, genetic distance among sample sites was measured using pairwise comparisons of G'_{ST}, and F_{ST}. Pairwise G'_{ST} and 95% confidence intervals were calculated using the diveRsity R package and pairwise comparisons of F_{ST} were calculated in Arlequin (Excoffier & Lischer, 2010; Keenan *et al.*, 2013). I tested for the statistical power to detect genetic differentiation for the sample sizes, number of loci and allele frequencies used in this study at five different expected levels of F_{ST} (0.001, 0.0025, 0.005, 0.01, and 0.05) using POWSIM (Ryman & Palm, 2006; Ryman *et al.*, 2006). POWSIM simulates the sampling of genes from a specified number of population with a set effective population size (2000 for this study) that have diverged by drift for *t* number of generations. Samples from the simulated populations are then used to test for genetic homogeneity using Fisher's exact test and Chi-Square tests. Power is then defined as the proportion of significant results obtained over multiple replicate simulations (2000 for this study). To estimate the

number of genetically distinct groups of rainbow smelt without *a priori* assumptions of population, two different clustering models were used. First, clustering was assessed using discriminant analysis of principal components (DAPC) which is a multivariate analysis that summarizes genetic differentiation between groups while overlooking within-group variation (Jombart, Devillard & Balloux, 2010). All DAPCs were conducted in R version 3.3.0 using the ADEGENET version 2.0.1 (Jombart, 2008; R Core Team, 2015). Second, Bayesian STRUCTURE analysis was run using the ParallelStructure package in R (Besnier & Glover, 2013) for each value of k = 1 – 5 with settings of 900,000 replicates and an initial burn-in of 100,000 replicates.

3.3.4. Demographic analysis

Rainbow smelt were sampled annually from 1985 to 2015 in the three major main basins of Lake Champlain by VTFWD. However, due to variability in the early sampling protocol, only data from 1990 to 2015 were used. Rainbow smelt were captured from three areas of the Main Lake (focused around Barber Point, Juniper Island, Valcour Island), one site in Malletts Bay, and one in the Northeast Arm (Figure 3.1). Sampling consisted of stepped oblique midwater trawling at night (Kirn & LaBar, 1991), between late July and early August. Each station was trawled four times with only one station being sampled per night. Trawls were deployed from 35 m to 10 m in 3-m steps of 5 min each.

Catch-per-unit-effort (CPUE) was expressed in terms of catch per 55 min of trawling. For each trawl replicate, all age-1 and older fish were counted and up to 200 fish were

measured (total length, mm), weighed (g) and otoliths were extracted for age estimation; whole otoliths were viewed at 30-70x magnification after clearing in 2:3 solution of glycerin and 70% ethyl alcohol (Kirn & LaBar, 1996). Due to high variability in age estimation of age 5+ smelt using this method, only age 1-4 smelt were used in our analysis.

Evaluation of demographic differences among basins focused on three principal metrics: age distribution, length-at-age, and catch-per-unit-effort (CPUE). Spearman rank correlations were used to compare basins across years because Shapiro-Wilk Normality tests (Royston, 1995) generally showed that data were not normally distributed and because the large magnitude of differences among years could bias non-rank based correlation methods, such as a Pearson's correlation. All analyses and graphics were conducted using R version 3.3.3 and the ggplot2 package version 2.2.1 (R Core Team, 2015; Wickham, 2009).

Variation in age distribution among basins was evaluated using chi-square analysis of the number in each age class summed across all years of data. Because age structure can be highly variable among years, depending on recruitment to age-1, the consistency in year class strength of age-1 fish among basins was evaluated using non-parametric Spearman rank correlations with annual mean number of age-1 fish as the response variable. I predicted that if Lake Champlain consisted of a single demographic stock of rainbow smelt, a strong positive correlation in the proportion of age-1 fish between any two basins would be evident.

Preliminary use of the von Bertalanffy growth equation showed that rainbow smelt generally did not have asymptotic growth in Lake Champlain, therefore differences in growth between basins, were evaluated using average length-at-age across all sampled years for age 1 to 4 fish and variation in length of age-1 individuals by year. To estimate differences in length-at-age for all age classes between basins, I first analyzed the entire dataset using analysis of covariance (ANCOVA) with mean length of fish as the response variable, basin as the principal factor, and age and year as covariates. Next, to evaluate trends across the time, I restricted the dataset to only age-1 fish which was the most abundant year class in most years at most sites and because early growth and mortality within the first year is often considered to be the most critical period for fish populations (Sifa & Mathias, 1987). I compared mean length of age-1 fish among basins using a 2-way ANOVA with length as the response variable, basin as the principal factor, and year as a covariate. I then used post-hoc Tukey HSD tests to detect comparisons with significant differences and evaluate the consistency of length-at-age-1 differences among basins.

If rainbow smelt growth is basin specific and not lake specific, one would expect there to be no relationship in age-1 length between basins across years, however if basins are interconnected, then yearly growth should be synchronous across years in between basins. To test if the length of age-1 fish was synchronous between basins across years, I used Spearman rank correlations to determine if the mean length of age-1 fish for a given year could be predicted by the mean length of age-1 fish for the same year in a different basin. In addition to synchrony in growth, length could be simply related to population

density. Therefore, to I tested whether age-1 length was correlated with smelt density within each basin with a Spearman rank correlation.

Variation in CPUE among basins was evaluated using a 2-way ANOVA with mean CPUE for a given year as the response variable, basin as the principal factor, and year as a covariate. To investigate which years and in how many years significant differences occurred between basins I re-ran the ANOVA using replicate trawls from each basin in the same year as the response variable and tested for significant differences using post-hoc Tukey HSD tests. The consistency of CPUE between basins across years was evaluated using Spearman rank correlation. Because CPUE can easily be driven by one or two strong year classes, a second set of correlations using CPUE of only age-1 rainbow smelt was conducted to assess whether age-1 CPUE alone might drive differences between basins. Significance for all tests was determined using alpha = 0.05.

3.4. Results

3.4.1. Genetic stock structure

Prior to subsequent analyses, loci Osmo45 and Omo3 were removed from the data due to inconsistencies in allele scoring and evidence of homozygosity excess indicating the presence of null alleles. The remaining six loci were generally in HWE following Bonferroni correction (corrected p-value = 0.01); however, Omo5 was significantly different from HWE expectations in the Northeast Arm samples, but was not found to have significant heterozygote or homozygote excess. Since this locus was in HWE at all other sites, it was included in all analyses. Genetic diversity was similar across all sites

and among all basins (Table 3.2). Observed heterozygosity ranged from 0.65 in the Northeast Arm and Barber Point to 0.67 in Malletts Bay, while allelic richness ranged from 8.52 in Valcour to 9.75 at Juniper Island. Effective population size of all sampled populations other than the Northeast Arm was found to be infinity (Table 3.3).

Tests of statistical power indicated that with our current sample sizes and set of loci the probability of detecting a genetic distance between two samples of F_{ST} = 0.005 was 92% and the probability of detecting a F_{ST} of greater or equal to 0.01 was 100%. Both F_{ST} and G'_{ST} estimates of genetic distance indicated no large genetic differences among any of the sampled sites, including those in different basins separated by at least one causeway (Table 3.3). G'_{ST} was generally small (global G'_{ST} = 0.03) and 95% CI always included 0. Interpretation of both STRUCTURE and DAPC indicated that a single, panmictic, lakewide population of rainbow smelt was the most likely genetic stock structure in Lake Champlain (Figure 3.2). STRUCTURE cannot directly estimate a single-population hypothesis; however, the delta K for all values of k 2 – 5 were small and posterior probabilities indicated that individual cluster membership was equally likely for all inferred cluster. DAPC also identified a single panmictic population as indicated by the high degree of overlap among sites when plotted (Figure 3.2).

3.4.2. Demographic stock structure

From 1990 to 2015 a total of 22,332 rainbow smelt were aged and measured from 676 separate trawls. Because the Main Lake is much larger than either Malletts Bay or the Northeast Arm, samples in the Main Lake were collected from multiple locations to get a

more complete estimate of population structure in the entire basin. Because the objective of this study was to identify differences among basins that are physically isolated by causeways, data from all reference stations in the Main Lake were combined annually to represent a single population of rainbow smelt.

The age distribution of rainbow smelt was skewed heavily, and age-1 to age-4 fish composed 98% of all fish aged and the remaining 2% was composed of age 5 and older fish and some YOY which are not fully recruited to the gear. When data were combined across all available years, age structure differed among basins ($X^2 = 169.41$; df = 6; p-value < 2.2e-16); based on the Pearson residuals of the chi-square test, the abundance of age-1 rainbow smelt was similar among all basins, while differences among basins were driven by age-3 and ange-4 (Figure 3.3). However, the effect was relatively small relating to only a 1 – 3% difference between the observed and expected number of individuals for any basin-by-age comparison. Cohorts of age-1 rainbow smelt appeared to be in synchrony among basins since the start of the dataset in 1990. The proportion of age-1 fish was positively correlated between the Northeast Arm and the Main Lake and between the Northeast Arm and Malletts Bay, but not between Malletts Bay and the Main Lake (Table 3.5).

Rainbow smelt length-at-age differed among basins ($p < 0.001$, $F_{2, 21644} = 3199.44$; Figure 3.4A; Table 3.4). Rainbow smelt were smaller in Malletts Bay than the Northeast Arm or the Main Lake at all ages. Length-at-age of rainbow smelt in the Main Lake and Northeast Arm also differed from each other at all ages but there was an interaction with age such that Northeast Arm rainbow smelt have a slower linear growth rate (11.15

mm/yr) compared to the Main Lake (16.35 mm/yr) or Malletts Bay (14.05 mm/yr) but a larger y-intercept (109.9 mm) than the Main Lake (99.3 mm) or Malletts Bay (90.5 mm). Differences between basins were fairly consistent for most of the 26-year dataset; however, a significant basin:year interaction was identified ($p < 0.001$, $F_{50, 21644} = 115.38$). Year-by-year comparisons of 9,305 age-1 rainbow smelt lengths suggested individuals from Malletts Bay were generally smaller than the other two basins in most years; length of age-1 rainbow smelt also varied significantly by year ($p < 0.001$, $F_{25, 9225} = 124.50$) and a basin:year interaction was identified ($p < 0.001$, $F_{48, 9225} = 48.33$; Figure 3.4B). Tukey HSD post-hoc comparisons indicated that age-1 rainbow smelt from Malletts Bay were significantly smaller than age-1 rainbow smelt in the Main Lake during 15 out of 26 years compared and only significantly larger in one out of 26 years. Overall, age-1 rainbow smelt in Malletts Bay were 12 mm smaller on average than Main Lake rainbow smelt. When compared to the Northeast Arm, Malletts Bay rainbow smelt were significantly smaller in 17 out of 26 years and larger only one of 26 years. Overall, Malletts Bay rainbow smelt were 16 mm smaller on average than Northeast Arm rainbow smelt. Age-1 rainbow smelt in the Main Lake were significantly smaller on average than Northeast Arm rainbow smelt in 8 out of 26 years and averaged 4 mm smaller than rainbow smelt in the Northeast Arm. No significant correlation in annual mean length at age-1 between basin pairs was identified (Table 3.5). Annual age-1 length and total CPUE in any basin was also not correlated between ($p > 0.6$ for all).

Total CPUE differed among basins ($p = 0.01$; $F_{2,72} = 4.47$; Figure 3.5; Table 3.4) and when years were combined CPUE was lower in the Main Lake (mean = 271, SD = 194)

than the Northeast Arm (mean = 818, SD = 895) or Malletts Bay (mean = 815, SD = 1080). However, CPUE also varied across sample years ($p = 0.008$, $F_{1,72} = 7.4$; Figure 3.5) and appeared to generally be driven by periodically high CPUE in the Northeast Arm and Malletts Bay associated with strong year classes, while CPUE in the Main Lake was much less variable. This interannual variability led to a significant interaction between year and CPUE ($p = 0.02$, $F_{2,72} = 3.76$). Tukey HSD post-hoc comparisons, of models run with each trawl as a replicate, indicated that CPUE in Malletts Bay was higher than the Main Lake in 4 of 26 years and higher than the Northeast Arm in 2 of 26 years, but smaller than the Northeast Arm in 3 of 26 years. CPUE was higher in the Northeast Arm than in the Main Lake in 6 of 26 years. Changes in CPUE across time were correlated between the Northeast Arm and Malletts Bay, but neither the CPUE in Northeast Arm or Malletts Bay were correlated with CPUE in the Main Lake (Table 3.5). The relationships in CPUE between basins were partially driven by strong age-1 cohorts in the Northeast Arm and Malletts Bay as indicated by the correlation between age-1 CPUE in the Northeast Arm and age-1 CPUE in Malletts Bay (rho = 0.81; $p < 0.01$) but no correlation between age-1 CPUE in the Main Lake and age-1 CPUE in either the Northeast Arm or Malletts Bay (rho = 0.25; $p = 0.230$ and rho = 0.30; $p = 0.138$).

3.5. Discussion

Genetic analysis indicates rainbow smelt in Lake Champlain consist of a single, genetically connected population with no evidence of significant pairwise genetic distance or genetic clustering, similar to slimy sculpin (Euclide *et al.*, 2017). However, differences in age structure, length-at-age, and CPUE among basins separated by

causeways indicate that growth and mortality of age-1 and older rainbow smelt may be basin-specific and that mixing of adults among basins is likely low. I hypothesize that this pattern is representative of strong larval or young-of-year dispersal but limited adult dispersal across causeways and suggest several possible explanations for variable demographics among basins despite the apparent genetic population connectivity.

3.5.1. Absence of genetic structure

Rainbow smelt in Lake Champlain had high genetic diversity but little to no genetic divergence between sites, indicating that rainbow smelt form a single genetic stock. Power estimates suggested that our sample size of individuals and loci genotyped at each site should have been sufficient to detect all but small levels of genetic distance (F_{ST} < 0.01). While all but two site pairwise comparisons had $F_{ST} < 0.01$, genetic distance of this scale and smaller would likely be biologically un-meaningful for the purpose of stock analysis in an abundant species such as rainbow smelt where loss of genetic diversity due to isolation is not a large concern (Hedrick, 1999). Therefore, while the use of larger sample sizes or additional loci may have increased statistical power, the detection of smaller levels of genetic distance would not change our interpretation of genetic stock structure even if identified. Therefore, I suggest that rainbow smelt in Lake Champlain should be considered a single genetic stock and discuss an ecological explanation for rainbow smelt connectivity among the three lake basins.

Low genetic population structure among basins can be explained by either populations size or gene flow. Rainbow smelt are an abundant species in Lake Champlain and

therefore likely have very large census populations size and our results indicate the effective population size is likely also high. Therefore, rainbow smelt in each basin could be physically isolated from one another but population size is sufficiently high too limit genetic drift (Gillespie, 2004). Alternatively, low genetic population structure could indicate that gene flow is sufficiently high across causeways to counteract the effects of genetic drift within each basin. In this scenario, dispersal through causeway openings must be possible. Basin connectivity could be maintained by adult dispersal. However, YOY and older rainbow smelt generally prefer temperatures cooler than 15°C and are abundant in waters deeper than 15 m and, in Lake Champlain, spawn in deep water (Marsden & Langdon, 2012; Simonin *et al.*, 2012). Given that all causeway openings are less than 10 m deep and reach temperatures of 20 - 25°C in the summer (Table 1.2), adult dispersal would need to take place when the lake is isothermal and would still force rainbow smelt into shallow water. Alternatively, population connectivity could be maintained by larval dispersal. Genetic structure of rainbow smelt in the St. Lawrence River estuary and along the Atlantic coast is maintained by larval dispersal and follows the member-vagrant hypothesis whereby the number of populations is equal to the number of larval retention sites - not spawning sites (Baby, Bernatchez & Dodson, 1991; Bernatchez & Martin, 1996; Kovach *et al.*, 2013). If rainbow smelt in Lake Champlain also follow the member-vagrant hypothesis, then all of Lake Champlain can be considered a single larval retention site where larval dispersal through causeway openings is not only possible, but high enough to maintain population connectivity.

For Lake Champlain to be a single larval retention site, planktonic larvae must passively drift through causeway openings. Water currents through causeway openings can be substantial, (e.g. 20 – 30 cm/s) which suggests that larvae could easily drift through openings (Myer, 1977). However, almost all the flow is out of the Northeast Arm and Malletts Bay into the Main Lake. During northerly winds, upwards of 99% of total flow was into the Main Lake (Myer, 1977). However, during southerly winds, flow direction reversed for Malletts Bay and 99% of the flow went into the basin and 72% of the flow from Malletts Bay flowed into the Northeast Arm. Flow directly between the Main Lake and the Northeast Arm did not reverse completely but 17% of flow direction was into the Northeast Arm (Myer, 1977). Therefore, while pelagic larvae likely drift through causeway openings, this drift may be primarily unidirectional, from the two smaller basins into the Main Lake. However, asymmetrical gene flow can be enough to maintain genetic diversity (e.g., Consuegra *et al.,* 2005; Morrissey *et al.,* 2009).

3.5.2. Presence of demographic structure

Overall, rainbow smelt age structure, length-at-age, and CPUE differed among the three main basins in Lake Champlain and length-at-age and CPUE between basins across the 26 years of sampling was not strongly correlated between basins. Additionally, the level of variance differed among basins, such that the two smaller basins had highly variable inter-annual CPUE compared to the Main Lake, despite the broad spatial heterogeneity that composed the Main Lake sample. In contrast to our genetic results, these differences indicate isolated stocks of rainbow smelt. Similar demographic differences characterize rainbow smelt from different zones in Lake Superior and Lake Erie (Luey & Adelman,

1984; Henderson & Nepszy, 1989). While stock differences in Lake Superior were attributed to adaptive separation between stocks isolated by high levels of predation and competition, stock differences in Lake Erie were attributed to limnological differences between sites. Given the genetic population connectivity observed in Lake Champlain, I suggest the differences in demography are more likely the result of limnological differences among basins such as productivity and prey abundance and composition.

Lower productivity could explain size differences among basins; rainbow smelt were smallest in Malletts Bay, and largest in the Northeast Arm. Malletts Bay is oligotrophic compared to the Northeast Arm and the Main Lake; mean chlorophyll of Malletts Bay is approximately 40% lower than the Northeast Arm and 20% lower than the Main Lake (LCBP, 2015). Similarly, the smaller sizes of rainbow smelt in the eastern basin of Lake Erie compared to the central basin were attributed to lower mean productivity in the eastern basin (MacCrimmon, Gots & Claytor, 1983). Low productivity in Malletts Bay would not, however, explain the significantly higher CPUE and larger inter-annual variability of rainbow smelt in Malletts Bay and the Northeast Arm compared to the Main Lake.

Variability in smelt CPUE among years and basin may be a consequence of differences in recruitment or larval distribution among basins. The variability of CPUE in the Northeast Arm and Malletts Bay was largely driven by years of high age-1 abundance. Differences in CPUE are possibly driven by variability in spawning within each basin leading to differences in the resulting cohort strength of age-1 fish the following year. However, very little is known about the spawning behavior or locations of rainbow smelt in Lake

Champlain which makes testing this hypothesis difficult. Alternatively, the high inter-annual variability in CPUE in the two smaller basins could reflect annual differences in larval dispersal into and out of each basin, early mortality due to competition or cannibalism, or variable abundance of predators. Larval smelt are planktonic, thus larval dispersal into and out of Malletts Bay and the Northeast Arm would occur due to current-driven advection through causeway openings. Flow through the causeway openings tends to flow westward from the Northeast Arm and Malletts Bay into the Main Lake, with only periodic wind-driven reversals in direction (Myer & Gruendling, 1979; Marsden & Langdon, 2012). Recruitment success of other species has been suggested to be affected by displacement of age-0 individuals (Dettmers *et al.*, 2005). Therefore, years of high or low age-1 abundance, e.g., 1995 in the Northeast Arm and 2003 in Malletts Bay, could be partially a result of advection during high wind events that occurred in spring of the previous year.

Differences in prey communities and abundance among basins may also result in differences in growth and abundance of rainbow smelt among basins. In Lake Champlain and the Great Lakes, age-1 and older rainbow smelt feed extensively on *Mysis diluviana* (Johnson *et al.*, 2004; Stritzel Thomson *et al.*, 2011). In Lake Champlain, however, *Mysis diluviana* are only abundant in the Main Lake, rare in the Northeast Arm, and possibly absent from Malletts Bay (Stockwell and Euclide, unpublished data). Therefore, differences in access to this important prey source could influence growth and mortality, resulting in demographic differences of rainbow smelt among basins.

Differences in the predator community among basins may also impact basin-specific rainbow smelt stocks. The primary rainbow smelt predators in Lake Champlain are lake trout (*Salvelinus namaycush*), Atlantic salmon (*Salmo salar*), and walleye. Lake trout and Atlantic salmon populations are supported entirely by stocking, and numbers stocked annually have been stable since the early 1990s. Thus, none of these major predators appear to have experienced major population fluctuations that would lead to intermittent changes in prey populations. However, differences in how predators are stocked could contribute to variable densities among basins. Lake trout are stocked only in the Main Lake and walleye are stocked only in the Main Lake and Missisquoi Bay, but the Malletts Bay population of walleye is naturally reproducing. Therefore, predator abundance may be variable among basins if predators do not or cannot actively redistribute among basins. Based on winter creel surveys lake trout appear to enter Malletts Bay and the Northeast Arm seasonally in the winter (Pientka, unpublished data). Of 93 lake trout tracked for three years in Lake Champlain using acoustic telemetry (Pinheiro, Stockwell & Marsden, 2017), one to nine tagged individuals were seen each week in Malletts Bay and one to three were seen each week in the Inland Sea, but none were detected in either basin between July and October-November (Marsden, unpublished data). Variability in the number of predators that enter the smaller basins in winter and spring could result in high variability in predatory reduction of rainbow smelt. However, our data show periodic peaks of rainbow smelt abundance, not years with unusually low abundance, so the predatory explanation seems unlikely.

Though rainbow smelt from different basins varied in length-at-age and CPUE, the proportion of age-1 rainbow smelt was correlated among basins which suggests synchrony in new rainbow smelt cohorts. This synchrony of cohorts supports the hypothesis that causeways limit post-larval but not larval dispersal within Lake Champlain. Later life-stage demographic traits, such as growth and overall CPUE, would depend on basin-level differences such as those described above, while new cohort strength may depend on lakewide larval abundance. Genetic connectivity among basins indicates that the Lake Champlain basins likely interact as a well-connected genetic sub-stocks whereby genetic diversity may be maintained by asymmetrical gene flow by larvae through causeway openings (Morrissey *et al.*, 2009). However, demographic independence among basins indicates that the ecological/recruitment processes within at least the Northeast Arm and Malletts Bay may be independent from the Main Lake and lack larval migration from the Main Lake, a process which is generally believed to stabilize populations (Macarthur & Wilson, 1967).

3.5.3. Conclusions

The present study indicates that analysis of stock structure using either molecular or demographic data alone would have misclassified rainbow smelt stock structure and lacked the nuance gained from a dual method strategy. Contradiction between demographic and genetic stock structure is not uncommon. While rainbow smelt demographic differences among regions in Lake Superior corresponded to genetic differences, this was not the case in Lake Erie (MacCrimmon, Gots & Claytor, 1983; Schreiner *et al.*, 1984). Additionally, two different ecotypes of rainbow smelt in Lac

Saint-Jean, Quebec, showed only modest genetic differentiation despite large morphological differences between ecotypes (Saint-Laurent, Legault & Bernatchez, 2003). Thus, demographic differences do not necessarily indicate genetically distinct fish stocks, and vice versa, emphasizing that caution should be used when using only a single method to identify new stocks or monitor existing stocks.

Our analysis suggests that although rainbow smelt CPUE appears to have declined in the Northeast Arm and Malletts Bay in the last decade, the lakewide rainbow smelt population genetic diversity remains high and genetic structure low. If smelt abundance continues to be suppressed in the smaller basins where gene flow from the Main Lake is less likely, overtime these basins populations may begin to show signs of genetic isolation from the Main Lake because genetic drift has a stronger effect on small populations (Gillespie, 2010). Historically, high inter-annual variability in abundance in the two smaller basins may have been offset by dispersal from the Main Lake, where CPUE has remained comparatively stable since 1990 when sampling began. The recent declines emphasize the need for continued monitoring of all three basins, and further investigation of potential causes of the demographic differences among basins.

Table 3.1: Characteristics of 8 microsatellites amplified in rainbow smelt. Shown are the marker name, forward and reverse primer sequence, fluorophore tail, amplified size range, annealing temperature (Ta) and citation for the source of the marker.

marker	primer (5' - 3')	florophore	size range	Ta	source
Osmo12	F: CTGTAATATTCCACTGCTGC	NED	157 - 193	55	Saint-Laurent *et al.* 2003
	R: CAAGTAGACAGTAGGGAGA				
Osmo16	F: GGATCTTGGATGAGAACAT	FAM	78 - 90	55	Saint-Laurent *et al.* 2003
	R: GGCTCTTTCATTACACAGG				
Osmo45	F: CTGTTGATAGATTGGCATC	HEX	193 - 263	55	Saint-Laurent *et al.* 2003
	R: CCCATTCAATTAGACAGTG				
Osmo157	F: CTTGCTTATGTAAAGGTGGG	FAM	228 - 264	55	Saint-Laurent *et al.* 2003
	R: GATCCACCAGTTCTCACA				
Omo1	F: CGGTCACGCAACTAACATCT	HEX	108 - 136	60	Coulson *et al.*, 2006
	R: CGGCTGGTTGGCTGTTTAT				
Omo3	F: GGATTTGCCATGTTGAAGCTA	HEX	170 - 230	60	Coulson *et al.*, 2006
	R: CACATGCACAACACAGTCCA				
Omo5	F: CTATGTGAACAGAAGCTGTGAAGAG	FAM	229 - 327	60	Coulson *et al.*, 2006
	R: TAAAGACACCTGCCGACTTG				
Omo11	F: CCTTGAGGCACTGAACCACT	FAM	152 - 204	60	Coulson *et al.*, 2006
	R: ACATGCACATGCAGGTAAGG				

Table 3.2: Site-specific summary statistics of rainbow smelt genotypes taken from six microsatellite loci grouped by basin and site in Lake Champlain. N = number of individuals sampled for genotyping, efN = mean number individuals genotyped across loci, H_O = observed heterozygosity, H_E = expected heterozygosity, F_{IS} = inbreeding coefficient, Ne = effective population size (lowest allele frequency used = 0.2) and jack knifed 95% CI, and AR = mean allelic richness across all loci based on minimum sample size of 32 individuals.

	N	efN	Ho	He	F_{IS}	Ne	AR
Main Lake							
Barber Point	33	30.83	0.65	0.64	-0.01	∞ (8-∞)	9.41
Juniper Island	32	29.67	0.66	0.65	0.01	∞ (64-∞)	9.75
Valcour	34	31.00	0.64	0.63	-0.01	∞ (103-∞)	8.52
Malletts Bay							
Malletts Bay	32	31.17	0.67	0.66	-0.02	∞ (61-∞)	9.29
Northeast Arm							
Northeast Arm	36	35.17	0.65	0.65	-0.01	32.3 (13-609)	9.14

Table 3.3: Pairwise G'_{ST} (below diagonal) and F_{ST} (above diagonal) estimated for rainbow smelt sampled from five sites in in Lake Champlain.

	Barber Point	Juniper Island	Valcour Is	Malletts Bay	Northeast Arm
Barber Point	-	-0.00429	-0.00741	0.00222	0.00285
Juniper Island	-0.0019	-	0.00037	-0.00676	0.00932
Valcour Is.	0.0128	0.0182	-	0.01866	-0.00512
Malletts Bay	-0.0103	-0.0041	-4e-04	-	0.01631
Northeast Arm	-0.0158	0.0065	0.0099	-0.0139	-

Table 3.4: ANOVA table for analysis comparing growth and CPUE among basins. "-" indicates that the effect was not calculated for the given response.

Effect		Response		
		length-at-age	legnth-at-age-1	CPUE
basin	N	21,945	9,305	78
	f-value	3199.4	1941.2	4.5
	p-value	<0.001	<0.001	0.010
year	f-value	338.4	124.5	7.4
	p-value	<0.001	<0.001	0.008
basin:year	f-value	115.4	48.3	3.8
	p-value	< 0.001	< 0.001	0.020
age	f-value	13862.8	-	-
	p-value	<0.001	-	-
basin:age	f-value	183.0	-	-
	p-value	< 0.001	-	-
age:year	f-value	55.6	-	-
	p-value	< 0.001	-	-
basin:age:year	f-value	18.1	-	-
	p-value	< 0.001	-	-

Table 3.5: Sample size of number of years compared (N), rho test statistic, and significance for Spearman correlations testing the between-basin relationships of proportion of age-1 fish, length at age-1, and catch-per-unit-effort (CPUE) across 26 years of trawling surveys.

		Main Lake : Northeast Arm	Main Lake : Malletts	Northeast Arm : Malletts
Prop Age-1	N	26	26	26
	rho	0.60	0.28	0.63
	p-value	<0.01	0.17	<0.01
Length at Age-1	N	26	26	26
	rho	0.38	0.33	0.29
	p-value	0.08	0.12	0.17
CPUE	N	26	26	26
	rho	0.12	0.06	0.60
	p-value	0.56	0.79	<0.01

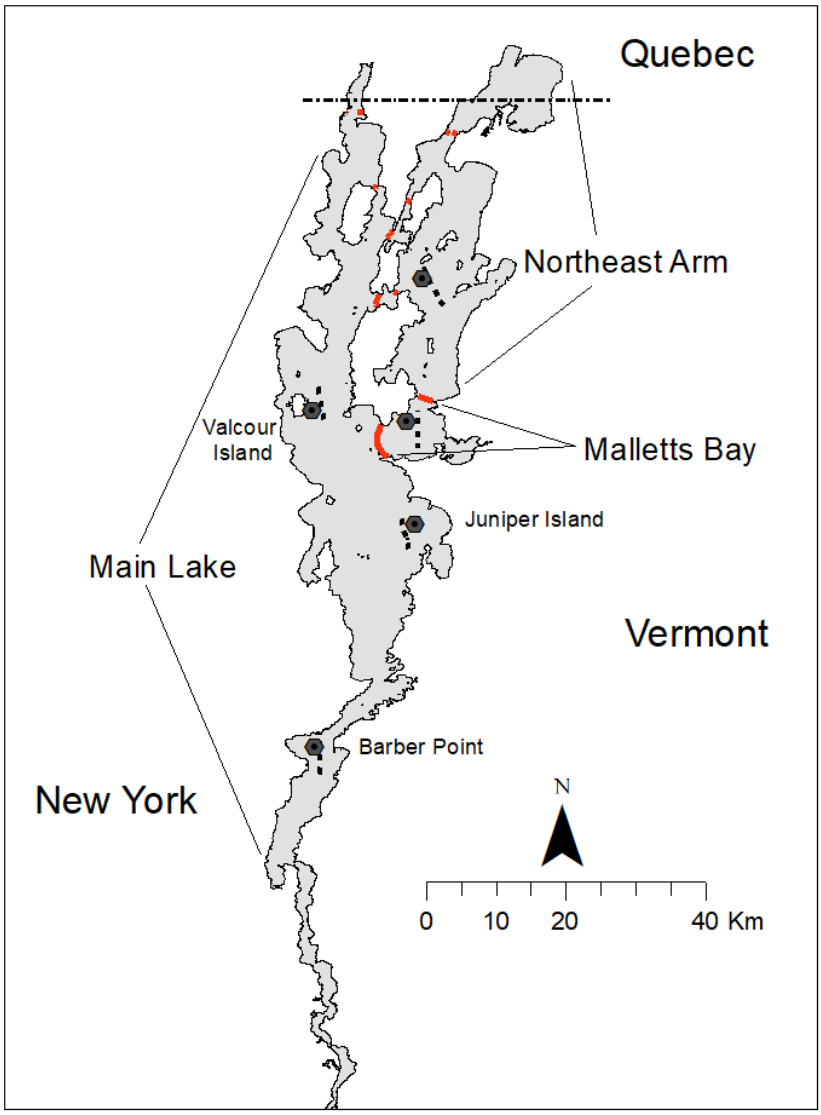

Figure 3.1: Locations of genetic samples (gray dots) and forage fish survey trawling paths (dotted lines) in Lake Champlain. Red lines indicate the location of a causeway.

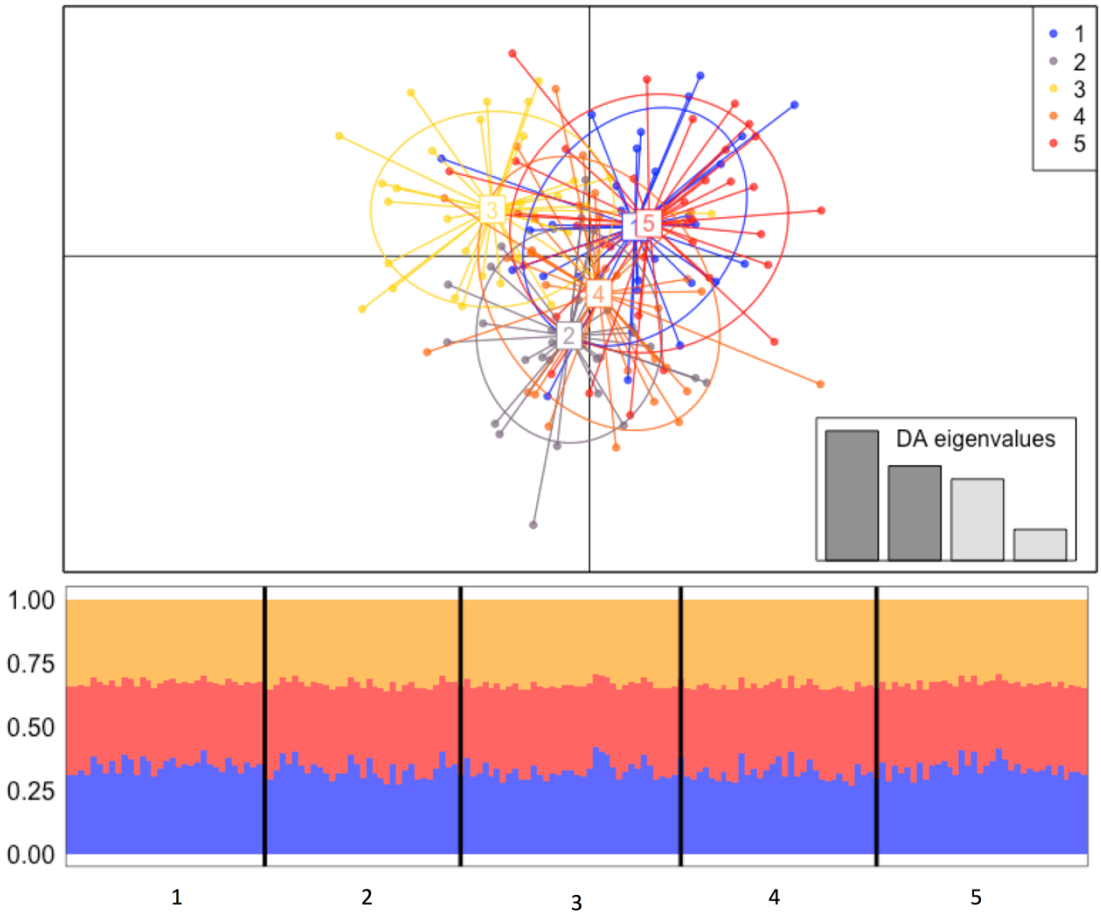

Figure 3.2: Clustering model outputs from DAPC (top) and STRUCTURE (k =3; bottom). Numbers indicate the five sites where rainbow smelt were sampled (1) Barber Point, (2) Juniper Island, (3) Valcour, (4) Malletts Bay, and (5) Northeast Arm. Each individual dot in the DAPC bi-plot represents a single genotyped individual and the color of the dot indicates the site the where the individual was sampled. The STRUCTURE barplot is a graphical representation of individual membership coefficient to each cluster (vertical bars). Colors represent different estimated clusters of a single admixed individual.

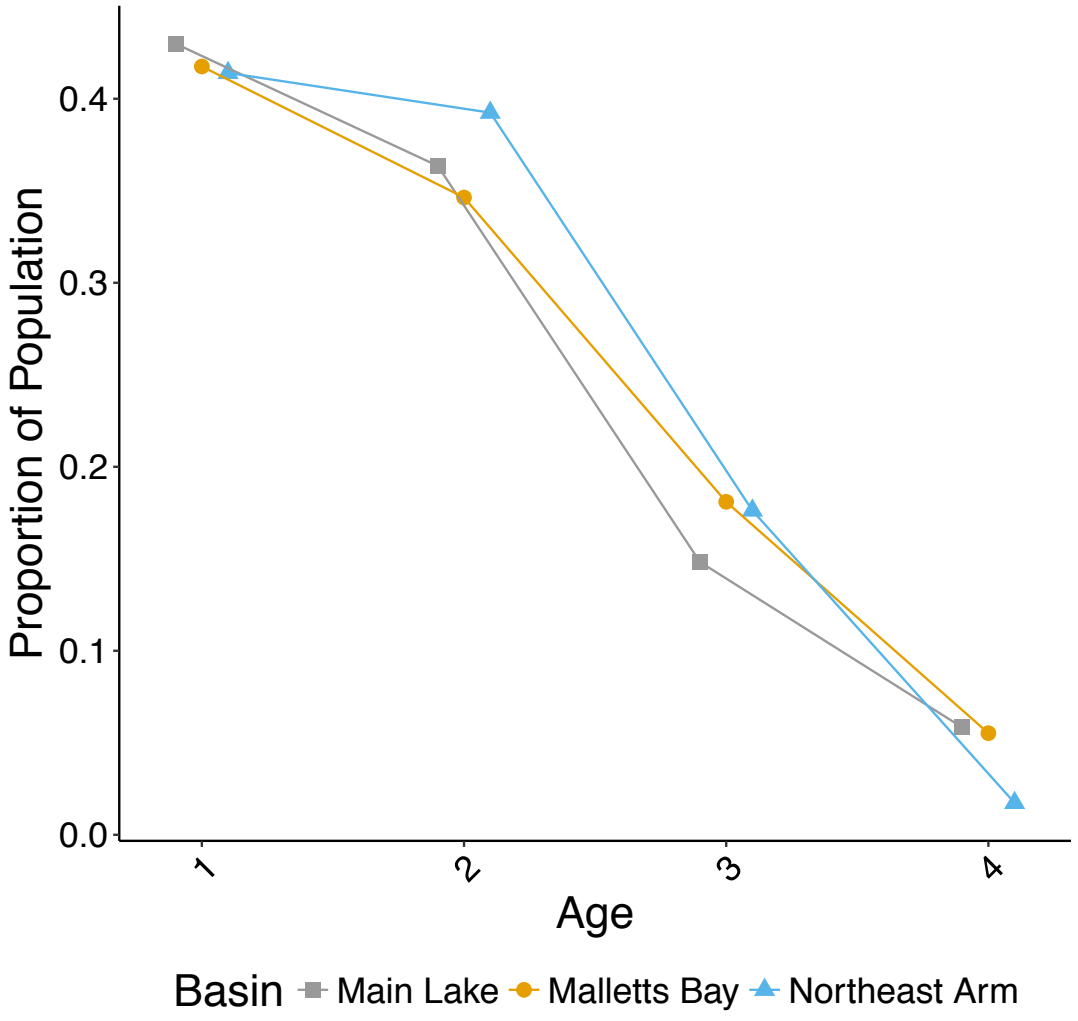

Figure 3.3: The proportion of rainbow smelt age 1 – 4 captured during forage fish surveys between 1990 – 2015 in the three partially isolated basins of Lake Champlain.

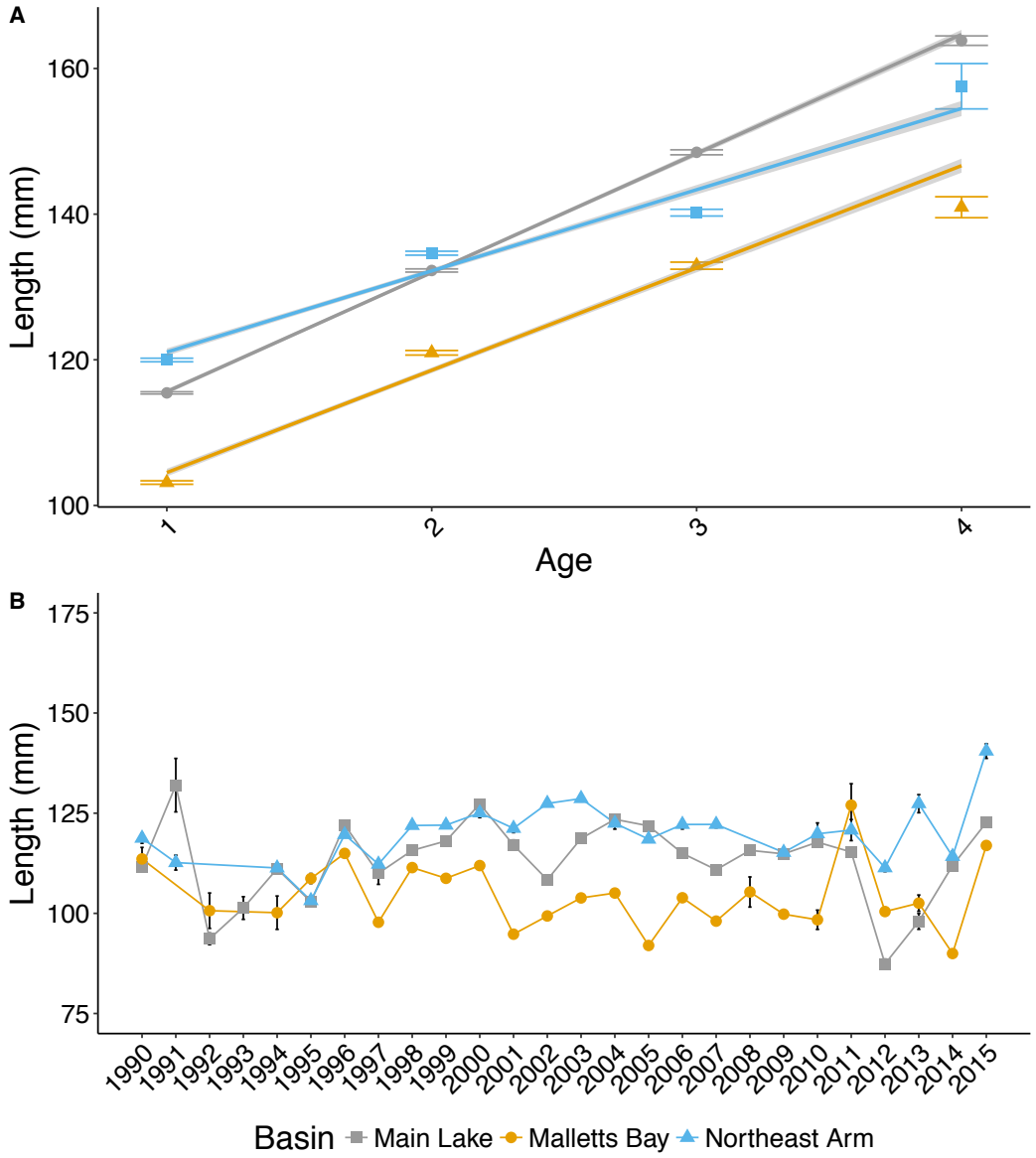

Figure 3.4: A) length-at-age of rainbow smelt averaged across 26 years of forage fish surveys. Lines represent line of best fit, gray background indicate 95% confidence intervals around line of best fit. B) average length of age-1 rainbow smelt per year in each Lake Champlain basin.

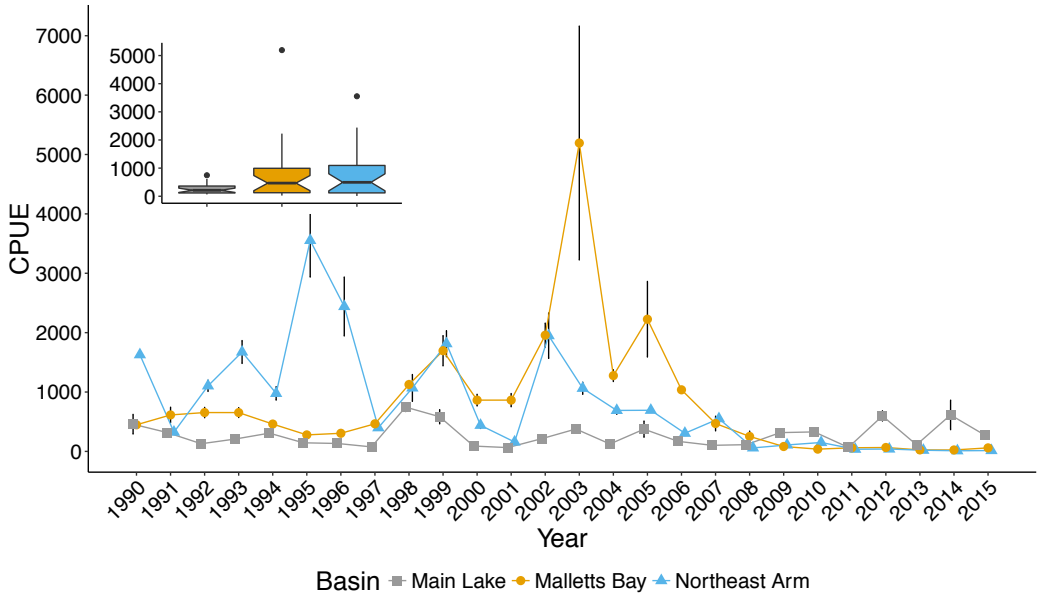

Figure 3.5: Total catch-per-unit-effort (CPUE) of rainbow smelt in each Lake Champlain basin for each year. Error bars represent standard error. Inset plot indicates the across-year CPUE for each basin (colors), lines indicate median values.

CHAPTER 4: GENETIC STRUCTURE OF LAKE WHITEFISH (*COREGONUS CLUPEAFORMIS*) IN LAKE CHAMPLAIN, VERMONT, 100 YEARS AFTER COMMERCIAL FISHERY CLOSURE

4.1. Abstract

Commercial fishing for lake whitefish in Lake Champlain closed in 1913 due to concerns about overexploitation. However, the historic whitefish population size is unknown and harvest statistics were not recorded. Lake trout, which were also commercially fished, disappeared from the lake by 1900; harvest may have significantly affected both species. In 2010, a growth analysis of lake whitefish found that populations were characteristic of an unexploited stock, suggesting that if the fishery had an impact, the population has recovered. I hypothesized that the genetic diversity of the population may have been reduced due to overfishing. Therefore, the objective of this study was to look for genetic evidence of a population bottleneck and describe the genetic diversity and population structure of lake whitefish in Lake Champlain. One hundred and fifty whitefish were collected on both sides of causeways that divide the northern portion of the lake into two basins. Fish were genotyped at 8 microsatellite loci; I evaluated genetic diversity and looked for evidence of a bottleneck by looking for heterozygosity excess with the program BOTTLENECK and running simulations under different overfishing scenarios. I conducted simulations to estimate how starting effective population size and fishing pressure in the 1900s would have affected genetic diversity observed 100 years later. Data suggest that lake whitefish have high genetic diversity compared to other lake whitefish populations, limited evidence of population sub-structuring and show no signs

of a recent bottleneck. Simulations suggest that even for a large effective population size of 10,000 individuals, a 50% - 90% reduction in population would have small impacts on diversity. These data provide a perspective on effects of a commercial fishery that was closed prior to population collapse, compared with Great Lakes whitefish populations that are currently recovering after overharvest collapsed their populations.

4.2. Introduction

Commercial fishing for lake whitefish (*Coregonus clupeaformis*) in Lake Champlain was closed in 1913 due to concerns of over-exploitation. Since the fishery closure follow-up to evaluate how the population has fared has been limited. Age and size structure, and estimates of growth and condition of adult fish from the two historic commercially harvested locations were evaluated in the early 1930s by Van Oosten and Deason (1939). From 2008 to 2010, an extensive growth and spawning assessment of lake whitefish found that populations exhibited characteristics of an unexploited population, suggesting that lake whitefish populations had fully recovered (Herbst, Marsden & Smith, 2011). However, the same study found almost no evidence of spawning at the two locations where lake whitefish were historically harvested; more recently, genetic barcoding indicated that many of the sampled larval fish may have been cisco (*Coregonus artedi*), not lake whitefish (Euclide, unpublished data). Therefore, lake whitefish reproduction may be lower than previously thought or may reproduce in different areas than historically harvested.

Lake whitefish populations may have recovered demographically, but genetic diversity lost during commercial harvest might take much longer to recover (Hutchings & Reynolds, 2004). While census size (Nc) of populations of fish tend to be large, many species have comparatively small effective population (Ne) sizes possibly due to variable reproductive success associated with high fecundity and early life stage mortality (Turner *et al.*, 2006; Hare *et al.*, 2011). Low Ne/Nc ratios can therefore become an issue in harvested populations and lead to low genetic diversity and reduced Ne (e.g., Hoarau *et al.,* 2005). Genetic drift increases as Ne decreases, eroding genetic diversity and limiting the adaptive potential of a population (Wright, 1931). In the face of increasing environmental change, assessing population diversity and managing fisheries for higher adaptive potential is important (Dudgeon *et al.*, 2006).

Since the closure of the lake whitefish fishery in 1913, Lake Champlain has experienced significant changes which may have influenced lake whitefish populations and degraded genetic diversity. Deforestation, shoreline development, and agricultural runoff have led to high sedimentation and eutrophication of Missiquoi Bay, which is believed to have been one of the largest spawning sites of lake whitefish in Lake Champlain (Figure 4.1; Marsden & Langdon, 2012). Additionally, when commercial fishing was greatest in the late 1800s and early 1900s, nine causeways were built connecting the northern islands of Lake Champlain to the mainland; these barriers may have restricted fish movement throughout the lake (Marsden & Langdon, 2012). Finally, as of 2017, 50 exotic species had colonized Lake Champlain, including alewife (*Alosa pseudoharengus*) which may be predators of larval lake whitefish, and zebra mussels (*Dreissena polymorpha*) which are a

low-quality prey for lake whitefish in the Great Lakes, but to a lesser degree in Lake Champlain (Marsden & Hauser, 2009; Herbst, Marsden & Lantry, 2013). If these changes reduced the population size and dispersal of lake whitefish in Lake Champlain, then the genetic diversity and structure of lake whitefish may have changed due to increased rates of genetic drift.

At its peak, commercial harvest in Lake Champlain was removing 24,000 – 40,000 kg of lake whitefish annually from Missisquoi Bay, and unreported amounts from other parts of the lake (Marsden & Langdon, 2012). The fishery was based primarily in fall and used beach seines to harvest fish as they aggregated to spawn. Harvest of spawning adults is generally unsustainable and can rapidly deplete populations through recruitment overfishing (e.g. Hutchings and Reynolds, 2004). However, because much of the fishery harvest in Lake Champlain was underreported, estimation of lakewide fishing pressure is difficult. Concurrently with the closure of the lake whitefish fishery in Lake Champlain, coregonids in the Great Lakes were in a state of overfishing which would eventually lead to the depletion of multiple coregonid species through the Great Lakes in the early to mid-1990s (Allan *et al.*, 2005; Eshenroder *et al.*, 2016). Therefore, if harvest in Lake Champlain was similar to harvest in the Great lakes, then by the time commercial fisheries in Lake Champlain were closed in 1913 lake whitefish population size in Lake Champlain may have already been substantially reduced.

Fall spawning aggregations of lake trout (*Salvelinus namaycush*) were also commercially harvested in Lake Champlain, and as with lake whitefish, harvest statistics were reported erratically and have not been compiled. Lake trout populations declined in Lake

Champlain throughout the 1800s and were extirpated by 1900, but the exact cause of the loss is unclear (Marsden & Langdon, 2012). Overharvest is one possible factor that could have led to the decline. For example, harvest was a major contributing factor to the collapse of lake trout across the Great Lakes in the 18th and 19th centuries (Hansen, 1999). Remnant populations of lake trout in Lake Superior showed signs of a lake-wide bottleneck and reduction in effective population size (Guinand *et al.*, 2003, 2012). If lake whitefish show signs of decreased genetic diversity and recent bottleneck, this could support the hypothesis that commercial harvest could also have contributed to extirpation of lake trout in Lake Champlain.

Loss of genetic diversity and population sub-structuring are two of the major potential consequences of habitat fragmentation and both effects are amplified in small or impaired population (Templeton *et al.*, 1990). I hypothesize that the construction of causeways while lake whitefish populations were likely at their lowest may have had a permanent effect on the population structure lake whitefish in Lake Champlain 100 years later. I conducted a genetic analysis of adult lake whitefish collected from both sides of causeways isolating the Main Lake of Lake Champlain from the Northeast Arm of the Lake Champlain to evaluate if historic overfishing and fragmentation has resulted in detectable population structure and reduced genetic diversity. I hypothesized that if commercial fishing and causeways had a significant role in shaping the genetic structure of lake whitefish, then (1) lake whitefish in the Main Lake would be genetically differentiated from lake whitefish captured in the Northeast Arm and (2) genetic diversity

of the lakewide lake whitefish population would be low, indicating the presence of a bottleneck.

4.3. Methods

4.3.1. Sample collection and microsatellite analysis

To evaluate the lakewide genetic diversity and structure, lake whitefish were sampled primarily in the Northeast Arm (Inland Sea and Missisquoi Bay) and the Main Lake (Burlington Bay, Grand Isle and South Lake) of Lake Champlain (Figure 4.1). Two individuals from Malletts Bay captured as bycatch for a different study were included in our analysis but Malletts Bay was not directly targeted in our sampling efforts. Adult lake whitefish were collected from the Inland Sea of Lake Champlain in 2008 using overnight sets of 1.8 m deep and 70.6–152.4 m long multi-panel gillnets with 7.6, 8.9, 10.2, 11.4, 12.7, 14, and 15.2-cm stretch mesh panels (Herbst, Marsden & Smith, 2011). Tissue samples of lake whitefish from Missisquoi Bay were collected and provided by Dr. Louis Bernatchez, Laval University, Quebec (Lu, Basley & Bernatchez, 2001). Adult whitefish from the Main Lake were collected as bycatch during bottom trawl surveys for lake trout during spring, 2016. Because whitefish in the Main Lake were captured eight years after samples in the Inland Sea, an additional 11 lake whitefish were collected in the Northeast Arm during 2015 bottom trawls to compare to 2008 samples to account for temporal

variation. All samples were either preserved in 95% ethanol or dried according to LaHood *et al.* (2008) for DNA extraction.

Samples of muscle tissue (Northeast Arm) were frozen in liquid nitrogen and reduced to a powder using a mortar and pestle before extraction; dried fin clips (Main Lake) were added directly to extraction tubes. DNA was extracted using the Puregene Quiagen extraction kit guidelines. After extraction, DNA samples collected from the Northeast Arm in 2008 were checked for degradation during storage using gel electrophoresis while samples collected in 2015 and 2016 were only checked for DNA concentration using a NanoDrop DNA analyzer. Samples were genotyped using polymerase chain reaction (PCR) at eight microsatellite loci previously identified for lake whitefish (BFW1, BFW2, Cocl-lav 28 (C28), Cocl-lav 45 (C45), Cocl-lav 68 (C68), Cocl-lav 6 (C6), Cocl-lav 4 (C4), Cocl-lav 23 (C23); Table 4.1) in 25 ul reactions containing primer-specific concentrations of forward and reverse primers (Patton *et al.*, 1997; Lu *et al.*, 2001; Rogers, Marchand & Bernatchez, 2004). Loci were amplified using a touchdown-based approach whereby the melting temperature (94°C) and elongation temperature (72°C) stayed the same for each cycle but annealing temperature was lowered by 0.5 or 1.0°C every 5 PCR cycles. All loci were amplified using one of two general programs: amplification of loci BFW1, BFW2, C23, and C6, PCR was initiated with a denaturing step of 94°C for 3 minutes followed by 33 cycles of 30 s at 94°C, 30 s at an annealing temperature (Table 4.1) which started at 60°C and decreased by one degree every five cycles, and ended with 30 s at 72°C. The final annealing temperature (55°C) was run for 8 cycles and followed by a final elongation at 72°C for seven minutes. The process for

loci C28, C45, C68, and C4, PCR was almost identical except the initial denature step was shortened to 30 s and annealing temperature was decreased by 0.5°C every 5 cycles from 62.5 to 59.0°C. Fragment analysis of PCR products was conducted in the University of Vermont Advanced Genome Technologies Core using an Applied Biosystems 3130 Genetic Analyzer and a LIZ 500 size standard and scored using GENEMAPPER software (Applied Biosystems).

4.3.2. Genetic diversity

All loci were assessed for the presence of null alleles with MICRO-CHECKER version 2.2.3 (Van Oosterhout *et al.*, 2004). Conformance to Hardy-Weinberg equilibrium (HWE) expectations at each locus, observed (H_O) and expected (H_E) heterozygosity, F_{IS} and allelic richness were estimated using the basicStats() function of the diveRsity package in R version 3.3.3 for each sampled site and then for all sites pooled to represent the total lake (Keenan *et al.*, 2013; R Core Team, 2015). HWE was calculated using exact testing and allelic richness was calculated using rarefaction and scaled to the smallest sample size. Very few individuals (<6) were collected from South Lake and Malletts Bay sites and were therefore excluded from allelic richness analysis. Any deviations from HWE following Bonferroni correction for multiple comparisons were assessed for heterozygote excess or deficiency using the diveRsity package for R. Private alleles were identified using GenAlEx (Peakall & Smouse, 2006, 2012). Contemporary effective population size was first calculated for each sampled location and then for the total lake using a both linkage disequilibrium and heterozygote excess methods in N_eESTIMATOR

(Do *et al.,* 2014) with minimum acceptable allele frequencies of 0.05, 0.02, 0.01, and 0.00.

4.3.3. Temporal stability of genetic diversity

Because samples were collected eight years apart, any genetic distance observed between Northeast Arm and Main Lake lake whitefish could be the result of slight changes in population-wide allele frequency over the eight years between sampling. Lake whitefish reach maturity around age five and live more than twenty years; therefore, the eight-year gap in sampling is less than a single generation and so was not predicted to have a large impact on observed genetic structure. However, to evaluate the amount of genetic distance than can be attributed to time between sampling events, three estimates of genetic distance were calculated. First, I conducted an analysis of molecular variance (AMOVA) to measure the amount of variation between samples of lake whitefish captured in the Northeast Arm in 2008 and samples of lake whitefish captured in the Northeast Arm in 2015. The AMOVA was conducted using a permutation test GenAlEx with 999 permutations. I further accounted for temporal differences by calculating values of pairwise genetic distance (F_{ST} and G'_{ST}) between 2008 and 2015 samples from the Northeast Arm. While G'_{ST} can bias genetic distance estimates making them appear higher than in reality, any values of G'_{ST} were always compared to estimates of F_{ST} which is less biased (Whitlock, 2011). If 95% confidence intervals around pairwise distance estimate included zero the difference was considered to be negligible.

4.3.4. Lakewide genetic structure

Possible genetic structure among sample sites was evaluated using pairwise comparisons of F_{ST} and G'_{ST}, and 95% confidence intervals calculated using the diveRsity R package. Significance was determined using confidence intervals whereby any pairwise estimate that did not include zero was considered significant. Two different approaches were used to evaluate genetic structure without *a priori* assumptions of population structure. First, variation among and within each drainage was assessed using STRUCTURE (Pritchard *et al.*, 2000) deployed through the ParallelStructure package for R (Besnier & Glover, 2013). Each estimate of k 1 – 5 was run through five replicate runs of 100,000 replicates and a 10,000 cycles burn-in. The most likely value of K was determined using posterior probabilities and deltaK and ln'(K) calculated in Structure Harvester (Evanno, Regnaut & Goudet, 2005; Earl & vonHoldt, 2012). Discriminate analysis of principal components (DAPC) was used as a second clustering estimator by evaluating overlap in DAPC bi-plots and proportions of successful reassignment based on the discriminant functions (Jombart, 2008; Jombart, Devillard & Balloux, 2010).

4.3.5. Bottleneck analysis

Evidence of a recent bottleneck was assessed using BOTTLENECK on the pooled dataset of 149 lake whitefish (Luikart & Cornuet, 1999). BOTTLENECK evaluates the presence of recent reductions in effective population size by comparing observed heterozygosity to simulated theoretical expected heterozygosity at population equilibrium. Because low-frequency alleles are lost during bottlenecks faster than heterozygosity is reduced, excess heterozygosity indicates a recent loss of genetic diversity. Tests were performed using both a stepwise mutation model (SMM) and the two-phase model of mutation (TPM)

which has been shown to be more suitable for microsatellite loci. Significance of heterozygosity excess following 1,000 iterations of the model was determined using one-sided Wilcoxon's signed-rank tests. The variance of TPM was set to 30 and proportion of SMM in TPM was set to 70% (Cornuet & Luikart, 1997). Because the exact effective population size and fishing pressure of lake whitefish in the 1900s is difficult to estimate, I simulated the loss of genetic diversity associated with different overharvest scenarios and effective population sizes in the program BOTTLESIM (Kuo & Janzen, 2003). BOTTLESIM is designed to simulate genetic bottlenecks in populations with overlapping generations based on prior allele frequency data to estimate the expected reductions of genetic diversity following a bottleneck event. I based our simulations off historic knowledge of commercial harvest in Lake Champlain and the present allele frequencies for the entire lake. Effective population size was set to either 10,000 or 2,000 which is likely significantly smaller than the actual census size (Nc) of lake whitefish in Lake Champlain; however, the Ne/Nc ratio in marine species is often 10^{-5}, and therefore a reasonable estimate for a large lake (Hare *et al.*, 2011). The percent reduction of effective population size was set to a 50, 75 or 90% reduction to simulate various over-fishing scenarios. All simulations were run for 1000 iterations using random mating, overlapping generations of 80%, and the age at maturity of 5 and maximum age of 25. To simulate the history of fishing in Lake Champlain as closely as possible, all simulations were run for 130 years, starting with 10 years of maximum Ne (10,000 or 2,000) followed by 120 years of a 50, 75, or 90% reduction in effective population size representing the time-period of highest reported harvest in the late 1800s and early 1900s to the present day

present day. BOTTLESIM assumes closed populations and no mutation. Both assumptions are reasonable given the low likelihood of migration between other systems and Lake Champlain and the relatively short time period over which simulations were run. However, given these assumptions our results represent a worst-case scenario.

4.4. Results

Locus C68 showed evidence of null alleles in Burlington Bay and Grand Isle, locus C6 showed evidence of a null allele at Grand Isle, and locus BFW2 showed evidence of a null allele at Missisquoi Bay. However, no consistent evidence of null alleles was found for any locus and all populations other than Grand Isle were in HWE following Bonferroni corrections; therefore, all loci were used in the following analyses. Grand Isle was the only sample site that was significantly out of HWE (Table 4.2). The divergence from HWE at Grand Isle was due to heterozygosity excess resulting from 11 of the 38 individuals genotyped having private alleles at least one locus and of the 11 individuals with private alleles, seven had private alleles at multiple loci (Table 4.3). The genotypes of all individuals with private alleles were re-analyzed and individuals GI_25 and GI_50 which had private alleles at four and five of the eight loci, respectively, were re-amplified and re-genotyped at each locus that showed private alleles. Following these quality checks, however, all private alleles appeared to be real. When individuals with more than two private alleles were removed from analysis, Grand Isle was in HWE, though still had a slight heterozygosity excess (Table 4.2). Because all private alleles appeared to be real, and not genotyping errors, all samples were included in the subsequent analysis. Power analysis indicated that, given the number of loci and sample sizes used, I should be able

to correctly identify genetic distances greater than 0.01 more than 98% of the time. Therefore, our sample design has sufficient power to detect all but relatively small levels of differentiation.

4.4.1 Inter-annual variation

Based on AMOVA results comparing 2015 Inland Sea samples to 2008 Inland Sea samples, 3% of variation was attributed to sampling date ($p = 0.01$). While AMOVA suggested that the amount of variation attributed to sampling was significantly greater than zero, confidence intervals of both G'_{ST} and F_{ST} included zero between 2015 and 2008 and were therefore functionally zero, which indicates that genetic distance between years was negligible. Because very little variance was explained by sampling date and pairwise distance estimates were both zero, 2015 and 2008 Northeast Arm samples were combined in all subsequent analyses.

4.4.2. Population sub-structuring

Only two pairwise G'_{ST} and F'_{ST} estimates among the six sampled sites were significantly greater than zero (Figure 4.2, Table 4.4). Both significant pairwise estimates were between the Inland Sea samples in the Northeast Arm and Main Lake sample sites. Pairwise distance estimates between lake whitefish from Malletts Bay and all other sites were high; however, only two individuals were genotyped from Malletts Bay and confidence intervals all included zero. Posterior probabilities from Bayesian STRUCTURE analysis indicated that there was very little support for all values of k which is indicative of a panmictic population. The program STRUCTURE does not

directly estimate panmixia (k = 1). However, there were no large peaks present when using second-order statistics such delta K or lnK suggesting no value of K was particularly explanatory. Additionally, all cluster assignment of all individuals became increasingly subdivided approximately proportional to the value of K which is characteristic of a single genetic cluster. Cluster analysis using DAPC indicated similarly low levels of genetic structure. Bi-plots of DAPC of lakewide lake whitefish samples supported the lack of population clustering as indicated by a high degree of overlap in DAPC bi-plots (Figure 4.3). The low high degree of overlap resulted in low individual reassignment accuracy (61%) to all sampling sites other than Malletts Bay.

4.4.3. Lakewide diversity and evidence of a bottleneck

Observed and expected heterozygosity ranged from 0.53 to 0.65 and 0.45 to 0.62 among sample sites and was 0.56 and 0.60 for the whole lake (Table 4.2). Allelic richness scaled to 21 individuals ranged from 4.18 to 5.95 among sample sites and was 5.09 for the whole lake. Effective population size ranged from 14.1 at Grand Isle to infinity for individual sample sites; however, bootstrapped confidence intervals at all sites other than Grand Isle included infinity (Table 4.2). Also, when individuals with two or more private alleles were removed from Grand Isle, Ne increased and the confidence interval included infinity. When samples were pooled, effective population size for the whole lake was estimated to be 139.7 (95% CI = 67.7 - 643.9). Inbreeding coefficient, F_{IS}, was negative for four of six site, but positive in Grand Isle (0.13), Missisquoi Bay (0.04), and the pooled lake samples (0.04).

No evidence was found of a recent bottleneck in Lake Champlain lake whitefish populations, as indicated by the lack of observed heterozygosity excess compared to simulated heterozygosity at any locus for either the SSM model or the TPM model ($SSM_{Wilcoxin}$ p = 1.00; $TPM_{Wilcoxin}$ p = 0.96). Simulations indicated that starting effective population size had a large impact on the observed loss in genetic diversity following a bottleneck. At an Ne of 10,000 individuals, loss of genetic diversity over 120 years ranged from 0.9% loss of observed alleles (OA) and 0.2% loss of H_O when populations were reduced by 50% to 14.2% in OA and a 0.9% reduction in H_O when populations were reduced by 90%. Alternatively, for a population size five times smaller (2,000 individuals), loss of genetic diversity ranged from a 14.3% loss in OA and 0.9% loss of H_O for a 50% reduction in population size to 39.8% in OA and a 4.0% reduction in H_O for 90% reduction in population size (Figure 4.4).

4.5. Discussion

We found limited evidence that commercial harvest in the late 1800s and lake causeways resulted in population sub-structuring and genetic bottleneck of lake whitefish in Lake Champlain. Genetic distance estimates supported hypothesis 1, that basins are genetically isolated. However, the genetic distance between lake whitefish captured at Main Lake sites and in Missisquoi Bay, which is in the Northeast Arm, were low and Bayesian cluster analysis did not identify any structure among basins. No evidence was found in support of hypothesis 2, that commercial fishing resulted in a bottleneck based on estimates of genetic diversity or Wilcoxon tests for heterozygosity excess between simulated and observed heterozygosity. Simulations suggest that the relative impact of

commercial harvest on observed genetic diversity today would depend largely on the effective population size at the time of harvest. However, at effective population sizes five times larger than I estimated in our study, a reduction in effective population size of 50% could have resulted in a similar reduction diversity as occurred following the collapse of lake trout in the Great Lakes (Guinand *et al.*, 2012).

Both G'_{ST} and F_{ST} indicated that there was modest, but non-zero genetic distance among lake whitefish collected from the Main Lake and those collected in the Inland Sea, but not between the Main Lake and Missisquoi Bay. While cluster analysis did not identify this same pattern, differences among basins could be too small to reliably detect using clustering techniques. STRUCTURE has been shown to have difficulty identifying the correct number of clusters when F_{ST} is small (< 0.02); the F_{ST} estimated in the present study slightly larger than this value (Chen *et al.*, 2007). DAPC generally performs as well as or better than STRUCTURE to identify clusters (Jombart, Devillard & Balloux, 2010). Therefore, the low, but positive genetic distance estimates between the Main Lake and the Inland Sea could indicate relatively recent reproductive isolation between basins.

Historically, commercial fishing occurred primarily at two sites, Missisquoi Bay in the Northeast Arm, and the southern portion of the Main Lake (Figure 4.1; Marsden & Langdon, 2012). The success of fall seining at these sites was presumably a result of large spawning aggregations at each location. Herbst, Marsden and Smith (2011) found high densities of coregonid larvae believed to be lake whitefish throughout the Lake Champlain in 2008, 2009, and 2010, however, recent re-identification of a subset of larvae using genetic barcoding suggests that many or all the larvae used to identify these

sites are cisco, not lake whitefish (George *et al.,* 2017, Euclide unpublished data). In the absence of any other observations of either spawning lake whitefish aggregations or larval lake whitefish, Missisquoi Bay and the South Lake may still be the primary spawning locations of lake whitefish. These areas are very distant from one another (> 100 km) and isolated by three causeways (Figure 4.1). Distance and lake causeways likely limited adult migration between basins and therefore between spawning sites. Increased physical isolation between spawning sites as a result of causeways combined with stronger effects of genetic drift as a result of depressed lake whitefish spawning stock abundance due to commercial harvest during the same period could have resulted in accelerated rates of genetic separation between Main Lake and Inland Sea fish.

Larval dispersal can be more important than adult dispersal in determining population connectivity for many species of fish (Pineda, Hare & Sponaugle, 2007). Coregonid larvae are pelagic, and known to drift long distances (Næsje, Jonsson & Sandlund, 1986). However, if the primary spawning sites of lake whitefish are at distal regions of the lake in basins that are isolated from each other by causeways, then larval dispersal between the Main Lake and Northeast Arm may be restricted. Strong currents in the Main Lake and the Northeast Arm likely mix larvae within each basin, but the narrow openings in the causeways and primarily unidirectional flow through causeway openings may inhibit larval drift between basins (Myer, 1977; McCormick *et al.*, 2008). Larval drift has been hypothesized as a potential explanation of the apparent genetic connectivity among Lake Champlain basins for slimy sculpin (*Cottus cognatus*) and rainbow smelt (*Osmerus mordax*; Euclide *et al.*, 2017, Euclide et al. unpublished data). However, slimy sculpin

and rainbow smelt likely spawn throughout the lake which could increase the likelihood of larval drift through causeway openings. Additionally, to our knowledge, neither slimy sculpin or rainbow smelt have been commercially harvested or experienced large declines in population abundance and therefore may be more robust to genetic drift than lake whitefish.

Genetic diversity was similar at all sites and in the pooled-lake sample when compared to genetic diversity in exploited lake whitefish populations in lakes Michigan and Huron (VanDeHey *et al.*, 2009; Stott, VanDeHey & Justin, 2010). Also, genetic diversity at loci BFW1, BFW2, and C23 in Lake Champlain was higher than in many unexploited populations of lake whitefish (Table 4.5; Lu *et al.*, 2001). I found no evidence of heterozygosity excess compared to simulated lakewide populations indicating that probability of a recent genetic bottleneck is low. Simulations indicated that at an Ne size approximately ten times the Ne estimated in our study and four times the upper confidence interval of the Ne estimated in our study for the entire lake, a reduction in population size greater than 50% would have caused a substantial loss of low-frequency alleles, but only modest decline in heterozygosity.

The number of loci used in the present study, however, may lack sufficient power to detect a bottleneck (Luikart & Cornuet, 1999). Overharvest generally decreases genetic diversity and can significantly bottleneck a population, but detecting changes in diversity can be difficult and require more markers than used in this study (Pinsky & Palumbi, 2014). A system wide assessment of *Coregonus hoyi* genetic diversity in the Great Lakes using 10 microsatellite loci and the same bottleneck analysis used in our study found very

little evidence of a bottleneck, despite a well-documented collapse of *C. hoyi* populations by the mid-20th century (Favé & Turgeon, 2008). Thus, non- detection of a bottleneck does not mean the effects of overfishing are not substantial. In Lake Superior, mean allelic richness declined more than 20% when lake trout collapsed between 1948 and 1959 (Guinand *et al.*, 2012). However, Guinand *et al.,* (2012) quantified the reduction in genetic diversity by comparing historic samples captured before and after the population collapsed, something that I was unable to do. The type of bottleneck analysis I could conduct here should be able to identify a large, recent, reduction in Ne, but without historic samples identification of smaller reductions in Ne is difficult. Therefore, without samples of lake whitefish from before commercial harvest occurred, I cannot conclusively say that over-fishing had no influence on genetic diversity, only that any change was not detectable in this study.

If I assume that lake trout were extirpated because of overharvest and that they were harvested at approximately the same intensity as lake whitefish, I would expect whitefish to have gone through a relatively severe drop in abundance due to fishing, mirroring that of lake trout. If this were the case, I should have seen depressed genetic diversity and strong evidence of a bottleneck in lake whitefish. However, our results suggest the opposite, lake whitefish have not experienced the strong bottleneck I would expect if harvest was sufficient extirpate a population. Therefore, if fishing pressure on lake trout was similar to lake whitefish, other factors besides harvest would be necessary to completely extirpate lake trout from Lake Champlain.

Based on our results, lake whitefish in Lake Champlain appear to form a genetically diverse, mostly unstructured lake-wide population and show no strong evidence of overharvest or genetic bottleneck. Demographically (Herbst, Marsden & Smith, 2011) and genetically (present study) the lake whitefish population appears to be in good condition with diverse age and length classes and equal or greater genetic diversity than other populations of whitefish and no signs of recent bottleneck. However, the lack of harvest data and preserved samples during and immediately following commercial harvest makes conclusively determining the impact commercial fishing had on lake whitefish difficult. Our study highlights the importance of monitoring populations and maintaining historic records for future research.

Table 4.1: Characteristics of the 8 microsatellites amplified in lake whitefish. Shown are the marker name, forward and reverse primer sequence, fluorophore tail, amplified size range, annealing temperature (Ta) and citation for the source of the marker.

marker	primer (5' - 3')	florophore	size range	Ta	source
BFW1	F: GATCAGAGAAATACACACAACGCATCAA	FAM	198 - 226	60 - 55	Lu et al. 2001
	R: CACGAGTCATTACCTTGGAGAC				
BFW2	F: GGGATACATCGGCAACCTCTG	FAM	145 - 165	60 - 55	Lu et al. 2001
	R: AAAAGAGTAACCCCTGACAGA				
CL23	F: GCTGTATGAGGATAGCATTC	FAM	250 - 284	60 - 55	Lu et al. 2001
	R: TGTGTTTTGCTGGATTACG				
C6	F: GCCATCATCCTCCCAGGAAAC	VIC	135 - 151	60 - 55	Rogers et al. 2004
	R: CAGGGAATCTGCACTGGAGC				
C28	F: ACAATAGCAGGCCATTCAGG	VIC	171 - 185	62.5 - 59	Rogers et al. 2004
	R: CCAATCTTCAAAGCCATTTCA				
C45	F: GAGTGACAGCAGGGAGCAG	VIC	237 - 255	62.5 - 59	Rogers et al. 2004
	R: GGCTCGGTTGAAAGTTGAGA				
C68	F: GTGTGTTACAAGTGGCTATG	PET	173 - 179	62.5 - 59	Rogers et al. 2004
	R: GTGATGGCTTTCAGAGGC				
C4	F: TGGTGTAATGGCTTTTCCTG	VIC	133 - 152	62.5 - 59	Rogers et al. 2004
	R: GGGAGCAACATTGGACTCTC				

Table 4.2: Site-specific summary statistics of lake whitefish genotypes taken from eight microsatellite loci in Lake Champlain. AR = mean allelic richness across all loci based on minimum sample size of 21 individuals, efN = mean number individuals genotyped across loci, H_O = observed heterozygosity, H_E = expected heterozygosity, F_{IS} = inbreeding coefficient, HWE = P-value for Hardy-Weinberg equilibrium test, HWE_{hom} and HWE_{het} = P-values for heterozygosity deficit and excess, Ne = effective population size (lowest allele frequency used = 0.2).

site	AR	efN	H_O	H_E	F_{IS}	HWE	HWE_{hom}	HWE_{het}	Ne
Burl. Bay	4.34	36.4	0.56	0.56	-0.032	0.02	0.93	0.15	173.6 (35.4 – ∞)
Grand Isle	5.95	32.3	0.53	0.62	0.130	0.00	0.81	0.00	14.1 (8.5 – 25.0)
Grand Isle (PA removed)	5.09	27.8	0.52	0.59	0.100	0.19	0.73	0.00	47.3 (17.8 – ∞)
Inland Sea	4.18	34.1	0.58	0.58	-0.027	0.23	0.88	0.50	∞ (47.5 – ∞)
Miss. Bay	4.63	19.9	0.55	0.57	0.039	0.29	0.73	0.16	∞ (35.4 – ∞)
S. Lake	NA	5.6	0.65	0.56	-0.166	1.00	0.92	0.99	NA
Mall. Bay	NA	1.9	0.63	0.45	-0.356	1.00	1.00	1.00	NA
Whole Lake Combined	5.09	130.1	0.56	0.60	0.040	0.00	0.92	0.00	139.7 (67.7 - 643.9)

Table 4.3: All individual genotyped lake whitefish and site of origin with at least one private allele present.

Site	No. Loci with Private Alleles	Loci with Private Alleles
Burl. Bay	1	BFW1
Grand Isle	1	BFW2
Grand Isle	4	C45 C23 C4 BFW2
Grand Isle	2	C45 BFW2
Grand Isle	5	C45 C28 C23 BFW2 BFW1
Grand Isle	3	C45 C23 C4
Grand Isle	1	C6
Grand Isle	2	C45 C23
Grand Isle	4	C45 C23 BFW2 BFW1
Grand Isle	1	C4
Grand Isle	1	C45
Grand Isle	2	C45 C4
South Lake	1	BFW2
Miss. Bay	1	C45
Miss. Bay	1	C4
Miss. Bay	2	C45 BFW1
Miss. Bay	1	C68
Inland Sea	1	BFW1
Inland Sea	1	C45

Table 4.4: F_{ST} (above diagonal) and G'_{ST} (below diagonal) for all sites sampled for whitefish in Lake Champlain. Comparisons significantly greater than zero are bolded.

	BB	GI	IS	MB	Miss	SL
BB		0.01	**0.032**	0.063	0.011	0.021
GI	0.023		**0.023**	0.043	0.010	0.007
IS	**0.062**	**0.051**		0.020	-0.002	0.024
MB	0.137	0.170	0.0483		0.054	0.080
Miss	0.021	0.026	-0.003	0.136		0.017
SL	0.037	0.031	0.0455	0.154	0.035	

Table 4.5: Mean number of alleles (Na), observed heterozygosity (Ho), and expected heterozygosity (He) of loci BFW1, BFW2 and C23 reported in Table 3 of Lu *et al* 2001 and the present study.

site	Na	Ho	He	source
Allagash	3.33	0.50	0.60	Lu et al 2001
Aylmer	7.00	0.67	0.70	Lu et al 2001
Carr	3.00	0.38	0.34	Lu et al 2001
Champlain	7.00	0.70	0.68	Lu et al 2001
Clear	3.33	0.53	0.44	Lu et al 2001
Cliff	3.67	0.56	0.50	Lu et al 2001
Crescent	4.33	0.38	0.39	Lu et al 2001
East	5.33	0.41	0.44	Lu et al 2001
Echo	3.67	0.58	0.49	Lu et al 2001
Harrow	5.00	0.45	0.43	Lu et al 2001
Haymock	4.67	0.45	0.56	Lu et al 2001
Indian	4.67	0.43	0.53	Lu et al 2001
Mira	4.00	0.67	0.63	Lu et al 2001
Poh_n_gamook	5.67	0.66	0.60	Lu et al 2001
Ross	3.33	0.41	0.37	Lu et al 2001
Rowe	2.33	0.33	0.33	Lu et al 2001
South	2.67	0.50	0.49	Lu et al 2001
Spider	3.33	0.50	0.52	Lu et al 2001
St. Francis	1.67	0.28	0.22	Lu et al 2001
T_miscouata	6.67	0.64	0.67	Lu et al 2001
Umsaskis	2.00	0.42	0.41	Lu et al 2001
Webster	4.33	0.49	0.57	Lu et al 2001
West Grand	3.33	0.45	0.37	Lu et al 2001
Burlington Bay	7.33	0.72	0.65	Present Study
Grand Isle	9.67	0.79	0.59	Present Study
Inland Sea	6.00	0.67	0.65	Present Study
Malletts Bay	2.33	0.42	0.67	Present Study
Missisquoi Bay	6.33	0.69	0.62	Present Study
South Lake	4.67	0.73	0.78	Present Study

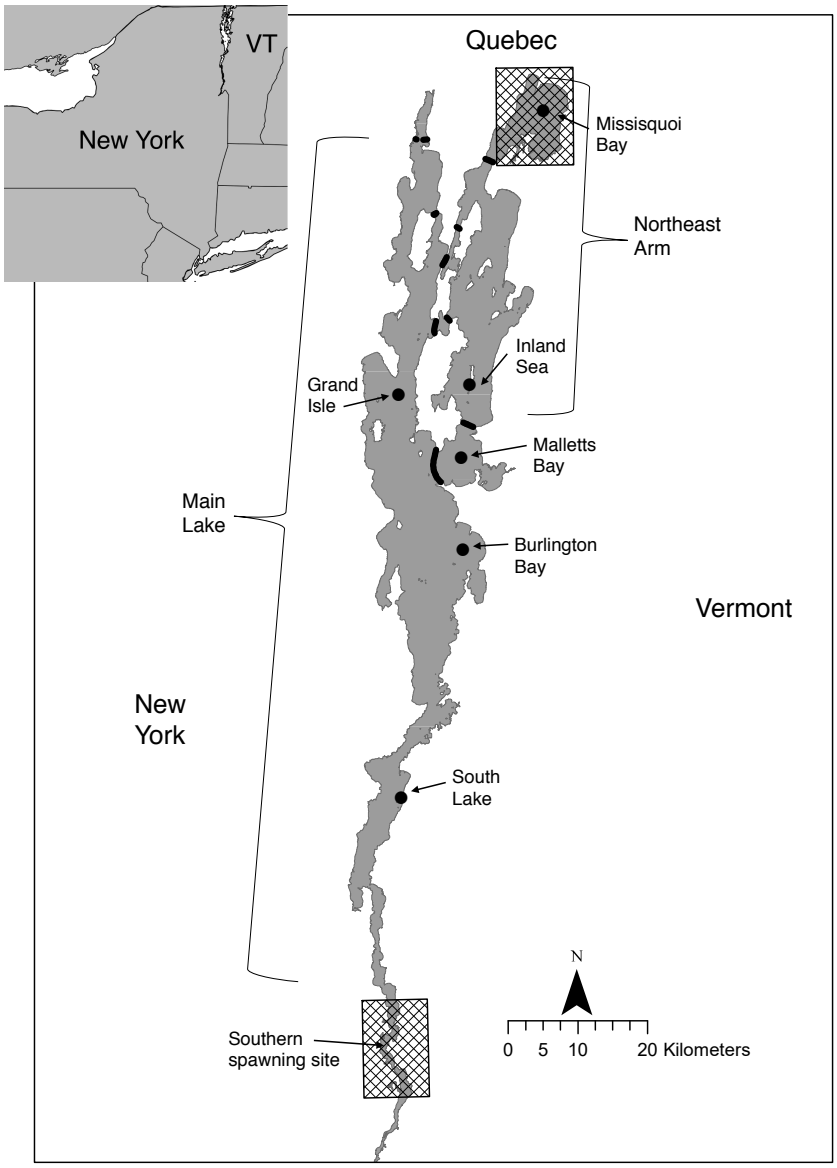

Figure 4.1: Locations of lake whitefish samples (dots), approximate locations of historic major fishing grounds (hashed boxes) and causeways (black lines). Major basins discussed in text are denoted using brackets.

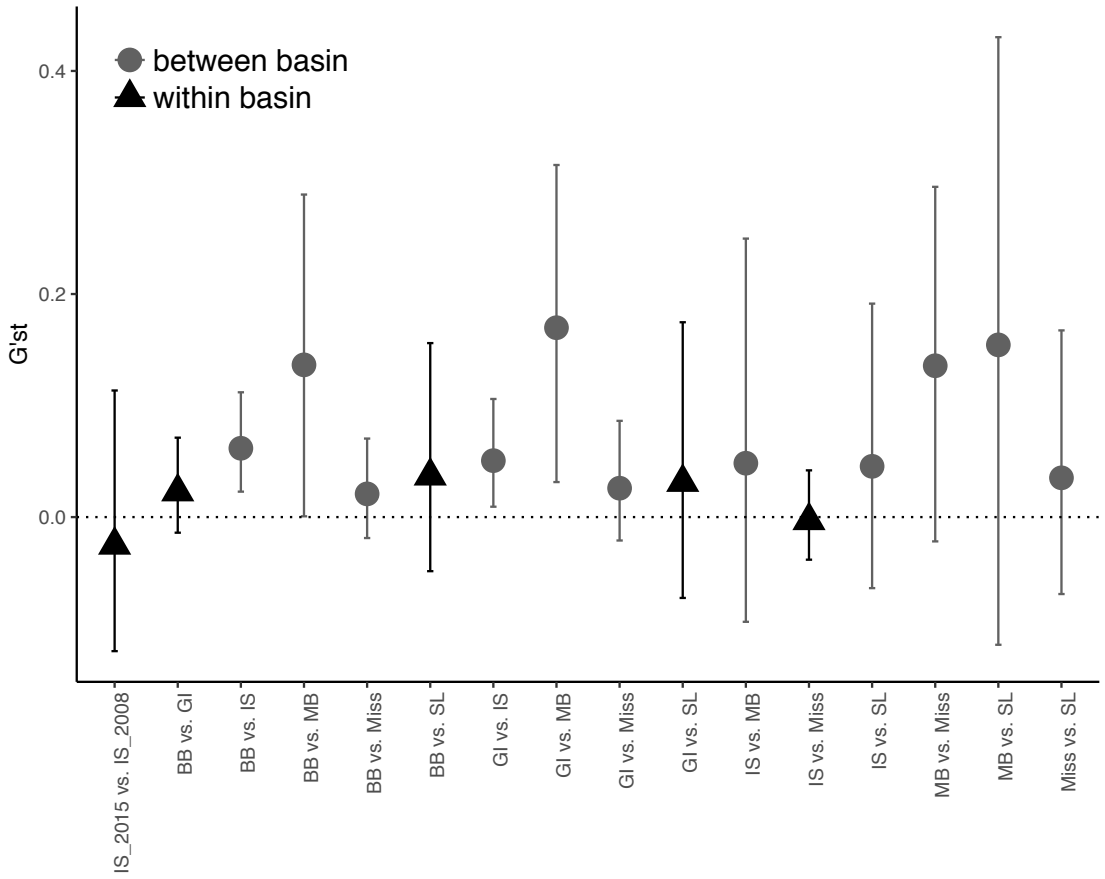

Figure 4.2: Pairwise genetic distance estimates (G'_{ST}) and 95% confidence intervals between 2008 and 2015 Inland Sea samples (IS), and among all sites sampled for whitefish in Lake Champlain: Burlington Bay (BB), Grand Isle (GI), Malletts Bay (MB), South Lake (SL), and Missisquoi Bay (Miss). Comparisons with confidence intervals including zero (dotted line) were not considered to be significant.

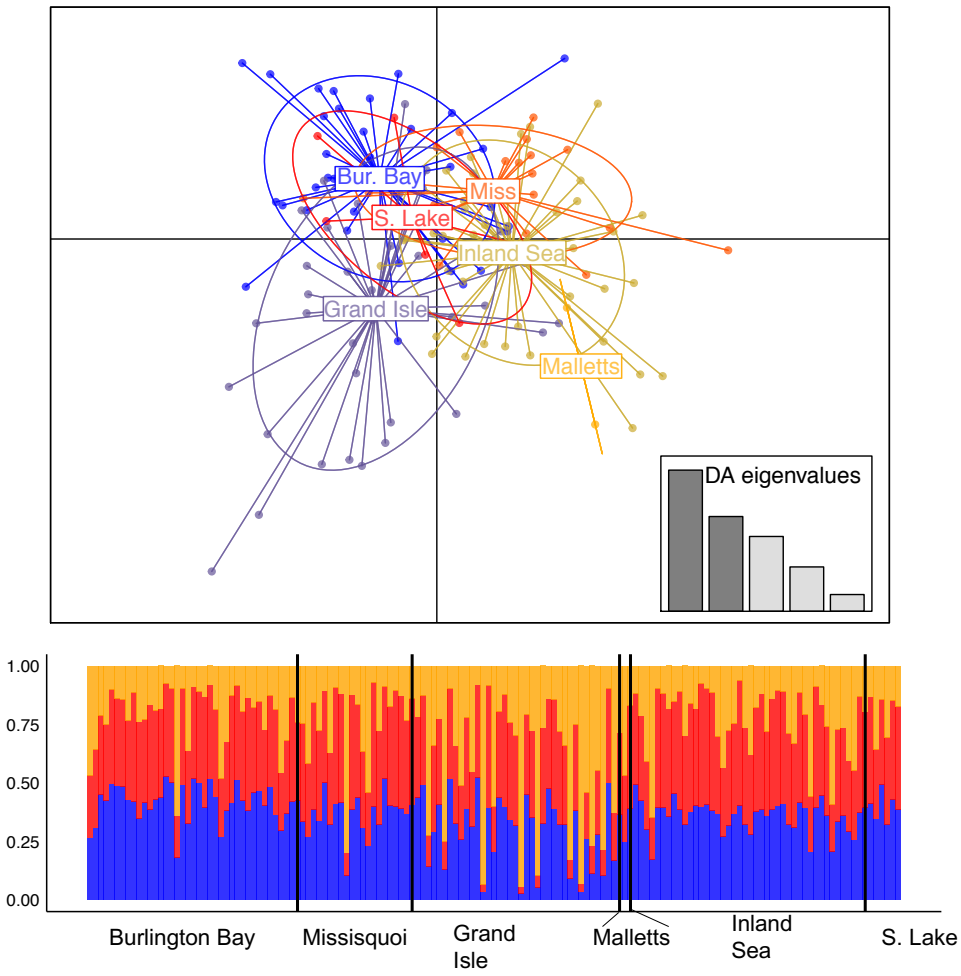

Figure 4.3: Genetic clustering of all whitefish sampled in Lake Champlain using discriminant analysis of principal components (top) and Bayesian STRUCTURE analysis with k = 3 (bottom). Each individual dot in the DAPC bi-plot represents a single genotyped individual and the color of the dot indicates the site the where the individual was sampled. The STRUCTURE barplot is a graphical representation of individual membership coefficient to each cluster (vertical bars). Colors represent different estimated clusters of a single admixed individual. Vertical black bars indicate breaks in sampled populations (x-axis).

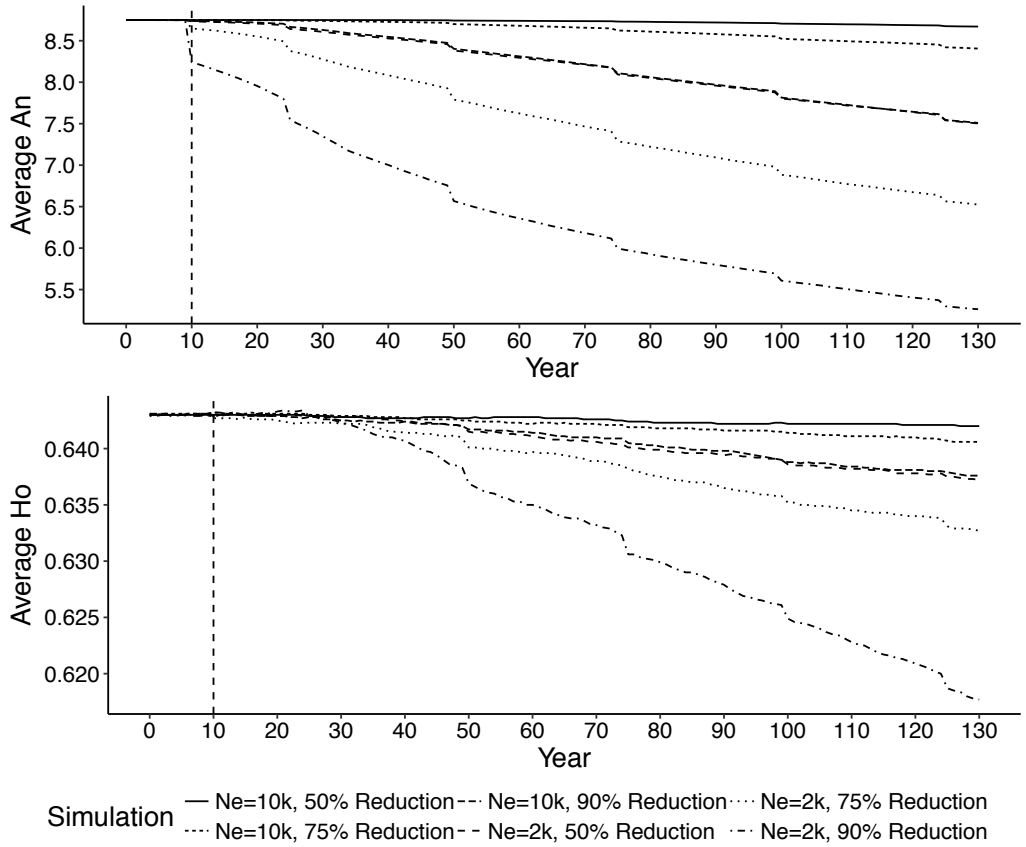

Figure 4.4: Time series of simulated average number of alleles (An) and observed heterozygosity (Ho) following a reduction of effectve population size from either 10,000 or 2,000 by 50%, 75% or 90% (line types). The simulated reduction in population size began after ten years (dotted line) and then population size was maintained at the reduced level for 120 years representing the time between peak lake whitefish harvest and present day.

CHAPTER 5: ROLE OF DRAINAGE AND BARRIERS IN THE GENETIC STRUCTURING OF A TESSELLATED DARTER POPULATION

5.1. Abstract

While population genetic structuring is easily identified, the causes of the structure can be difficult to determine. Habitat fragmentation in aquatic systems has often been identified as a major source of increased population structure and decreased genetic diversity in fish, including benthic resident species such as darters. However, these findings are often not replicated across natural and manmade barriers and come from endangered or threatened populations where the genetic structure is likely already compromised due to small population size. To evaluate the factors involved in structuring a healthy darter metapopulation, I genotyped 506 tessellated darters from 18 sites in three different river drainages and one large lake. Sites were all in the same watershed but separated from one another by one or more of three different types of barriers: dams, natural fall lines and causeways. I found that while diversity and allele frequency varied largely by drainage, within drainage variation was minimal even across multiple barriers. No single barrier type appeared to be more formidable than any other. Our results indicate that healthy populations of darters may naturally be structured by drainage, but likely disperse across barriers to retain drainage-wide homogeneity.

5.2. Introduction

Issues associated with habitat fragmentation are at the forefront of modern conservation planning in both terrestrial and aquatic systems (Haddad *et al.*, 2015). Aquatic systems are particularly vulnerable to the loss of connectivity as a consequence of habitat fragmentation. The construction of dams and culverts in riverine systems often interrupts hydrology (Ligon, Dietrich & Trush,1995; Shaw *et al.*, 2016) and blocks fish migrations (Dynesius & Nilsson, 1994). Loss of connectivity in rivers can have negative effects on both migratory (Junge *et al.*, 2014) and resident fish populations (Peacock *et al.*, 2016), leading to population declines and loss of genetic diversity (Winston, Taylor & Pigg, 1991; Meldgaard, Nielsen & Loeschcke, 2003).

Barriers in aquatic systems range from large hydroelectric dams and waterfalls to smaller low-head dams, weirs, culverts and natural cascades. In the United States, large dams often receive the most public attention as a source of fragmentation, but small dams less than 15 m high outnumber large dams almost 18 to 1 and impound three to four times more water in aggregate than large dams (Rosenberg, Mccully & Pringle, 2000). Because even a 1-m tall barrier is impassible to many fish, the relative impact of small dams on stream connectivity is high.

Though anthropogenic alterations such as dams can negatively influence species that inhabit rivers by decreasing connectivity and increasing genetic distance among populations (Helfman, 2007), most lotic systems are naturally fragmented by waterfalls that may have isolated populations for thousands of years. For example, populations of

cutthroat trout (*Oncorhynchus clarkii clarkia*) in rivers along the coast of Alaska fragmented by natural waterfalls show clear signs of asymmetric gene flow and high population structure above and below waterfalls (Whiteley *et al.*, 2010). Determining the impact of anthropogenic habitat fragmentation relative to natural fragmentation may help predict the future influence of dams and identify natural levels of population structure across barriers.

Much of what is known about river fragmentation comes from research focused on migratory and/or adfluvial fish such as salmonids. However, fragmentation also impacts stream residents such as perch, darters, and catfish (Leclerc *et al.*, 2008; Beneteau, Mandrak & Heath, 2009; Sotola *et al.*, 2017). Species respond to fragmentation differently; for example, upstream gene flow for bullhead (*Cottus gobio*) was completely blocked by small dams in the Sense River in Switzerland, causing substantial genetic structure (Junker *et al.*, 2012), whereas, populations of blue sucker (*Cycleptus elongates*) in the Missouri River experienced only minimal changes in genetic diversity and showed no strong genetic structure across 3,000 km of river fragmented by six dams (Bessert & Orti, 2008). Therefore, studying how barriers influence population structure in multiple species continues to be important to understand the consequences of habitat fragmentation.

Darters (Percidae) are a particularly good species group for examining effects of barriers, as they have life history traits that make them sensitive to habitat fragmentation. Over 140 species of darter are present in North America and are common residents in most freshwater environments (Kuehne & Barbour, 2015). Darters prefer benthic habitats and

tend to have relatively limited dispersal ability, so they are vulnerable to issues commonly associated with dams, including pollution, habitat loss, and reduced population connectivity. Consequently, darters are a disproportionately endangered group, with 44% of darters listed as vulnerable, threatened or endangered (Helfman, 2007; Jelks *et al.*, 2008).

Decreased connectivity due to dams has had genetic consequences for many threatened or endangered species of darters and is believed to contribute to population declines (Beneteau, Mandrak & Heath, 2009; George, Neely & Mayden, 2010; Sterling *et al.*, 2012). However, most species of darters evolved in naturally fragmented environments and disperse only short distances even in connected regions of streams (Dammeyer, Phillips & Bonner, 2013). Therefore, case studies evaluating population structure in healthy populations of darters across both natural and manmade barriers is important to begin to identify the range of genetic variation that can be present in a darter populations, while not overstating the generalization of observations (Richardson *et al.*, 2016). Tessellated darters (*Etheostoma olmstedi*) are found in Lake Champlain and its tributaries, and are considered to be "abundant" in Vermont (Vermont National Heritage Inventory, 2017). Populations tessellated darters are not exploited, and the only anthropogenic activity that may have affected stream populations was an increase in sedimentation during a period of deforestation in the1800s (Marsden & Langdon, 2012); populations are likely to have recovered from any effects during this period, as streams have steadily increased in substrate quality during subsequent reforestation (Wang *et al.*, 1997; McBride, Hession & Rizzo, 2008).

Our objectives were to describe the level of genetic structure in a healthy population of darters and identify the relative influence of natural versus manmade fragmentation on the genetic structure and diversity of darter populations. I analyzed genetic data collected from tessellated darters sampled across three types of barriers (lake causeways, dams and natural fall lines) throughout the Lake Champlain watershed in Vermont. I structured our analysis to evaluate five hypotheses: 1) tessellated darter populations are genetically structured among Lake Champlain drainages by distance and by barriers; 2) genetic diversity decreases with distance from Lake Champlain which is presumed to have the highest genetic diversity; 3) both natural and manmade barriers increase population structure and decrease genetic diversity; 4) movement across instream barriers is primarily downstream, while movement across lake barriers is similar in both directions; 5) the magnitude of a barrier's effect on diversity and structure is related to barrier age and type.

5.3. Methods

5.3.1. Study location

The study was conducted in the Lake Champlain watershed, which spans 21,326 km^2. Lake Champlain is long (193 km) and narrow (20 km at the widest point), spans the border between New York and Vermont, USA, and Vermont and Quebec, CA and drains north into Quebec, Canada. Three large islands naturally divide the northern portion of Lake Champlain into eastern and western arms (Figure 14). Seven causeways built between 1800 and 1900 link the islands to the mainland and isolate the lake further into

four major basins: the Main Lake and a northeastern arm which is subdivided into Missisquoi Bay at the north end, the Inland Sea in the center, and Malletts Bay at the south end. All the causeways have one or two shallow openings (1 – 9 m deep) that allow some flow of water and passage of boats and fish. The three tributaries to Lake Champlain sampled in this study drain into three lake basins: Lewis Creek (southern Main Lake), Indian Brook (Malletts Bay), and the Missisquoi River (Missisquoi Bay). These tributaries all contain populations of tessellated darters and have one dam and a natural waterfall within the study area (Table 5.1). Indian Brook is the smallest stream, with a drainage area of 16.8 km^2 and mean discharge of 0.5 $m^3 s^{-1}$. Lewis Creek has a moderate size drainage of 200 km^2 and a mean discharge of 3.1 $m^3 s^{-1}$. The Missisquoi River is one of the largest tributaries to Lake Champlain with a drainage area of 2201.5 km^2 and mean discharge of 35 $m^3 s^{-1}$. The height of dams was taken from the height reported in the Vermont Dam Inventory managed by the Vermont Department of Environmental Conservation. Because the fall lines are partially eroded and form multiple cascades, a single height measurement would not be descriptive of the barrier. Therefore, the height of fall lines was defined by first creating a path of the entire cascade region of the fall line as determined visually in the field and then confirmed using topography in Google Earth. Next, the elevation profile of the entire path was used to identify the 20-m section with the steepest slope and defined the height of the fall line as the change in elevation across the steepest 20 m section of total path because this section was most likely to be the greatest barrier to migration. These measurements confirm that

all fall line heights were equivalent to dam heights and therefore reasonable barriers to tessellated darters.

5.3.2. Fish sampling and genetic analysis

Fish were captured using a combination of beach seines, dip nets, and benthic trawls at 18 sites throughout Lake Champlain and the three tributaries (Figure 5.1). Specifically, I targeted populations separated by two causeways in the lake, and by a natural fall line and dam in each of the three tributaries, allowing comparison between populations separated by a causeway, dam, fall line, dam and fall line, or no barrier (i.e., distance alone). The sampling strategy also allowed comparisons between tributaries relative to lake populations, and downstream relative to upstream populations. Individuals were killed in the field and preserved in 95% ethanol. In the laboratory, fish were placed in 2-ml centrifuge tubes filled with fresh 95% ethanol for storage, generally within 24 hr of sampling.

DNA was extracted from samples using a 5% Chelex-100 suspension. For each sample, approximately 1 mm^3 of muscle tissue was placed in 200 µl PCR tube with 150 µl of 5% Chelex-100 solution and 5 µl Proteinase-K (Qiagen). Samples were incubated at 55°C for 8 hr followed by 99°C for 10 min, 37°C for 1 min, and 99°C for 10 min and held at 4°C or frozen at -20°C for polymerase chain reactions (PCRs). PCR was conducted for 12 microsatellite loci previously identified for the *Etheostoma* genus; D1, Eo4, Eo6, Eo7 (DeWoody *et al.*, 2000), Eca46EPA, Eca49EPA (Tonnis, 2006), C2, C6, D116 (Switzer, Welsh & King, 2008), Ebl3, Ebl6 (Beneteau, Mandrak & Heath, 2007) and Esc26b

(Gabel *et al.*, 2008). Loci C2, EO7, and Eca46EPA were found to be monomorphic after genotyping 99 individuals and were removed from future analysis. Loci were amplified in multiplex reactions when possible in 12.5 µl reactions containing 6.25 µl 2X Taq DNA Polymerase Master Mix (New England BioLabs Inc.), 0.8 µM μl^{-1} fluorescently labeled forward and unlabeled reverse primer, and DNA template. The general PCR program used was 95°C for 2 min, 30 cycles at 95°C for 30 s, 20 s at marker-specific annealing temperature (Table 5.2), 68°C for 30 s followed by a final extension of 68°C for 10 min. Fragment analysis of PCR products were analyzed in the University of Vermont Advanced Genome Technologies Core using an Applied Biosystems 3130 Genetic Analyzer and a ROX 500 size standard and scored using GENEMAPPER software (Applied Biosystems).

5.3.3. Statistical analysis

All loci were assessed for the presence of null alleles with MICRO-CHECKER version 2.2.3 (Van Oosterhout *et al.*, 2004). Conformance to Hardy-Weinberg equilibrium (HWE) expectations at each locus using exact testing, observed (H_O) and expected (H_E) heterozygosity, F_{IS} and allelic richness scaled to the smallest population and using rarefaction to account for differences in sample size were estimated using the basicStats() function of the diveRsity package in R version 3.3.3 (Keenan *et al.*, 2013; R Core Team, 2015). Any deviations from HWE following Bonferroni correction for multiple comparisons were assessed for heterozygote excess or deficiency. Effective population size of each sampled location was calculated using a linkage disequilibrium method in N_eESTIMATOR (Do *et al.*, 2014) with minimum acceptable allele frequencies of 0.02.

Asymmetrical upstream and downstream migration across barriers was evaluated for all drainages separately using the experimental divMigrate() function in the diveRsity package in R which uses the method described in Sundqvist et al. (2016). In brief, this method works by generating a hypothetical pool of migrants for a given pair of populations and then estimates a measure of genetic differentiation between each population and the hypothetical pool. This directional genetic differentiation can then be used to estimate relative levels of migration. After migration was estimated among all sites, a two-way analysis of variance (ANOVA) was used to determine if migration differed across different barrier types and upstream vs. downstream. To evaluate genetic clustering without *a priori* assumptions of population, two different approaches were used. First, variation among and within each drainage was assessed using STRUCTURE (Pritchard *et al.*, 2000) deployed through the ParallelStructure package for R (Besnier & Glover, 2013). STRUCTURE was run hierarchically, first on the complete dataset and then on sites within each drainage. Each dataset was examined separately through five replicate runs of 100,000 replicates and a 10,000 cycles burn-in at k = 1- 5. Discriminate analysis of principal components (DAPC) was used as a second clustering estimator and run hierarchically like STRUCTURE (Jombart, 2008; Jombart, Devillard & Balloux, 2010). Clusters were identified as overlapping groups in DAPC bi-plots. Possible genetic structure among sample sites was evaluated further using pairwise comparisons of G'_{ST}, and 95% confidence intervals calculated using the diveRsity R package. Because G'_{ST} is standardized and therefore performs better for loci with multiple alleles and is not an estimate that is dependent on single-step mutation model which are sensitive to issues of

homoplasy common in microsatellite loci, I chose G'_{ST} as my estimate of genetic distance (Hedrick, 1999, 2005; Sefc, Payne & Sorenson, 2007). However, standardized estimates of genetic distance can bias migration estimates by inflating distance estimates and therefore should not be used as an estimate of gene flow (Hedrick, 2005).

We tested for the statistical power to detect genetic differentiation at five different expected levels of F_{ST} (0.001, 0.0025, 0.005, 0.01, and 0.05), given the sample sizes, number of loci and allele frequencies used in this study, using POWSIM (Ryman & Palm, 2006; Ryman *et al.*, 2006). POWSIM simulates the sampling of genes from a specified number of population with a set effective population size (2000 for this study) that have diverged by drift for *t* number of generations. Samples from the simulated populations are then used to test for genetic homogeneity using Fisher's exact test and x^2-tests. Power is then defined as the proportion of significant results obtained over multiple replicate simulations (2000 for this study).

To evaluate how drainage, upstream-distance, number of barriers and barrier type impacted genetic diversity (H_E, H_O, and allelic richness), I used a series of variance and covariance analyses (ANOVA and ANCOVA). Differences in genetic diversity among basins and upstream distance were evaluated using a two-way ANOVA with the diversity estimate as the response variable and drainage and upstream distance as the predictor variables. To test if barrier type influenced the change in genetic diversity from downstream to upstream populations, the change in diversity was calculated between every two pairs of sites within the same drainage as the difference between the downstream diversity estimate and the upstream diversity estimate for a given pair of

sites. Next, differences in the change in diversity (H_E, H_O, and allelic richness) across five barrier types (no barrier, causeway, dam, fall line, dam and fall line) was assessed using an ANOVA with pairwise change in diversity as the response variable and barrier type as the predictor variable. The pairwise change in diversity was used as the response variable rather than point estimates of diversity themselves to directly assess the influence of barriers on diversity while partially controlling for effects of upstream distance and variation in diversity among drainages. Any significant effects were investigated using Tukey honestly significant tests. For all statistical tests, significance was determined based on an alpha level of 0.05.

5.3.4. Generalized Linear Models

To determine how drainage, distance, number of barriers, barrier type and barrier age impacted genetic distance (G'_{ST}), I used a generalized linear models (GLM) approach. Unlike more traditional approaches such as partial Mantel tests to a single predictive variable, GLM can combine multiple predictors and likelihood statistics can be employed to compare among models (Storfer *et al.*, 2007). Landscape features were chosen to limit collinearity and models were purposefully kept simplistic, comparing only a single feature in addition to a null model of isolation by distance (IBD) at a time. Models were fit using the glm() function in the stats package in R with pairwise G'_{ST} as the response variable and one or more landscape features as the predictor variable and assuming a Gaussian distribution. Because G'_{ST} is standardized, it cannot be used as an estimate of gene flow. However, our goal was to identify the relative influence of landscape features on genetic distance, not estimate migration among sites. Therefore, using a standardized

method such as G'_{ST} allows for comparison of genetic distance while controlling for differences in genetic diversity throughout the study system that would influence non-standardized estimates of genetic distance (Hedrick, 1999, 2005).

Eight total models in two broad categories and were run to describe genetic distance of tessellated darters. Category 1 included three null models of genetic distance across the total study area (global models hereafter). Model 1 was our null global model and evaluated the influence of isolation by waterway distance (IBD) on genetic distance among all sampled sites. Geographic distance was measured in meters as the shortest distance via water between any two site pairs. Model 2 evaluated the influence of IBD and total number of barriers on genetic distance among all sampled sites. Model 3 evaluated the influence of IBD and a random effect of drainage comparison (a factor indicating the two drainages involved in the pairwise estimate of distance) on genetic distance among all sampled sites. The purpose of model 3 was to determine if other unmeasured differences among drainages explained more variance than distance alone. Category 2 models limited the dataset by removing pairwise comparisons between drainages and analyzing only within-drainage pairwise comparisons (referred to as within-drainage models hereafter). Six within-drainage models were evaluated. Model 4 was our null within-drainage model and evaluated the influence of just IBD on genetic distance within each drainage, ignoring the presence or absence of barriers. Model 5 evaluated the influence of IBD and barrier type (no barrier, dam, causeway, fall line, or combination of a dam and a fall line) on genetic distance. Model 6 assumed all barrier types were equal and evaluated the influence of IBD and total number of barriers (0-2) on

genetic distance. Model 7 evaluated the influence of IBD and barrier age on genetic distance measured as the age of the oldest barrier in years separating two populations (0 – 12,000) and Model 8 assumed genetic distance was drainage-specific, and evaluated the influence of IBD and drainage size (km^2) on genetic distance. To account for variation in units among predictors, all parameter estimates were standardized by dividing them by two standard deviations (Gelman, 2008). All but our two null models included only distance and a single additional predictor to avoid issues associated with collinearity between barrier metrics which can confuse model interpretation (Zuur, Ieno & Elphick, 2010).

Model selection was conducted separately in each of the two model categories and was based on three principal metrics. First, models were ranked using Akaike's Information Criterion (AIC) whereby a larger absolute value AIC indicates more support for a given model (Akaike, 1992). Second, to test if added predictors improved a model beyond that of a null model of isolation by distance, I used likelihood ratio tests calculated using the anova() function in the stats package of R. Third, the adjusted R^2 was calculated for each model to provide a directly interpretable metric of the variance explained by each model. To help with independent model interpretation, null and residual deviance and residual degrees of freedom were also reported, but not used directly in model selection.

5.4. Results

The reported heights of dams and estimated heights of fall lines were roughly equivalent. Therefore, all barriers were considered to be effective barriers to tessellated darters. A

total of 482 tessellated darters was sampled during July and August 2016 and an additional 24 darters were collected during August 2017. Tessellated darters were successfully sampled from all targeted locations other than above and below the natural fall line in the Missisquoi River where tessellated darters have been reported to be less common possibly due to the presence of fantail darters (*Etheostoma flabellare;* Rich Langdon personal communication). Thus, only samples above and below the dam in Missisquoi River were evaluated. Evidence of null alleles was found in 9 out of 162 locus-site comparisons. However, no locus was identified to have null alleles in more than 3 of 18 populations and there were no consistent deviations from HWE among loci or within populations following Bonferroni corrections. Because evidence of null alleles and deviations from HWE was infrequent and inconsistent, the complete dataset was analyzed for population analysis moving forward. Tests for statistical power indicated the probability of detecting genetic differences of F_{ST} of 0.005 and greater was 100% (all simulations detected differentiation; Table 5.3). Therefore, the current loci and sample sizes should be sufficient to detect all but small differences which are likely not biologically meaningful in the context of this study and therefore interpretation of their effect should be avoided (Hedrick, 1999; Richardson *et al.*, 2016).

Allelic richness, H_E, and H_O differed significantly among sampled drainages (ANOVA $p < 0.001$ for all comparisons) and were consistently higher in Lake Champlain and Missisquoi River than sites in Indian Brook or Lewis Creek (Table 5.4; Figure 5.2). In contrast, effective population size was estimated to be infinity for at least one site in every drainage and the jackknifed confidence interval included infinity in all but three

sites, with no clear pattern by drainage. F_{IS} was variable but generally low (range = -0.09 – 0.14, mean = 0.02) across all sites. Allelic richness, H_E, and H_O also decreased slightly with distance upstream from Lake Champlain. When Lake Champlain was included in the analysis, allelic richness, H_E, and H_O all a had significant negative relationship with upstream distance (Figure 5.3); however, when only river populations were analyzed, only allelic richness maintained a significant negative relationship with upstream distance, though a negative, non-significant, relationship was still apparent between H_E and H_O and upstream distance.

Allele frequencies differed among sampling drainages. STRUCTURE and DAPC analysis revealed three distinct clusters grouped by sampling drainage (Figure 5.4). Lake Champlain and Missisquoi River samples clustered into a single, admixed group while Lewis Creek and Indian Brook formed separate, more divergent populations with very little overlap with other clusters. Lewis Creek and Indian Brook clusters had higher definition than the Missisquoi and Lake Champlain cluster as indicated by the high density of points along discriminant function 1 of the DAPC analysis (Figure 5.3). Estimates of pairwise G'_{ST} corroborated observed clusters whereby G'_{ST} values between pairs of drainages were much higher than within drainages (Table 5.5).

The influence of barriers on genetic diversity and population structure was less defined than the influence of drainages. Cluster analysis conducted within each drainage did not show any clustering that would indicate the presence of more than a single, panmictic population within each drainage. Estimates of pairwise G'_{ST} corroborated the observed lack of clusters whereby G'_{ST} values between pairs of sites within the same drainage were

universally low and confidence intervals almost always included zero. Within drainages, allelic richness, H_O, and H_E did not change as the number of downstream barriers increased ($p > 0.1$ for all; Table 5.3). The change in allelic richness differed among barrier types ($F_{4,35} = 4.645$, $p = 0.0041$) and was significantly greater across fall lines and the combination of dams and fall lines than across causeways (Tukey HSD $p = 0.008$ and 0.015) but similar among all other barrier types. The same main effect was found for H_O ($F_{4,35} = 2.731$, $p = 0.0445$) and H_E ($F_{4,35} = 6.804$, $p = 0.000367$). However, Tukey HSD test revealed no significant pairwise differences in H_O among barrier types (Tukey HSD $p > 0.05$ for all) but did reveal that H_E was significantly greater across fall lines and the combination of dams and fall lines than across causeways or dams (Tukey HSD $p =$ 0.0023, 0.0400, 0.0036, and 0.0464 respectively; Figure 5.4). The change in diversity from downstream to upstream of a barrier was greater across fall lines than dams, but similar to populations separated by causeways or no barrier at all (Figure 5.4). Overall, estimated migration was higher in the downstream direction (mean = 0.45; SD = 0.23) than upstream (mean = 0.35; SD = 0.15) for river samples ($p = 0.014$) but was similar in both directions across causeways for lake samples ($p = 0.78$). The relative amount of estimated migration did not vary by barrier type ($p = 0.77$).

5.4.1. Generalized Linear Models

Of the three global models of genetic distance, Model 3 which contained the predictors of waterway distance and a random effect, basin combination, performed significantly better than the other two models (Table 5.6) and appeared to predict almost all the variation among sites (adjusted $R^2 = 0.97$). Model 2, which included the total number of barriers

separating two sites as a predictor, performed slightly but not significantly worse than our null IBD model (Model 1). Models 1 and 2 explained identical amounts of variation (adjusted $R^2 = 0.21$), further indicating that the number of barriers between two sites did not substantially influence genetic distance. Of the five within-drainage models of genetic distance, no predictor was found to significantly improve the performance from the null model of IBD (Table 5.6). However, this does not indicate that the IBD model explained a high amount of variation in genetic distance (adjusted $R^2 = 0.02$). Additionally, there was low overall null deviance in G'_{ST} within drainages, and therefore little deviance for any predictive variable to explain (Table 5.6).

5.5. Discussion

Tessellated darters in the Lake Champlain watershed were characterized by a high amount of variation among drainages but low variation in genetic diversity and allele frequency within drainages. Populations within individual drainages maintained genetic connectivity even across strong dispersal barriers and had limited loss of diversity with upstream distance and increased fragmentation. These findings are indicative of distinct sub-populations residing in river drainages with exchange of individuals across barriers within drainages.

Tessellated darter populations had drainage-specific genetic diversity. Estimates of allelic richness, $H_{E,}$ and H_O were more than twice as high in Lake Champlain and Missisquoi River than in Indian Brook and more than 50% higher than Lewis Creek. The observations are consistent with a patch size hypothesis of genetic diversity whereby

genetic diversity increases with area occupied by the population (Vellend, 2003). The Missisquoi River drains over 80 times the area of land and discharges 70 times more volume of water than Indian Brook, and is 11 times larger in drainage area and discharge than Lewis Creek. If the size of the drainage is proportional to the population size and patch size, our results are consistent with other studies. Knaepkens *et al.* (2004) found that observed and expected heterozygosity of European bullhead (*Cottus gobio*) nearly doubled as patch size doubled from 3000 to 6000 m. Additionally, Vellend (2005) used simulations to evaluate how genetic diversity varied with patch size that and found that not only did genetic diversity increase with patch size, but that the relationship was stronger for common species than rare species. Therefore, because tessellated darters are common in all four of the drainages I analyzed, the relationship between patch size and genetic diversity may have a larger effect size and therefore be more detectable in our study compared to studies in which populations sizes are small.

Not surprisingly, drainage also had a large influence on the population structure of tessellated darters and drainage combination was the strongest predictor of genetic distance in our global model. Drainage often explains much of the variation in other darter species; for example, greenside darter populations (*Etheostoma blennioides*) in Ontario were structured by drainage (F_{ST} = 0.079) and similar results were found for the fountain darter (*Etheostoma fonticola*), Okaloosa darters (*Etheostoma okaloosae*) and others (Beneteau, Mandrak & Heath, 2009; Austin *et al.*, 2011; Olsen *et al.*, 2016). In addition to drainage effects, I found that waterway distance had a moderate effect on genetic distance at a global scale and almost no effect of distance among sites within

basins. Similarly, distance explained 40-85% of genetic divergence among drainages in a recent invasion of greenside darter populations (Beneteau, Mandrak & Heath, 2009). The strong divergence of the greenside darters in that study may be partially explained by a strong founder effect related to the recent invasion. While distance explained about 20% of the variation of G'_{ST} in global models in the present study, the IBD pattern showed a notable break in suggesting that other, unmeasured difference among drainages also influence genetic distance. The observed break in the IBD pattern is exemplified by the apparent lack of genetic divergence between of Missisquoi River and Lake Champlain darters but large genetic divergence between Indian Brook and Lake Champlain darters. The Missisquoi River is 44 km from the closest Lake Champlain population I sampled, while Indian Brook empties into the lake only 10 km from the nearest Lake Champlain sample site. If distance alone predicts genetic distance, darters from Indian brook should be genetically more similar to Lake Champlain darters than I observed, while Missisquoi River darters should be genetically more distant. These patterns could indicate that Missisquoi River functionally acts as a continuation of Lake Champlain, while smaller drainages like Indian Brook and Lewis Creek contain isolated sub-populations with little migration to or from Lake Champlain. Overall, our results suggest that, in a large, stable population of tessellated darters, genetic structure and diversity may be almost entirely determined by river drainage, with low migration between sub-populations regardless of distance or physical barrier, partially refuting hypothesis 1 that distance and barriers influence population structure.

Within drainages, neither natural nor man-made fragmentation had a large influence on the genetic structure and diversity of darter populations, giving no support to hypotheses 3 and 5. Because all but one within drainage pairwise G'_{ST} estimate had a 95% confidence interval that included zero, the level of genetic distance among sites within drainages was functionally zero. Therefore, the inability to detect clusters of individuals within drainages or explain variance in pairwise distance across different barrier types was not surprising. However, the lack of genetic distance among fish separated by barriers in our study is in direct contrast to research on many other species including yellow perch (*Perca flavescens*), bull trout (*Salvelinus confluentus*), and log-perch (*Percina caprodes*), where dams were one of the strongest predictors of population structure (Leclerc *et al.*, 2008; Meeuwig *et al.*, 2010; Roberts, Angermeier & Hallerman, 2013). I assessed population structure across three dams ranging from 37 to 117 years old, of which all had a height of at least 1 m and formed strong upstream barriers for small fish such as tessellated darters (Porto, McLaughlin & Noakes, 1999). If the barriers I evaluated truly isolated darter populations, our power analysis indicated that even at a relatively large effective population size (2000 individuals), significant genetic distance should be detectable after 20 generations of isolation and drift. Given that tessellated darters likely mature at 1-2 years old and only live to age 4 or 5 years old, even 37 years of isolation could be enough to result in population structure (Fahy, 1954). Barriers of similar age and size have been shown to result in observable genetic structure in populations of other small fish, some with abundant populations. For example, the European chub (*Squalius cephalus*) had higher genetic differentiation and a larger decline

in allelic richness across regions separated with many small weirs or large dams than in un-fragmented sections (Gouskov & Vorburger, 2016). Other species, such as the Yazoo darter (*E. raneyi*), with compromised or endangered populations also show signs of increased genetic distance among sites separated by dams (Sterling *et al.*, 2012). However, the effect of multiple small dams on European chubs was relatively small, indicating that migration across barriers was possible. Also, much of the difference among Yazoo darter populations could be explained by strong bottlenecks associated with small population size. Therefore, the impact of a barrier may be more strongly linked to life history and population demography of a species than the age or size of the barrier.

Though dams often influence population structure of fish, there are many examples where they do not. Mottled sculpin (*Cottus bardi*), which are common in the Nantahala River (North Carolina, USA), show patterns of strong isolation by distance across just 5 km, but very little evidence of any isolation by barrier (Lamphere & Blum, 2012). The population structure of six species of fish in the Truckee River of California and Nevada was found to be significantly structured by barriers during a low-flow year, but the structure disappeared the following year when high river discharge re-distributed fish and broke down the observed structure (Peacock *et al.*, 2016). These examples suggest that small, instream barriers do not necessarily result in genetic differentiation of fish populations, even if they limit fish movement. For tessellated darters, downstream migration across barriers may be sufficient to homogenize populations. Especially, if upstream populations are large enough to reduce the effects of genetic drift. I found very

low levels of genetic distance among sites within drainages and evidence of strong downstream migration, supporting hypothesis 4. Additionally, I found only a small decrease in genetic diversity with upstream distance (hypothesis 2), indicating that upstream populations are not suffering from stronger genetic drift or inbreeding than downstream populations. Therefore, darter populations may be resistant to the influence of barriers if some dispersal is possible, even if dispersal is uni-directional.

5.5.1. Implications for barrier management and fish conservation

Instream barriers have been a conservation concern and focus of research for decades, with the general consensus that dams and other barriers have long-term, negative effects on genetic diversity (Helfman, 2007). As interest in barrier removal continues to grow (Mclaughlin *et al.*, 2013), identifying the highest-impact barriers to target for removal and understanding the potential impacts of new barriers is increasingly important. However, efforts to identify and predict the influence of barriers on fish populations has had mixed success; some investigators have found a strong relationship between barrier type and connectivity (Gouskov & Vorburger, 2016) and others found only limited relationships between barriers and connectivity (Chick, Pegg & Koel, 2006). Our research supports a growing number of studies that indicate many populations of fish may be resistant to the effects of habitat fragmentation and are able to maintain population connectivity across barriers. However, predicting which taxa or populations are most sensitive to habitat fragmentation can be problematic (McLaughlin *et al.*, 2006). Therefore, I suggest future studies of aquatic fragmentation focus on assessing the

influence of a common barrier on multiple taxa, rather than multiple barrier types on a single taxon as I presented here.

Our results indicate that high population structure between drainages and variable genetic diversity may be normal for darters and therefore sufficient for a sustainable population. Many darter species are endangered and are the focus of population restoration or reintroduction (e.g., Shute, Rakes & Shute 2005; Olsen *et al.*, 2016). Our results provide a baseline level of genetic structure and diversity for a non-endangered species of darter and can therefore be used to help establish target conservation goals for endangered darters with similar ecology. Although I did not find that barriers had an influence on the population structure of tessellated darters, many studies on other threatened or endangered species have found that barriers can have a large effect on the dispersal, diversity, and genetic structure of populations (e.g., Austin *et al.*, 2011; Beneteau *et al.*, 2012; Roberts, Angermeier & Hallerman, 2013). Therefore, the influence of habitat fragmentation may be species-specific and amplified by small population sizes inherent in endangered species.

Table 5.2: Basic characteristics of the seven barriers in the Lake Champlain basin evaluated in this study. FL – natural fault line, CW = causeway, YBP – years before present.

Barrier name	latitude	longitude	type	yr. built	YBP isolation	height (m)	river drainage area (km^2)	Mean discharge (m3 s-1)
Lewis Creek FL	44.2600	-73.212631	fall line	NA	12000	3.8	200	3.1
Lewis Creek Dam	44.27867	-73.177211	dam	1980	37	3.96	200	3.1
Indian Brook FL	44.51477	-73.12766	fall line	NA	12000	5.5	17	0.5
Indian Brook Dam	44.541807	-73.152637	dam	1900	117	3.65	17	0.5
Missisquoi Dam	44.920591	-73.127902	dam	1920	97	5.79	2202	35.0
Outer Malletts CW	44.564793	-73.311199	causeway	1899	98	0	21326	NA
Sandbar CW	44.631246	-73.256109	causeway	1850	167	0	21326	NA

Table 5.2: Characteristics of 12 microsatellites amplified in tessellated darters. Shown are the marker name, forward and reverse primer sequence, fluorophore tail, amplified size range, annealing temperature (Ta) and citation for the source of the marker.

marker	primer (5' - 3')	florophore	size range	Ta	source
D1	F: CTCATCCATATTGCCTTGAGAGG	HEX	148 -164	49	DeWoody et al. 2000
	R: CTAACATTACATTGCTATTGAG				
EO4	F: CAGAGAAGATGTTTGCACTTC	FAM	96 - 124	56	DeWoody et al. 2000
	R: GTGAGGAGGGATAGCAGGC				
EO6	F: AACAGATGATGCTCAGTGG	HEX	153 - 179	56	DeWoody et al. 2000
	R: ATCGACGACATACGAGTTCTG				
EO7	F: ACTGTGCTGTTGAGAAATGC	FAM	monomorphic	49	DeWoody et al. 2000
	R: ACTGACCTTGTTTCAATGAG				
Eca46EPA	F: CTAAGCATGGTTTGGTTTGTGA	FAM	monomorphic	49	Tonnis 2006
	R: CCTTTTTTCCAGTGTCAGTGTCATTT				
Eca49EPA	F: AGATGGATGGATGGCTTGACGTA	FAM	138 - 178	49	Tonnis 2006
	R: GTGCTGAAGAAAAAGGCAACA				
EosC2	F: GCTCTCACAAACACACACAAAC	HEX	monomorphic	56	Switzer et al 2008
	R: ATCGACTCAACCCCAGATTAG				
EosC6	F: AAAGCCTGAGGGACAATTACAC	HEX	224 - 232	49	Switzer et al 2008
	R: CCTTTGCTGGTAAATCTCACAC				
EosD116	F: GCTGCCGACAGTGAAATAATAC	FAM	217 - 273	56	Switzer et al 2008
	R: GTGCATGTTTGTTGTGTTATGG				

Ebl3	F: CTGCTCTAAAGGATGAGTAACTGG	HEX	317 - 347	60	Beneteau et al 2007
	R: ATGTTCCCAAACTGTGGTGGT				
Ebl6	F: TATCATCCCATCGTCTGTCG	HEX	262 - 300	56	Beneteau et al 2007
	R: TGGCCCAAACAACAAGCTG				
Esc26b	F: TTCATACACGGTGCACTCACAT	FAM	309 - 401	60	Gabel et al 2008
	R: GCACAACATATGTCGTTAAGCTCC				

Table 5.3: Power results (proportion of significant tests) for X^2 - test and Fisher's exact tests run using POWSIM at various levels of expected F_{ST}. All simulations used effective population sizes of 2000 individuals and were replicated 2000 times.

Expected F_{ST}	X^2	Fisher's Exact
0.000	0.077	0.072
0.001	0.410	0.332
0.0025	0.921	0.873
0.005	1.000	1.000
0.010	1.000	1.000
0.050	1.000	1.000

Table 5.4: Number of tessellated darters genotyped (N), mean effective sample size (efN), observed heterozygosity (Ho), expected heterozygosity (He), inbreeding coefficient (F_{IS}), allelic richness (AR), and estimated effective population size (Ne).

	N	efN	Ho	He	F_{IS}	AR	Ne
Lake Champlain							
ChIS	39	33	0.619	0.662	0.069	5.174	360.5 (44.5 - ∞)
ChML1	24	23.333	0.609	0.645	0.025	5.164	∞ (96.4 - ∞)
ChMalE1	12	10.889	0.568	0.635	0.094	5.488	∞ (17.5 - ∞)
ChMalE2	24	22.333	0.588	0.638	0.094	5.229	∞ (127.8 - ∞)
ChMalW	35	33.889	0.604	0.664	0.073	5.512	2678.3 (101.8 - ∞)
ChML2	13	12.56	0.69	0.66	-0.05	5.12	19.5 (10.4 - 56.9)
Indian Brook							
IBADAF	48	41.333	0.297	0.327	0.083	2.491	65.3 (19.7 - ∞)
IBADBF	24	19.89	0.34	0.34	-0.02	2.68	∞ (13.4 - ∞)
IBBDBF	47	41.33	0.36	0.35	0.03	2.67	50.4 (21.6 - 485.7)
Lewis Creek							
LADAF1	23	22.667	0.49	0.479	-0.03	3.479	223 (26.3 - ∞)
LADAF2	24	24	0.477	0.454	-0.04	3.432	∞ (74.1 - ∞)
LBDAF1	24	22	0.48	0.48	0.02	3.6	35.5 (12.5 - ∞)
LBDAF2	12	11	0.49	0.45	-0.09	3.51	∞ (15.6 - ∞)
LBDAF3	12	10.56	0.4	0.44	0.14	3.32	7.9 (2.5 - 46.2)
LBFBD1	23	21.22	0.51	0.48	-0.08	3.73	44.9 (16.0 - ∞)
LBFBD2	24	23.67	0.54	0.52	-0.02	3.66	∞ (33.7 - ∞)
Missisquoi River							
MissAD	48	43.11	0.6	0.63	0.04	4.91	549.2 (82.3 - ∞)
MissBD	50	44.44	0.65	0.65	-0.01	5.18	∞ (121 - ∞)

Table 5.5: Estimates of pairwise G'_{ST} calculated among all sites sampled in the Lake Champlain basin.

	ChIS	ChML1	ChMalE1	ChMalE2	ChMalW	ChML2	IBADAF	IBADBF	IBBDBF
ChIS									
ChML1	0.02								
ChMalE1	0.0183	-0.0033							
ChMalE2	0.0084	0.0089	0.0184						
ChMalW	0.0226	7e-04	0.0188	-0.0062					
ChML2	0.001	0.0171	0.0525	-0.0154	-0.0025				
IBADAF	0.363	0.2335	0.327	0.3523	0.3445	0.3685			
IBADBF	0.3597	0.2222	0.3017	0.3407	0.3314	0.3648	-0.0052		
IBBDBF	0.3741	0.2323	0.3022	0.3581	0.3366	0.3677	0.0436	0.0219	
LADAF1	0.1918	0.2567	0.2286	0.2414	0.271	0.3148	0.4952	0.4822	0.5458
LADAF2	0.208	0.2988	0.2733	0.2643	0.2919	0.3227	0.5659	0.5615	0.6189
LBDAF1	0.189	0.2441	0.2083	0.2427	0.2689	0.3041	0.4764	0.4709	0.5367
LBDAF2	0.2064	0.2671	0.2311	0.2586	0.2913	0.3334	0.4753	0.4643	0.5365
LBDAF3	0.213	0.2658	0.2206	0.2538	0.2953	0.3491	0.4881	0.4744	0.5376
LBFBD1	0.1836	0.2351	0.1884	0.2219	0.2481	0.3017	0.4876	0.4782	0.5418
LBFBD2	0.1702	0.2468	0.184	0.2182	0.2539	0.272	0.5144	0.5075	0.559
MissAD	0.0266	0.01	-0.008	0.0169	0.0199	0.0482	0.3501	0.3353	0.3303
MissBD	0.0567	0.0152	-0.0032	0.035	0.0301	0.0528	0.3333	0.3098	0.2869

Table 5.5 continued

	LADAF1	LADAF2	LBDAF1	LBDAF2	LBDAF3	LBFBD1	LBFBD2	MissAD
ChIS								
ChML1								
ChMalE1								
ChMalE2								
ChMalW								
ChML2								
IBADAF								
IBADBF								
IBBDBF								
LADAF1								
LADAF2	0.0028							
LBDAF1	-0.004	0.0101						
LBDAF2	-0.013	0.0112	-0.0249					
LBDAF3	-0.0187	0.0167	-8e-04	-0.0273				
LBFBD1	-0.0046	0.0015	-0.0056	-0.0091	-0.0097			
LBFBD2	0.0187	0.0315	-0.0027	-0.0048	0.0212	0.0135		
MissAD	0.2178	0.2374	0.2139	0.2383	0.2208	0.1832	0.2001	
MissBD	0.2651	0.3017	0.2726	0.2986	0.2729	0.2384	0.2586	0.002

Table 5.6: Models used to describe connectivity of tessellated darters across the Lake Champlain basin and within individual drainages. Model selection metrics included: Akaike Information Criteria (AIC), residual degrees of freedom (RDF), residual deviance, null deviance, adjusted R2, and likelihood ratio test chi-square p-value (LRT p).

Model ID	Model	Rank	AIC	RDF	residual deviance	null deviance	adj_R^2	LRT p
Global models								
Model 1	*G'st* ~ dist	2	-417.34	151	0.563	0.723	0.216	
Model 2	*G'st* ~ total barriers+distance	3	-415.38	150	0.563	0.723	0.211	0.84
Model 3	*G'st* ~ basin combination+distance	1	-914.12	142	0.019	0.723	0.971	**< 0.01**
Within-drainage models								
Model 4	*G'st* ~ distance	2	-297.31	38	0.001	0.001	0.017	
Model 5	*G'st* ~ barrier type+distance	3	-296.90	34	0.001	0.001	0.091	0.13
Model 6	*G'st* ~ drainage area+distance	5	-295.31	37	0.001	0.001	-0.010	0.98
Model 7	*G'st* ~ isolation time+distance	4	-295.76	37	0.001	0.001	0.002	0.52
Model 8	*G'st* ~ total barriers+distance	1	-298.81	37	0.001	0.001	0.075	0.07

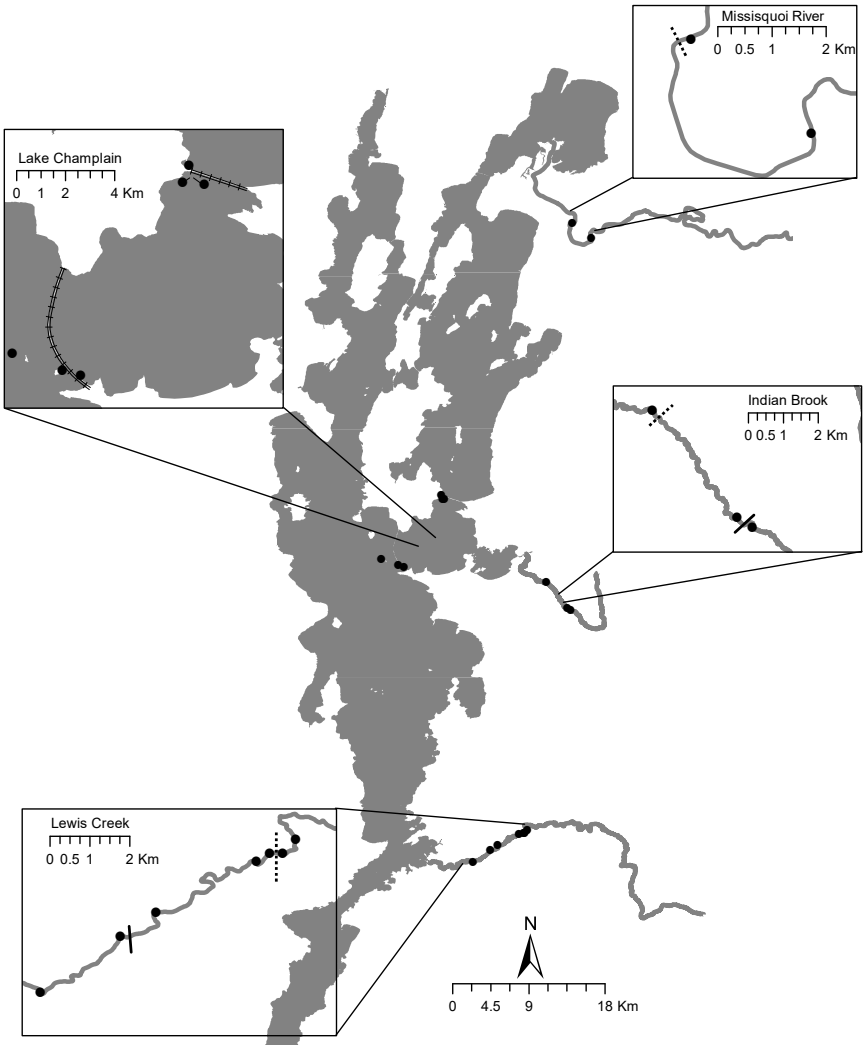

Figure 5.1: Sampling sites (black dots) for tessellated darters collected from Lake Champlain and three Lake Champlain tributaries (Missisquoi River, Indian Brook, and Lewis Creek). Three types of potential barriers to darter dispersal are indicated in inset maps: fall lines (solid lines), dams (broken lines) and causeways (double line with hash marks).

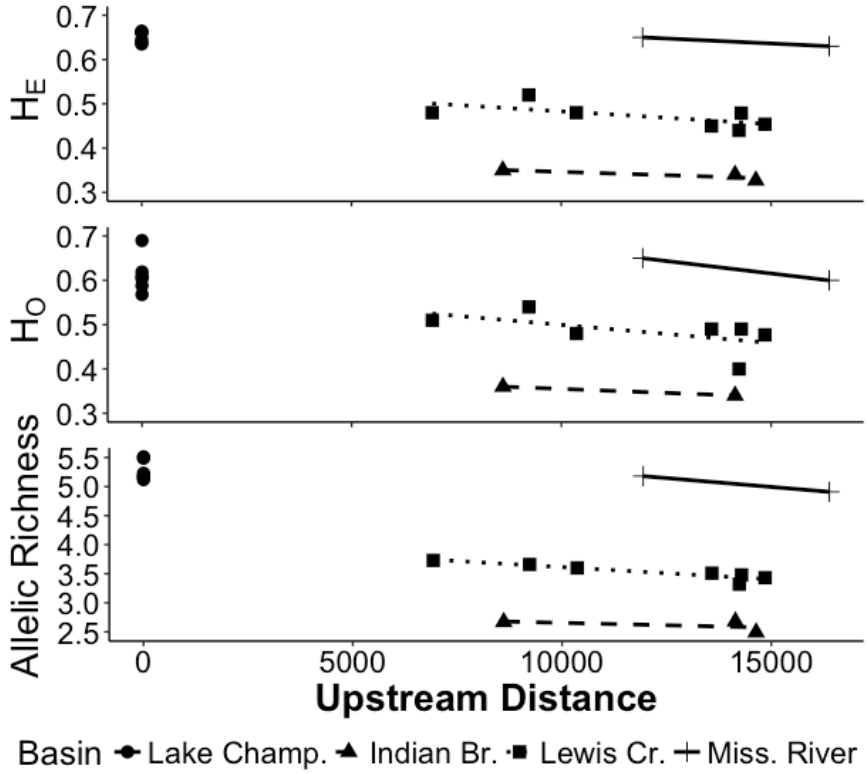

Figure 5.2: Average observed (H_O) and expected (H_E) heterozygosity, and allelic richness for tessellated darters collected from Lake Champlain, Indian Brook, Lewis Creek and the Missisquoi River as a function of upstream distance from Lake Champlain. Each dot represents a single sample location.

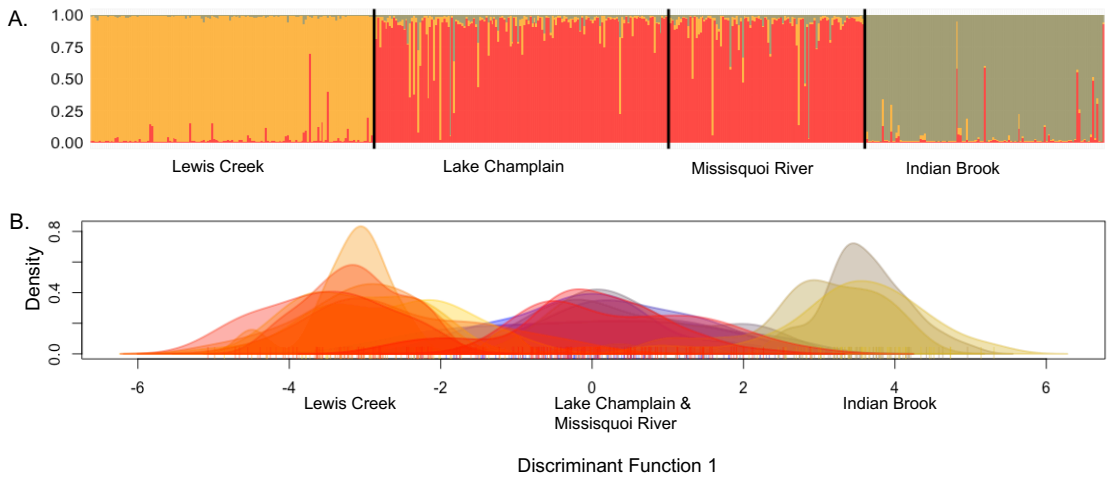

Figure 5.3: Two types of cluster analysis of tessellated darters sampled from 18 sites. (A) barplot of STRUCTURE results for the most likely number of clusters (k = 3). Each bar represents a single individual with color representing the relative likelihood an individual is from a given colored cluster, vertical black lines indicate separation between drainages. (B) Clustering of darters along the most descriptive discriminant function of a DAPC. Colored peaks refer to specific sampling locations in the drainages Lewis Creek (oranges), Lake Champlain and Missisquoi River (reds and blues), and Indian Brook (beige).

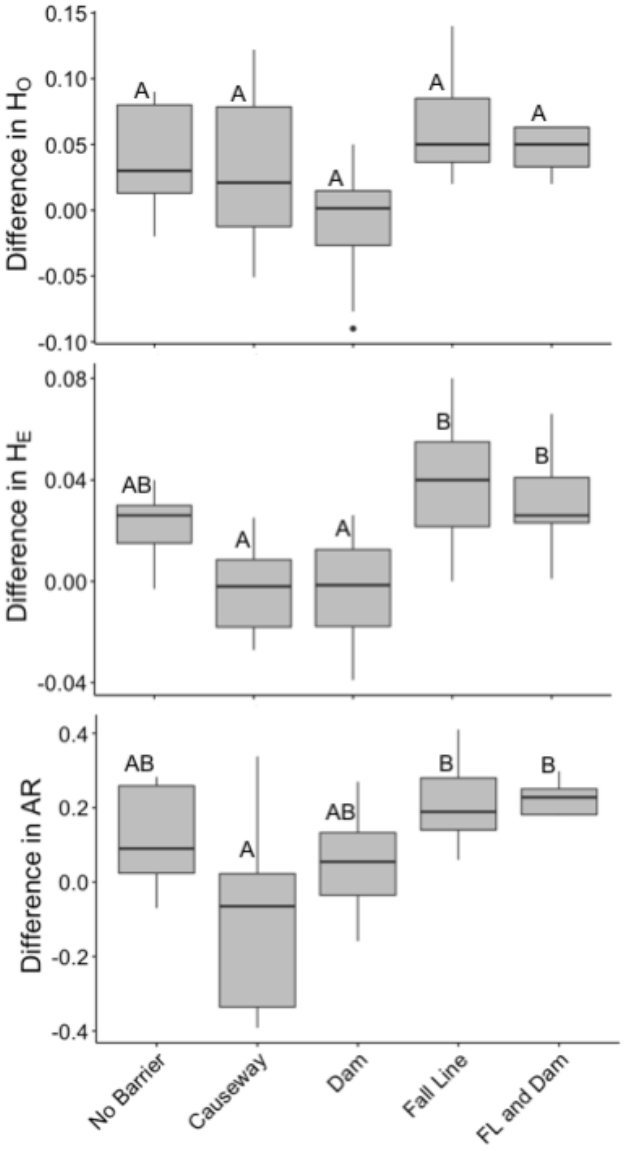

Figure 5.4: Average change (downstream to upstream) in observed (H_O) and expected (H_E) heterozygosity, allelic richness (AR) between sites within drainages for tessellated darters collected on either side of five barrier treatments (x-axis). FL = fall line.

CHAPTER 6: SUMMARY AND CONCLUSIONS

Habitat fragmentation has diverse effects on populations in both terrestrial and aquatic environments. In lotic systems, habitat fragmentation often results in the loss of genetic diversity and increase in population sub-structuring among populations (e.g., Wofford, Gresswell & Banks, 2005). Much less is known about the effect of habitat fragmentation in lentic systems, where fragmentation is less common. The primary objective of my dissertation was to identify and describe the impact of habitat fragmentation on fishes in a large, fragmented lake and to identify patterns in genetic structure among species and types of fragmentation. To accomplish this objective, I conducted population genetic assessment of four species of fish native to Lake Champlain, Vermont: slimy sculpin, rainbow smelt, lake whitefish and tessellated darters. Slimy sculpin, rainbow smelt and lake whitefish were chosen because they varied in adult dispersal from low (slimy sculpin) to high (rainbow smelt) but all prefer deep, cool water and were therefore likely to be dispersal-limited by the warm, shallow causeway openings. Tessellated darters were chosen as a fourth species to evaluate barrier differences because they are common in both lentic and lotic habitats. I found that manmade barriers (causeways and dams) had no influence on the genetic structure of three of the four species and only a small influence on the genetic structure of the fourth, lake whitefish. Genetic distance between darters sampled on either side of three different barrier types (causeways, dams and fall lines) was also consistently low but barrier-type did influence genetic diversity. The population structure within a given drainage was low in both streams and the lake;

however, stream populations appeared to be genetically distinct from lake populations in two of three comparisons indicating that there may be limited gene flow between Lake Champlain and its tributaries.

In both terrestrial and lentic systems, increased genetic sub-structuring and decreased genetic diversity are a common consequence of habitat fragmentation (Templeton *et al.*, 1990). Therefore, the widespread panmixia observed was unexpected and suggests that either dispersal across all barriers evaluated must be possible at some life stage or that individual basins support large enough populations that the effect of genetic drift is small, and therefore not enough time has passed for populations to genetically diverge (Gillespie, 2004). However, slimy sculpin, rainbow smelt, and lake whitefish were all chosen specifically because their habitat preferences make adult dispersal through lake causeways unlikely. The demographic population sub-structuring identified in rainbow smelt suggests that dispersal through causeways is likely partially restricted as I predicted (Chapter 3). No direct estimates of migration were made in any chapter; however, I hypothesize that larval transport is partially responsible for the apparent lake-wide genetic connectivity of slimy sculpin, rainbow smelt and lake whitefish. Rainbow smelt and lake whitefish both have known planktonic larval stages which can determine population structure (Næsje *et al.*, 1986; Kovach *et al.*, 2013) and both have been seen in spring icthyoplankton tows in Lake Champlain (Euclide and Marsden, unpublished data). Though, recent evidence suggests that whitefish larval densities may be lower than previously thought (Euclide unpublished data). No planktonic stage has been reported for

slimy sculpin; however, I found that slimy sculpins have negligible genetic distance across 65 km in Lake Champlain and 230 km in Lake Ontario, suggesting a significant dispersal phase in their life history (Chapter 2). Because adult sculpins have small home ranges and move only short distances throughout their adult life (Gray, Cunjak & Munkittrick, 2004; Hudy & Shiflet, 2009) adult dispersal alone is unlikely to be enough to maintain lake-wide connectivity. Future research should evaluate demographic differences in other species among basins to test whether the differences in demography identified in smelt are typical in slimy sculpin and lake whitefish. In addition to a demographic study, field and laboratory experiments should be conducted to test my hypothesis that larval drift contributes to genetic connectivity among basins.

Habitat fragmentation research often focuses on threatened or endangered species with impaired populations. None of the species I studied are currently listed as threatened or endangered or subject to major fishing pressure. Therefore, even if the populations I sampled are completely or partially isolated, genetic drift may be too weak cause populations to diverge genetically. The three basins of Lake Champlain are each large enough to support self-sustaining populations. Thus, even very little migration among basins may be enough to maintain panmixia. However, if populations are reduced in the future by extrinsic factors such as overharvest or habitat loss and degradation, then populations may begin to show signs of genetic sub-structuring among basins of the lake as the mutation/drift equilibrium changes (Gillespie, 2010). Of the four species I studied, only lake whitefish, which were commercially harvested in the 1900s, showed evidence

of population structure among basins, supporting the hypothesis that reduced population size could increase population sub-structuring in Lake Champlain (Chapter 4). Rainbow smelt CPUE has declined in Malletts Bay and the Northeast Arm since the invasion of alewife in the early 2000s (Chapter 3). If the population of rainbow smelt continues to decline, rainbow smelt could begin to show signs of population sub-structuring due to causeways.

Lake fragmentation by causeways is a rare and understudied type of habitat fragmentation. The relative rarity of other studies of lake fragmentation make it difficult to generalize the results observed here to other systems. However, I found little evidence that barrier type had a substantial impact on the amount of population sub-structuring present within populations (Chapter 5). This finding does not necessarily indicate that barrier-type has *no* influence on population structure but does indicate that species-specific traits may be more important than barrier traits for predicting species' response to fragmentation. Chapter 5 also highlights the importance of accounting for the natural landscape structure when evaluating species' responses to habitat fragmentation. Incorporating the underlying fragmented, dendritic nature of freshwater systems in sample design and analysis is critical to determine whether the observed genetic structure is the result of a manmade barrier, such as a dam or causeway, or simply the result of low natural migration between two sites.

The four studies presented here are the first direct genetic analysis of lentic habitat fragmentation to my knowledge. These results emphasize the importance of comparative

studies and the need for continued monitoring and assessment for a diversity of species. Comparing the influence of causeways on multiple species provided much stronger evidence that lake causeways are not a major barrier to fish gene flow than if only a single species was used. Additionally, by comparing my data with results from other lakes, barrier types, and lotic and lentic systems, I could draw more general conclusions. Sculpin genetic structure in Lake Champlain was similar to sculpin genetic structure in Lake Ontario, indicating that low genetic structure of sculpin may be common in large lakes. The comparative study design enabled me to conclude that the lack of structure around a novel barrier (causeways) was similar to that of well-studied barriers (dams and fall lines). Though habitat fragmentation is less common in lentic than in lotic habitats, the inclusion of uncommon types of fragmentation, such as causeways, and a wide range of taxa is important in habitat fragmentation research. Aquatic habitats are increasingly fragmented worldwide (Grill *et al.*, 2015). To predict how fragmentation will impact populations research needs to include not only what type of barriers are most impactful and what species are sensitive to fragmentation, but also what types of barriers have the least impact and what species are robust to fragmentation.

CHAPTER 7: BIBLIOGRAPHY

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