ADDRESSING KEY CONSERVATION PRIORITIES IN A DATA POOR SPECIES

by

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DEDICATION PAGE

For my family.

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ABSTRACT

Conserving biodiversity is suggested to be one of the most important challenges being faced by the global community. The field of conservation biology has been developed to examine the threats that drive species to low abundance, the dynamics of species in low abundance and the methods to rebuild abundance. Typically, assessing these issues requires substantial data inputs; however we are often faced with situations where little information exists. In this thesis, I addressed several key conservation priorities in the endangered Atlantic Whitefish (*Coregonus huntsmani*), a data poor species, which has been restricted to one watershed for most of the past century.

Using molecular genetic markers Atlantic Whitefish were determined to be a distinct and basal species within the genus. Population size was suggested to be low and the incidence of inbreeding high as genetic effective population size was among the lowest of any fish species examined and genetic diversity was 2-6 times lower than regional congeners.

Through laboratory experiments environmental threats to the persistence of Atlantic Whitefish were examined. Overall, Atlantic Whitefish were tolerant to a broad range of environmental conditions and were capable of surviving in harsher environments than many other regional species. Furthermore, their persistence in current habitats will likely not be influenced by the assessed environmental conditions. As part of this work, a suite of methods and metrics to compare thermal sensitivity across a range of finfish species were assessed.

In order to inform recovery efforts, I developed simulation models to evaluate habitat suitability for translocation of Atlantic Whitefish. As part of this work, I examined the role of incorporating variability in species response, environment and / or life history into simulations. The results showed that the inclusion of multiple sources of variability altered the perception of optimal habitats; however, several watersheds offered suitable translocation habitats.

Throughout this thesis I explored novel tools to address some of the key issues facing conservation programs of data poor species. This work is not only applicable to the conservation of Atlantic Whitefish, but also outlines some of the potential tools useful in addressing conservation priorities in other species.

LIST OF ABBREVIATIONS USED

AIC(c) – Aikaike Information Criterion (corrected)

An - number of alleles

Ar – allelic richness

COI – cytochrome oxidase subunit I

COSEWIC – Committee on the status of endangered wildlife in Canada

EM – Elliott Model

He – expected heterozygosity

Ho – observed heterozygosity

IAM – infinite alleles model

mtDNA – mitochondrial DNA

Nc – census population size

Ne – effective population size

OCLTT – oxygen and capacity limited thermal tolerance hypothesis

PCR – polymerase chain reaction

PGLS – phylogenetic least squares regression

PKM – Parker Model

PM – polynomial model

QM – quadratic model

RM – Ratkowsky model

SARA – Species at Risk Act

SMM – stepwise mutation model

SU – Southern Upland region of Nova Scotia

TPM – two phase mutation model

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CHAPTER 1: INTRODUCTION

1.1 Conservation Biology

Conserving biodiversity has been put forth as one of the most important challenges currently faced by the global community (Rockström *et al.* 2009). Estimates put the loss of biodiversity at rates between 3 and 10% per decade (Stork 2010), despite the fact that the scientific community has achieved the capabilities to look for species in previously under studied habitats (Snelgrove and Smith 2002) with tools that will differentiate some the most cryptic species (Hebert *et al.* 2004). Diversity losses at all levels of biological organization result in decreased resilience (Chapin *et al.* 2000; Reusch *et al.* 2005). At the ecosystem level, losses in diversity decreases the efficiency of ecosystem functioning and services (Worm *et al.* 2006). The factors implicated in loss of biodiversity have largely been anthropogenic and include landscape transformation (Gonzalez *et al.* 2011), over exploitation (Dulvey *et al.* 2003), pollution (McNeely 1992) and the introduction of alien species (Mack *et al.* 2000).

Often, species conservation is put in the context of the reliance of society on the specific ecosystem services and products they provide. Although this is an important point for garnering public support, there are often broader implications of species loss. From an ecosystem perspective, losses in species diversity will cause the breakdown of trophic linkages (Ehrlich and Ehrlich 1981) as the most basic service provided by species is that of a consumer and a producer, transferring energy between trophic levels and connecting components within a system's food web. When any component of an ecosystem becomes dramatically reduced in abundance or is lost, other species begin to over-accumulate and the entire ecosystem may move toward a new productivity regime (*e.g.* Frank *et al.* 2005). Aside from the ecosystem considerations, species have intrinsic

value as they represent distinct evolutionary lineages shaped by the selective forces that have allowed them to persist into the present.

The field of conservation biology has been developed in response to the growing concern over the demise of species. Conservation biology is a synthetic field of study which brings together many aspects of ecology and biology with the goal of preserving biological diversity and maintaining productive populations. Throughout conservation biology there has been substantial effort put toward understanding the mechanisms that drive species to low abundance, the dynamics of species in low abundance and the methods to rebuild abundance (reviewed by Caughley 1994). These points can be summarized in three main questions that compel many conservation efforts:

1. How / when did the species abundance decline to a low level?
2. Is the species able to persist at its current level of abundance?
3. Can we restore this species to its historical or prehistorical level?

Addressing these questions requires intensive study, a long time series of data or both. Typically, determining when a species decreased in abundance would be assessed using a long time series of survey data (Wilson *et al*. 2011). This type of data would also be useful for direct determination of some of the correlative or causative factors that have influenced the population through that time period and permit the impacts of relative threats to be assessed.

Determining the capability of a species to persist at current abundance levels requires information on the present status of the species, the prevalence of the threats that originally caused the species decline and the identification of any new threats that may develop (Prugh *et al*. 2010). As part of determining the species status, indices of genetic diversity relative to historical levels or conspecific populations are useful as the

level of genetic diversity may be correlated population persistence and the ability to adapt to new and changing environments (Willi *et al.* 2006).

The restoration of a species to its historical range or level of abundance is often the ultimate goal of conservation. There are several methods available to provide an opportunity for restoration to occur; however their effectiveness will depend on the type of threat that caused the species demise (Miller *et al.* 2002), the status of the reduced population (Male and Bean 2005) and the species life history and biology (Sheller *et al.* 2006; Baker *et al.* 2009). In some circumstances, removing the pressure caused by key threats may be sufficient to allow the species to rebound from low population sizes (Frank *et al.* 2011). More often, however, intensive, multifaceted conservation programs are required. Some of the approaches used by conservation programs include restoration of lost habitat (Budy and Schaller 2007), supplemental restocking of captive reared individuals (Ingram *et al.* 2011), and translocation of individuals to new or previously inhabited areas (Parker 2008).

The time required for recovery of species that have been reduced to only a few individuals will be substantial as the remaining genetic diversity may be low, and it will take many generations to return to diversity levels approximating those found within the historical gene pool (Altukhov 1995). A similar long term commitment to recovery will be required for species that are not amenable to captive rearing, have long generation times and / or do not produce many viable offspring.

Increasingly, species requiring conservation have very little baseline information with which to build a conservation and recovery plan. This is particularly true as the rate of species discovery is increasing and more newly identified species are suggested to have low abundance (Kohler *et al.* 2005) or are likely present in reduced numbers of populations (Pinhal *et al.* 2012). Without baseline information regarding the species demographic history, biology or physiology, it is difficult to understand or quantify the

threats that caused the populations decline let alone implement a successful rebuilding program. This problem was the focus of my thesis:

How do we best obtain the data needed to conserve species when very little historical or present day information is available on species biology, response to threats, or abundance?

The focal species used throughout the thesis is the endangered Atlantic Whitefish (*Coregonus huntsmani*); however most of the methods and tools used or developed here can be applied to the conservation of other species. Before outlining the thesis I provide a brief introduction to the Atlantic Whitefish and describe the paucity of historical information available to make conservation decisions.

1.2 Atlantic Whitefish *Coregonus huntsmani*

Atlantic Whitefish are an anadromous fish endemic to Nova Scotia, Canada. They are part of the *Coregonus* species complex which is found throughout the north temperate and polar zones of North America, Europe and Asia. Atlantic Whitefish were only recently described as a distinct species (Scott 1987), as they were historically misidentified as the other regional *Coregonus* species, Lake Whitefish (*C. clupeaformis*) or were identified as either Sault Whitefish (*C. labridoricus*) or Acadian Whitefish (*C. canadensis;* Piers 1927; Livingstone 1953). Both morphometric and genetic analysis supported their distinct species status (Edge *et al.* 1991; Bernatchez *et al.* 1991a, b). Atlantic Whitefish were thought to be historically widespread, but extensive dam building without effective fish passage across most of Nova Scotia likely extirpated populations before they were ever identified (Bradford *et al.* 2010). At the time of species identification extant populations were only present in the Tusket-Annis River (herein Tusket) and the Petite Riviere both in southwestern Nova Scotia (Edge 1984). The

Tusket population made upstream anadromous migrations in late autumn to spawn (Edge and Gilhen 2001). Despite directed sampling efforts within the Tusket watershed over the past two decades, the last positively identified Atlantic Whitefish from this watershed was in 1982 (Bradford *et al.* 2004). The Tusket population is believed to be extirpated. The potential threats that were implicated in the demise of the Tusket River Atlantic Whitefish were acidification of the watershed through the deposition of acid rain, the illegal introduction of non-indigenous piscivorous fish Smallmouth Bass (*Micropterus dolomieu*) and Chain Pickerel (*Esox niger*), poaching, and the loss of preferred habitat through the construction of dams (Edge and Gilhen 2001). None of these threats have ever been quantified.

Within the Petite Riviere watershed three lakes, Hebb, Milipsigate and Minamkeak, are currently inhabited by Atlantic Whitefish. These lakes are isolated from the sea by a dam with no upstream fish passage, which has been in place for most of the past 100 years (Bradford *et al.* 2004). There have been no documented instances of anadromous migrations in the Petite Riviere population, however, individuals have been found in nearby estuaries on rare occasions (Edge and Gilhen 2001). Recent laboratory and hydroacoustic tracking studies show the Petite Riviere population retains the ability to make anadromous migrations, as larval stages are capable of tolerating sea water and juvenile fish show a strong preference for seawater (Cook and Bentzen 2009; Cook *et al.* 2010a; Cook and Bradford unpublished data). There is regional variability in pH levels across Nova Scotia and the Petite Riviere has not suffered the same pH declines due to acid rain as the Tusket River. The population abundance of the Petite Riviere population has never been quantified, but is assumed low as the total area of their current habitat is 16km^2 (Bradford *et al.* 2010).

As a result of the species' reduced distribution and presumed low abundance, it was assessed by the Committee on the Status of Endangered Wildlife in Canada

(COSEWIC) as Endangered in 1984 (Edge 1984) and again in 2000. More recently, under Canadian federal legislative act, the Species At Risk Act (*SARA*) Atlantic Whitefish has been listed as Endangered and protected from direct or indirect harmful acts (DFO 2006). Concerns over the genetic fitness and diversity of Atlantic Whitefish have been raised due to their single population status, migration restrictions and small area of available habitat as this is the sole source of individuals for restorative repatriation and translocation. The presumed historical, present day and future threats on the Petite Riviere include the loss of preferred habitat, the presumed low population size, the newly introduced Smallmouth Bass and Chain Pickerel, low pH and the future threat of warming temperatures. Due to their amenability to culture, relatively high fecundity and short generation times it would appear Atlantic Whitefish should respond to positively to recovery programs.

1.3 Thesis Outline

In this thesis I take steps toward obtaining the information to help guide the conservation of this little studied species. Initially, I provided further support of the taxonomic uniqueness of Atlantic Whitefish by generating two phylogenetic trees from mitochondrial DNA COI sequences and microsatellite loci for a subset of species from the subfamily Coregoninae, including members of the genera *Coregonus, Prosopium* and *Stenodus*. Using these same microsatellite markers, I inferred the species recent and historical demographics through the estimation of long and short term effective population sizes and through methods designed to detect a population bottleneck. Further I assessed the current levels of genetic diversity compared to other regional *Coregonus* species to provide information on their ability to persist in their current state (Chapter 2).

Next, I developed a method for comparing species thermal sensitivity (Chapter 3). This method sets the framework for identifying potential habitat ranges and impacts of a changing climate for species where limited data are available.

In Chapter 4, I assessed the impact of the environmental threats of low pH and temperature to Atlantic Whitefish using laboratory studies. These studies were designed to assess the persistence of the species in its current habitats and provide information useful for habitat evaluation to guide repatriation and translocation efforts. For egg and yolk sac larvae, reaction norms were developed comparing the sensitivity of different spawning pairs to low pH. I used the method developed in Chapter 3 to examine the thermal sensitivity of Atlantic Whitefish in comparison to previously published results from other members of the Salmonidae family. Further, I examined the interaction of temperature and pH on juvenile growth rates and made a determination of low pH's effect on thermal sensitivity. Additionally, in Chapter 4 I genotyped microsatellite loci for individuals showing different phenotypic responses to environmental threats to detect any differential changes in genetic makeup base on observed allele frequencies.

In Chapter 5, I used simulation modeling in combination with the results obtained in Chapter 4 to explore the options for the repatriation of Atlantic Whitefish in different lacustrine habitats across Nova Scotia given their environmental characteristics, including proximity to salt water, bathymetric features, pH and temperature. Chapter 5 also examined the importance of incorporating variability in environmental, species responses and different life history strategies on the outputs of simulation models. In Chapter 6, I briefly discussed the key findings of the thesis, their implications for the recovery of Atlantic Whitefish and for the broader field of conservation biology.

CHAPTER 2: GENETIC DIVERSITY, EFFECTIVE POPULATION SIZE AND EVOLUTIONARY STATUS OF THE ENDANGERED ATLANTIC WHITEFISH *COREGONUS HUNTSMANI*

2.1 ABSTRACT

In conservation biology genetic markers provide a means to describe a species past evolutionary relationships, recent demographic changes and remaining evolutionary potential. This is particularly important for endangered species when little is known about their history. Here, the phylogenetic status, recent and historical effective population size, and current levels of genetic diversity were described for an endemic Canadian anadromous fish, the Atlantic Whitefish (*Coregonus huntsmani*). The current global range of Atlantic Whitefish consists of three small semi-natural lakes (16km^2 total area) located within a single watershed in the Province of Nova Scotia. The lakes have not been accessible from the sea for much of the past century. The effects of being landlocked at low abundance on genetic fitness have not been previously investigated for this species. Estimates of genetic diversity and effective population size were made using 15 microsatellite loci. For comparative purposes, several populations of congeners (*C. clupeaformis* and *C. artedii*) were genotyped for a subset of the same microsatellites. Additionally, a phylogenetic tree of Atlantic Whitefish along with 15 other members of the subfamily Coregoninae was generated using a segment of the mitochondrial COI gene.

Results show that Atlantic Whitefish occupy a basal position within the *Coregonus* genus. Furthermore, the phylogenetic tree suggests that the genus *Coregonus* is paraphyletic, as *Stenodus leucichthys* was more closely related to the rest of the *Coregonus* branch than is the Atlantic Whitefish. Atlantic Whitefish's genetic diversity (heterozygosity) was 10-35% lower than any of the other species/populations examined; however, there was no evidence to support a recent population bottleneck. Long- and short-term effective population size estimates were 91 and 18 individuals,

respectively, suggesting the population abundance has declined. Genetic diversity and effective population size estimates for Atlantic Whitefish were among the lowest for any fish species examined to date; however, recent laboratory studies suggest they remain tolerant to a range of environmental factors and possess significant intraspecific plasticity.

2.2 INTRODUCTION

In conservation biology, two measures of genetic status can be key considerations for species of concern. The first is the phylogenetic position of the species, as it describes the evolutionary relationships of the focal species to related species, provides a measure of its distinctiveness and what would be lost in terms of evolutionary diversity should it go extinct. The second is genetic diversity, as it is thought to influence population persistence and the ability to adapt to new and changing environments or evolutionary potential (Willi *et al.* 2006). Both theoretical and practical studies suggest that in small populations genetic diversity is reduced through the combination of random genetic drift and the breeding of closely related individuals (Frankham 1995a). The loss of genetic variation and resultant increase in homozygosity can increase expression of deleterious recessive alleles and eliminate fitness improvements from over-dominant loci, which can result in inbreeding depression (Keller and Waller 2002). Inbreeding depression has been shown to reduce fitness and increase extinction risk in both natural and cultured populations (Newman and Pilson 1997; Saccheri *et al.* 1998; reviewed by Crnokrak and Roff 1999), with the expression of deleterious alleles accounting for much of the decreased fitness (Charlesworth and Charlesworth 1999). Over time, the negative impact of inbreeding depression may be reduced as deleterious alleles are purged from the population through natural selection (Frankham *et al.* 2001). Despite this, evolutionary potential may remain low as genetic

diversity continues to be limited. Furthermore, the effects of inbreeding depression will be exacerbated in stressful environmental conditions (Armbruster and Reed 2005), and may reduce species' capacity to adapt to future climate change (Rice & Emery 2003).

Determining when and how rapidly population abundance has declined can help illuminate the causes of the decline and the potential genetic impacts. Traditionally, estimating changes in abundance has required a long time series of survey data. Often these data are not available or may not provide sufficient resolution to depict important abundance changes. The predictive framework of genetic theory associated with the implicit relationship between genetic diversity and population size can be used to recreate part of a population's demographic and abundance history (*e.g.* Saillant *et al.* 2004). Even if a good time series of survey data is available, the use of genetic data may provide more information on the underlying dynamics of populations, as abundance may be better described by effective rather than census population sizes (Luikart *et al.* 2010). The effective size of a population is defined as the size of an ideal population experiencing the same rate of genetic change as the focus population (Crow and Kimura 1970). Real populations do not adhere to idealized characteristics and thus effective population sizes are generally less than census population sizes. Meta-analytic studies suggest census population size (Nc) may be 2 – 10x higher than genetic effective population size (Ne), as variance in either reproductive success and population size or skewed sex ratio will decrease the effective number of individuals in the population (Frankham 1995b; Nunney 1995).

The Ne of a species or population can be estimated on different time scales by exploiting different characteristics of genetic markers. Coalescent or long term Ne estimates are described by the relationship between Ne, genetic diversity (θ) and mutation rate (μ) as $\theta=4Ne\mu$, and represent the harmonic mean of Ne over about 4Ne generations (Hare *et al.* 2011). Contemporary estimates of Ne can be measured by

either the temporal change in allele frequencies (Jorde and Ryman 1995) or the nonrandom association of alleles (Hill 1981). As conservation targets for Ne, Franklin (1980) suggested a 50 : 500 rule with 50 representing the minimum Ne to reduce the impact of inbreeding depression and 500 being the Ne to maintain sufficient evolutionary potential. Others have suggested much higher Ne's are required (Lynch and Lande 1998).

Rates of population decline can be inferred from the comparison of long and short term genetic effective population sizes; however, rapid declines in population size through bottlenecks may not be identified, or adequately characterized through this method. Further, recent population bottlenecks will decrease long term Ne estimates, as methods put greater weight on more recent generations (Beerli 2009). It is important to identify bottlenecks, as extinction risk will increase shortly thereafter because the loss of genetic variation may occur too rapidly for the purging of deleterious alleles to be effective (Luikart *et al.* 1999). Conversely, with effective management some of the longer term genetic consequences of small population sizes may be averted in populations affected by recent bottlenecks, as substantial genetic diversity might be maintained since rare alleles will be lost faster than overall genetic diversity (Cornuet and Luikart 1996). By exploiting the differential loss of genetic information, a statistical test for recent (2-4 Ne generations) population bottlenecks has been developed and successfully used in some species (Spencer *et al.* 2000; Al-Rabah'ah and Williams 2004; but see Hoffman *et al.* 2011).

Atlantic Whitefish are a member of the genus *Coregonus* which are distributed throughout the north temperate and polar regions of North America, Europe and Asia. Although Atlantic Whitefish were thought to be historically widespread, the species was only recently given distinct species status (Scott 1987) at which point extant populations were only documented in two watersheds, the Petite Riviere and the Tusket River, both

in Nova Scotia, Canada (Locations 1 and 9 respectively; Figure 2.1). The Tusket population was anadromous, and made regular upstream migrations in autumn. Anadromy on the Petite Riviere was never documented; however, historical data suggest that dams with inadequate fish passage pre-date the description of the species and may have caused the demise of an anadromous contingent (Bradford *et al.* 2010). The surviving population of Atlantic Whitefish has been landlocked in three semi-natural oligotrophic lakes for most of the past 100 years (Bradford *et al.* 2004). Despite sampling efforts over the past decade, there have been no observations of Atlantic Whitefish in the Tusket River since 1982 (Bradford *et al.* 2004). This population is assumed extirpated. The demise of the Tusket River population was suggested to be due to decreases in environmental pH caused by acid precipitation, unauthorized introductions of the predatory Smallmouth Bass (*Micropterus dolomieu*) and Chain Pickerel (*Esox nigris*), poaching, and loss of habitat through inefficient fish passage around a hydroelectric dam located near the head of tide (Edge and Gilhen 2001). The abundance of Atlantic Whitefish in the Petite Riviere has not been satisfactorily estimated but is considered to be generally low owing to the small (16km^2) quantity of aquatic habitat available. The Petite Riviere has not been as severely affected by acid precipitation and predatory fish were not detected in the system until 2010. As a result of the species' reduced distribution and presumed low abundance, it was assessed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) as endangered in 1984 (Edge 1984) and again in 2000. More recently, under Canadian federal legislative act, the Species At Risk Act (*SARA*) Atlantic Whitefish has been designated endangered and protected from direct or indirect harmful acts (DFO 2006). Concerns over the genetic fitness and diversity of Atlantic Whitefish (*Coregonus huntsmani)* have been raised due to their low number of populations, migration restrictions and small area

of available habitat as this is the sole source of individuals for restorative repatriation and translocation.

Previous genetic analyses of Atlantic Whitefish based on mitochondrial DNA (mtDNA) analysis of three specimens, and isozyme analysis of 20 specimens, confirmed morphometric analysis that showed Atlantic Whitefish are genetically divergent from other Coregonid species and represent an ancient lineage (Behnke 1972; Bernatchez *et al.* 1991a; b). These studies were not designed to quantify genetic variation within the species. The purpose of the current work was to extend the assessment of the phylogenetic status of Atlantic Whitefish, estimate their present genetic diversity, estimate contemporary and long term effective population sizes, and investigate the likelihood that the species has experienced a bottleneck. To accomplish these goals Atlantic Whitefish were genotyped using a suite of microsatellite loci, and a segment of the cytochrome oxidase mitochondrial gene (COI) was sequenced for comparison with published values for other Coregoninae species. To provide a better context for measures of genetic diversity in Atlantic Whitefish, the genetic diversity in other whitefish species including Lake Whitefish (*Coregonus clupeaformis*) from 9 local populations, Lake Ontario, and one lake from Nunavut as well as a single Cisco (*Coregonus artedi*) population from Lake Ontario were assessed using the same genetic markers.

2.3 METHODS

2.3.1 Sample Collection And DNA Extraction

The Atlantic Whitefish adipose fin clips and scales ($N = 169$) were obtained from Hebb ($N = 140$), Milipsigate ($N = 17$) and Minamkeak lakes ($N = 12$; Table 2.1, Figure 2.1) during 2002, 2004 and 2007 were used as DNA sources. As previous work found no genetic differentiation among the three lakes (Murray 2005), and most samples were

from Hebb Lake, all samples were considered to represent a single population. Samples of Lake Whitefish were collected from 11 locations: 8 from Nova Scotia, the Saint John River (New Brunswick), Lake Ontario (Ontario) and MacAlpine Lake (Nunavut) (Table 2.1; Figure 2.1). Cisco were collected from Lake Ontario. All fin clips were preserved in 95% ethanol at room temperature until DNA was extracted. Genomic DNA was extracted from 8-10 scales or ~5mg of fin tissue using the either DNeasy DNA Extraction Kit (Qiagen) or following Elphinstone *et al.* (2003). DNA was stored at -20°C until analysis.

2.3.2 Microsatellites

Fifteen microsatellite markers amplified in Atlantic Whitefish; of these, 12 also amplified in Lake Whitefish and 11 in Cisco (Table 2.2). Polymerase chain reaction (PCR) protocols followed methods described in Murray (2005). Microsatellite alleles were scored manually through comparisons to allele ladders run on each gel. The allele ladders were prepared for each locus by pooling PCR products from individuals selected to encompass the full suite of allele sizes, which were calibrated alongside a 60-400 bp fluorescent ladder (Promega). Negative and positive controls and duplicate samples were used to improve scoring accuracy. Microsatellite loci were validated to not possess large allele dropout, null alleles and amplification stutter using Microchecker v 2.2.1 (van Oosterhout *et al.* 2004). Microsatellite data for Lake Whitefish and Cisco and a subset of the Atlantic Whitefish were provided by Murray (2005).

2.3.3 COI

COI sequences were obtained from a subset of samples from each of Atlantic Whitefish, six Lake Whitefish populations and the Cisco population. The mitochondrial

COI region analyzed here was first PCR amplified using the FishR1 and FishF1 primers (Ward *et al.* 2005), under thermocycling conditions of denaturation at 94°C for 30s, annealing at 55°C for 30s and extension at 72°C for 60s, repeated 30 times. The resultant PCR product was size fractionated in 1.5 % TAE agarose gels, at 6 volts/cm. Amplified products were then excised from the gel, and purified using MiniElute Gel extraction kits, following procedures specified by the manufacturer (Qiagen). Approximately 50-200 ng of purified COI PCR product was then sequenced with the same primers, using BigDye ™ Terminator cycle sequencing chemistry, under procedures specified by Applied Biosystems, and by size fractionating and detecting cycle sequencing products on an MJ Basestation automated fragment analyzer (MJ bioworks). Sequences were aligned by ClustalW in MEGA v.4 (Tamura *et al.* 2007) using the default settings for gap opening and extensions resulting in a 659bp length of the COI gene.

2.3.4 Diversity Estimates

Tests for departures from Hardy Weinberg equilibrium (HWE) and linkage equilibrium were performed in Genepop version 4.1 (Rousset 2008) with P-values compared against a Bonferroni corrected alpha level (Rice 1989). Using both the full set of microsatellite data as well as the set of polymorphic loci, standard methods were used to calculate observed and expected heterozygosity (H_o; H_e) and mean number of alleles (An; averaged across loci). Allelic richness (Ar) was calculated using rarefaction to provide unbiased estimates of allelic richness when sample sizes differ between groups (HP-Rare; Kalinowski 2005). Within species or population COI genetic diversity was estimated as the total number of haplotypes within the group and the mean number of nucleotide substitutions per site within population haplotypes π (Nei 1978).

2.3.5 Bottleneck

Atlantic Whitefish genotypes were tested for evidence of a recent population bottleneck using the program Bottleneck (Piry *et al.* 1999). This method compares heterozygosity levels with the number of alleles, as theory predicts that during recent population bottlenecks heterozygosity declines more slowly than the loss of alleles, leading to excess heterozygosity relative to equilibrium expectations (Cornuet and Luikart 1996, Luikart and Cornuet 1998). Expected heterozygosity levels at mutation drift equilibrium will differ depending on the mutation model and although microsatellites are thought to mutate primarily following a stepwise mutation model (SMM, Kimura and Ohta 1973) some evidence favors the infinite alleles model (IAM, Kimmel *et al.* 1998). As such, bottleneck tests were performed using the SMM, IAM, as well as a two phase model (TPM), which combined the mutation models at 70% SMM and 30% IAM.

2.3.6 Effective Population Size (Ne)

Microsatellite data were used to estimate Ne for Atlantic Whitefish, Lake Whitefish and Cisco. Several methods have been proposed for estimating Ne from genetic data, each having different assumptions and/or measures Ne at different time scales. Two single sample estimates of Ne were used for all species. The first method was based on linkage disequilibrium or the non random association of alleles between neutral loci (herein Ne_{LD}; Hill 1981) and was implemented in LDNe (Waples and Do 2008). This method estimates effective number of parents contributing to the sample assuming no immigration has occurred. Confidence intervals were estimated by the jackknifing approach (Waples and Do 2008). The second single sample method estimates long term Ne (herein Ne_{θ}) assuming populations were at drift-mutation equilibrium, genetic variation (θ) is constant and related to Ne as $\theta = 4N_e\mu$ where μ was

the per locus mutation rate. θ can be estimated from multiple locus heterozygosity levels (H) (Ohta and Kimura 1973)

$$\theta_H = \frac{1}{2} - \frac{1}{2(1-\hat{H})^2}$$ Equation 2.1

This method assumes a stepwise mutation of neutral loci in a closed population. As θ_H generally overestimates θ, a bias correction suggested by Xu and Fu (2004) based on sample size was applied. Variance estimates for θ_H were made using a jackknifing procedure across all loci. Direct estimates of μ were not available; however, estimates of between 10^{-4} and 10^{-3} mutations/locus/generation for microsatellites have been published (Shaklee and Bentzen 1998; Yue *et al.* 2007). To incorporate the uncertainty in μ into Ne estimates a resampling procedure was performed on the jackknifed estimates of θ_H where μ was sampled 100,000 times from a uniform [10^{-4}, 10^{-3}] distribution. Confidence limits for Ne were calculated as the 95% quantiles of the resultant distribution. The distributions of Ne were highly skewed due to the nonlinear relationship between μ and Ne; as such the difference between the point estimate of Ne and the upper limit were greater than that to the lower estimate.

The third estimate of Ne was a temporal method (herein Ne_{JR}) where Ne was calculated using estimates of genetic drift (F) between successive generations using the method of Jorde and Ryman (2007). This method accounts for biases associated both with rare alleles (equation 2.2) and small sample sizes (equation 2.3):

$$F = \frac{\sum_{j=1}^{k}\sum_{i=1}^{a}\left(x_{ji} - y_{ji}\right)^2}{\sum_{j=1}^{k}\sum_{i=1}^{a} z_{ji}\left(1 - z_{ji}\right)}$$ Equation 2.2

$$F' = \frac{F\left(1 - \frac{1}{4\tilde{n}} + \frac{1}{4N}\right) - \frac{1}{\tilde{n}} + \frac{1}{N}}{\left(1 + \frac{F}{4}\right)\left(1 - \frac{1}{2n_y}\right)}$$

Equation 2.3

where x_{ji}, y_{ji}, z_{ji} are the frequencies of the i^{th} allele from the j^{th} locus in generation 1 and 2 and the mean of x_{ji}, y_{ji} respectively. In equation 2.3, n_y was the sample size for the second sample, the harmonic mean was denoted $\tilde{n}$ and the population size (N) was set to 2000 (Bradford *et al.* 2010). This sample size correction is more sensitive to $\tilde{n}$ than actual estimates of N (Jorde and Ryman 2007).

For species with discrete generations the relationship between Ne and F is:

$$Ne_{JR} = \frac{t}{2F'}$$

Equation 2.4

Using this model on species with overlapping generation's results in negatively biased Ne estimates (Jorde and Ryman 1995; Waples and Yakoto 2007; Palstra and Ruzzante 2008). Incorporating a correction factor (C) for overlapping generations and generation time (G) yields an unbiased estimate of Ne with the relationship between Ne and F as:

$$Ne_{JR} = \frac{C}{2GF'}$$

Equation 2.5

Determining C required the development of a life table incorporating age or stage specific survivorship (l_i), fecundity (b_i) and their product, p_i, the probability of a gene being inherited by a parent of age i. Based on these demographic parameters, C can be estimated as:

$$C = \frac{f_{1,1}(t) + f_{1,1}(t+1) - 2f_{1,2}(t+1)}{f_{1,1}(t+1) - f_{1,1}(t)}$$ Equation 2.6

where $f_{i,j}(t)$ is an estimate of the genetic drift variance between age classes i and j for cohort t. Estimates of the $f_{i,j}$ (t) can be determined by initially setting each to 0 and iterating through:

$$f_{1,1}(t+1) = 1 + \sum_{i=1}^{k}\sum_{j=1}^{k} p_i p_j f_{i,j}(t)$$

$$f_{i,i}(t+1) = \frac{1}{l_i} - \frac{1}{l_{i-1}} + f_{i-1,j-1}(t) \quad for\ 1 < i \le k$$ Equation. 2.7

$$f_{1,j}(t+1) = \sum_{i=1}^{k} p_i f_{i,j-1}(t) \quad for\ 1 < j \le k$$

$$f_{i,j}(t+1) = f_{i-1,j-1}(t) \quad for\ 1 < i < j \le k$$

until a constant value of C is obtained (Jorde and Ryman 1995). Generation time G was estimated by (Felsenstein 1971):

$$G = \sum i \cdot p_i$$ Equation 2.8

Confidence intervals on Ne_{JR} were produced through jackknifing across all alleles. All temporal Ne calculations were implemented through a custom R function (validated against data provided by Jorde and Ryman (1995; 1996) and is available from the author upon request).

Ne_{JR} was only applied to Atlantic Whitefish as temporal samples were not available for the other species or populations. Samples collected during 2004 and 2007 were used in analyses. To ensure sufficient sample sizes only cohorts with >15 individuals were used in analyses (Palstra *et al.* 2007). Details on the development of an Atlantic Whitefish life table are given in Appendix A: Chapter 2.

2.3.7 Genetic Diversity And Habitat Area

The relationships between each genetic diversity measure or effective population size with habitat size were evaluated using generalized linear models. For proportional estimates such as expected heterozygosity, a binomial error function was used, for all others a Gaussian error function was used. For each relationship, model diagnostics were performed including plots of residuals against predictor variables to assess independence of results, residuals against fitted values to assess constant variance, and Cook's distance plots to determine the relative leverage of each datum (Zuur *et al.* 2009). Potential outliers or influential data points were removed and relationships reanalyzed to determine if model fits were significantly altered.

2.3.8 Divergence And Phylogeny

Population and species levels of differentiation were measured for both the microsatellite and COI sequence data. For microsatellites, between group F_{ST} and R_{ST} values were calculated using FSTAT version 2.9.3.2 (Goudet 1995). Percent sequence divergence estimates were calculated as the average number of nucleotide substitutions per site between populations using the COI data (Dxy), implemented in DNAsp version 5.10.1 (Rozas *et al.* 2003).

Phylogenetic trees based on microsatellite and COI data were generated independently. For the microsatellites, following the recommendations of Takezaki and Nei (1996), combinations of distance measures were used to generate the tree topology. The branching topology was based on the Cavalli-Sforza and Edwards' (1967) geometrically based chord distance, whereas the length of the branches was calculated using $(\delta\mu)^2$ (Goldstein *et al.* 1995), which is based on the stepwise model of microsatellite mutation. Cavalli-Sforza and Edwards' (1967) distances were calculated

using Gendist in PHYLIP (v. 3.62; Felsenstein 1989) and the $(\delta\mu)^2$ distances were calculated using POPTREE2 (Takezaki *et al.* 2010). The topology and branch lengths combined using the USER tree option in PHYLIP. Finally, statistical support for the tree topology was evaluated by bootstrapping across loci (1000 replicates) using Populations. The tree was visualized using Treeview (v1.6.6, Page 1996).

COI sequence data were available in GenBank for 15 additional members of the subfamily Coregoninae, including 12 *Coregonus* species and the closely related *Stenodus leucichthys, Prosopium coulterii* and *P. cylindraceum* (see Appendix A: Chapter 2 for accession numbers). Unique haplotypes from sequences generated above were aligned with those obtained from GenBank using the same procedure listed above. A rooted phylogenetic tree was generated by maximum parsimony (MP) using MEGA v 4.0, with Atlantic Salmon (*Salmo salar*) as the out-group. The MP tree was obtained using the Close-Neighbor-Interchange algorithm with search level 2 (Nei and Kumar 2000) in which the initial trees were obtained with the random addition of sequences (10 replicates).

2.4 RESULTS

Global tests indicated that there were no significant departures from HWE or linkage disequilibrium for any populations or loci tested. There were some within population or within loci departures from HWE as BWF1 showed marginal deviations from HWE for the all the Lake Whitefish populations (minimum P=0.012), and both the SRJ and LO were not in HWE (P=0.02 and P=0.031, respectively); however, after adjusting the critical alpha level through Bonferroni corrections, none of these departures were significant. There were no systemic evidence of genotypic linkage disequilibrium across populations or within species (P=0.07).

2.4.1 Genetic Diversity And Effective Population Size

Atlantic Whitefish exhibited lower genetic diversity than any of the Lake Whitefish or Cisco populations. In particular, average observed and expected heterozygosity (Ho, He) at polymorphic loci were 0.27 and 0.29, respectively, substantially lower than the least genetically diverse Lake Whitefish population, SBL, for which both Ho and He were 0.39 (Table 2.3). There was only one COI haplotype in Atlantic Whitefish compared with 9 over all the Lake Whitefish populations and two within the single Cisco population examined (Table 2.3).

The genetic diversity estimates of An, Ar and He as well as long term effective population size (Ne_{θ}), were positively correlated with lake area ($P<0.05$; Table 2.4). Diagnostic tests of residuals from generalized linear models showed that for the significant relationships Atlantic Whitefish (AW) and the Lake Whitefish population EL were consistently identified as potential outliers. AW had lower genetic diversity than expected based on habitat area and EL had higher diversity than expected based on area. Atlantic Whitefish was a significant leverage point, indicating it altered the overall regression parameters in the relationship between He and lake area (Table 2.4; Figure 2.2). The lack of relationship between Ne_{LD} and habitat size may have been due to the inability of the method to distinguish effective population size from infinity for the larger habitat sizes (data not shown) for those populations with estimates greater than 50 individuals. The relationship between COI haplotype diversity and lake size was not explored due to the lack of informative data points, as few populations were sequenced and few haplotypes were identified.

The Ne estimates for Atlantic Whitefish were Ne_{JR}= 18, Ne_{LD}=38 Ne_{θ}=91 with overlapping confidence intervals for both short term estimates (Ne_{JR} and Ne_{LD}) and between the long term estimate and the linkage disequilibrium estimate (Ne_{θ} and Ne_{LD};

Figure 2.3). Based on the relationship between habitat size and long term effective population size for Lake Whitefish, Atlantic Whitefish should have a Ne_{θ}=590 given their lake size, which was more than six times higher than that estimate. This comparison assumes that the two species share a similar relationship between lake size and carrying capacity.

2.4.2 Bottleneck

There was no evidence of heterozygosity excess in Atlantic Whitefish for any mutation model tested (SMM P=0.98; IAM P=0.53; TPM P=0.68). A heterozygosity deficit was detected using the SMM (P = 0.03); given that this was the most conservative mutation model used it is likely that this deficit comes as a result of a previous bottleneck within the population (Cornuet and Luikart 1996). The absence of heterozygosity excess using all three models suggests that Atlantic Whitefish are at mutation drift equilibrium. Assuming that it takes between 2Ne and 4Ne generations for populations to reach mutation drift equilibrium (Cornuet and Luikart 1996; Nei and Li 1979) and given the lower limit in short term effective population size estimates (Ne_{LD}, Ne_{JR}) from above, a bottleneck for Atlantic Whitefish has not occurred in the last ~28 generations (112 years with generation time of 4; see Appendix A: Chapter 2).

2.4.3 Divergence And Phylogeny

Both F_{ST} and R_{ST} values for the interspecific comparisons were highly significant and ranged between 0.71-0.81 and 0.71-0.99, respectively (Table 2.5), with the Cisco : Atlantic Whitefish F_{ST} and R_{ST} being lower (0.71 for both statistics) than any Atlantic Whitefish : Lake Whitefish comparisons (minimum 0.73 for F_{ST}; 0.76 for R_{ST} Table 2.5). In contrast, the COI Dxy estimates showed that Atlantic Whitefish and Lake Whitefish

were more closely related than Atlantic Whitefish were to Cisco, however the differences were small as sequence divergence was 3.8% for Atlantic Whitefish : Cisco and 3.7% for Atlantic Whitefish : Lake Whitefish. Across all Coregoninae species Atlantic Whitefish is least divergent from *Coregonus autumnalis* (Table 2.6, Table 2.7).

Atlantic Whitefish represent a basal lineage within the genus *Coregonus* based on both the COI sequence data and the microsatellite data (Figure 2.4 and 2.5). Moreover from the COI sequence data, a specimen from another genus *Stenodus leucichthys* is more closely related to the rest of the *Coregonus* branch than are the Atlantic Whitefish, suggesting the genus is paraphyletic (Figure 2.4).

2.5 DISCUSSION

Atlantic Whitefish represent a basal lineage of the genus *Coregonus* which has species throughout the North Temperate and Polar Regions of North America and Eurasia. Atlantic Whitefish currently possesses very low levels of genetic diversity and likely has for more than 100 years. The reduced genetic diversity likely resulted from population size reductions through the loss of preferred habitat from the blockage of upstream fish passage and residence in three small oligotrophic lakes (Bradford *et al.* 2004). Long term estimates of effective population size of 91 individuals (95% CI 51, 401) were higher than the current estimates of between 18 (95% CI 14, 37) and 38 (95% CI 14, 141) individuals based on temporal changes in allele frequencies and linkage disequilibrium based methods, respectively. Long term effective population size estimates were higher than current estimates, which is contrary to other studies on species similar to the Atlantic Whitefish which were displaced by glaciation, as populations expanded once they are able to recolonize formerly glaciated habitats (Guiher and Burbink 2008).

There was no evidence to support a recent population bottleneck in Atlantic Whitefish; however, the possibility of a bottleneck prior to the detectable time period (>2Ne generations before present ~100 years) or the gradual erosion of genetic diversity over time cannot be ruled out. Still, it was likely that a bottleneck occurred prior to the detectable time period given the extremely low genetic diversity estimates in Atlantic Whitefish coupled with the construction of a dam prior to 1926 lacking upstream passage which may have reduced any anadromous component of the population (Bradford et al. 2010). Further supporting this hypothesis, Demontis *et al.* (2009) indicated heterozygosity was lost faster and was maintained at lower levels following bottlenecks than during prolonged inbreeding. Undetected bottlenecks would result in the long-term Ne estimate being downwardly biased as this method assumes that the population to be in mutation drift equilibrium throughout the measured time (Crow and Kimura 1971). Atlantic Whitefish have thus, likely suffered larger population declines than indicated by the Nes reported here.

Single species genetic diversity estimates generally can not be compared across studies as differences will arise due to several factors including the choice of genetic marker, location of loci within the genome (Primmer *et al.* 1997), allele size range (Garza *et al.* 1995), and species biogeographical history (Bernatchez and Wilson 1998; but see McCusker and Bentzen 2010). In the current study, I attempted to account for some of these influential factors by using comparable microsatellite loci and segment of COI across regional *Coregonus* species, including Lake Whitefish and Cisco, which have been subjected to the same history of glaciation. In an attempt to standardize genetic diversity measures on the basis of population size, I used a surrogate, lake size to account for the differences in genetic diversity based on available habitat. In other species, lake size has been shown to be directly proportional to population size as abundance – occupancy relationships suggest that the larger the overall habitat size the

greater the probability of encountering suitable habitats (Gaston *et al.* 2000). Following that trend, here I showed significant positive relationships between most measures of genetic diversity or effective population size and lake size for Lake Whitefish. Given that relationship, the genetic diversity found in Atlantic Whitefish was well below what would be expected based on habitat size, further supporting the notion of recent population declines. This assertion was based on the assumption that the habitat size - carrying capacity relationship would be similar between in Lake and Atlantic Whitefish and although I do not have conclusive evidence to support this assumption, it has been shown elsewhere that both Lake and Atlantic Whitefish preferentially utilize specific depth habitats (Cook *et al.* in review; Baldwin and Polacek 2011). This suggested that although the calculation of specific lake volume or total habitat area encompassed by the species-specific depth preference may improve these relationships, the current results can not be discounted.

The contemporary effective population size estimates for Atlantic Whitefish of between 18 and 38 individuals are among the smallest reported for single populations of fish, let alone entire fish species and provide support for the extremely small population size of the species. Moreover, the estimate presented here is likely an overestimate of Ne as genetic differences between the three lakes of Atlantic Whitefish would increase the perceived diversity. As comparisons, a single small population of Northern Pike (*Esox lucius*) was estimated to have an Ne of 48 individuals (Miller and Kapuscinski 1997), similarly four Swedish Brown Trout (*Salmo trutta)* populations had Ne estimates of 52- 480 (Jorde and Ryman 1996). Even the endangered species *Nototropis mekistocholas* and *Moxostoma hubbsi* effective population size estimates were between 85 and 513 (Saillant *et al.* 2004) and 107 – 568 individuals (Lippe *et al.* 2006) respectively. The latter was also among Canada's endangered freshwater fish species (DFO 2007).

All of the short term Ne estimates for Atlantic Whitefish fall below both suggested levels of the 50:500 rule of thumb for population viability, suggesting Atlantic Whitefish are at danger of inbreeding and loss of evolutionary potential due to low genetic diversity (Franklin 1980). Nonetheless, recent work has suggested that Atlantic Whitefish may still possess evolutionary potential as familial differences in tolerance to low pH were evident (Chapter 4). Furthermore young life stages may be capable of enduring environmental changes as they possess ability to tolerate a wide range of environmental conditions. In particular, Atlantic Whitefish were tolerant to low environmental pH, as they performed well at levels lower than they have ever experienced based on the results from paleolimnological pH reconstructions (Ginn *et al.* 2008) and are less thermally sensitive than other locally occurring diadromous species (Chapter 4).

The ability of Atlantic Whitefish to tolerate broad environmental conditions and continue to survive in their current environment may be due to their persistence at low genetic diversity for numerous generations. Theory suggests that inbred species or populations that manage to avoid extinction and persist at low numbers for numerous generations reduce their mutational load through purging of deleterious recessive alleles (Frankham *et al.* 2001). Purging occurs during inbreeding events as the frequency of individuals homozygous for deleterious alleles will increase and are removed from the population through natural selection. The role of purging as a mitigating measure against inbreeding depression has been under debate (Byers and Waller 1999; Crnokrark and Barrett 2002; Boakes *et al.* 2007). Recent evidence suggests purging can reduce the impacts of inbreeding depression and improves fitness with several generations of inbreeding (4-6 generations Larsen *et al.* 2011; <19 generations Swindell and Bouzat 2006). That said, purging will only be effective in removing alleles which are actively selected against over multiple generations (Hedrick 1994; Willis 1999). Purging will also

be ineffective against selective pressures occurring only stochastically as deleterious recessive alleles may not be effectively removed from the population.

The distinct and basal phylogenetic status of Atlantic Whitefish supported the information provided elsewhere (Bernatchez *et al.* 1991b; Hubert *et al.* 2008). In earlier phylogenetic studies, Atlantic Whitefish was suggested to be monophyletic with *C. clupeaformis* / *C. lavaretus* complex and polyphyletic with the *C. artedii* / *C. autumnalis* complex (Bernatchez *et al.* 1991b). Here, I showed a clear basal position of the Atlantic Whitefish node location using both microsatellite data and COI sequence data. The difference between previous work and that shown here may be attributed to the different genetic marker used, as restriction fragment polymorphisms (RFLP) were used in the earlier study. Due to the presence / absence nature of RFLP's, they are useful in determining if differences exist between species, however inferring phylogenies may be problematic as they are prone to homoplasy, where a restriction site may be lost by one or several mutations, thereby masking the true level of divergence between species. And although microsatellites may also be influenced by homoplasy, particularly between species, as the number of repeat units is constrained within loci (Estoup *et al.* 1998), they still contain more information than the RFLP markers and perform better for phylogenic reconstructions (Smith *et al.* 1997). Sequence data may provide the best option for generating phylogenies as the there is information in every base pair change. By using the COI segment in the current work the phylogenetic relationships between species were depicted using a section of the mitochondrial COI gene. The use of only the COI gene in this context relies on the assumption that the phylogeny inferred from the gene is the same as phylogeny of the species. Ideally, multiple genes or entire genomes would be used in the reconstruction of phylogenies (Gontcharov *et al.* 2004). COI sequence data have recently been advocated for the identification of fish species (Ward *et al.* 2005) and has been successfully used to reproduce phylogenetic

relationships in some groups but not all (Russo *et al.* 1996; Tobe *et al.* 2010). This was evident in the presented COI phylogeny which could not resolve relationships between *C. zenthicus*, *C. nigripinus*, *C. hoyi*, and some haplotypes of *C. artedii*, which have been shown to be distinct species in previous analyses (Bernatchez *et al.* 1991b), although this species complex is known to be difficult to characterize (Turgeon *et al.* 1999; Turgeon and Bernatchez 2001 a, b). That said, the basal position of the Atlantic Whitefish shown here for two separate phylogenies was supported by more evidence than any of the previous phylogenetic relationships or the morphometric studies.

The distinctive species status of Atlantic Whitefish was supported here by both mitochondrial COI and nuclear microsatellite data. The minimum percent sequence divergence between Atlantic whitefish and any other Coregonine was 3.3%, which was greater than the percent divergence for any of the other within genus pairwise divergence estimates. Similarly the microsatellite F_{st} and R_{st} values were higher between Atlantic Whitefish and *C. clupeaformis* or *C. artedii* than between *C. clupeaformis* and *C. artedii*. The level of sequence divergence required for a provisional species status has been suggested to be 10x the intraspecies divergence estimate (Hebert *et al.* 2004). Atlantic Whitefish possess a single COI haplotype and thus can not be assessed using this criterion. However, 10x the mean within species COI divergence estimates from all *Coregonus* haplotypes examined here yield an estimate of 1.1%, indicating all species examined, including Atlantic Whitefish were distinct species. The percent sequence divergence from the currently presented COI data were consistently higher for than the previously reported RFLP data, which is likely a reflection of the aforementioned influence of homoplasy in the latter genetic marker.

Overall, Atlantic Whitefish possessed very limited genetic diversity and were characterized by an extremely low genetic effective population size. That said, there was no evidence of a recent genetic bottleneck, suggesting Atlantic Whitefish have been at

low diversity levels for much of the past century. I provided further support for the unique evolutionary status of Atlantic Whitefish at the base of the *Coregonus* genus. Despite the limited genetic diversity, work from elsewhere suggest Atlantic Whitefish has been shown to possess significant tolerance to and plasticity in their response to environmental conditions (Chapter 4).

Table 2.1: Site description for the Cisco, Atlantic and Lake Whitefish populations used in the present genetic analysis. Lake indicates which lake the fish were captured, abbreviation indicates the name used for that population throughout the paper, Area is the habitat area in km^2. N_{Micro} and N_{CO1} indicate the number of samples genotyped using each method respectively.

Species	Province	Watershed (Reference for Figure 1)	Lake	Abbreviation	Area	N_{Micro}	N_{COI}
Atlantic Whitefish	Nova Scotia	Petite Riviere (1)	Minamkeak,Milipsigate, Hebb	AW	16.5	169	61
Lake Whitefish	Nova Scotia	Mira River (2)	Mira River	MR	35.5	32	29
		Hardy-Gabarus (3)	MacIntyres	MI	0.72	64	
			MacLeods	MC	0.74	49	
		St. Mary's River (4)	Eden	EL	2.23	45	
		Little River (5)	Scots	ScL	0.76	46	
		Musquodoboit (6)	Shaw Big	SBL	0.82	32	
			Gibraltar	GL	0.85	70	
		Mushamush River (7)	Little Mushamush	LM	4.4	50	11
		Medway River (8)	Little Ponhook	LP	0.81	58	26
			Shingle	ShL	4.7	36	
		Tusket River (9)	Kempt Back	KL	3.3	52	8
			Mink	ML	1.4	23	
	New Brunswick	Saint John River (10)	Saint John River	SJR	435.5	40	8
	Ontario	Great Lakes (11)	Ontario	LO	10,300	25	18
	Nunavut	Perry River (12)	MacAlpine	MA	447	40	
Cisco	Ontario	Great Lakes (11)	Ontario	LOC	10,300	30	30

Table 2.2: Characteristics of 15 microsatellite loci used in this study; size ranges for alleles in each species are given in base pairs, and (-) indicates weak or no amplification.

Locus ID	Repeat Sequence 5'-3'	Reference	Source species	Atlantic Whitefish	Lake Whitefish	Cisco
Chu1	$(GGAT)_{15}$	Murray 2005	*C. huntsmani*	145-169	117	117
Chu4	$(CCAT)_{21}$	Murray 2005	*C. huntsmani*	214-222	-	-
Chu6	$(CCAT)_6N_{32}(CCAT)_8$	Murray 2005	*C. huntsmani*	175	107-111	115
Chu16	$(CAGA)_8(CGGA)_4$ $CAGA)_8$	Murray 2005	*C. huntsmani*	224-232	168-380	-
Chu19	$(CCAT)_{19}$	Murray 2005	*C. huntsmani*	174	112-134	120-124
BWF1	$(GA)_{16}$ N_{95} $(TG)_{13}$	Patton *et al.* 1997	*C. nasus*	221-223	190-225	191-197
BWF2	$(CA)_{25}$	Patton *et al.* 1997	*C. nasus*	194	148-164	148-164
Cisco90	$(AC)_{10}$ ATAT $(AC)_3$	Turgeon *et al.* 1999	*C. artedi*	108	100-128	100-108
Cisco157	$(GT)_{17}$	Turgeon *et al.* 1999	*C. artedi*	159-163	141-181	145-163
Cisco200	$(GT)_{45}$	Turgeon *et al.* 1999	*C. artedi*	199-211	209-279	195-259
Cocl23	$(GT)_8$	Bernatchez 1996	*C. clupeaformis*	240	248-273	258-299
Cocl-Lav41	$(CT)_{36}$	Rogers *et al.* 2004	*C. clupeaformis*	169-175	-	-
Cocl-Lav49	$(GT)_{17}$	Rogers *et al.* 2004	*C. clupeaformis*	164	168-212	162-204
Cocl-Lav68	$(CA)_{11}$	Rogers *et al.* 2004	*C. clupeaformis*	173	175-179	179-209
Cocl-Lav72	$(GT)_{23}$	Rogers *et al.* 2004	*C. clupeaformis*	187-191	-	-

Table 2.3: Genetic diversity estimates from microsatellite and COI sequence data. For the microsatellite data An was mean number of alleles, Ar is allelic richness, He is expected heterozygosity, Ho is observed heterozygosity. From the COI data, the number of haplotypes and the associated haplotype diversity were reported. For population abbreviations see Table 2.1.

	Microsatellites										COI	
Species/Population	All Loci					Polymorphic Loci						
	N loci	An	Ar	He	Ho	n loci	An	Ar	He	Ho	Haplotypes	Haplotype Diversity
AW	15	2.13	1.61	0.16	0.15	8	3.12	2.15	0.29	0.27	1	0
EL	11	5.92	3.31	0.46	0.46	9	7.56	3.82	0.61	0.61	--	--
GL	11	3.92	2.36	0.28	0.29	9	4.5	2.49	0.34	0.35	--	--
MI	11	3.5	2.54	0.34	0.34	8	4.75	3.12	0.5	0.51	--	--
MC	11	4.92	2.87	0.36	0.34	8	6.88	3.56	0.53	0.51	--	--
MR	11	5.08	3.45	0.53	0.53	10	5.9	3.7	0.63	0.63	1	0
ScL	11	2.5	2.12	0.36	0.35	8	3.25	2.53	0.54	0.53	--	--
LM	11	4.75	2.96	0.42	0.41	9	6	3.39	0.56	0.54	2	0.22
ShL	11	3.5	2.29	0.29	0.3	8	4.75	2.77	0.44	0.45	--	--
LP	11	5.58	2.91	0.38	0.38	8	7.87	3.62	0.57	0.56	1	0
SJR	11	5.82	3.64	0.58	0.59	11	5.82	3.64	0.58	0.59	2	0.5
ML	11	3.08	2.5	0.36	0.36	10	3.5	2.65	0.43	0.43	--	--
KL	11	4.08	2.43	0.31	0.32	7	5.62	2.69	0.47	0.47	2	0.286
SBL	11	2.33	2.06	0.26	0.26	8	3	2.45	0.39	0.39	--	--
MA	11	4.58	3.29	0.51	0.52	11	4.91	3.29	0.56	0.57	--	--
LO	11	7.17	4.37	0.62	0.62	11	7.72	4.36	0.67	0.67	1	0
LOC	11	7.27	3.83	0.56	0.5	9	8.67	4.11	0.68	0.61	2	0.52

Table 2.4: Relationship between genetic diversity or effective population size estimates and the logarithm of lake area for *Coregonus* species. An = number of alleles, Ar = allelic richness, He = expected heterozygosity, Ne_{LD} = effective population size estimated by linkage disequilibrium methods, Ne_{θ} = effective population size estimated by genetic diversity. For population abbreviations see Table 2.1.

Response	N Populations/Species	Intercept	Slope	slope p-value	Potential Outliers
An	17	4.81	0.25	<0.05	AW, EL, LP
Ar	17	2.9	0.12	<0.01	AW, EL, LP
He	16	-0.11	0.07	<0.001	AW† EL,GL
Ne_{LD}	11	239.5	-12.4	0.41	AW,EL,LOC†
Ne_{θ}	16	367.2	79.6	<0.001	AW,EL,MR,LO†

†high leverage data points removed from regressions

Table 2.5: Genetic differential measures of F_{ST} (lower triangle) and R_{ST} (upper triangle) from microsatellite markers calculated between population pairs among three species. All F_{ST} and R_{ST} values are significant (α = 0.001). Interspecific comparisons were italicized. For population abbreviations see Table 2.1.

Population or species	EL	GL	MI	MC	MR	SCR	LM	SL	LP	SJR	ML	KL	SB	MA	LO	*LOC*	*AW*
EL		0.55	0.51	0.47	0.37	0.50	0.52	0.51	0.53	0.34	0.43	0.55	0.48	0.48	0.36	*0.62*	*0.85*
GL	0.48		0.53	0.46	0.29	0.26	0.28	0.54	0.54	0.20	0.40	0.39	0.43	0.40	0.29	*0.67*	*0.92*
MI	0.40	0.45		0.20	0.36	0.46	0.38	0.56	0.57	0.37	0.44	0.53	0.49	0.41	0.36	*0.65*	*0.88*
MC	0.36	0.46	0.03		0.31	0.49	0.32	0.52	0.51	0.32	0.42	0.56	0.53	0.32	0.31	*0.65*	*0.86*
MR	0.21	0.31	0.29	0.25		0.26	0.22	0.41	0.36	0.10	0.29	0.30	0.36	0.29	0.16	*0.51*	*0.81*
SCR	0.39	0.38	0.42	0.41	0.24		0.34	0.62	0.58	0.22	0.36	0.41	0.33	0.44	0.30	*0.61*	*0.88*
LM	0.37	0.37	0.31	0.28	0.21	0.29		0.53	0.47	0.23	0.49	0.44	0.43	0.35	0.29	*0.53*	*0.82*
SL	0.38	0.55	0.53	0.52	0.30	0.56	0.47		0.28	0.34	0.42	0.48	0.65	0.50	0.36	*0.71*	*0.94*
LP	0.40	0.48	0.48	0.46	0.26	0.49	0.40	0.16		0.41	0.50	0.46	0.63	0.55	0.43	*0.66*	*0.91*
SJR	0.19	0.33	0.31	0.29	0.10	0.25	0.24	0.32	0.32		0.16	0.24	0.31	0.32	0.19	*0.42*	*0.85*
ML	0.26	0.45	0.38	0.36	0.20	0.39	0.39	0.41	0.41	0.15		0.22	0.44	0.42	0.32	*0.69*	*0.96*
KL	0.41	0.47	0.47	0.45	0.26	0.38	0.39	0.51	0.43	0.28	0.27		0.44	0.50	0.39	*0.72*	*0.99*
SB	0.42	0.54	0.55	0.53	0.31	0.36	0.43	0.58	0.52	0.28	0.43	0.46		0.58	0.43	*0.71*	*0.88*
MA	0.37	0.52	0.43	0.42	0.34	0.46	0.41	0.47	0.43	0.32	0.40	0.48	0.52		0.08	*0.58*	*0.82*
LO	0.35	0.41	0.38	0.37	0.25	0.34	0.31	0.45	0.39	0.23	0.35	0.41	0.40	0.15		*0.42*	*0.76*
LOC	*0.44*	*0.56*	*0.53*	*0.50*	*0.40*	*0.52*	*0.47*	*0.56*	*0.51*	*0.38*	*0.49*	*0.56*	*0.57*	*0.40*	*0.33*		*0.71*
AW	*0.73*	*0.79*	*0.77*	*0.77*	*0.74*	*0.77*	*0.76*	*0.82*	*0.77*	*0.74*	*0.81*	*0.80*	*0.82*	*0.74*	*0.74*	*0.71*	

Table 2.6: Pairwise Dxy estimates from mitochondrial COI sequences obtained between Atlantic Whitefish, Cisco and Lake Whitefish. Only interspecific comparisons (italicized) were significantly different (P<0.05). For population abbreviations see Table 2.1.

	AW	LOC	MR	LP	LM	SJR	LO
AW							
LOC	*0.0381*						
MR	*0.0369*	*0.0207*					
LP	*0.0369*	*0.0207*	0.0000				
LM	*0.0371*	*0.0210*	0.0002	0.0002			
SJR	*0.0374*	*0.0213*	0.0005	0.0005	0.0008		
LO	*0.0369*	*0.0207*	0.0000	0.0000	0.0002	0.0005	
KL	*0.0372*	*0.0210*	0.0003	0.0003	0.0006	0.0009	0.0003

Table 2.7: Mean Dxy divergence estimates from COI sequence data of Coregonines.

	C. huntsmani	*C. sardinella*	*C. zenithicus*	*C. autumnalis*	*C. nasus*	*C. laurettae*	*C. kiyi*	*C. hoyi*	*C. clupeaformis*	*C. artedi*	*C. lavaretus*	*C. pidschian*	*S. leucichthys*	*P. coulterii*
C. sardinella	3.8													
C. zenithicus	4	1.1												
C. autumnalis	3.3	0.8	0.7											
C. nasus	4.5	1.6	2	1.8										
C. laurettae	4.1	1.3	0.5	0.7	2.1									
C. kiyi	4	1.1	0	0.7	2	0.5								
C. hoyi	4.1	1.2	0	0.7	2	0.6	0							
C. clupeaformis	3.7	1.9	2.3	2.1	1.2	2.4	2.3	2.3						
C. artedii	3.8	1.1	0.1	0.5	1.9	0.4	0.1	0.2	2.2					
C. lavaretus	4.3	1.8	2.2	2	1.1	2.3	2.2	2.3	1	2.1				
C. pidschian	3.8	1.7	2.2	1.8	1.1	2.3	2.2	2.3	0.5	2.1	0.7			
S. leucichthys	3.6	3	3.1	2.9	3.3	3.2	3.1	3.2	3.2	3	3.3	3.2		
P. coulterii	16.6	15.4	15.2	15.7	15.5	15.7	15.2	15.1	15.3	15.3	15.7	15.6	15.1	
P. cylindraceum	12.1	12	11.5	11.8	12.6	12	11.5	11.5	13	11.7	13.5	13.2	12.1	14.2

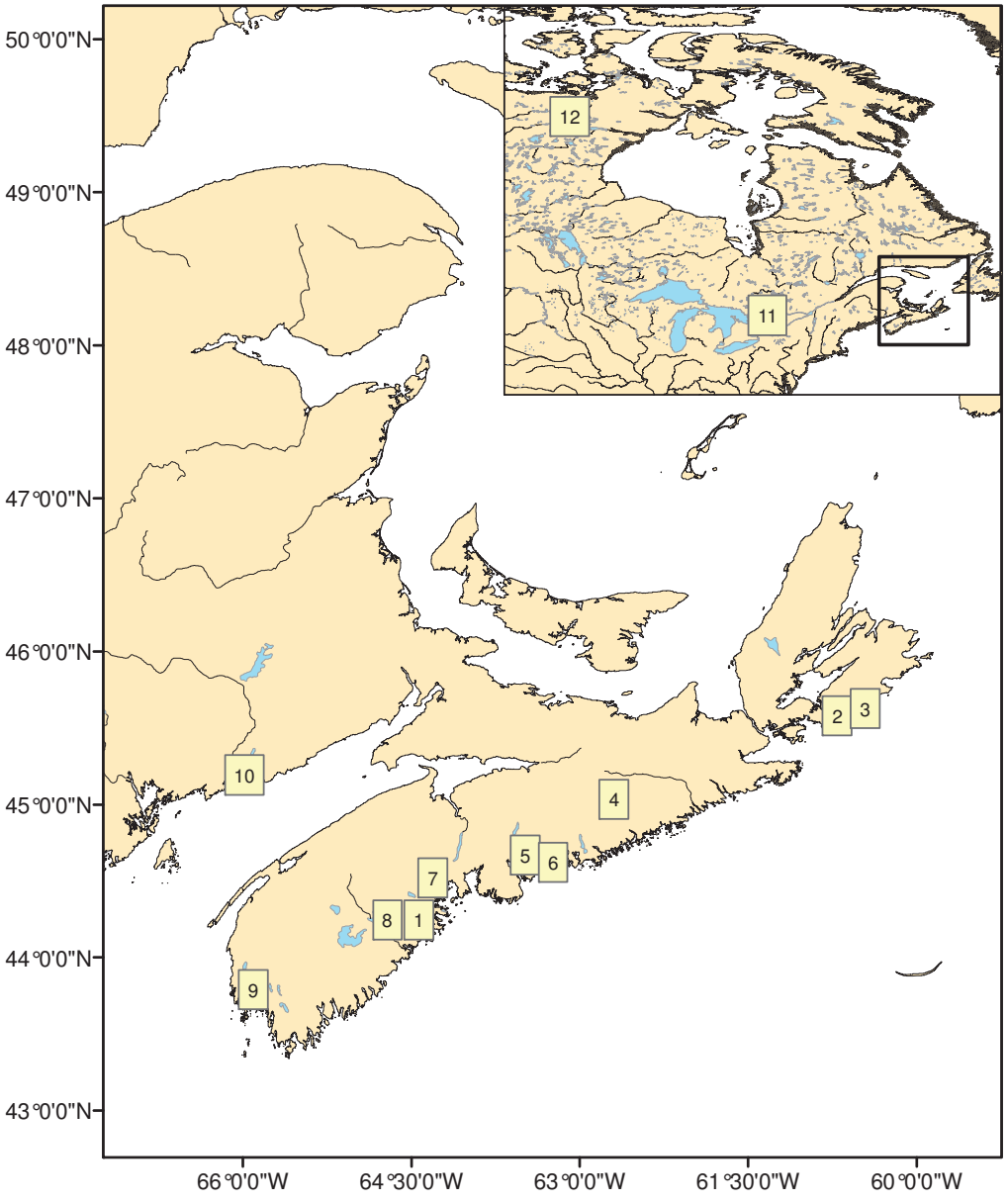

Figure 2.1: Map of sample locations for Atlantic Whitefish (1), Lake Whitefish (2-12), and Cisco (11) used in this study. Numbers represent sample locations which are described in Table 2.1.

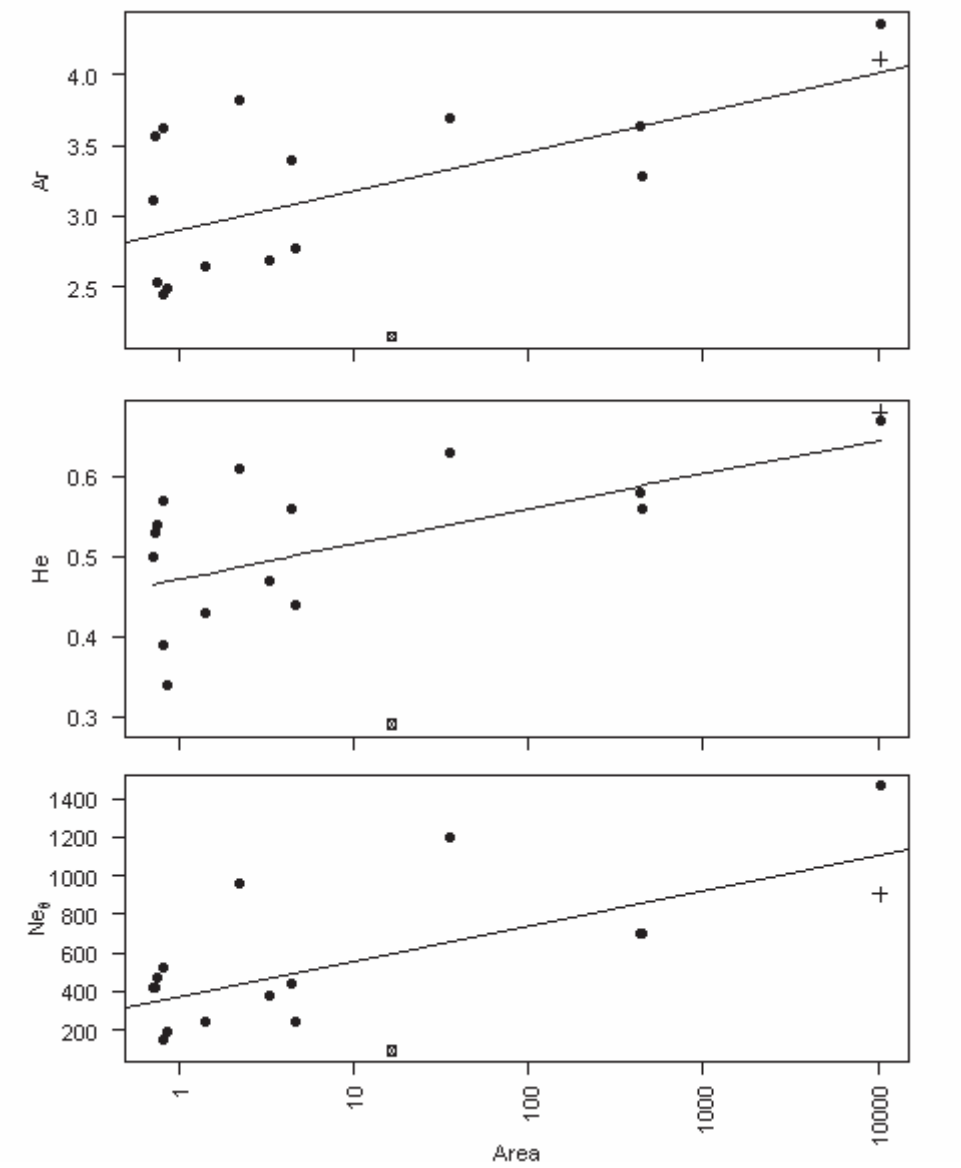

Figure 2.2: Relationship between genetic diversity measures of allelic richness (Ar), expected heterozygosity (He), and long term effective population size (Ne_θ) with the natural log of lake area (km^2) for Atlantic Whitefish (crossed box), Cisco (plus sign) and Lake Whitefish (filled circle).

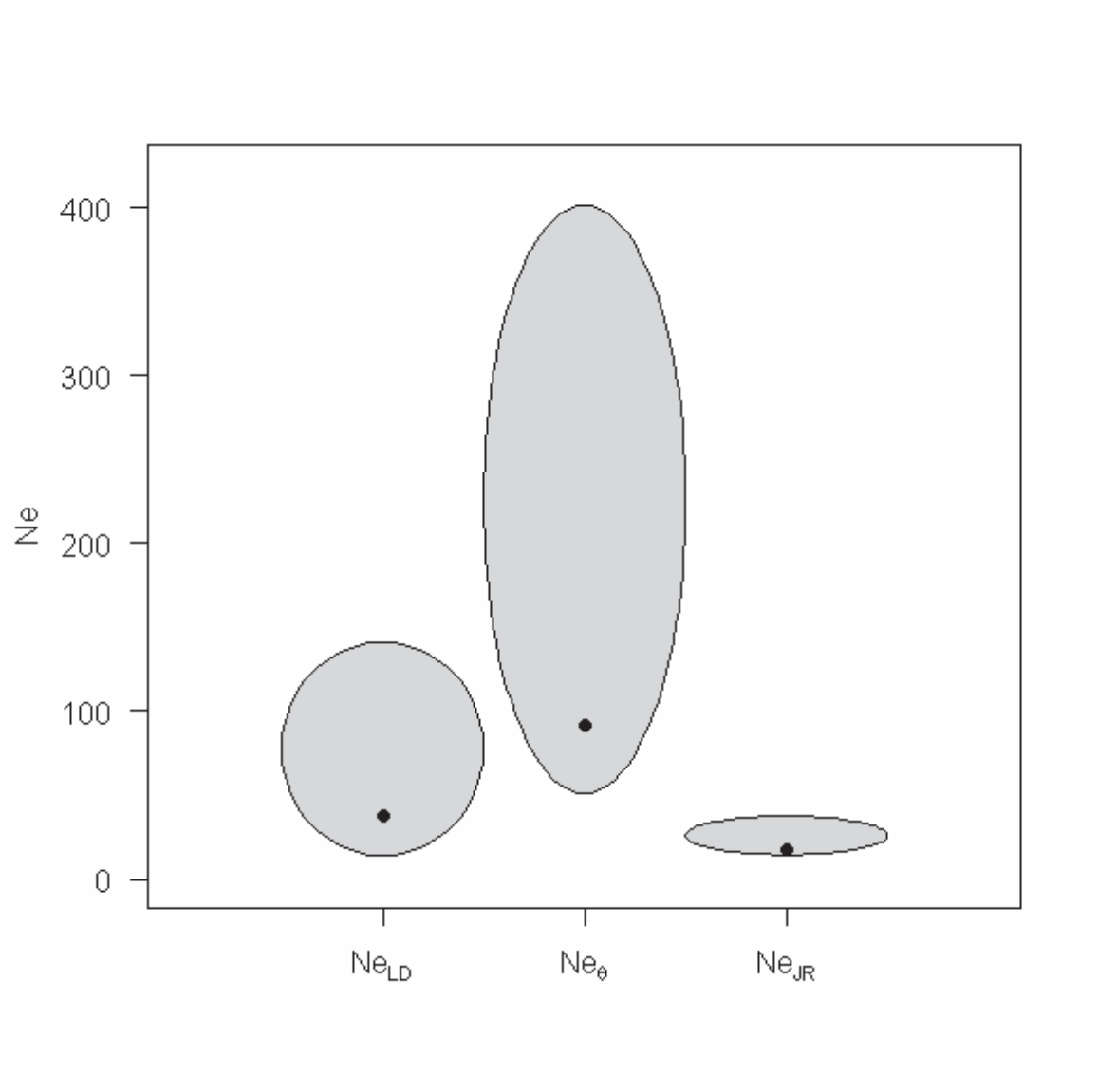

Figure 2.3: Estimates of effective population size in Atlantic Whitefish from the linkage disequilibrium method (Ne_{LD}), genetic diversity (Ne_{θ}) and Jorde and Ryman's temporal method (Ne_{JR}).

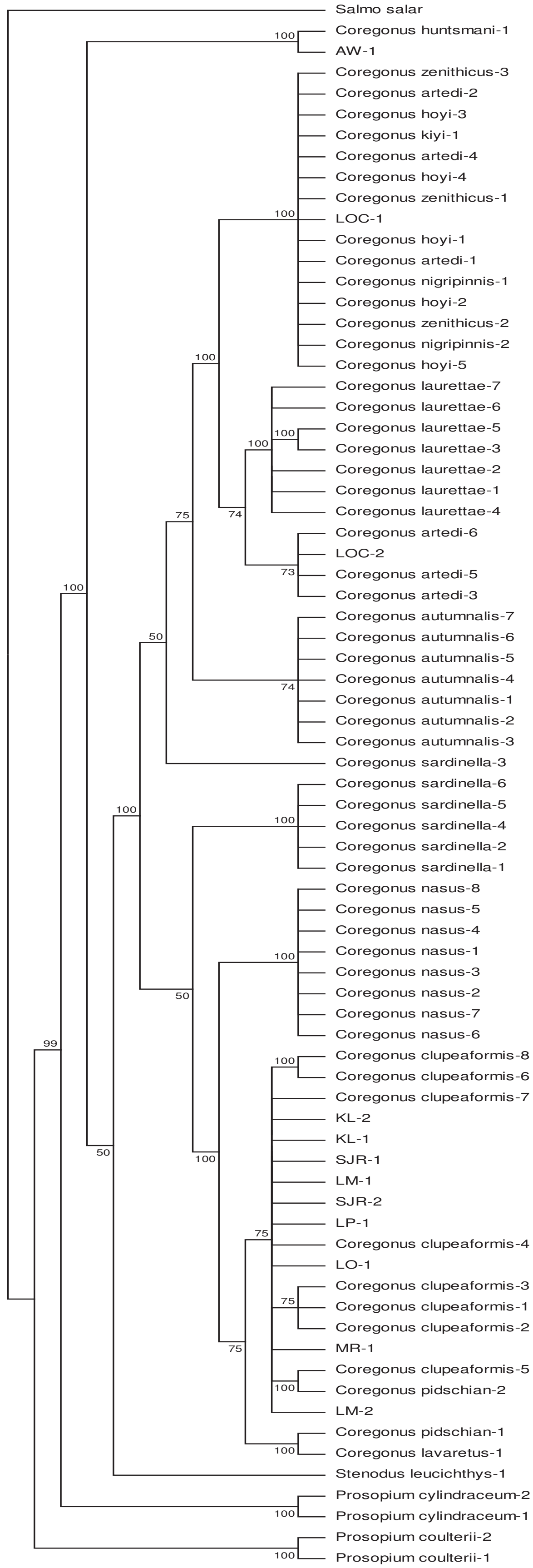

Figure 2.4. Evolutionary relationships of *Coregonus*, *Prosopium* and *Stenodus* species inferred using the Maximum Parsimony method. The consensus tree inferred from 408 most parsimonious trees is shown. Branches corresponding to partitions reproduced in less than 50% trees are collapsed. The percentage of parsimonious trees in which the associated taxa clustered together were shown next to the branches.

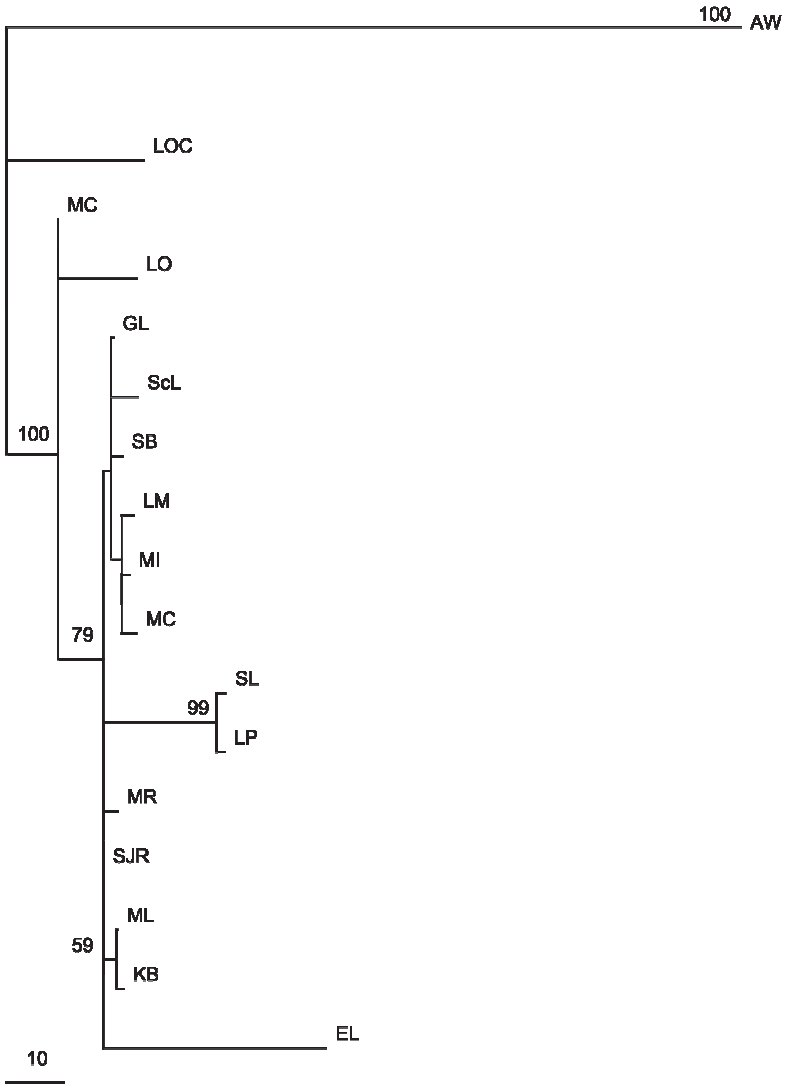

Figure 2.5: Population dendrogram based on microsatellite loci for Atlantic Whitefish (AW), 15 populations of Lake Whitefish (see Table 2.1 for abbreviations), and one population of Cisco (LOC). This is a hybrid tree generated from Cavalli-Sforza and Edwards' (1967) chord distance, and branch length was calculated using Goldstein et al's (1995) $(\delta\mu)^2$, Percent bootstrap support was shown for each node. Nodes receiving <50% support were collapsed.

CHAPTER 3: METHODS AND METRICS FOR COMPARATIVE THERMAL SENSITIVITY IN FISH

3.1 ABSTRACT

Temperature is considered one of the main environmental factors controlling fish populations. Mechanisms have been identified to describe physiological responses to temperature; however, few broad scale systematic or comparative analyses of both temperature growth relationships and thermal sensitivity of fish have been undertaken. Here, data describing the temperature growth relationships of temperate fish species from a range of habitats and life history characteristics were compiled from previously published studies and comparative analysis performed to assess modeling approaches and sensitivity metrics. Further, comparative analyses were performed to determine the relationships between thermal sensitivity and life history parameters. Phylogenetic generalized least squares regressions were used to compare relationships across species. Within species comparisons were done using least squares regression or permutation tests of bootstrapped results.

Results suggest a common model can be used to describe performance-temperature relationships across a broad range of fish species. A single integrative metric of thermal sensitivity can be used to encompass the thermal sensitivity of stage-specific temperature growth relationships. Through interspecific comparisons, thermal sensitivity was suggested to increase in coldwater species and in species with either a high asymptotic body size (L_∞) or are long lived. Intraspecific comparisons showed that thermal sensitivity changed through ontogeny such that early life stages were more sensitive than subadults across most of the species tested. Results are discussed in relation to relevant physiological mechanisms.

3.2 INTRODUCTION

Temperature is considered one of the main environmental factors controlling growth and productivity in fish populations (Fry 1971). Among the responses studied, temperature has been shown to influence biogeography (Pörtner 2002), growth (Brett 1979), survival rates (Cook *et al.* 2006), development (Fonds 1979), spawning time (Hutchings and Myers 1994), swimming performance (Bernatchez and Dodson 1985), immune function (Alcorn *et al.* 2002) and foraging success (Bystrom *et al.* 2006) through its influence on physiological processes. Although the majority of fish species are exposed to natural temperature fluctuations associated with diurnal, seasonal, annual and multidecadal processes, recent information suggests warming at global and local scales is occurring at rates faster and temperature variability is greater than previously documented (IPCC 2007). To date, the current temperature changes have resulted in changing phenology (Hughes 2000) and poleward shifts in distribution (Perry *et al.* 2005). Further alterations are expected as climate forecasts predict temperatures to continually increase in some regions by 1.8-4.0℃ over the next century (IPCC 2007). As species distributions change in response to climate changes, ecosystem structure and function are being affected (Takasuka *et al.* 2007; Ficke *et al.* 2007; Cheung *et al.* 2008; Rijnsdorp *et al.* 2009). In order to better understand contemporary distributions and to predict population level responses to changing climates, the thermal niche and thermal sensitivity of individual fish species should be studied and compared.

Species exhibit a range of thermal tolerance due to constraints on molecular, cellular and systematic processes which can be defined as their thermal niche (Farrell *et al.* 1996; Pörtner 2001; Somero 2004). Assessments of the response of ectotherms to changes in temperature should consider both high and low extremes as physiological process are inhibited at both ends of the spectrum. Species' thermal bounds are

thought to be defined by past evolutionary processes and contemporary habitats (Algar *et al.* 2009; Hall and Thatje 2009; Eliason *et al.* 2011), which result in a gradient of thermal niche breadths from broad generalist eurytherms (*e.g. Anguilla anguilla*; Sadler 1979) which can thrive in a wide range of temperatures, to specialist stenothermic species that can only tolerate a narrow range of temperatures (*e.g. Pachycara brachycephalum*; van Dijk *et al.* 1999). Within species, thermal niches differ among populations (Eliason *et al.* 2011), through ontogeny (Bjornsson and Steinarsson 2002) and in interaction with other environmental factors (Pörtner 2008; Chapter 4).

Approaches to obtaining data for describing the thermal niche of fishes have included using distributional data from surveys of natural populations, as well as laboratory studies under controlled conditions. Using data collected from wild populations, methods have generally identified species' thermal bounds through the correlation of current distributions with environmental variables (e.g. Righton *et al.* 2010). The disadvantage of this approach lies in its reliance on contemporary distributions to define "bioclimatic envelopes"; whereas, in fact, distributions are often influenced by other biotic and abiotic factors such as harvesting, small population sizes or barriers to dispersal (Davies *et al.* 1998; Thomas *et al.* 2001). It is also difficult to identify the full breadth of a species' bounds using field data, as the thermal extremes are very rarely observed. Furthermore, survey data often fail to account for the thermal preferences or requirements of all life stages, as complete distributional data are generally not available. To overcome such problems, many efforts have shifted to laboratory or fish culture settings where temperature effects can be examined singly and in combination with other biotic and abiotic factors.

In the laboratory, thermal bounds are generally described by a thermal tolerance or a critical thermal measure (*sensu* Fry 1956), which defines the temperatures resulting in death or severe impairment (reviewed in Lutterschmidt and Hutchinson 1997).

However, other performance measures, including those mentioned above, are impaired at a narrower temperature range than those defined by thermal tolerance. And although there is a relatively predictable relationship between thermal tolerance, thermal optima and preference (Jobling 1981), it is the narrower range of sublethal responses that are required to define thermal bounds, as they will be more closely tied to recruitment and other population processes (Rice *et al.* 1993; van Dijk *et al.* 1999). One disadvantage of using controlled studies is the difficulty in incorporating the environmental and biotic variability inherent in natural systems, which is important to understand potential habitat use (Righton *et al.* 2010).

From laboratory collected data several models have been developed to describe the relationship between performance and temperature across a range of species. The most commonly used models are the quadratic (*e.g.* Buckel *et al.* 1995), polynomial (*e.g.* Bjornsson *et al.* 2007), Elliott model (Elliott *et al.* 1995), Ratkowsky model (Ratkowsky *et al.* 1983) and Parker model (Parker 1974). As the relationship between many performance measures and temperature follow a similar pattern, all of the models share a similar characteristic shape with an upper maximum where performance is optimized and downward slopes toward an upper and lower minimum (Angilletta *et al.* 2003; Englund *et al.* 2011; Figure 3.1). The greatest difference between the models is in the degree of flexibility in shape around both the optimum and upper/lower tails. Several of the models have been directly compared (Forseth *et al.* 2001); however, there is no consensus on the best or most biologically relevant model to use.

From model outputs two measures of thermal sensitivity are generally calculated. The first termed the 'tolerance range' is defined as the linear difference between the upper and lower critical temperatures and represents the total range of positive performance. The second, termed 'performance breath' is the linear difference between arbitrarily chosen performance levels both above and below the maximum (e.g. 80% of

maximum; Huey and Stevenson 1979) which provides information on the thermal range of optimal performance. In determining thermal sensitivity both measures should be considered simultaneously, as species may have either narrow performance breadth and wide tolerance range or wide performance breadth and narrow (relatively) tolerance range (Figure 3.2), each yielding different conclusions of species sensitivity.

Here, I explored the thermal sensitivity of fishes and attempted for define the 'best' model for depicting performance-temperature relationships. I also investigated the currently used metrics of thermal sensitivity as well as developed new metrics integrating the tolerance range and performance breadth. I examined whether any interspecific patterns of thermal sensitivity with available life history or biology measures could be detected. Finally, I made intraspecific comparisons to examine patterns in thermal sensitivity and body size. For this work I used data from previously described species-specific thermal performance curves from temperature growth experiments. Results were discussed in relation to physiological mechanisms where appropriate.

3.3 METHODS

3.3.1 Data

Growth was the performance measure used in the current analysis as it is both a highly integrative metric and it represents the outcome of a broad number of physiological processes (Weatherly and Gill 1987) and the measurements are easily comparable across studies. Data were compiled from primary literature sources that reported growth across a range of temperatures. For inclusion in analyses, growth measurements were required at levels both above and below the species optimum, such that decreased growth rates were observed at either end of the range. Both length and weight growth data sets were included. Only temperate species were included in the

current analysis, as tropical and polar species have been shown to have developed adaptations that are known to influence thermal niche (Pörtner and Peck 2010). Where possible, raw data were obtained otherwise, data were acquired from published tables or captured through digitization of graphs using WinDig version 2.5. When growth was reported as mean (μ) and standard deviation (σ), data were generated through iteratively sampling a N (μ,σ) distribution for the reported number of experimental units until the randomly selected data's mean and standard deviation matched those reported. The frequency of use of the five different growth temperature models in the original papers was calculated from the reports used here.

For each species, life history and biological characteristics including the von Bertalanffy coefficients K and $L\infty$ (Bertalanffy 1938), latitudinal range (maximum – minimum latitude), environment (freshwater, marine, diadromous), maximum age and temperature range were collected from the online repository of fish information, Fishbase, for comparison with thermal sensitivity (Frosese and Pauly 2011; www.Fishbase.org; accessed September 10, 2011).

Similar to previous environmental performance and tolerance models, the response, growth, was standardized for comparison between species (Slatkin and Lande 1976; Gilchrist 1995). Previous standardization methods used models with a constant area under the curve, leading to a trade-off between performance breadth and maximum performance as in the Huey and Hertz (1984) 'jack-of-all-trades' comparisons. Here, performance was standardized within each dataset such that each measurement (p_i) was related to the maximum value ($p_i \cdot \max(p_i)^{-1}$). This method allowed for the comparison of thermal performance in terms of breadth (distance between the minimum and maximum) as well as performance area (area under the curve- see below).

3.3.2 Models, Fitting And Thermal Sensitivity

The functional relationships between temperature and growth were explored using five previously published models applied to each dataset. Common notation was applied across models with T_i representing the experimental temperature, G_i standardized growth rate at T_i, Tm optimum temperature, Tu upper growth temperature and Tl lower growth temperature. Not all models yield estimates of Tm, Tu or Tl across all parameter space. An example plot of all five models fitted to the same dataset is shown in Figure 3.1.

3.3.2.1 Quadratic Model (QM)

Quadratic models have been used in multiple forms including the standard non-monic model (ax^2+bx+c) as well as other simple derivations. For this analysis, the vertex parameterization which permits the direct estimation of Tm was used:

$$G_i = 1 + w(T_i - Tm)^2 \qquad \text{Equation 3.1}$$

where *w* scales the relationship between observed growth and Tm. Tu and Tl were estimated through the calculation of roots for equation 3.1.

3.3.2.2 Polynomial Model (PM)

Polynomial models from previous studies were generally cubic due to the inherent nature of the data. As such, only the cubic model was examined here with the model following the standard polynomial form:

$$G_i = aT_i^3 + bT_i^2 + cT_i + d \qquad \text{Equation 3.2}$$

where a, b, and c are polynomial coefficients and d is a constant. Tu and Tl were estimated using the polyroot function in the statistical platform R (version 11.1), with only real roots reported. Tm was estimated as the local maxima between Tu and Tl.

3.3.2.3 Elliott Model (EM)

The Elliott model is a two phase regression model, used extensively on freshwater and anadromous species and is defined as:

$$G_i = \left(\frac{T_i - T_{\lim}}{Tm - T_{\lim}} \right) \quad where \begin{cases} T_{\lim} = Tl \text{ if } T_i \le Tm \\ T_{\lim} = Tu \text{ otherwise.} \end{cases} \qquad \text{Equation 3.3}$$

Unlike previously published versions of this model a body size scalar, cW^{-b} was removed as growth rates were standardized prior to fitting regressions.

3.3.2.4 Ratkowsky Model (RM)

The Ratkowsky model was originally proposed to describe bacterial growth in relation to temperature (Ratkowsky *et al.* 1983); it has since been used in modeling fish growth-temperature relationships. This model has the form:

$$G_i = d(T_i - Tl)\left(1 - e^{g(T_i - Tu)}\right) \qquad \text{Equation 3.4}$$

where d is the growth coefficient for temperatures between Tl and Tm and g accounts for the downward curvature from Tm to Tl. This model directly estimates Tl and Tu. Tm can be obtained from parameter estimates by solving for the maximum value as (Jonsson *et al.* 2001):

$$\ln(1 + g(Tm - Tl)) = -g(Tm - Tu) \qquad \text{Equation 3.5}$$

3.3.2.5 Parker Model (PKM)

Parker Model was designed to describe the metabolic responses of aquatic organisms to environmental variables. PKM is in the form:

$$G_i = \left[\left(\frac{T_i}{Tm} \right) \times \left(\frac{Tu - T_i}{Tu - Tm} \right)^{\left(\frac{Tu - Tm}{Tm} \right)} \right]^d \qquad \text{Equation 3.6}$$

This model proportions growth into regions above and below Tm and uses the shape parameter, d, to account for the curvature in the model. Tl is undefined for this model as it reaches an asymptote at low abscissae. As such, areal thermal sensitivity metrics requiring Tl were determined by setting Tl =0; linear thermal sensitivity metrics requiring Tl were not estimated as they would be upwardly biased (Figure 3.2; see section on Thermal Sensitivity below). PKM in its current form is only applicable for the interval $T_i[0,\infty]$, however, transformations of T_i (such as conversions to °K) could be applied to account for positive growth at $T_i < 0$. T_i transformations were not explored in the current analysis as positive growth was not observed at temperatures <0°C for any dataset.

3.3.2.6 Model Fitting And Choice Of Best Fit

All models were fit to data sets using maximum likelihood estimation with a Gaussian negative log likelihood objective function within the *optim* function in R. Standard Akaike Information Criteria (AIC) and a penalized AICc were calculated for each model as:

$$AIC = 2k - 2\ln L \qquad \text{Equation 3.7}$$

$$AICc = AIC \cdot \frac{2k(k+1)}{n-k-1} \qquad \text{Equation 3.8}$$

where k is the number of parameters, L is the likelihood from the best fit model, and n is the number of observations used to fit the model. The AICc adds an extra penalty for the number of parameters in the model, which was important as several of the datasets contained relatively few observations and the models had different numbers of parameters (Burnham and Anderson 2002).

The five fitted models were compared within each dataset by a combination of methods. First, the precision of the parameter estimates (or derived estimates) of Tm, Tu and Tl were examined by comparing each model's output against the median of all

five models. Next, models with the lowest AIC and AICc values were chosen; with values ±2 considered equivalent (Burnham and Anderson 2002). Finally, models directly estimating biologically relevant parameters and following realistic patterns were given preference due to the ease of estimating the standard errors of biologically relevant parameters and the increased value for estimating thermal sensitivity (see below). All best fit models, parameter estimates, sensitivity measures and species information were provided in Appendix B: Chapter 3.

3.3.2.7 Thermal Sensitivity

Two types of thermal sensitivity metric were calculated, one representing the often measured linear temperature range between successive critical temperatures (*sensu* Huey and Stevenson 1979; Gilchrist 1995) and the other being a new measure representing the area under the curve between those same critical temperatures. The linear temperature range (*L*) sets the bounds on positive growth with groups possessing a large *L* having decreased sensitivity to temperature changes. The areal temperature range (*A*) integrated the *L* with the curvature of the model such that two groups may have a similar *L* but divergent *A* by possessing either a sharp or flat curve around the Tm (Figure 3.2). *A* was calculated by numerical integration of each fitted model between the two critical temperatures using the integrate function in R (version 11.1).

Three pairs of critical temperature points were chosen. The first, Tl:Tu defined the broad scope thermal sensitivity. This pair of temperatures covered the total linear range (LT) and total area under the curve (AT) (Figure 3.2). The second, Tm:Tu represented the upper temperature sensitivity, narrow upper linear ranges (ULT) or small upper areas (UAT) indicated limited ability to tolerate increasing temperatures. The third set of temperature points represented the range of optimum temperatures for growth and were calculated as the upper and lower temperatures that result in 75% of maximum

growth. Narrow or small values of optimum linear temperatures (OLT) and optimal areal temperatures (OAT) indicated the performance was highly influenced by temperature changes.

3.3.2.8 Model Evaluation and Sensitivity Metric

In order to compare models across all datasets to determine the best to use for future studies the within dataset model selection criteria identified above were compiled for all models across all datasets. In particular, data were compiled to determine the proportion of datasets where Tm, Tl and Tu were estimated within ±2℃ of the median of all five models, the proportion of fits where AIC and AICc's were within ±2 of the minimum value, the number of biologically relevant parameters and the number of instances the model was selected as the best fit across all data sets. Further comparisons of the best fit models were made to determine if specific models performed better for specific taxonomic groups or size ranges.

Thermal sensitivities were compared by calculating correlation coefficients between each pair of metrics.

3.3.3 Interspecific Comparisons

The relationships between each of the thermal sensitivity metrics and each temperature parameter (Tu,Tm,Tl) or life history / biological characteristic (K, $L\infty$, maximum age, latitudinal range, temperature range and environment) were explored using either phylogenetic generalized least squares regression for the continuous variables (PGLS; Butler and King 2004) or phylogenetically independent contrasts for the categorical variable (environment). These methods accounted for the lack of independence between data points from different species by integrating their phylogenetic relationships into the covariance structure of models. In continuous

variables, the evolution of states was assumed to follow a Brownian process thus allowing for construction of nodal values (Felsenstein 1985). Relationships between categorical variables cannot be estimated in this way unless all daughters within a node fall within the same category. As this was not the case for the categorical variable used here, independent contrasts were made by identifying nodes with daughter variables that differ in category (example in Appendix B: Chapter 3; Burt 1989). Each daughter node was only used in one independent contrast per categorical comparison.

The phylogenetic relationships between species were determined through the creation of a neighbor joining tree of COI sequences downloaded from Genbank (Appendix B: Chapter 3). The tree was generated in MEGA 4.0 (Tamura *et al.* 2007) assuming a Kimura two parameter substitution model (Kimura 1980). COI sequence data were available for 19 of 25 species. PGLS was implemented through the nlme and ape packages in R whereas the independent contrasts were performed using the brunch function in the R package caper. PGLS results using the COI phylogeny were compared to those using a phylogeny based on taxonomy assuming equal branch lengths between groups (Pinsky et al. 2011) using the anova function in R. Where multiple model fits were available for the same species, the median level of each response was used. Standard linear regression diagnostics were performed; these tests included testing for normality of the residuals using quantile plots, heteroscedascitiy using standardized residuals versus model fits, quality of fits using model fits against observed values and leverage using Cook's distance. Influential data points were omitted and analyses reran.

3.3.4 Factors Affecting Thermal Physiological Parameters

Data were available to determine the effect of fish size on thermal sensitivity. Thermal sensitivities were compared across size classes using bootstrapping and a permutation test. This procedure randomly resampled the residuals from the original

fitted model $\hat{\varepsilon}_j$ and added these deviations to the original growth data (y_i) such that a synthetic response variable (y_i') was created for each bootstrap iteration as, $y_i' = y_i + \hat{\varepsilon}_j$ (Wu 1986). This method was used as it maintains the information contained in the explanatory variable, temperature, which was important for this analysis as some datasets contained few observations at each temperature. Each model was bootstrapped 1000 times and the density distributions of thermal parameters and sensitivities were compared between factor levels by permutation tests (Good 2005).

Species with more than five body size classes included in analysis were initially assessed using linear regression; however, if threshold changes (break points) in the slope of the relationship between thermal parameters or sensitivity and the factor were observed, the models were analyzed following the method described by Davies (1987). This process iteratively examined the linear model for changes in slope across the independent variable, calculates a Wald statistic for each iteration, and provides an approximate P-value for the largest statistic. A statistically significant ($P<0.05$) Davies test indicated the presence of a break point. Those datasets with significant Davies tests were reanalyzed using segmented regression analysis. This type of analysis allowed for direct estimation and statistical testing of thresholds (break-points) and regression parameters (slopes) of relationships on either side of the threshold. Statistical tests were implemented in R (version 11.1) using the lm function and the segmented package.

Species-specific data will not be discussed as the goal of this effort was to identify patterns and processes associated with thermal sensitivity, not to raise concern over particular species because data were available for only a relatively small number of species in the current analysis.

3.4 RESULTS

Models were fit to 101 datasets from 25 species covering seven orders of fish including Gadiformes (4 species), Salmoniformes (5), Anguilliformes (1), Cypriniformes (1), Perciformes (9), Pleuronecteformes (4) and Siluriformes (1). From these data, a broad range of thermal profiles were examined as optimum temperatures (Tm) and upper temperatures (Tu) ranged between 8.0 - 31.4 ℃ and 13.5 - 39.4℃, respectively (Figure 3.3 and 3.4). Accompanying these thermal profiles, a broad range of sensitivity levels for each metric were observed (Figure 3.4). A survey of the published results used in this analysis showed that the QM was the most commonly fit model in the source publications (propotion = 0.71; Figure 3.5) and weight growth data were used far more frequently than length growth data (0.95; data not shown).

3.4.1 Model choice

RM and PKM were the most frequently chosen best fit models with proportions of 0.61 and 0.30 respectively (Table 3.1). These two models were chosen based on their ability to precisely predict Tm, Tu and TI (in the RM only) as well as producing physiologically realistic curvature patterns (Figure 3.1). The RM model also had the lowest AIC values for most of the model fits (0.55). PKM did not perform well when data above the optimum was not well defined resulting in unreasonable Tu values (>50℃ or <0.5℃ above Tm). Additionally, PKM had a slight positive bias for the AT thermal sensitivity metric as TI was undefined and set to 0.

EM like PKM often poorly estimated Tu and TI when growth above or below the optimum was not well defined by the data. Additionally, EM was not generally chosen due to the unrealistic nature of the sharp peak at the optimum level (Table 3.1; Figure 3.1). Comparisons showed areal thermal sensitivities (AT, OAT, and UAT) estimated by

the EM were significantly lower due to the biphasic linear response with no curvature about Tm ($P<0.01$); as such, EM results were not included in comparisons of areal thermal sensitivity.

QM had the lowest AICc's (0.93) for most of the datasets because it had the fewest parameters (2) of any of the five models (Appendix B: Chapter 3). QM was not chosen as the overall best fit model as it often did not provide good fits for Tu and Tl simultaneously and did not consistently predict precise Tm values due to its rigid nature (Figure 3.1). PM had the lowest AIC's for a large proportion of the datasets (0.54), as many only measured growth around the optimum leaving the upper and lower tails undefined. Similar to the QM, PM generally did not fit both Tu and Tl, however PM did precisely predict Tm (Table 3.1). In addition, PM did not contain any thermal parameters and often did not result in estimates of either Tl or Tu as real roots could not be found. None of the models showed any difference in their ability to fit either specific body sizes or taxonomic groups (Table 3.1).

3.4.2 Sensitivity Metric

As expected, there were strong positive relationships between the various measures of thermal sensitivity; however some patterns in the metrics did emerge (Figure 3.6). The areal metrics provided an integrative measure of thermal sensitivity as the correlation coefficients were higher (range $r = 0.76 – 0.91$) than those within linear metrics ($r = 0.67 – 0.80$). Furthermore, the information contained in the areal metrics encompassed that provided by linear metrics as correlations were very high for similar pairs of critical temperatures (0.94 AT:LT; 0.97 OAT:OLT; 0.97 UAT:ULT). Overall, AT provides the best depiction of thermal sensitivity; however, incorporating the upper tolerance range (UAT) may provide some additional information as UAT was less

strongly correlated with AT (r = 0.76) than the OAT : AT comparison (r = 0.91; Figure 3.6).

3.4.3 Interspecific comparisons

Warm water species possessed lower thermal sensitivity across all metrics as the slopes for all comparisons increased with Tu and Tm (Table 3.2; Figure 3.7). However, only the relationships between sensitivity and Tu were statistically significant ($P<0.01$), with the exception of Tu – LT, despite the strong relationship between Tm and Tu ($P<0.001$). A significant relationship was also evident between thermal sensitivity metrics and von Bertalanffy's $L\infty$ ($P<0.05$) or maximum age such that species with a high $L\infty$ or older maximum age were more thermally sensitive (Figure 3.7), notwithstanding the lack of relationship between $L\infty$ or maximum age and Tm or Tu (Table 3.2).

No significant relationship existed between thermal sensitivity metrics and any of lower temperature (Tl), von Bertalanffy K, temperature range, or latitudinal range (Table 3.2). In addition, no statistically significant differences in thermal sensitivity among inhabited environments were observed, although freshwater species were generally the least sensitive and had the highest Tm and Tu, with diadromous and marine species displaying similar sensitivity (Figure 3.8). For all comparisons the PGLS results did not differ between the COI phylogeny and the taxonomically based phylogeny (results not shown).

3.4.4 Body Size Comparisons

Thermal sensitivity decreased with increasing body size up to between 10 and 30% of $L\infty$ as there were statistically significant positive relationships between thermal sensitivity parameters and body size across the species examined (Figure 3.9). Beyond

this body size a zero or slightly negative relationship was prevalent. Within species all sensitivity parameters, with the exception one species' ULT, showed similar patterns across body sizes.

3.5 DISCUSSION

Thermal performance data on a range of fish species were compiled and compared using the same suite of thermal sensitivity metrics and models. Results suggested that two models were more informative in describing thermal sensitivity, and that the two types of thermal sensitivity metric examined showed similar patterns. Across species, thermal sensitivity increased with lower optimum and upper temperatures (Tm, Tu), and large asymptotic body sizes ($L\infty$) or long lived fish. Combining results of intraspecific changes in temperature growth relationships through ontogeny indicated young life stages were more sensitive to temperature changes. An important consideration for the following discussion was the number of species represented in the analysis. Available information was collected for 25 temperate zone species from seven taxonomic orders, which represents a relatively small proportion of the current global inventory of fish species >31,000 within >60 taxonomic Orders (Eschmeyer *et al.* 2010). The inclusion of tropical and polar zone species would undoubtedly influence the results (Stillman 2003; Tewksbury *et al.* 2008; Deutsch *et al.* 2008) as no extreme stenotherms were included in this analysis. That said, many of the recent changes in marine species' distributions have been identified in temperate zones (Rijnsdorp *et al.* 2009). Moreover, providing a synopsis of the available data and methodologies sets the basis for moving forward in comparative studies of thermal sensitivity and may spur on the rapid collection and compilation of data.

Of the five models compared, those developed for describing the relationship of environmental variables with either bacterial growth in the Ratkowsky Model (RM) or

aquatic organism metabolism in the Parker Model (PKM) provided the best fits and most information on temperature growth relationships. In previous studies, the quadratic model (QM) was most often used for modeling temperature-growth relationships (*e.g.* Buckel *et al.* 1995; Baras *et al.* 2002). The choice of QM was likely driven as the purpose for many of these studies was to define an optimum temperature for growth in aquaculture (Imsland *et al.* 1996; Bjornsson and Steinarsson 2002) and the QM model provided the best fit for many of the datasets using AICc criteria, in addition to providing reasonable estimates of Tm. Results shown here indicate QM does not provide good estimates of thermal sensitivity and depending on the shape of the relationship may not predict Tm precisely.

For describing temperature growth relationships, RM and PKM prevailed, as they were intrinsically flexible but provided enough rigidity in the shape of models around the critical levels Tm, TL and Tu. Moreover, they followed biologically and physiologically realistic patterns, including a single maximum and an asymmetric skew toward low temperatures (Huey and Kingsolver 1989). The biggest difference between the PKM and RM was in how they model the maximum or minimum growth temperatures. The RM model continues on a downward trajectory as the tails pass through zero growth, whereas the PKM reaches an asymptote at one or both tails as growth approaches zero. In results shown here, the RM generally fit best, as the majority of individual datasets contain few, if any, data points with negative growth at either low or high temperatures. The PKM may prove to be the best model for future studies as datasets covering the full range of temperature-performance relationships suggest that growth at the low extreme will asymptote rather than continue downward (Malloy and Targett 1991; Edsall *et al.* 1993; Baras *et al.* 2002). This relationship will be particularly true if length growth rather than weight growth is used, as losses in body length generally only occur after periods of extended stress or malnutrition (Huusko *et al.* 2011).

Previous model comparisons were restricted to the RM and EM for Atlantic Salmon (Forseth *et al.* 2001). In their work the RM was largely chosen as the curvature around Tm was suggested to be more realistic than EM's biphasic linear nature. They suggested that perhaps the EM would be more suited to an individual fish's response across a range of temperatures. Similar to the idea of reaction norms where the responses of genotypes within a species are compared across a range of environmental variables (reviewed by Pigliucci 1996), species and populations are more likely to exhibit some plasticity in response to temperature, which will be better represented by the range of Tm's as provided by RM's curvature.

To date, thermal sensitivity has mainly been calculated through linear 'tolerance' and 'performance' ranges (Huey and Stevenson 1979). Here using area under the thermal performance curve metric (AT) the characteristics of these two measures were combined. Despite the strong correlation between linear and areal measures I advocate the use of the areal metrics as they provided more information on overall thermal sensitivity in a single measure. Results showed that there was no great advantage to subdividing AT into various performance ranges, however, with the concerns over rising global temperatures, the information contained in the upper area (UAT) may prove to be a good estimate of sensitivity to warming temperatures.

The mechanistic basis for many of the results shown here, particularly the relationships between thermal sensitivity and upper temperature fall within the newly developed and perhaps unifying concept describing temperature performance relationships termed oxygen and capacity limited thermal tolerance (OCLTT; Pörtner 2001). Briefly, OCLTT attributes the decline in performance at upper and lower temperatures to the capacity limitations of oxygen delivery systems to organs and mitochondria. In cold water, aerobic capacity and decreased production of ATP in muscle mitochondria becomes limiting to circulation and ventilation, whereas in warm

water excessive oxygen demand causes a decrease in body level oxygen concentration that can not be compensated. To alleviate the issues of aerobic capacity in cold water, cold adapted species have evolved higher mitochondrial densities or have changed the functional properties of mitochondria such that they can function better in the cold (Johnston *et al.* 1998). These adaptations have the disadvantage that resting and standard metabolic rates are higher at a given temperature to cover the increased cost of mitochondrial synthesis and proton leakage (Rolfe and Brand 1997; Fangue *et al.* 2009). Higher metabolic rates decrease metabolic scope reducing the upper thermal limits thereby increasing the thermal sensitivity of cold water species as was shown here and elsewhere (Brett 1970; Peck and Conway 2000).

Ontogenetic changes in thermal niche of fish have been reported for many species with the general consensus that decreases in both thermal tolerance and thermal optima occur in later life stages. This pattern is attributable to decreasing mass-specific metabolic rates with increasing body size (Duston *et al.* 2004; Bjornsson and Steinarsson 2002; Imsland *et al.* 1996). The decreased thermal sensitivity from small body sizes up to 10-30% of maximum body size coupled with the decrease or plateau thereafter closely match the changing pattern of metabolism and consumption through ontogeny (Post and Lee 1996; Bochdansky and Leggett 2001). It should be noted that the full picture of thermal niches and body size was not depicted and what was shown here likely represents an underestimate of thermal sensitivity as data were available for only late-larvae / early juvenile to subadult life stages. These results do not cover the most sensitive life stages, which are during the eggs-early larvae as well as during maturation and spawning (King *et al.* 2007). The decreased thermal range in newly hatched and early feeding fish is due to their energetic and developmental constraints from decreased foraging ability (Miller *et al.* 1988), poor food conversion (Zambonino Infante and Cahu 2001), and insufficient capacity of internal organs (Pörtner *et al.* 2004).

The increase in thermal sensitivity during maturation and spawning is due to the increased oxygen demand of developing gonads which narrows limits set by the OCLTT (Pörtner and Farrell 2008) such that in years in which temperatures are outside the required thermal window, spawning will either be protracted or skipped (Hutchings and Myers 1994; Takasuka *et al.* 2007).

One the strongest relationship's with thermal sensitivity was for L_∞, such that species with large L_∞ were more sensitive to temperature change. Coupled with this was the similar, although weaker, relationship between thermal sensitivity and the maximum age of species. While the mechanistic bases for these relationships are unknown, potential explanations exist. First, species with large L_∞ and high maximum age are characterized by having large length at maturity, and large clutch size (Winemiller and Rose 1992). Following life history theory, large, long-lived species with repeat reproduction show increased lifetime fitness, compared to small, short-lived species, in stochastic environments (Schaffer 1974), as they have potential for a larger number of spawning bouts which, given their large clutch size, makes each individual growing and spawning season less important to the fish's overall lifetime reproductive success. This pattern suggests that long lived species may have adapted a narrow thermal niche. Similar hypotheses were made following a recent meta-analysis of longevity, demographic parameters and climate variability, as results suggested longer lived species were more resilient to climate driven changes due to the persistence of adult life stages and the ability to reproduce over several years (Morris *et al.* 2008). However, the data compiled by Rijnsdorp *et al.* (2009) on the current changes in species distribution or population responses to climate change in the northeastern Atlantic indicated that five of eight of the species affected were long lived and four of those five had maximum body sizes greater than 70cm (L_∞ Froese and Pauly 2011). The increased thermal sensitivity of large L_∞ fish is important to study further in light of changing climate as these species

also possess a higher susceptibility to extinction risk due to their life history characteristics and the harvesting pressures they experience (Reynolds *et al.* 2005; Olden *et al.* 2007).

In the current analysis, data sets were restricted to laboratory based studies as to examine the direct influence of temperature on growth and to not introduce any of the extraneous, unobserved or uncontrolled variables inherent to survey or field collected data. Further, laboratory studies are important for populations or species that have small or reduced population sizes or reside in restricted habitat ranges as the tolerable environmental variables may be broader than those in which they currently dwell (Algar *et al.* 2009; Hall and Thatje 2009). That said, there are benefits to performing comparative analyses and validating lab based results of thermal sensitivities in natural settings, as species specific sensitivity will be affected by the interactions of numerous biotic and abiotic variables. Combining results from laboratory experiments with field observations would allow for more informed decisions regarding the impacts of climatic factors on population parameters. However, the transportability of laboratory results to natural environments has been questioned (Sloman and Armstrong 2002; Swanson *et al.* 2005), particularly when results are obtained from populations that have been reared in captivity for multiple generations and have been subjected to advertent or inadvertent selection (Hena *et al.* 2005). Moreover, studies have shown marked differences in physiological and ontogenetic rates when directly comparing laboratory and field studies (Gozlan *et al.* 1999). One option for combining these two types of study is to use a Bayesian approach where results from laboratory studies can be used as the priors for spatial and habitat modeling of species distributions in the wild (Bal *et al.* 2011). Another option would be to use individual or simulation based modeling with the environmental responses taken from laboratory studies and the variability in environmental parameters taken from natural environments (e.g. Holker and Breckling 2002; Chapter 5).

The incorporation of phylogenetic data into comparative analysis has become increasingly frequent (*e.g*. Pinsky *et al.* 2011) due to the increased availability of genetic sequence data, familiarity with analysis and the availability of methods for analysing mixed effects models or specifying distinct error structures (Butler and King 2004). Incorporating phylogeny into the analyses presented here changed inferred patterns for many comparisons, particularly those that were marginally significant prior to phylogenetic corrections, reinforcing the importance of using these relationships in comparative analysis. In the current work, the phylogenetic relationship of species were depicted using a single section of the mitochondrial COI gene, as sequence data for this gene were available for 19 of 25 species examined. The use of only the COI gene in this context relies on the assumption that the phylogeny inferred from the gene is the same as phylogeny of the species. Ideally, multiple genes or entire genomes would be used in the reconstruction of phylogenies (Gontcharov *et al.* 2004); nonetheless, the COI-based phylogeny used here corresponded to expected systematic relationships, and adequately described the relationships among species, as when results were compared to those using a phylogeny based on taxonomy with equal branch lengths there were no significant differences between model results.

Temperature growth relations have a mechanistic basis which can be used as the foundation for studying the impacts of climate change on the thermal sensitivity of performance. In the current analysis the relationship of thermal sensitivity with growth was suggested to be influenced by a species optimum and upper temperatures and asymptotic body size L_∞ and maximum age. Within species thermal sensitivity was indicated to be influenced by life stage. The use of laboratory studies depicted similar patterns of temperature- growth relationships that can be described using one of two previously published models. New metrics of thermal sensitivity integrating performance breadth with the model's curvature were developed and should be explored in further

studies. Additional work should focus on identifying significant interactive relationships and the important climatic variables being altered under the current climate change scenarios. Overall, the methods presented here provide tools to examine the relative sensitivity of species or life stages to temperature and should be considered in future study.

Table 3.1: Summary of information on combined model selection criteria. Tm, Tl and Tu represent the proportion of fitted data sets with model estimates within ± 2℃ of the median of all five models. AIC and AICc represents the proportion of fitted datasets within ±2 of the minimum AIC or AICc for each specific dataset. Selected indicates the proportion of instances specific models were chosen as the best fit. Thermal parameters indicated the number of biologically meaningful parameters (Tu, Tl and Tl) estimated in model fitting. Taxonomic Orders and size classes detail the number of occurrences in the best fit models.

Model	Tm	Tu	Tl	AIC	AICc	Selected	Thermal Parameters	Taxonomic Orders (Total=7)	Size classes (Total=5)
QM	0.58	0.32	0.52	0.17	0.93	0.03	1	2	1
PM	0.79	0.25	0.26	0.54	0.00	0.02	0	1	0
EM	0.81	0.22	0.65	0.04	0.04	0.04	3	3	2
RM	0.87	0.46	0.84	0.55	0.04	0.61	2	6	4
PKM	0.84	0.25	--	0	0.01	0.30	2	6	5

Table 3.2: Slopes of relationships between thermal niche parameters, life history parameters and thermal sensitivity metrics including observed temperature range in nature (Temp. range) and species latitudinal range (Lat. Range) from linear regression analysis using phylogenetic generalized least squares regression. Slopes in italics represent outputs after influential data points were removed from regressions. Astrices represent statistically significant relationships (P<0.1*; P<0.05**; P<0.01***).

Metric	Parameter							
	Tm	Tu	Tl	K	Linf	Max. Age	Temp. Range	Lat. Range
LT	0.42	*0.34*	-0.05	10.5	-0.04***	-0.03	-0.01	0.01
OLT	0.12	*0.15***	-0.01	3.7*	-0.02***	-0.06*	0.04	0.02
ULT	0.04	*0.31***	-0.10	4.64	-0.02*	0.03	0.06	0.01
AT	0.21	*0.21***	-0.06	3.19	-0.02***	*-0.09***	0.07	0.02
OAT	0.08	*0.12***	-0.01	1.77	-0.01***	-0.01	0.02	-0.01
UAT	0.01	0.14**	-0.03	2.01	-0.01*	-0.02	0.03	-0.02
Tm	--	0.72***	*1.04****	7.59	-0.01	-0.02	0.07	0.08
Tu	--	--	*1.14****	10.81	-0.02	0.01	0.15	0.12
Tl	--	--	--	4.54	0.01	0.05	-0.02	0.15

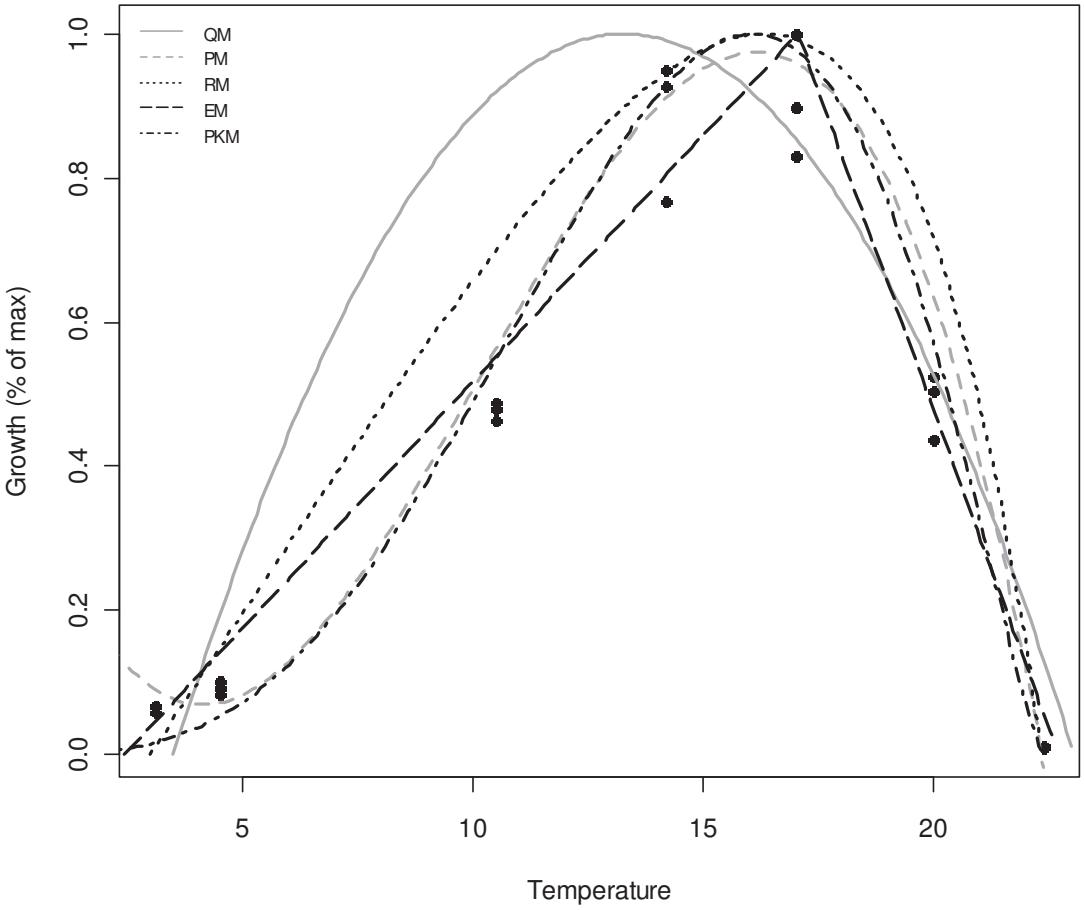

Figure 3.1: Model fits for five proposed temperature growth relationships to the same data set. See text for abbreviations. Symbols represent the raw data values used to fit the models.

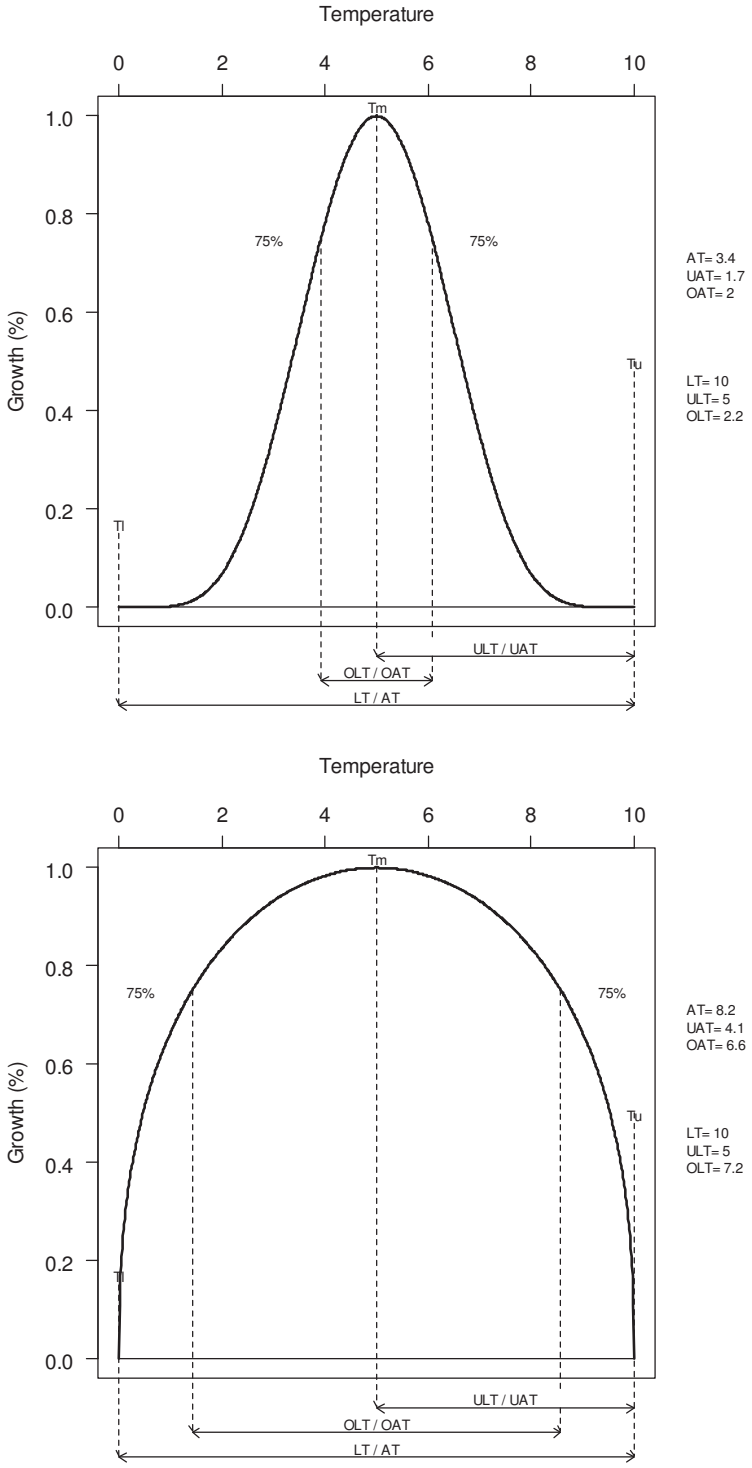

Figure 3.2: Comparison of temperature – growth curves for two hypothetical species showing similar linear temperature tolerance (LT) and different areal thermal tolerance (AT). Abbreviations are defined in text.

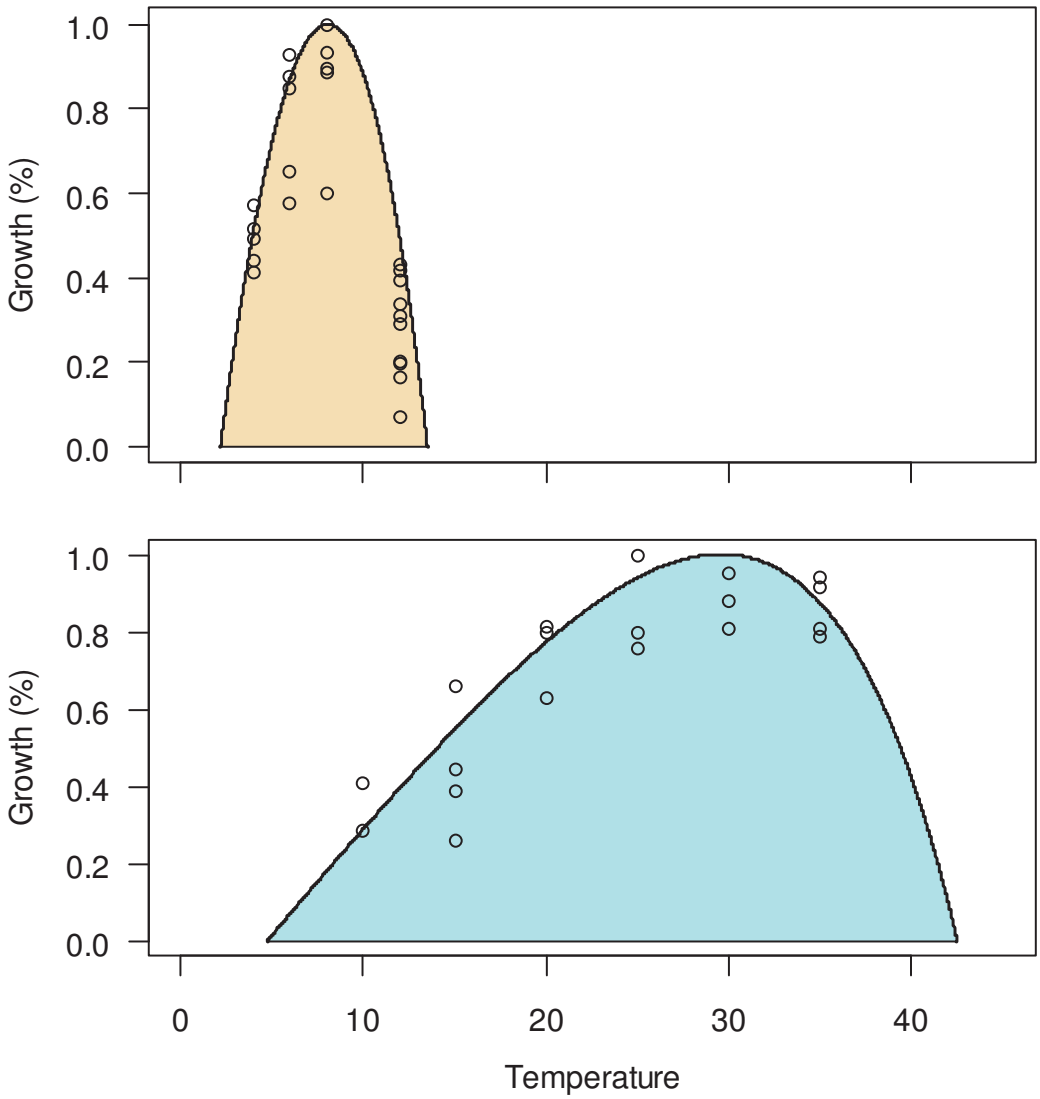

Figure 3.3: Example plots of RM for two different species showing markedly different thermal biological profiles and sensitivities. Open symbols represent raw data points used in model fitting.

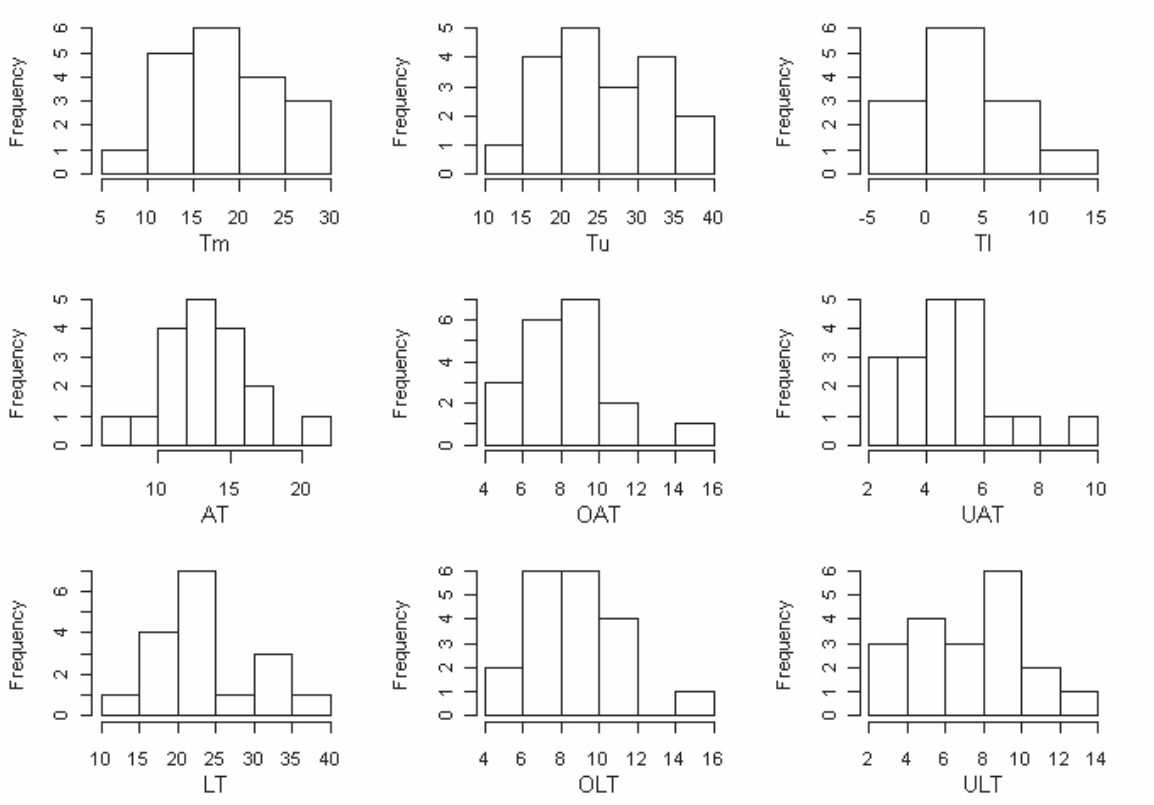

Figure 3.4: Histogram of median species specific critical thermal temperatures and thermal sensitivity metrics from best fit models. Sample size in Tl histogram was reduced as Tl could not be estimated from PKM. LT and AT from PKM models were estimated with Tl=0.

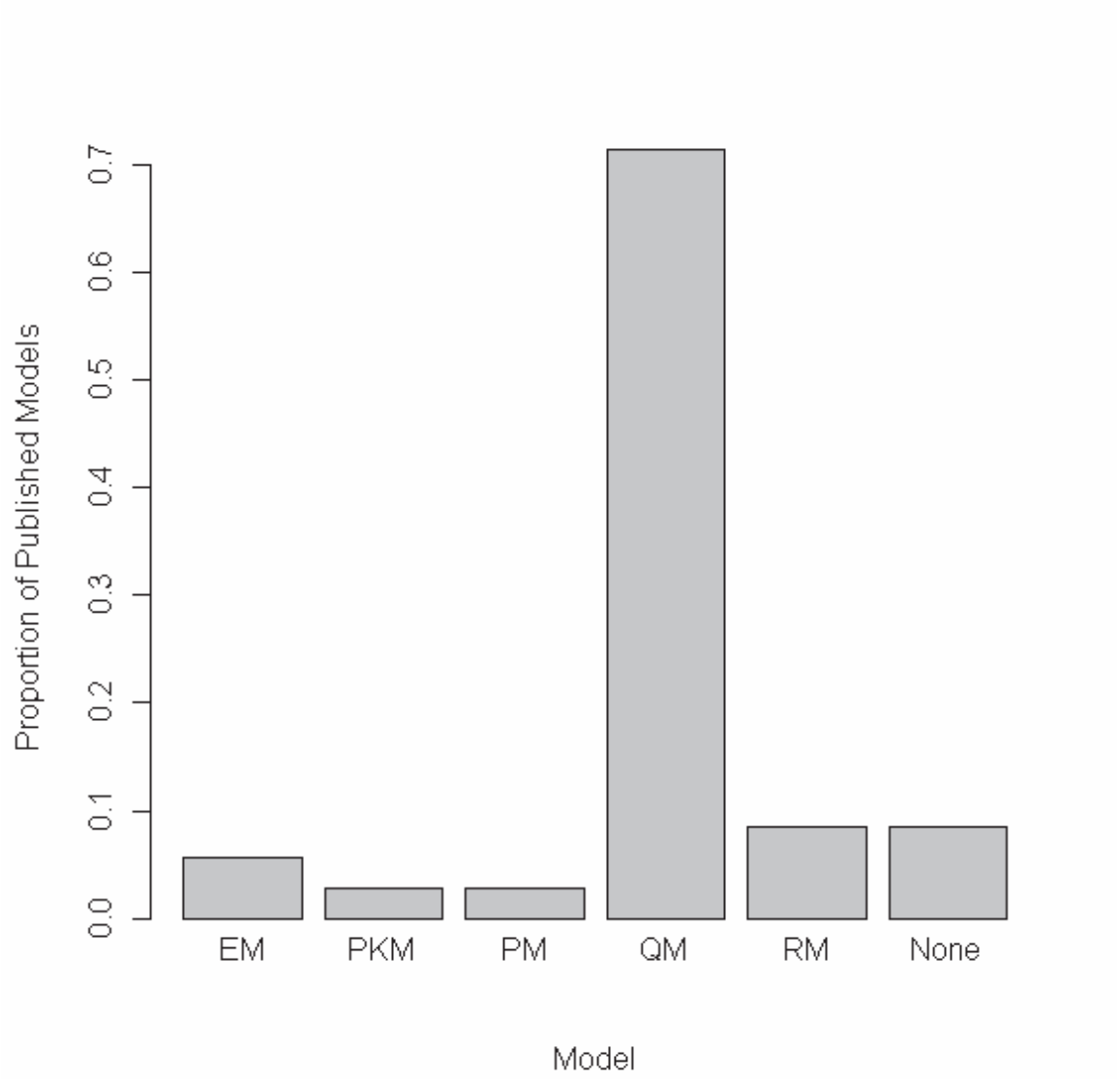

Figure 3.5: Proportion of studies selecting specific models for describing temperature growth relationships.

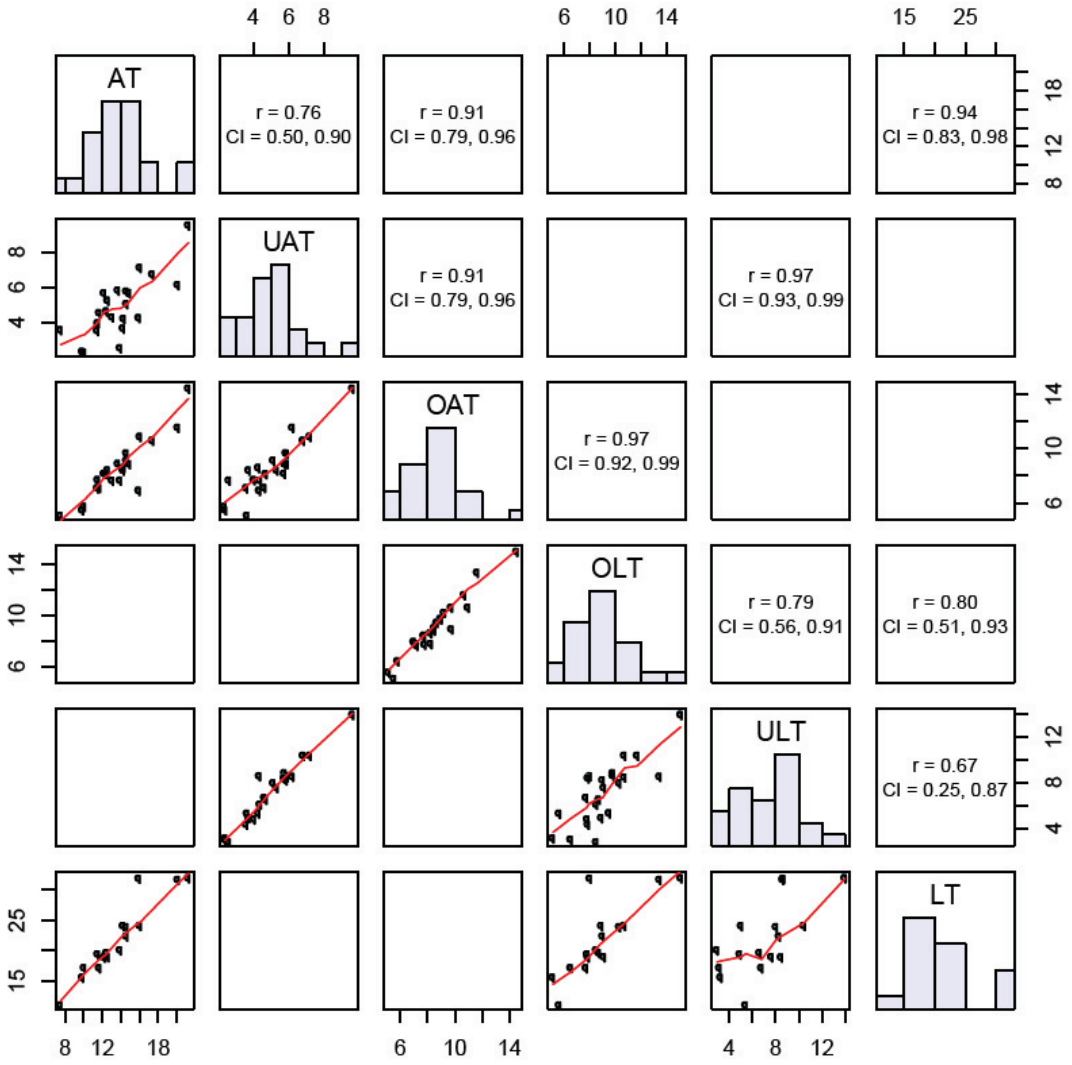

Figure 3.6: Applicable comparisons of area and linear thermal sensitivity metrics. Lower matrix shows plots of raw data with lowess smoother through data points. Diagonal represents the histograms of data points for each metric. Upper matrix represents the Pearson correlation coefficient with 95% confidence intervals.

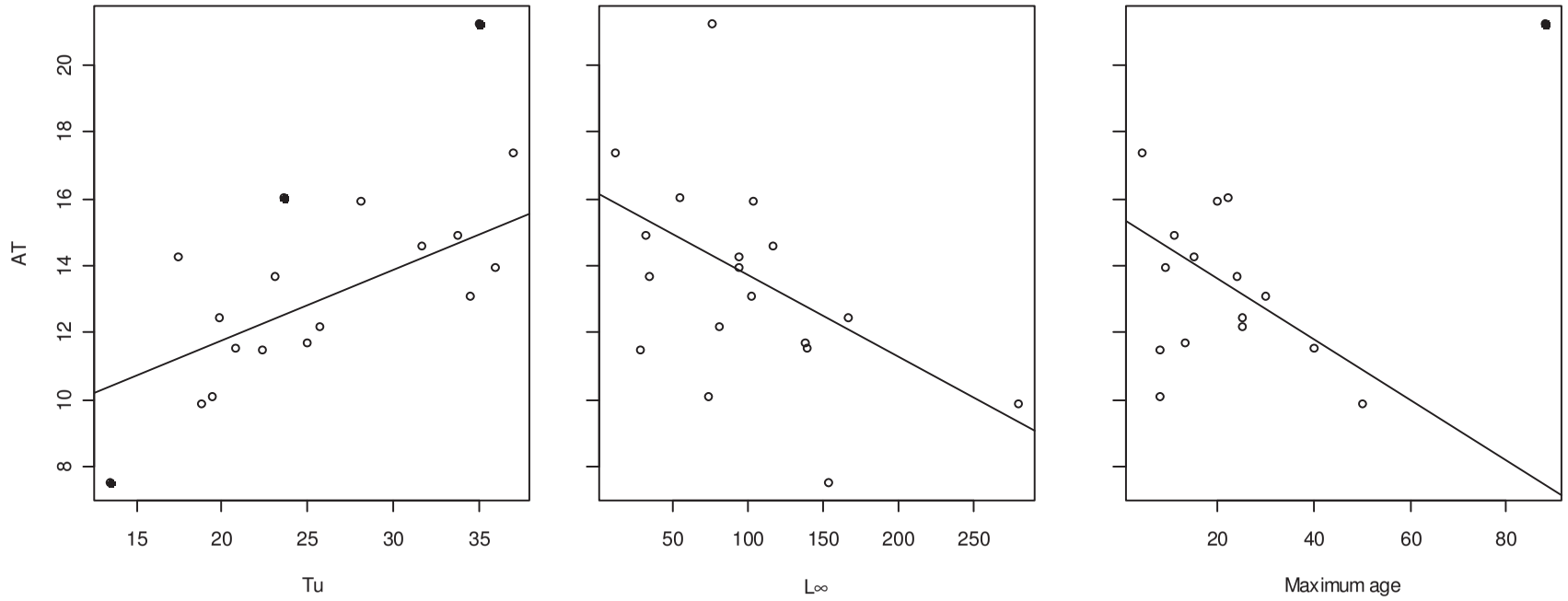

Figure 3.7: Statistically significant comparison of the thermal sensitivity metric AT with upper performance temperature (Tu - ℃), asymptotic maximum body size (L∞ - cm) or maximum age (years) using phylogenetic generalized least squares. Solid lines represent relationships PGLS regression best fit lines. Filled symbols represent influential points removed from regression analysis.

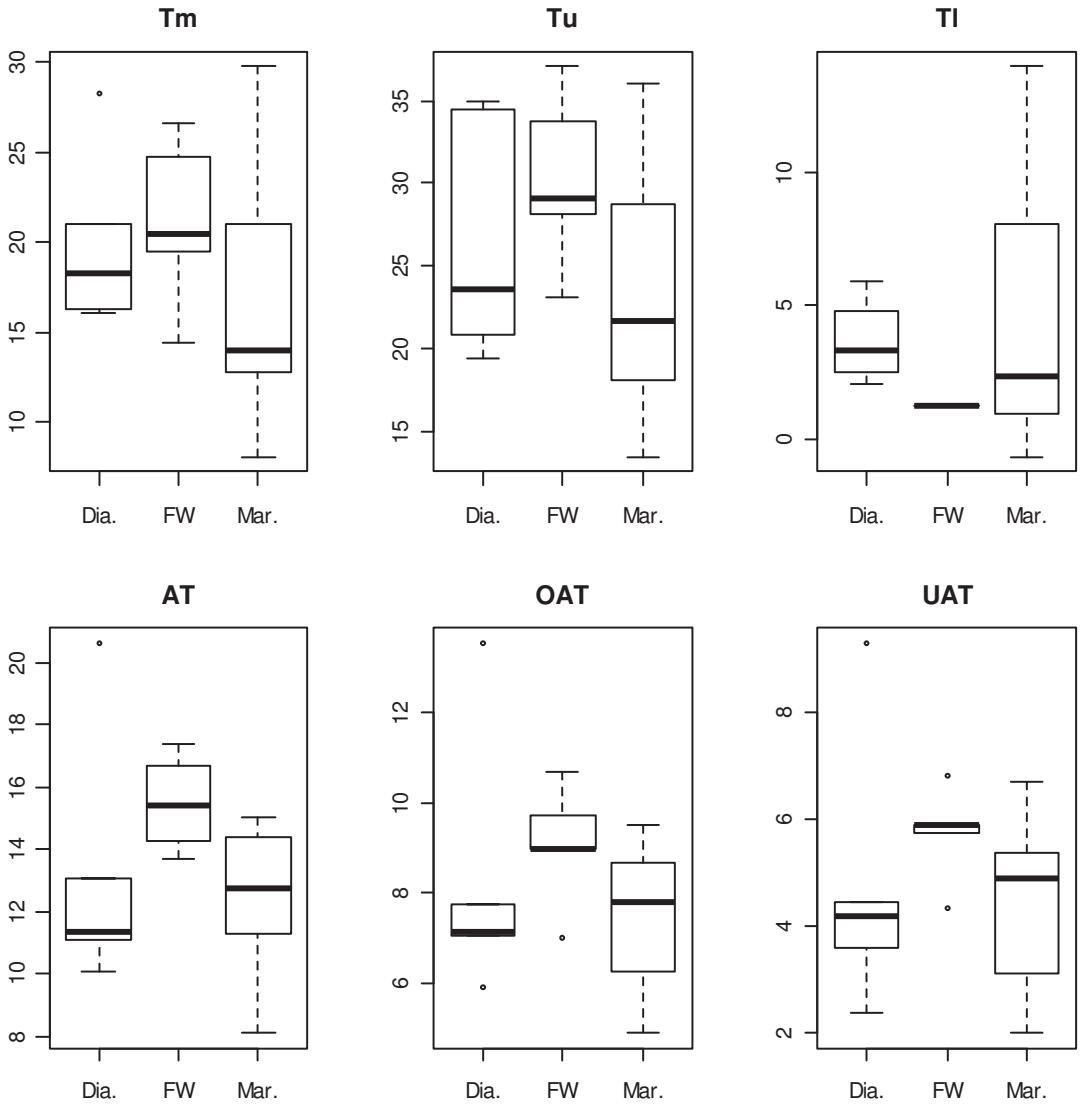

Figure 3.8: Comparison of three thermal sensitivity metrics and thermal niche parameters for diadromous (Dia.), freshwater (FW) or marine (Mar.) species.

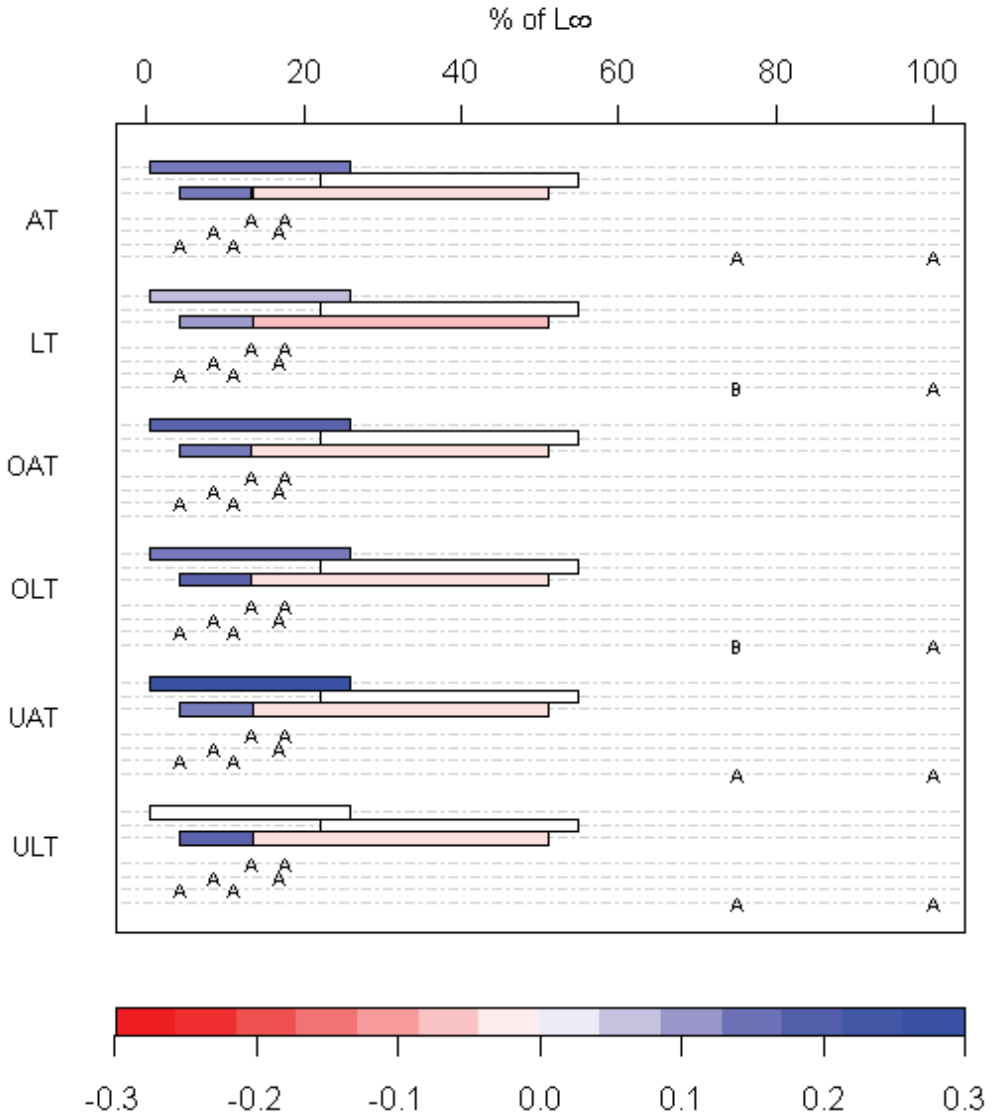

Figure 3.9: Slopes of relationships between thermal sensitivity and body size for seven fish species. For each of three species and six thermal sensitivity metrics horizontal coloured bars represent statistically significant slopes between thermal sensitivity parameters and body size. The length of each bar represents the range of body sizes included in the estimate of slope. Blue bars represent decreasing sensitivity across size ranges whereas red bars represent increasing sensitivity. For four other species where only limited body size groups were available, estimates of thermal sensitivity were compared by permutation tests. Within a species and metric, body sizes sharing the same letter were not statistically different (P>0.05).

CHAPTER 4: RESPONSES TO PERCEIVED ENVIRONMENTAL THREATS IN THE ENDANGERED ATLANTIC WHITEFISH *COREGONUS HUNTSMANI*

4.1 ABSTRACT

Identifying and evaluating the threats to the persistence of endangered species are key components to conservation programs. The endangered anadromous Atlantic Whitefish have been restricted to a single population in three land-locked lakes in southwestern Nova Scotia, Canada. The only other documented population on the Tusket River has recently been extirpated which was thought to be due to a combination of factors including loss of preferred habitat through damming, low pH from acid rain, poaching, and the illegal introduction of fish predators. Similar to other species, the future threat of climate warming may affect the persistence of Atlantic Whitefish. The relative importance of these threats has never been assessed.

The impact of low pH on Atlantic Whitefish was assessed using a series of laboratory experiments. As pH sensitivity was known to change through early ontogeny, these experiments were designed to determine the effects of low pH on survival, development and growth through the early life stages of Atlantic Whitefish. To begin to define the thermal niche of Atlantic Whitefish the interactive effects of temperature and pH on growth of juvenile Atlantic Whitefish were studied. Furthermore, the thermal sensitivity of Atlantic Whitefish was compared to other members of the family Salmonidae where previously published results where available.

Results suggest that current pH regimes will not influence the persistence of Atlantic Whitefish in the Petite Riviere and that low pH was not the sole contributing factor to the loss of the Tusket River population. With projected increases to pH on the Tusket River simulation model results suggest survival rates should improve dramatically should repatriation experiments be attempted.

Atlantic Whitefish were less sensitive to low pH and have intermediate thermal sensitivity compared to other similar species. Experimental results suggest a strong increase in thermal sensitivity with decreasing pH as growth rate and optimum temperature for growth were both reduced when pH declined below 4.75. Current temperature regimes will likely not influence the growth and productivity of Atlantic Whitefish, however, with the projected future warming scenarios or in low pH environments, this may change. By the restoration of anadromy to the Petite Riviere Atlantic Whitefish, the impacts of warming may be mitigated as more thermal refugia will be available.

4.2 INTRODUCTION

Conservation practices seek to identify the historical, contemporary and future threats to species persistence. Threats may come from a variety of anthropogenic, interspecific, and / or environmental factors and result in negative impacts to populations. Examining the impacts of threats on small and inbred populations is of particular value as these groups are thought to be more susceptible to negative impacts due to their reduced genetic diversity and implied reductions in evolutionary potential and ability to meet the demands of changing environments (Frankham 1995a). Genetic diversity decreases in small populations through genetic drift and the increased probability of breeding between related individuals. Inbreeding results in fitness decreases, or inbreeding depression, as higher genetic homozygosity levels allow for the expression of deleterious recessive alleles as well as the loss of fitness improvement from overdominant loci (Charlesworth and Charlesworth 1999). The length of time a population spends in an inbred state will affect the level of inbreeding depression displayed, as populations that have been inbred for many generations will have some

deleterious recessive alleles purged through natural selection (Hedrick 1994). Purging will only be effective for alleles that are strongly tied to fitness or are genetically linked to alleles under selection (background selection) for multiple consecutive generations (Crnokrak and Barrett 2002). The impacts of inbreeding depression are more pronounced under stressful or stochastic conditions (Armbruster and Reed 2005).

Determining which threats have been most influential in a species decline often begins as a broad overview of potential factors, with the prime candidates being identified from available information on factors known to have changed concomitantly. From there, a suite of correlational analyses and directed measures can winnow the list down to the most likely threats. Understanding the relative impact of the threats on population persistence should be addressed both on a threat by threat basis as well as in combination as complex patterns of responses can emerge. Further, threats need to be assessed across multiple life history stages as responses may change (Marcus and Brown 2003).

In the short term, some anthropogenic and interspecific threats can be reduced through human intervention. For example, fishing pressures or illegal capture and retention of individuals can be reduced by fishery restrictions or enhanced protection programs, respectively. Interspecific competition or predation can be mitigated through selective culling programs (Sanz-Aguilar *et al.* 2009). Similarly, environmental threats may be reduced through the translocation of individuals to new and more suitable environments, or through *in situ* habitat enhancements such as the application of calcium carbonate to reduce the impact of acidification in rivers (Watt 1986). However, for effective long term conservation it is important to understand how and at what level threats become detrimental to the species in question. Moreover, to restore species to their natural ranges and for successful translocation, work needs to be done to

determine whether historical threats remain, their relative importance and if they can be mitigated.

Atlantic Whitefish are a member of the genus *Coregonus* which are distributed throughout the north temperate and polar regions of North America and Eurasia. Although Atlantic Whitefish were thought to be historically widespread, by the time it was recognized as a distinct species (Scott 1987) extant populations were only documented in two watersheds, the Petite Riviere and the Tusket River, both in Nova Scotia, Canada. Both rivers are located in south-western Nova Scotia. In the Tusket River, Atlantic Whitefish were anadromous whereas in the Petite Riviere they were predominantly freshwater resident and are thought to have been landlocked for most of the past 100 years (Bradford *et al.* 2004). Almost as soon as the species was identified, it was noted to be in decline and the last positively identified Atlantic Whitefish specimen on the Tusket River was in 1982 (Bradford *et al.* 2004). The Petite Riviere population resides in three semi-natural lakes covering a total area of ~16km^2 and is presumed in low abundance due to limited habitat availability. Recent genetic analyses showed that the Petite Riviere Atlantic Whitefish population has extremely low genetic diversity and small genetic effective population size, and likely suffered a population bottleneck around the time the population became landlocked (Chapter 2). This remaining population has had a low effective population size for much of its recent history, suggesting the possibility of inbreeding depression and lack of evolutionary potential to survive in stressful environments (Chapter 2).

The factors that contributed to the loss of the anadromous Tusket River population were thought to include decreased environmental pH, the reduction of available habitat through the construction of a hydroelectric dam with inadequate fish passage, unauthorized introductions of non-native predators, and poaching (Edge and

Gilhen 2001). Here, I will focus on abiotic environmental threats of pH and temperature to Atlantic Whitefish.

pH declines in some of Nova Scotia's watersheds have been recorded since they were initially measured in the 1950's (Wiltshire and Machell 1981) and were suggested to be a key threat in the decline of a number of Atlantic Salmon populations (Watt 1987). The acidification of Nova Scotia watersheds resulted from the long range transmission, and subsequent deposition, of industrial sulphate emissions originating from the Ohio Valley, northeastern United States and Ontario and Quebec in Canada (Clair *et al.* 2002). The buffering capacity of local bedrocks (granite, gneiss and pyritic slate; Roland 1982) is naturally low and sources of carbonate are scarce (Watt *et al.* 2000). Both current and paleo- limnological studies have revealed variation in baseline pH's, both among and within watersheds (Figure 4.1), in many cases generated by localized pockets of carbonate rich glacial till (drumlin fields) remaining from the Wisconsinan deglaciation (Clair *et al.* 2007; Ginn *et al.* 2007; 2008).

The Petite Riviere has not been subjected to the same level of pH decline as other watersheds (Figure 4.1), including the Tusket River. The higher buffering capacity of the native soils against pH depression may be a significant factor that has favoured the persistence of Atlantic Whitefish in this system. Based on current observations and future projections of sulphate emissions, pH levels have stabilized within regional watersheds and pH levels in the most acidified watersheds are expected to increase over the next several decades (Clair *et al.* 2004). The projected increase for the Tusket River should bring mean annual pHs >5.0 (Clair *et al.* 2004).

In other regions that have suffered similar decreases in pH, impacts on fishes have been exacerbated by increased aluminium (Al) concentrations, as Al solubility increases with decreasing pH (Driscoll 1984; Gensemer and Playle 1999). Although total Al levels can be high in most acidified Nova Scotian watersheds, fish do not suffer the

same negative effects as high levels of organic acids chelate the Al rendering it less toxic (Lacroix and Kan 1986).

Within acidified watersheds, pH tends to follow a relatively predictable pattern with the lowest and most variable pH levels in spring and autumn as a result of increased runoff from freshets or melt water (Figure 4.1). Both of these periods represent critical periods for Atlantic Whitefish, and salmonids in general, as these are the seasons for spawning and hatching, which are regarded as some of the more sensitive life stages (Farmer 2000). The impacts of seasonal fluctuations and episodic drops in pH have also been shown to be important to survival of Atlantic Salmon cohorts (Lacroix and Townsend 1987). There has never been a formal assessment of the response of Atlantic Whitefish to low pH.

Increasing climate variability and global temperature increases (IPCC 2007) are additional threats that are common to the Atlantic Whitefish and many other species world wide. Although there is no evidence to support temperature changes within the Petite Riviere, or the larger Northwest Atlantic for that matter (Casey and Cornillon 2001), increases are expected (Chmura *et al.* 2005). To date, temperatures have changed by varying amounts within a region; however, mean global temperature has increased by 0.6°C since 1900 and climate forecasts predict temperatures to continually increase (IPCC 2007). With a projected 2°C global temperature increase by 2060 (IPCC 2007), sea surface temperatures around Nova Scotia are expected to increase by 1.5°C (Chmura *et al.* 2005).

In other fish species, the recent temperature increases have resulted in changes in both growth and phenology (Hughes 2000), as well as shifts in geographic distribution (Perry *et al.* 2005). Temperature affects fish through its influence on physiological rates including growth (Brett 1979), survival rates (Cook *et al.* 2006), development (Fonds 1979), spawning time (Hutchings and Myers 1994), swimming performance (Bernatchez

and Dodson 1985), immune function (Alcorn *et al.* 2002) and foraging success (Bystrom *et al.* 2006). Most fish have specific thermal niches which maximize the efficiency of these processes. Thermal niches are thought to be shaped both by evolutionary history and contemporary processes. The breadth of a species thermal niche can be considered as a proxy for thermal sensitivity (Chapter 3), as species with a wide thermal breadth will be less sensitive to temperature changes. There was no information on the thermal niche of Atlantic Whitefish.

The purpose of this work was to assess the effects of environmental threats on the viability of Atlantic Whitefish in their current environment and under future scenarios. To that end, laboratory based experiments were designed to study the effect of pH on both survival through early ontogeny, and growth of post-hatch larvae through to juveniles. For the earliest egg and larval stages, spawning groups were maintained separately to assess the variability in response among mating groups of Atlantic Whitefish. Spawning groups displaying different responses were genotyped at several microsatellite loci to determine if genetic differences could be described. Thermal niche breadth was examined in juvenile Atlantic Whitefish and was compared with Salmonidae species to determine their relative thermal sensitivity. I explored the interactive effects between the two environmental threats, low pH and temperature, on thermal breadth of juvenile Atlantic Whitefish. Using the results from the laboratory experiments, I compared thermal profiles from within the Petite Riviere lakes and estuary with the thermal sensitivity estimates for Atlantic Whitefish at different pH levels to estimate the potential impacts of climate warming. The effect of low pH on species persistence in the Petite Riviere and the relative impact low pH had on the demise of the Tusket River population was assessed using a simulation model developed in Chapter 5.

4.3 METHODS

All fish used in these experiments were the F1 progeny of wild captured Atlantic Whitefish from the Petite Riviere. Spawnings were performed through the dry fertilization technique (Piper *et al.* 1982) at the Mersey Biodiversity Facility in Milton, Nova Scotia. Unless otherwise stated, fish were transferred to Dalhousie's Aquatron facility as eyed eggs and were hatched and reared in pH 7.0 until use in experiments. Individual fish were only ever used in a single experiment.

4.3.1 Experimental Laboratory

The experimental laboratory was located in the Dalhousie Aquatron facility. Fresh water supply was obtained from Halifax tap water (originating in Pockwock Lake) after passage through two sequential dechlorinators. The lab had 15 experimental tanks (0.66×0.41cm; W×D), supplied by water from five header tanks (0.56×0.30×0.36cm; L×W×D) that each served three experimental tanks. Within each header tank pH levels were continuously measured using multichannel electronic temperature and pH devices (Consort bvba), which controlled the dose rate of dilute H_2SO_4 delivered by dosing pumps (Pulsatron Idex Corp.). Temperature was controlled in the experimental tanks through the addition of immersion heaters and the mixing of heated and ambient freshwater. Treatment pHs in all experiments were achieved by raising or lowering the pH from acclimation levels at a maximum rate of 1.0 pH unit per day.

4.3.2 pH Effects on Egg Viability

The effect of pH on post-fertilization survival was assessed using eggs from two female Atlantic Whitefish retained in separate batches. Each batch was fertilized with the milt collected from two males. Eggs and milt were allowed to mix for 60 s then split

evenly yielding 4 samples of approximately 1100 eggs each. The fertilized eggs were rinsed and water hardened in freshwater of either pH 5 or 7 for three hours prior to transfer to treatment pHs. Eggs from each sample were further divided into groups of 35 individuals, placed into screened incubation pots and moved to experimental tanks. Treatment pHs were 4.1, 4.3, 4.5, 5.0 and 7.0. Each combination of female x water hardening pH x incubation pH was replicated six times. Eggs were exposed to treatment pHs at mean (standard deviation) water temperatures of 5.3 (0.2) ºC for 2 weeks. Two weeks post fertilization eggs were examined using a dissecting microscope to assess survival.

4.3.2.1 Statistical Analyses

Survival data was analyzed as a generalized linear model assuming a binomial distribution with main effects of female, water hardening and rearing pH as well as all potential interactions examined. Post hoc tests were performed using Tukey adjusted least square means.

4.3.3 Low pH on Hatch Success And Yolk Sac Larvae Survival

Fertilized eggs from five separate spawnings, each consisting of the eggs from one female and milt from 2-3 males, were incubated to eyed stage in pH 5.2 and ambient water temperatures. Treatment pHs were 4.0, 4.3, 4.6, 5.2 and 7.3. Eggs from each spawning were separated into three batches for each pH treatment, resulting in 75 batches of 15-20 eggs. Mortalities were counted and removed once daily. Experiments were continued for eight days after 50% hatch date for each spawning group. During this period, dead larvae were removed daily. At the end of the experiment all larvae were euthanized by MS222 overdose and measured.

4.3.3.1 Statistical Analyses

Time to hatching was modeled using a logistic model (equation 4.1) fit to the daily cumulative hatch profiles for each treatment pH. In this analysis all spawning groups were combined as there were no detectable differences between groups for cumulative hatch curves (data not shown). In equation 4.1, *Hatch* is cumulative hatch, *a* is the hatch rate on day 0, *b* is the maximum hatch rate, *D* is the experimental day, D_{50} is the date that 50% of the maximum hatch occurred and *H* is the rate of increase in hatch. The cumulative hatch models were compared across all pH combinations through the addition of incremental parameters (Δ) and an indicator variable (X_i) as in equation 4.2, with all other parameters as in equation 4.1. This method compares all regression parameters for significant deviations between each pair of models (Bates and Watts 1988). Statistically significant Δ's ($P<0.05$) indicate differences between the models being compared.

$$Hatch = a + \frac{b - a}{1 + \left(\frac{D}{D_{50}}\right)^{H}} \qquad \text{Equation 4.1}$$

$$Hatch = \left(a + \left(\Delta a X_1\right)\right) + \frac{\left(b + \left(\Delta b X_1\right)\right) - \left(a + \left(\Delta a X_1\right)\right)}{1 + \left(\frac{D}{\left(D_{50} + \left(\Delta D_{50} X_1\right)\right)}\right)^{\left(H + \left(\Delta H X_1\right)\right)}} \qquad \text{Equation 4.2}$$

The effects of pH and family, as well as their interaction, on overall hatch rate or larval survival were investigated separately with binomial generalized linear models. To compare the genotypes of surviving and non-surviving larvae groups of 50 individuals were genotyped at five polymorphic microsatellite loci, Chu1, Chu4, Chu16, Cocl-lav72 and BWF1, following the protocols outlined in Chapter 2. Genotypes were compared

between groups using an analysis of molecular variance (AMOVA) with the AMOVA function in the R-package ade4.

4.3.4 Low pH On Survival And Growth Of Post Yolk-Sac Larvae And Juveniles

The effects of low pH on survival and growth on three different life stages of feeding Atlantic Whitefish were examined using sequential experiments. The first experiment used post yolk-sac larvae Atlantic Whitefish with an initial mean (standard deviation) total length of 24 (0.3) mm. Larvae were separated into groups of 20 individuals and were maintained in triplicate batches at treatment pH's of 3.9, 4.1, 4.3, 5.0 and 7.0 and a constant temperature of 14.0ºC for eight days. Fish were fed to apparent satiation 4-5 times daily, with mortalities removed as observed. Survival and growth rates on a per tank basis, as well as metamorphic stage (scale coverage- early metamorphosis (10-40%), mid-metamorphosis (40-70%), late metamorphosis (70-90%), juvenile (>90%)), were assessed at the end of the experiment.

The second experiment used metamorphosed Atlantic Whitefish with an initial mean (standard deviation) total length of 39 (3.3) mm. Groups of 25 fish were reared in triplicate for each treatment pH of 4.0, 4.2, 5.0 and 7.0 at a common temperature of 18.0ºC. Fish were fed to apparent satiation 2-3 times daily with mortalities removed as observed. Survival and growth rates were assessed at the end of the 15 day experiment.

The third experiment used juvenile Atlantic Whitefish initial total length of 69 (8.0) mm. Groups of 25 fish were reared in triplicate for each treatment pH of 4.0, 4.2, 4.7, 5.0 and 7.0 at a temperature of 20.0ºC. Fish were fed to apparent satiation twice daily. Survival and growth rates were calculated at the end of the 16 day experiments.

4.3.4.1 Statistical Analyses

The effect of pH on growth or survival was modeled using either Gaussian or binomial generalized linear models, respectively. Within each treatment pH the proportion of fish surviving to a discrete stage of metamorphoses was assessed via a randomization procedure. This analysis compared the observed metamorphic stage data within a pH treatment to 1000 random samples of data across all pH's to determine if the proportions significantly differed from a random sample.

4.3.5 Assessment of pH Impacts on Persistence

Using the stage specific pH – survival responses collected from the above experiments a simulation model was developed in Chapter 5. This model incorporates observed seasonally variable pH levels and temperature variability with the variability in modeled pH – mortality rate regression parameter estimates to predict survival from spawning to the end of the first year of growth. The simulation model was fully described in Chapter 5.3.2.2 with the model incorporating the full suite of variability used here (Table 5.2 model 7). I examined the effects of the seasonal pH profiles from the Petite Riviere and the Tusket River to determine the relative impacts on survival. In addition, I estimated future survival potential in the Tusket River using the projected pH increases (Clair *et al.* 2004).

4.3.6 Temperature x pH Growth Experiments

Interactive effects of pH and temperature on growth of juvenile Atlantic Whitefish were assessed using treatment temperatures of 3.1, 4.5, 10.5, 14.2, 17, 20, 22.4°C and treatment pHs of 4.0, 4.2, 4.75, 7.1. Not all combinations of temperature and pH could be maintained at the same time, due to the constraints of the laboratory; therefore, a

series of three sequential experiments were performed. Each experiment examined growth at all treatment pHs and either two or three treatment temperatures. Specifically, temperatures of 3.1 and 4.5°C were maintained in experiment 1, 10.5, 14.2 and 17°C were maintained in experiment 2 and 20 and 22.4°C were maintained in experiment 3. Twenty fish were exposed to each temperature x pH combination. Fish were fed three times daily to apparent satiation. Across all experiments the mean (standard deviation) initial body size of fish was 98 (1.6) mm with no statistically significant differences in body size between experiments (P>0.05). All experiments lasted 10 days. All fish were measured at the end of each experiment.

4.3.6.1 Statistical Analyses

Growth data derived from each treatment pH-temperature combination was fitted to two nonlinear models that have been used to define the temperature-growth function for a variety of fish species (Chapter 3). The first model, the Parker model (Parker 1974) has the form;

$$G_i = a\left[\left(\frac{T_i}{Tm}\right)\times\left(\frac{Tu - T_i}{Tu - Tm}\right)^{\left(\frac{Tu-Tm}{Tm}\right)}\right]^d \qquad \text{Equation 4.3}$$

where G_i is the growth rate at a temperature (T_i), a is the maximum growth rate, Tm is the optimum growth temperature, Tu is the upper growth temperature and d is the scaling parameter. The second model was the Ratkowsky model (Ratkowsky *et al.* 1983), which has the form,

$$G_i = w(T_i - Tl)\left(1 - e^{g(T_i - Tu)}\right) \qquad \text{Equation 4.4}$$

where Tl was the lower growth temperature Tu was the upper growth temperature, w was the growth coefficient for temperatures between Tl and Tm and g accounted for the downward curvature from Tm to Tl. Optimum temperature, Tm, was be obtained from parameter estimates by solving for the maximum value as (Jonsson *et al.* 2001):

$$\ln(1+g(Tm-Tl))=-g(Tm-Tu) \qquad \text{Equation 4.5}$$

Models were fit to data sets using maximum likelihood estimation with a Gaussian negative log likelihood objective function within the *optim* function in R (R version 2.13). The best fit model to each data set was evaluated with standard Akaike Information Criteria (AIC) calculated as:

$$AIC=2k-2\ln L \qquad \text{Equation 4.6}$$

where k is the number of parameters, L is the likelihood from the best fit model. From the best fit model an estimate of thermal sensitivity was measured as the area under the temperature growth curve (Chapter 3) across the entire range positive growth (Tl to Tu) using the integrate function in R.

Thermal sensitivity, optimum temperature, upper temperature and maximum growth rates were compared using bootstrapping and permutation tests. This procedure randomly resampled the residuals from the original fitted model $\hat{\varepsilon}_j$ and added these deviations to the original growth data (y_i) such that a synthetic response variable (y_i') was created for each bootstrap iteration as, $y_i' = y_i + \hat{\varepsilon}_j$ (Wu 1986). This method was used as it maintained the information contained in the explanatory variable, temperature, which was important for this analysis as datasets contained few observations at each temperature. The density distributions of thermal parameters and sensitivities were

derived for each model from 1,000 bootstrapped samples and were compared between pH levels by permutation tests (Good 2005).

4.3.6.2 Thermal Sensitivity and Climate Projections

Using the best fit growth temperature model for each pH, I estimated the temperatures representing the range of optimum growth temperatures, calculated as the upper and lower temperatures that result in 75% of maximum estimates growth. I compared these temperatures to the cumulative distribution of observed summer temperatures (May 11 – September 30) collected from two locations in the Petite Riviere, one representing the freshwater lentic habitat and the other representing the estuarial temperatures to determine the proportion of time temperatures were above the optimal growth range. To assess the impact of climate change I compared the proportions to those under the predicted 1.5°C increase by 2060 (Chmura *et al.* 2005). The optimum temperature range in estuarial waters was calculated from the pH 7.0 model, as work from elsewhere suggested Atlantic Whitefish growth was not affected by natural salinity levels (Cook *et al.* 2010a).

4.3.7 Comparisons of Thermal Sensitivity Within Salmoniformes

Thermal characteristics of optimum growth temperature, upper growth temperature and overall areal thermal sensitivity (AT; Chapter 3) of juvenile Atlantic Whitefish were compared to those characteristics in other juvenile Salmoniformes species at the same life stages to determine their relative sensitivity. For these comparisons only the Atlantic Whitefish temperature-growth relationship for pH 7.0 was used. Temperature – growth data was available for Brook Trout (*Salvelinus fontinalis;* McCormick *et al.* 1972), Atlantic Salmon (*Salmo salar*; Jonsson *et al.* 2001; Elliott and

Hurley 1997), Arctic Charr (*Salvelinus alpinus;* Larsson *et al.* 2005) and Sockeye Salmon (*Oncorhynchus nerka*; Brett 1974). Where possible, multiple data sets within a species were used in the comparisons.

Prior to model fitting the growth data within each data set were scaled to have a maximum growth rate of one (Chapter 3). This procedure was done to remove the interspecific differences in growth potential as it will significantly alter the measurement of thermal sensitivity but has no effect on optimum or maximum temperature (Chapter 3). Following the same procedures given above both the Parker Model and Ratkowsky models were fit to each data set, with the best model chosen by AIC criteria. The same bootstrapping and permutation tests were used to evaluate differences between optimum temperature, upper growth temperature and thermal sensitivity; however when multiple data sets were available from the same species, the outputs from the bootstrapping procedure from each were combined to generate the distribution of species-specific parameter estimates and thermal sensitivities.

4.4 RESULTS

4.4.1 pH Effects On Egg Viability

Post fertilization egg survival was affected by pH, both at the water hardening and subsequent rearing stages as shown by the significant main effects from the generalized linear models (Table 4.1). Overall, egg survival decreased with decreasing pH and eggs fertilized in pH 5 had lower survival rates than those fertilized in pH 7 (Figure 4.2). The largest decrease in survival was between pH 4.5 and 4.3 as overall mean (standard deviation) rates decreased from 0.76 (0.07) to 0.22 (0.13). Interestingly, no differences in survival were observed between fertilization and survival rates for eggs fertilized in pH 5 or 7 and reared in pH 7 (Figure 4.2). Differences in egg survival were

apparent between the two females used in this experiment, particularly at low pH, as female 1's eggs fertilized in pH 7 showed significantly higher survival rates than female 2 (Figure 4.2).

4.4.2 Low pH On Hatch Success And Yolk Sac Larvae Survival

Hatch success of Atlantic Whitefish was affected by the interaction of low pH and spawning group, as divergent responses between the spawning groups at low pH resulted in two clusters (Table 4.1; Figure 4.3). Specifically, one cluster of two spawning pairs showed comparatively higher hatching success in pHs <4.75. The largest decrease in hatch success differed between these two clusters, as the more pH tolerant cluster showed greatest decreases between pHs 4.3 and 4.1 and the less tolerant cluster had the greatest difference between pH 4.5 and 4.3 (Figure 4.3). Although this study was not designed to detect maternal effects, there were no differences between length, weight, age and years in captivity across females used in matings (data not shown).

Not only were the overall hatch rates decreased in low pH, the time courses of hatch rates were also affected by low pH's as median hatch date (D_{50}) was delayed by ~1 day in pH of 4.3 and ~3 days in pH 4 relative to pH of 7.0 (Table 4.2). Moreover, the rate of increase in cumulative hatch (*H*) was significantly lower in pHs <4.6 (Table 4.2). In addition, variance in parameter estimates increased with pH declines suggesting plasticity in responses between individuals (Table 4.2)

Two interesting results were displayed from the successfully hatched larvae maintained in respective treatment pHs. First, there were no significant differences among spawning groups for larval survival (Table 4.1). Second, the only pH level where survival was significantly reduced was for pH 4.3 (Figure 4.4). Although larval survival did not significantly differ statistically between spawning groups, at pH 4.3, the survival

of larvae from those pH sensitive groups identified during egg stage was markedly lower at a rate of 0.71 (0.07) compared to 0.95 (0.04; data not shown).

4.4.3 Low pH On Survival And Growth Of Late Larvae And Juveniles

pH affected the survival and growth of all three stages of Atlantic Whitefish tested in this section; however the patterns changed with increased development and body size (Figure 4.5). Survival rates of metamorphosing larvae displayed the characteristic pattern of marked decreased survival at pH <4.3 (Figure 4.5a); however, growth rate exhibited no trend with the lowest growth rate occurring at pH 4.3 and the highest growth at pH 4.0. Additionally, the fish at pHs <4.3 possessed later metamorphic stages at the end of the experiment than those at pH 4.3 (Figure 4.6). Juvenile stages of Atlantic whitefish showed characteristic decreases in both survival and growth with reduced pH, with growth being affected at higher pHs than survival. Larger juveniles were more tolerant to low pH for survival (Figure 4.5b, c). Specifically, the largest decrease in survival for early juvenile fish occurred between pHs of 5.0 to 4.2 whereas larger juveniles did not show this same decrease until pHs decline below 4.5. In addition, survival remain substantially higher in larger fish 0.84 compared to 0.37 even at pH 4.0 (Figure 4.5). Growth rates between experiments were not directly comparable the experiment on smaller fish did not include pH 4.5, which was a critical pH level for these comparisons.

4.4.4 Assessment of pH Impacts on Persistence

Model simulations suggested that there was little influence of pH on survival within the Petite Riviere as median survival estimates were 0.92, and had little variability with first and third quantiles of survival at 0.90 and 0.94 (Figure 4.7). The survival estimates in the Tusket watershed were lower and more variable than the Petite with a

median and first and third quantiles of 0.65, 0.60 and 0.69, respectively. With the projected improvement in pH for the Tusket River, the survival estimates increased markedly with a median and first and third quantiles of 0.88, 0.85 and 0.89, respectively (Figure 4.7).

4.4.5 Interspecies Comparisons Of Thermal Sensitivity

The temperature growth relationship of Atlantic Whitefish was best described by the Parker Model and followed the predicted patterns of temperature growth relations shown for other species (Chapter 3). Atlantic whitefish juveniles had a higher optimum growth temperature at 19.1℃ than either Brook Trout (14.8℃), Sockeye Salmon (16.1℃), or Arctic Charr (15.3℃) but were similar to Atlantic Salmon (18.1℃; Figure 4.8). Upper growth temperatures for Atlantic Whitefish at 22.9℃ differed only from Sockeye Salmon with an estimate of 19.1℃. The differences between optimum and upper temperatures were the smallest for Atlantic Whitefish (3.8℃) and Sockeye Salmon (3.0℃). The most to least thermally sensitive species were (Figure 4.8),

Sockeye Salmon>Atlantic Salmon>Arctic Charr>Atlantic Whitefish>Brook Trout

The distributions for some thermal parameters and thermal sensitivity in some species were quite broad. This occurred as a result of one of two processes, either there were multiple divergent data sets included in the species distribution, or the model was not well resolved for that estimate. For example, the distributions for Atlantic Salmon were broad as there were five populations included, whereas the Brook Trout model only contained a single data set, however, the model was not well resolved for upper growth temperatures (Figure 4.8).

4.4.6 Temperature x pH Growth Experiments

The relationship between temperature and growth followed the characteristic relationship regardless of pH and all data sets were best fit with the Parker Model. Both maximum growth rate and optimum temperature decreased with decreased pH. Specifically, maximum growth decreased significantly between pH levels of 4.75 and 4.2 (Figure 4.9; 4.10). Optimum growth temperatures exhibited a stepped pattern, decreasing by approximately 1°C with each successive decrease in experimental pH levels (Figure 4.10). Thermal sensitivity increased with decreased pH, similar to maximum growth temperature, as the area under the curve decreased and thermal sensitivity increased significantly between pH levels of 4.75 and 4.2 (Figure 4.9; 4.10). Upper growth temperatures were not significantly affected by lower pH.

4.4.7 Assessment of Thermal Sensitivity In Relation to Climate Change

The temperatures representing the optimum growth range changed with pH, as the upper temperature level decreased from 22.2°C in pH 7.0 to 19.3°C in pH 4.0 (Table 4.3). In fresh water, the observed summer temperatures in the current regime exceeded the upper optimum temperature at a proportions between 0.33 and 0.65 depending on the pH, whereas only estuarial water only exceed the upper at a proportion of 0.03 (Table 4.3, Figure 4.11). Under the projected temperature increases the proportion of time the freshwater temperatures were above the upper optimum increased to between 0.54 and 0.73 (Table 4.3; Figure 4.11). There was a similar increase in the proportion of time estuarial water temperatures were above the upper to 0.09, however it remained substantially lower than the freshwater proportions.

4.5 DISCUSSION

Despite the low genetic diversity currently present in Atlantic Whitefish, the species showed remarkable tolerance to low pH and had lower temperature sensitivity than other related outbred species. From the results presented here, assessing only the direct impacts of low pH on Atlantic Whitefish, it does not appear that current pH levels will influence the persistence of Atlantic Whitefish in the Petite Riviere, nor that the lower pH in the Tusket was the sole contributing factor to the extirpation event that occurred during the 1970's and 1980's, as was likely the case with native Atlantic salmon in other regional systems (Watt *et al.* 1983). Furthermore, neither current pH nor the predicted increase to >5.0 for the Tusket River (Clair *et al.* 2004) for the ensuing decades would result in a total loss of Atlantic Whitefish during the freshwater stage should an effort to repatriate the species to the Tusket be attempted.

The thermal sensitivity estimates suggest that Atlantic Whitefish possess an intermediate level of thermal sensitivity compared with other Salmonidae. Despite the effect of low pH on thermal sensitivity, the range of pHs in neither the Petite Riviere nor Tusket River should significantly increase thermal sensitivity. However, thermal sensitivity will increase in low pH environments. Currently, the freshwater temperatures are above the optimum range for growth of Atlantic Whitefish during part of the summer months. The proportion of the summer growing season above the optimal range increased with predicted warming trends over the next 50 years. Although the temperatures used here do not represent the full suite of available thermal habitats within the lakes, such as the cooler thermally stratified areas, there are very likely to be implications of warming on the growth and productivity of Atlantic Whitefish. Similar predictions have been made for other anadromous species (Jonsson and Jonsson 2009). Through the restoration of anadromy of Atlantic Whitefish on the Petite Riviere

this threat may be alleviated as temperatures in the estuary and coastal waters will remain cooler and provide more thermal refugia for Atlantic Whitefish.

4.5.1 Genetic Diversity, Inbreeding And Interspecies Comparisons

The inherent tolerance of Petite Riviere Atlantic Whitefish to low pH appears to have persisted for at least 100 years, since the population has been landlocked in its current, well-buffered habitat (Bradford *et al.* 2004; Ginn *et al.* 2008). And although we cannot assess whether Atlantic Whitefish have lost fitness through the historical population bottleneck, low genetic diversity or general low abundance it is important to note that they maintain the ability to survive in the range of conditions characteristic of regional environments. Furthermore, these experiments showed that tolerance to low pH and temperature sensitivity was comparable to that in other Salmonidae, and also evidence of significant differences between spawning groups of fish in tolerance to low pH.

Theory predicts that the loss of alleles conferring traits not under selection and not at fixation, will be a function of initial allele frequency, population size and time, such that small populations will lose alleles faster than large populations (Hartl and Clark 1997). Similar to the these studies, work on the salt tolerance of Atlantic Whitefish suggested that they maintain the ability to move between fresh and salt water at early life stages with very little increase in mortality and no impact on growth (Cook and Bentzen 2009; Cook *et al.* 2010a), despite having had no exposure to salt water in nature since they were landlocked. Perhaps the persistence of this low pH and salt tolerance is the result of hitchhiking selection or a selective sweep, where the alleles conferring low pH or salt tolerance are linked to another fitness related trait that is currently under selection (*e.g.* Bradbury *et al.* 2011).This hypothesis could be explored

by the creation of a genetic linkage map for Atlantic Whitefish combined with determination of the genetic components conferring both low pH and salt tolerance.

Although I could not assess the tolerance and performance of Atlantic Whitefish in low pH relative to that of an outbred population of the same species, comparisons can be made with other species sharing biogeographic history or common ancestry to gauge relative sensitivity of the sole surviving Atlantic Whitefish population. In terms of pH tolerance, all stages of Atlantic Whitefish tested were more tolerant than any of the regional Atlantic Salmon populations that have been investigated. Specifically, most early life stages of Atlantic Salmon show reduced survival or decreased performance at pHs below 5.0 (Lacroix 1985; Lacroix *et al.* 1985; Peterson and Martin Robichaud 1986; Fraser *et al.* 2008) whereas the responses of Atlantic Whitefish were generally not affected until pHs were below 4.6. The pH tolerance of American eel elvers was markedly higher than that of Atlantic Whitefish as survival of the former species was not reduced at pHs as low as 4.0 (Reynolds 2010). The only other regional species studied for low pH tolerance was Brook Trout, which showed responses similar to those of Atlantic Whitefish, with decreased juvenile survival at pHs <4.3, albeit in short term experiments (Hurley *et al.* 1989). Atlantic Whitefish showed pH tolerance similar to that of several of the more acid tolerant European congeners examined, however there are marked differences between species. Related low pH tolerant species include anadromous European whitefish (*C. lavaretus lavaretus*), which show decreased fertilization, hatch and larval survival at pH's of 5.0, 4.5 and 4.5 (without Al), respectively, (Keinanen *et al.* 2003) as well as *C. pallasi, C. wartmanni* and *C. peled* all of which had a low pH LD50 of 4.5 (Rask *et al.* 1988). In contrast, larval Vendace (*C. albula*) were particularly pH sensitive with markedly lower survival at pHs <5.5 (Duis and Oberemm 2000).

The thermal sensitivity of Atlantic Whitefish was intermediate to that of other outbred Salmonidae species. By comparison, the most thermally sensitive species described here was the Sockeye Salmon, which showed marked differences in thermal sensitivity between populations (Eliason *et al.* 2011) and has shown decreased production accompanying the reported climate changes occurring to date (Hinch *et al.* 1995; Rand *et al.* 2006 Farrell *et al.* 2008; Martins *et al.* 2011). There have not been reported population effects from warming in any of Atlantic Salmon, Brook Trout or Arctic Charr; however, each of these species are under threat from expected increased warming trends (Jonsson and Jonsson 2009).

4.5.2 Ontogeny Of Low pH Tolerance

The effects of pH through early development of Atlantic Whitefish were complex. Low pH affects the ion and acid base balance across most life stages, which can lead to either the complete (lethal) or partial (sublethal) disruption of physiological processes. Changes in pH tolerance have been attributed to improvements in ion regulatory systems, as well as improved buffering capacity through increased size (Wood and McDonald 1982). In Atlantic Whitefish, the most to least pH sensitive life stages were as follows: hatching >early larvae> post-fertilization >metamorphosis >early juvenile >late juvenile. The greater sensitivity of the earliest life stages to low pH is particularly important as these stages naturally occur when environmental pHs will be at their lowest and most variable levels (Figure 4.1). Although it does not appear that low pH is a threat to the persistence of Atlantic Whitefish in the Petite Riviere or an absolute impediment to the reintroduction of the species to the Tusket River. That said, the range of pHs characteristic of potential translocation habitats should be examined prior to stocking. As in other species, the egg and early larval stages of Atlantic Whitefish were the most sensitive to low pH. Within these stages, hatching was shown to be the most sensitive,

as decreased pH resulted in both delayed and reduced hatching success. Similar patterns of delayed hatching have been noted in other species (Atlantic Salmon, Peterson *et al.* 1980; Rainbow Trout, Kwain and Rose 1985), which have been attributed to the decreased effectiveness of the proteolytic hatching enzyme, chorionase, in low pH (Waiwood and Haya 1983). Ineffective chorinase results in either the inability of embryos to break through the chorion leaving the embryo encapsulated, or after initial rupture, the chorion is not weakened enough to allow for complete extrication of embryos and only the tail and trunk protrude. In the latter case, the embryo frequently dies from damage to the yolk sac (Kwain and Rose 1985) or reduced oxygen supply. The decreased hatch rate in low pH was not only due to the ineffectiveness of hatching enzymes, but the direct inability of embryos to survive in low pH, as differences in survival between spawning groups were shown without differences in timing to hatch. As further evidence, those embryos that were capable of hatching from the pH sensitive spawning groups did not have as high larval survival rates as those hatched from the pH resistant groups.

Although eggs were not fertilized in a full suite of low pH environments, there was a significant interaction between post-fertilization pH and rearing pH, suggesting that eggs fertilized in neutral pHs have higher survival rates when exposed to lower pHs during development. The impact of pH on fertilization and egg survival has been shown on all other species studied including Atlantic Salmon, European Whitefish (*Coregonus lavaretus*) and Brown Trout (*Salmo trutta*), where the decreased survival was attributed to the high sensitivity of the newly formed embryo and the rapid uptake of low pH water into the egg's vitelline space in a process called water hardening (Alderdice 1988). Once water hardened, the chorion provides some protection from low pH as the periviteline fluid within eggs is maintained at a higher pH than their surrounding environment (Day and Garside 1977; Eddy and Talbot 1985). The mechanism causing this higher pH is unknown however, as chorions of most fishes are relatively permeable to ions (Peterson

and Martin Robichaud 1993). The results presented here suggest that there is a limit to the protection provided by the egg chorion as some pH sensitive embryos reared in pH 4.3 that survived to hatch, died once they were fully exposed outside of the egg. Comparatively, sensitive embryos reared in pH 4.0 did not survive to hatch as indicated by the higher larval survival in this pH.

Early larvae are generally considered to be one of the more sensitive life stages due to their under-developed ion regulating ability, as their integument is highly permeable and ion regulatory cells in the gills are not fully developed (Sayer *et al.* 1993). In addition, early larvae have very little internal buffering capacity due to their relatively small body size.

There appear to be complex interactions between lethal and sublethal responses during metamorphoses at low pH. In particular, the individuals showing the highest resistance to low pH also possessed the fastest growth and development. Perhaps, this was indicative of the physiological advantages conferred to individuals possessing the capacity for rapid ontogenetic development; i.e., early metamorphosis may result in better growth and survival. It is apparent that the staging method chosen may have direct implications for pH tolerance as the level of scale covering indicates the switch from the characteristic thin and ion permeable larval integument to the juvenile thickened and less permeable integument. Alternatively, or perhaps in addition, individuals that have completed metamorphosis have the advantage of numerous physiological changes that may benefit growth, increased efficiency of both digestive enzymes and metabolism (Forstner *et al.* 1983), for example. In sublethal pHs, survival rates did not decline but growth and development were retarded, which may prove detrimental in natural environments through increased susceptibility to predation (Rice *et al.* 1987).

The responses to low pH in juvenile stages followed the typical patterns of decreased survival and growth at low pH with growth being affected at higher pHs than

survival. The ion regulatory systems of these two sizes of juvenile fish used in experiments were expected to be very similar. The improved tolerance in the later juvenile stage can likely be attributed to the larger body size and resultant greater reserves of ions to buffer the low internal pHs (Wood 1989).

4.5.3 Temperature Sensitivity

The pH x temperature growth relationships shown here demonstrates the importance of studying the interaction between environmental factors. Although significant relationships between temperature growth relationships and body size or food ration have been shown in several species (Brett *et al.* 1969; Imsland *et al.* 1996), the interactions with abiotic factors have rarely been examined. The physiological basis for the characteristic shape of the temperature growth relationship has recently been described by the oxygen and capacity limited thermal tolerance hypothesis (OCLTT; Pörtner 2001). Briefly, OCLTT attributes the decline in growth at both upper and lower temperatures to the capacity limitations of oxygen delivery systems to organs and mitochondria. In cold water, aerobic capacity and decreased production of ATP in muscle mitochondria becomes limiting to circulation and ventilation, whereas in warm water excessive oxygen demand causes a decrease in body level oxygen concentration that cannot be compensated. The changing temperature growth relationship and increased thermal sensitivity with low pH also falls within the mechanistic scope of the OCLTT. In low pH, active ion active uptake is substantially decreased, which results in an increased passive efflux of ions through displacement of intracellular Ca^{+2} ions with H^{+} ions on the gill's binding sites (Wood and McDonald 1982). The loss of ions causes a reduction in blood volume as water moves from extracellular to intracellular fluids. Additionally, red blood cells swell, which in combination of the decreased blood volume, causes an increase in both viscosity and arterial pressure and a slowing of blood flow

(Wood and McDonald 1982; Wood 1989). The slowing of blood decreases the availability of oxygen to tissues thereby decreasing the temperature required for OCLTT to become limiting; as a result, temperature sensitivity was increased and optimum temperature decreased.

Unfortunately, the temperature sensitivity shown here is only for a single life stage, which will not describe the full sensitivity of a species. Ontogenetic changes in thermal niche of fish have been reported for many species, with the general consensus that decreases in both thermal tolerance and thermal optima occur in later life stages as mass specific metabolic rates decrease with increasing body size (Duston *et al.* 2004; Bjornsson and Steinarsson 2002; Imsland *et al.* 1996). The juvenile thermal sensitivity results presented here probably represent an underestimate of the thermal sensitivity of Atlantic Whitefish, as these results do not cover the most sensitive life stages, which are eggs-early larvae and maturation - spawning (King *et al.* 2007). That being said, the thermal sensitivity results were compared with similar stages of other species and the same body size across the pH experiments.

Atlantic Whitefish have maintained tolerance to low pH despite being landlocked in a moderate level pH environment at low abundance for much of the past century. The thermal sensitivity of Atlantic Whitefish was similar to other members of the family *Salmonidae*. It would appear that environmental pH did not play a large role in the demise of the Tusket River, assuming that both populations had similar pH tolerances. Furthermore, the current pH levels on the Petite Riviere should not affect the persistence of this population. Although I could not directly assess the impacts of low genetic diversity on the environmental responses of Atlantic Whitefish, comparisons with other outbred species show that they do not possess any greater reduction in survival or growth in relation to stressful environmental conditions. These results should be useful in identifying the most suitable habitats for translocation of Atlantic Whitefish, as regional

pH differences will affect survival potential. Future temperature increases may affect the growth and productivity of Atlantic Whitefish in their current lakes; however this threat may be alleviated through the restoration of anadromy and access to more thermal refugia.

Table 4.1: Generalized linear model results for the effect of pH on survival and growth of Atlantic Whitefish through early ontogeny.

Life stage	Response	Error	Model Coefficient	Estimate	Error	P-value
Water hardening	Survival	binomial	Female	24.45	6.26	<0.001
			Hardening pH	6.68	1.55	<0.001
			Rearing pH	10.40	2.16	<0.001
			Female x Hardening pH	-4.45	1.03	<0.001
			Female x Rearing pH	-5.30	1.40	<0.001
			Hardening pH x Rearing pH	-1.40	0.35	<0.001
			Female x Hardening pH x Rearing pH	0.96	0.23	<0.001
Hatching success	Survival	binomial	pH	3.57	0.99	<0.01
			Spawning Group	-2.88	1.20	<0.05
			pH x Spawning Group	0.66	0.28	<0.05
Larvae	Survival	binomial	pH	1.98	0.55	<0.001
			Spawning Group	-0.10	0.14	0.44
			pH x Spawning Group	Not significant		
Pre-metamorphosis	Survival	binomial	pH	11.475	1.58	<0.001
Pre-metamorphosis	Growth	Gaussian	pH	-2.13	0.121	<0.05
Early juvenile	Survival	binomial	pH	4.678	0.5056	<0.001
	Growth	Gaussian	pH	0.4444	0.08996	<0.001
Juvenile	Survival	Binomial	pH	6.25	1.396	<0.001
	Growth	Gaussian	pH	0.3684	0.119	<0.001

Table 4.2: Parameter estimates for the logistic model showing the change in hatch rate with time for each treatment pH. Parameter estimates for model comparisons are shown as the absolute difference between the two models (incremental parameters), significant differences ($P<0.05$) in model parameters are indicated by *.

		Parameter (S.E.)				Model Statistics	
		B	A	D_{50}	H	R^2	F
pH Model	4.0	28.6 (16.5)	1.0 (1.8)	11.1 (4.1)	3.6 (2.4)	0.74	49.4
	4.3	69.9 (7.2)	0.8 (1.4)	9.2 (0.8)	3.7 (0.3)	0.99	512
	4.6	100 (5.4)	3.0 (3.2)	8.0 (0.2)	5.9 (1.1)	0.96	385
	5.2	100 (3.8)	3.8 (3.0)	7.9 (0.2)	6.8 (0.9)	0.95	516
	7.3	100 (5.8)	2.7 (2.9)	7.8 (0.2)	7.9 (1.2)	0.96	584
Comparison	4.0-4.3	41 (14.2)*	3.1 (2.4)	2.8 (0.8)*	0.1 (0.2)	0.97	925
	4.0-4.6	79.1 (9.4)*	1.5 (1.2)	3.0 (0.9)*	2.1 (0.7)*	0.99	2107
	4.0-5.2	76.4 (9.5)*	1.9 (1.4)	3.1 (0.9)*	2.8 (0.9)*	0.99	2517
	4.0-7.3	74.8 (8.2)*	1.2 (1.1)	3.3 (1.7)*	3.9 (0.8)*	0.99	3447
	4.3-4.6	43.4 (7.2)*	0.2 (0.4)	1.2 (0.7)	2.1 (0.5)*	0.99	2190
	4.3-5.2	41.2 (10.3)*	3.0 (1.7)	2.1 (1.1)	3.1 (0.5)*	0.99	1387
	4.3-7.3	42.8 (8.7)*	1.7 (0.8)	1.3 (0.5)	4.0 (0.7)*	0.99	1873
	4.6-5.2	5.3 (2.8)	0.8 (1.1)	0.1 (1.3)	1.1 (0.6)	0.97	231
	4.6-7.3	4.1 (2.1)	0.4 (0.2)	0.4 (0.1)	1.8 (1.0)	0.99	1734
	5.2-7.3	6.4 (3.2)	1.1 (3.2)	0.5 (0.9)	0.9 (0.6)	0.97	1132

Table 4.3: Predicted optimum temperature range for growth of Atlantic Whitefish juveniles reared in four different pH levels. pH specific temperatures are related to the proportion of days in summer where temperatures exceed the upper temperature within both freshwater and estuarial environments. For comparison, the proportion of days is also calculated with a 1.5℃ increase projected by 2060 (Chmura *et al.* 2005). No results were presented on low pH models in estuarial temperatures as the appropriate temperature growth model was assumed equivalent to that at pH 7.0.

		pH for Growth Model			
Measure / Location	Metric / Time period	7.0	4.75	4.2	4.0
Optimum Temperature Range	Lower	14.3	14.1	12.9	12.6
	Upper	22.2	22.0	21.7	19.3
Freshwater Temperatures	Current	0.33	0.38	0.44	0.65
	Projected	0.54	0.55	0.58	0.73
Estuarial Temperatures	Current	0.03			
	Projected	0.09			

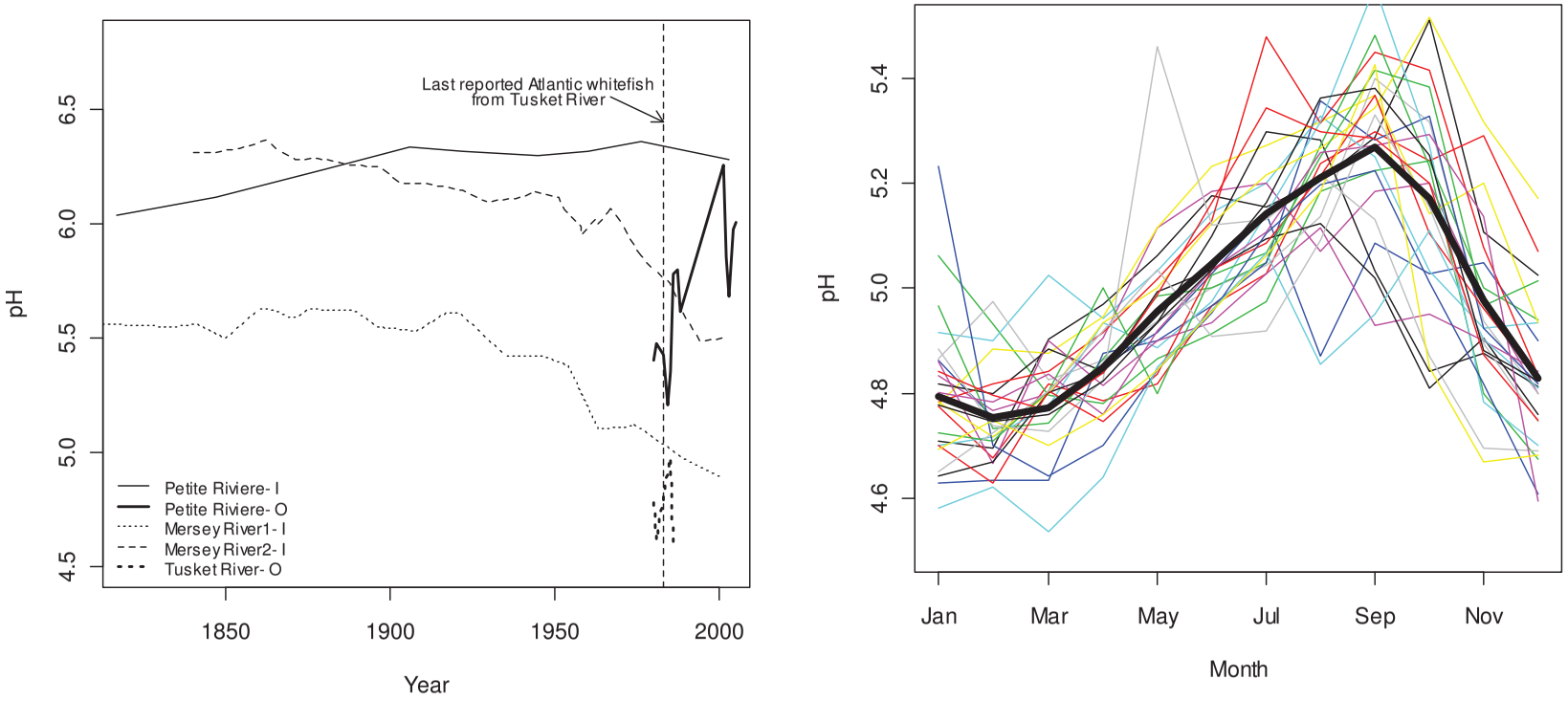

Figure 4.1: (Left) Time series of pH from inferred (I) paleolimnological diatom records or observed data (O) for watersheds in south western Nova Scotia (modified from Ginn *et al.* 2007; 2008). Two lakes within the Mersey River watershed are shown to display the different baseline pH levels within a watershed. (Right) Intra-annual pH trends from the Mersey River pH data for 26 years separately and overall mean (thick black line) (data from T. Clair).

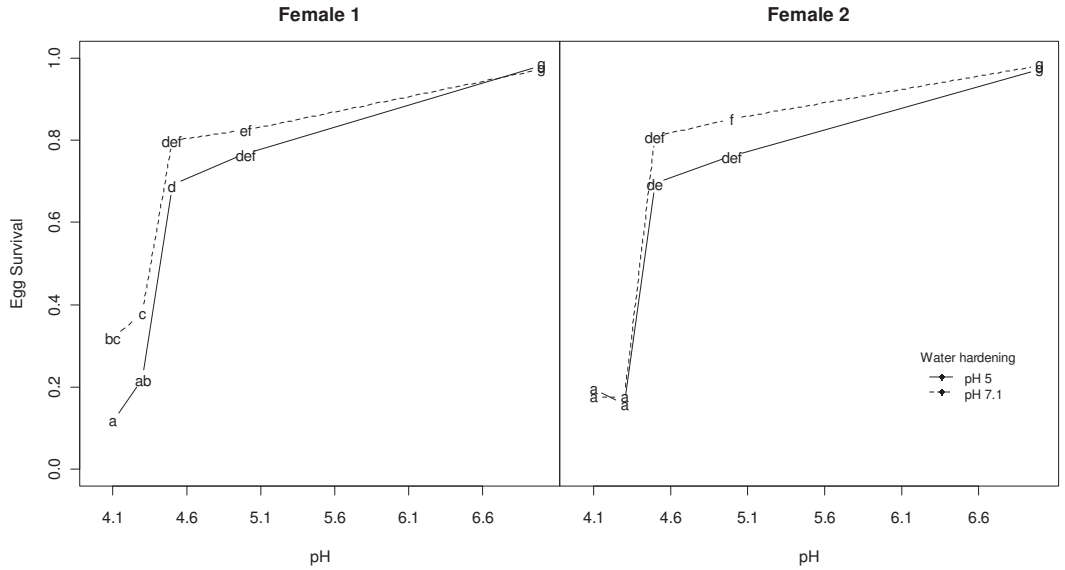

Figure 4.2: Effect of low pH on mean survival of eggs of two female Atlantic Whitefish water hardened at two different pH's and reared at five different pH levels. Across both plots points sharing same letter are not significantly different (P<0.05).

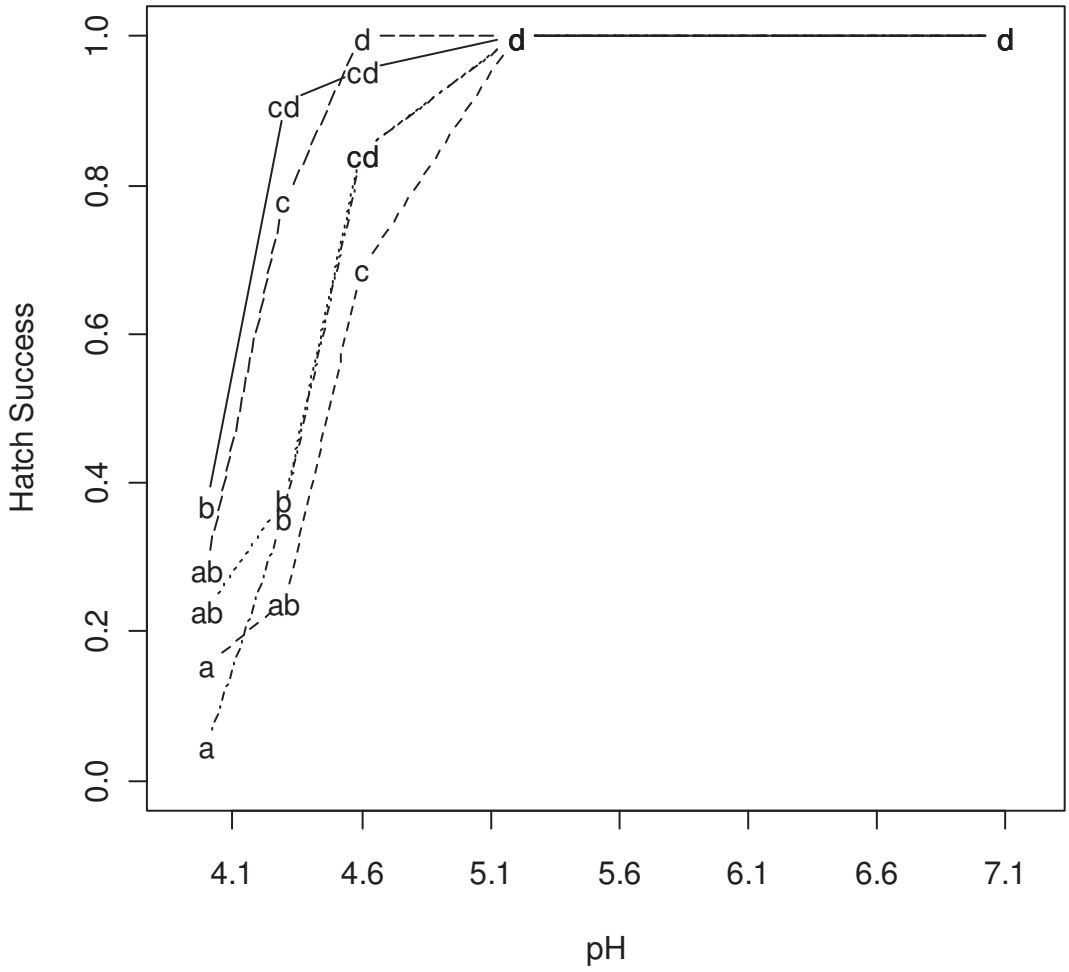

Figure 4.3: Effect of low pH on mean hatching success of the eggs from five Atlantic Whitefish spawnings reared at five different pH levels. Each line represents a unique spawning group. Across all spawnings, points sharing same letter are not significantly different ($P<0.05$).

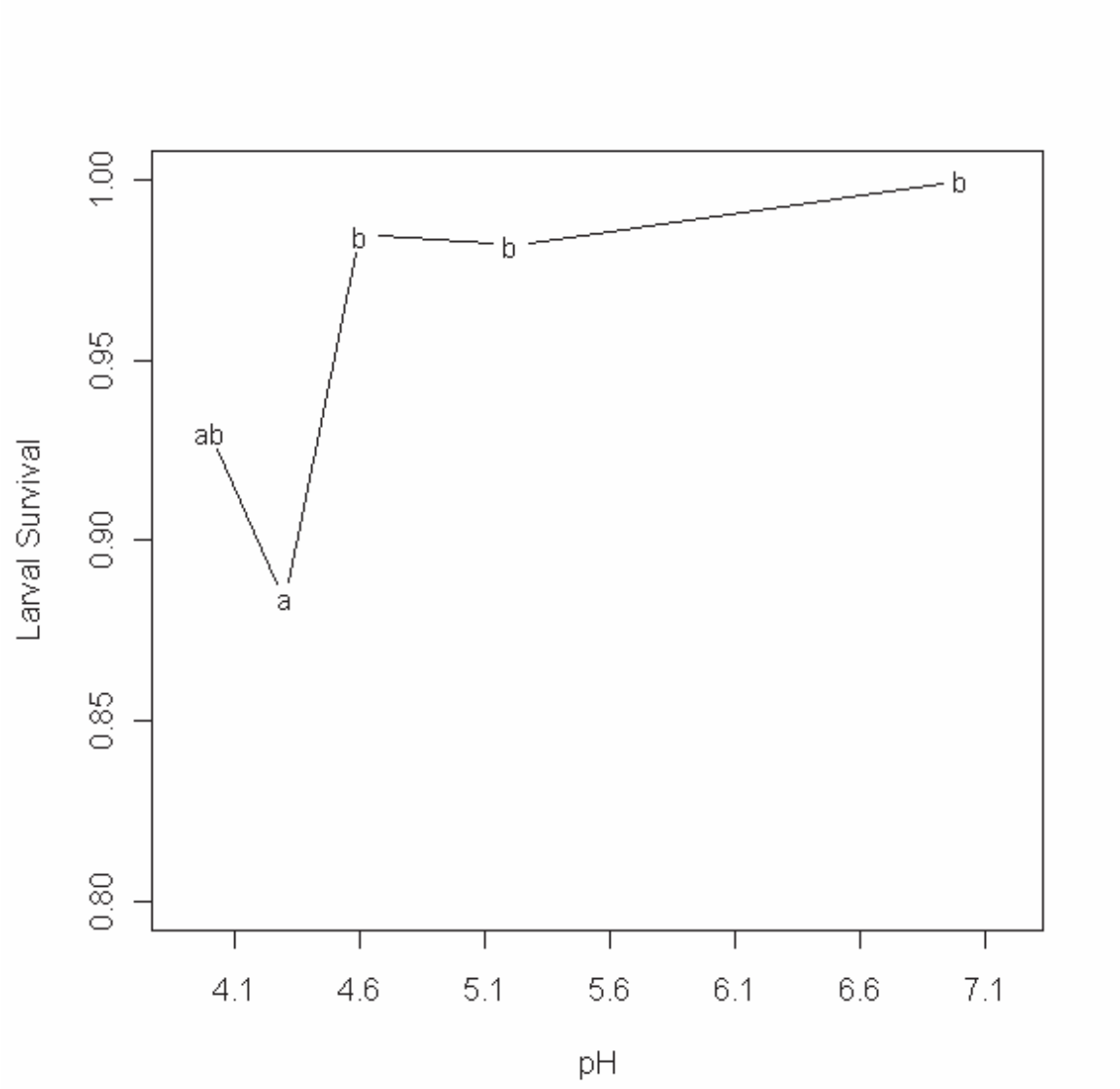

Figure 4.4: Effect of low pH on mean larvae survival for individuals that were capable of hatching in low pH water. Points sharing same letter show no significant differences (P>0.05).

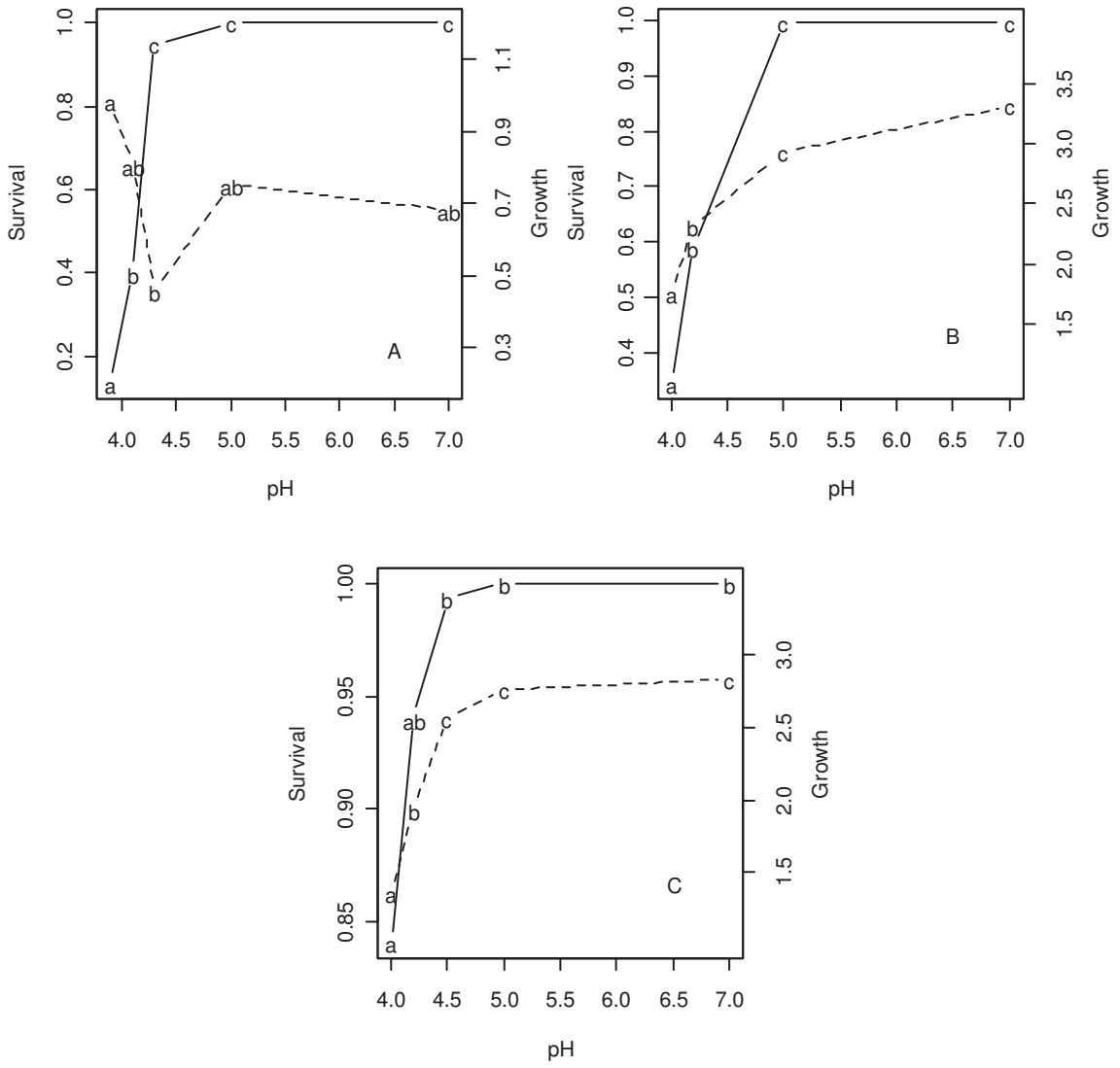

Figure 4.5: Means of survival (solid lines) and growth (dashed lines) of three stages of young Atlantic Whitefish exposed to low pH. (A) represents premetamorphosis larvae, initial size 24mm; (B) represents early juveniles, initial size 39mm; (C) represents juveniles, initial body size of 69mm. Within plots and lines, groups sharing the same letter are not significantly different (P>0.05).

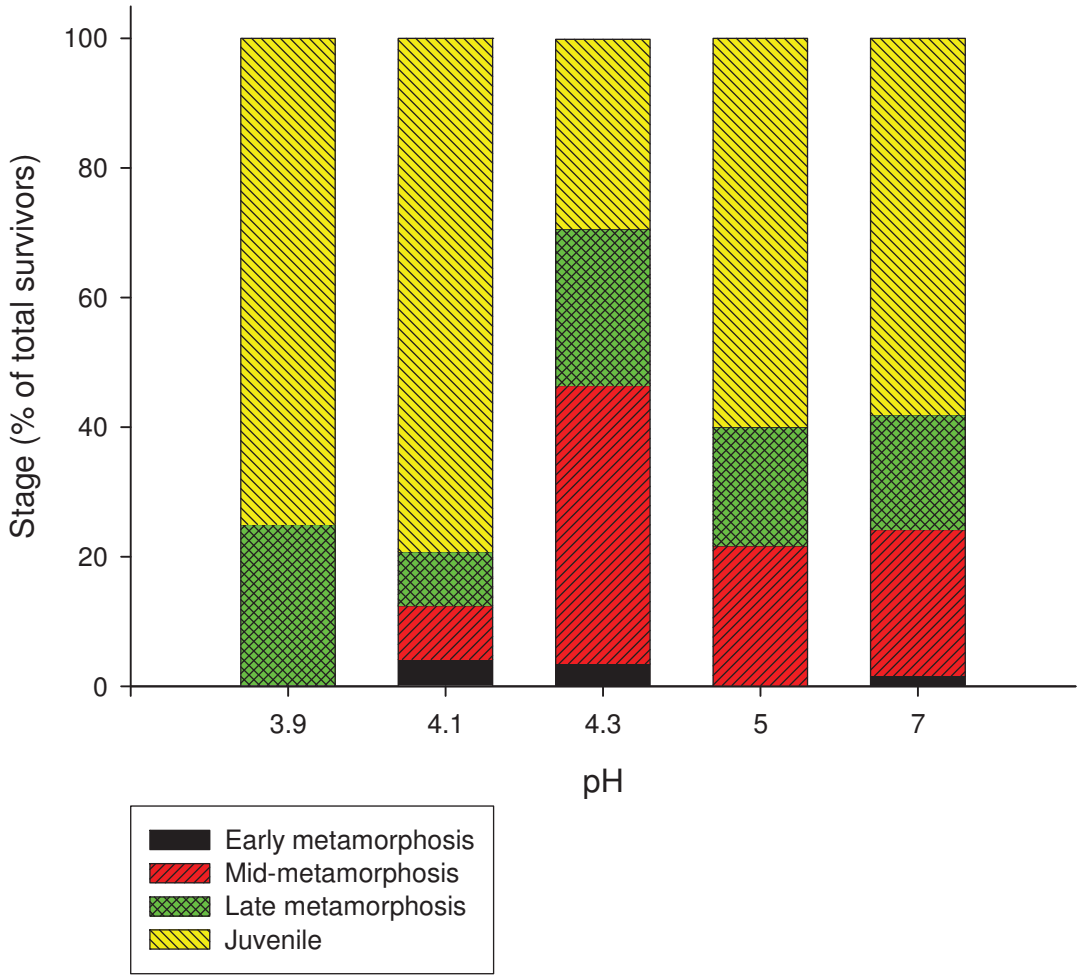

Figure 4.6: Stage of metamorphosis (early, mid, late, juvenile) of survivors from an experiment to determine the effect of low pH on survival and growth of pre-metamorphosis Atlantic Whitefish.

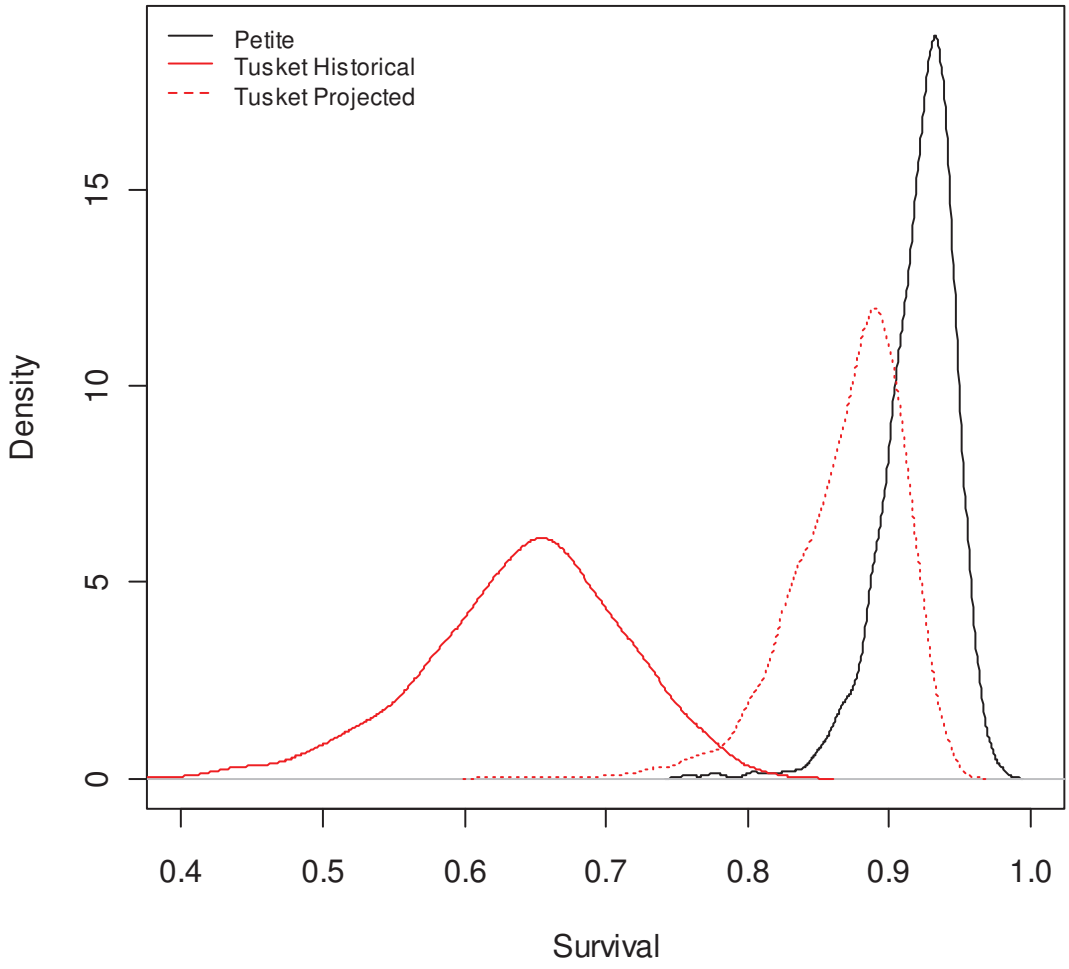

Figure 4.7: Estimates survival probabilities of Atlantic Whitefish to the end of their first growing season based on seasonal pH profiles from the Petite Riviere, the Tusket River and the projected pH levels on the Tusket River based on the results of Clair *et al.* (2004).

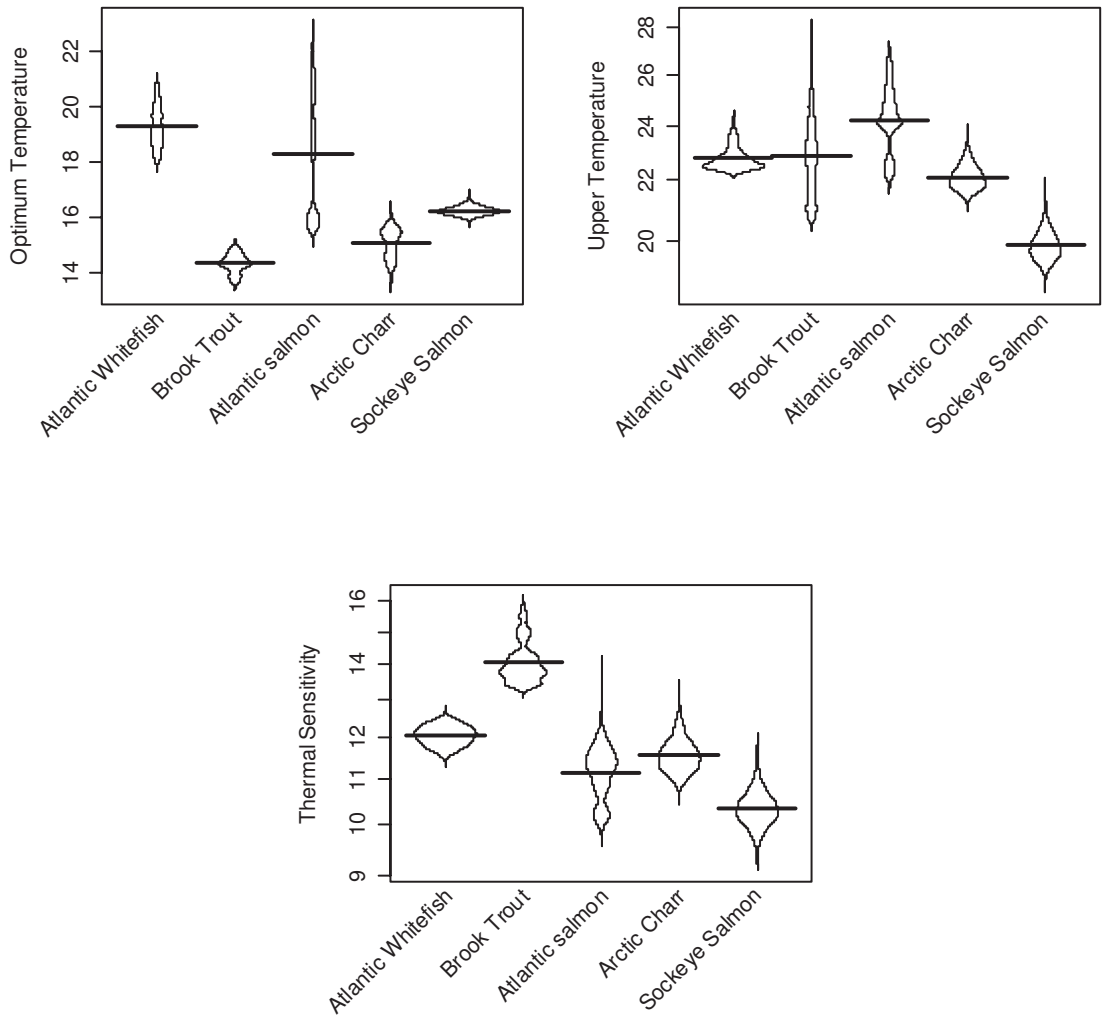

Figure 4.8: Beanplot comparisons of thermal parameters and sensitivity from bootstrapped models describing the standardized temperature-growth relations for Atlantic Whitefish, Atlantic Salmon, Brook Trout, Arctic Charr, Sockeye Salmon.

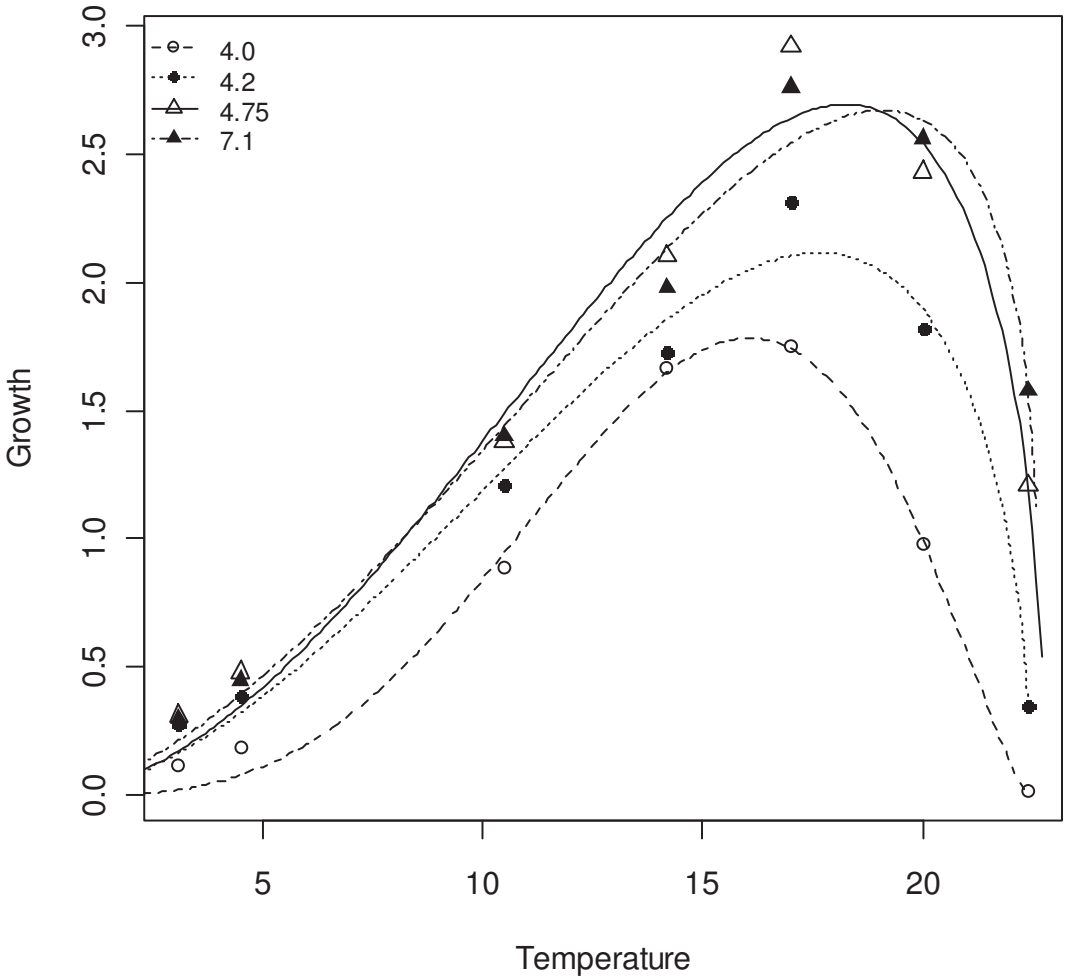

Figure 4.9: Juvenile temperature growth relations as affected by low pH levels of 4.0, 4.2, 4.75, and 7.1 in Atlantic Whitefish. Points represent raw data and lines represent best fit nonlinear regression models.

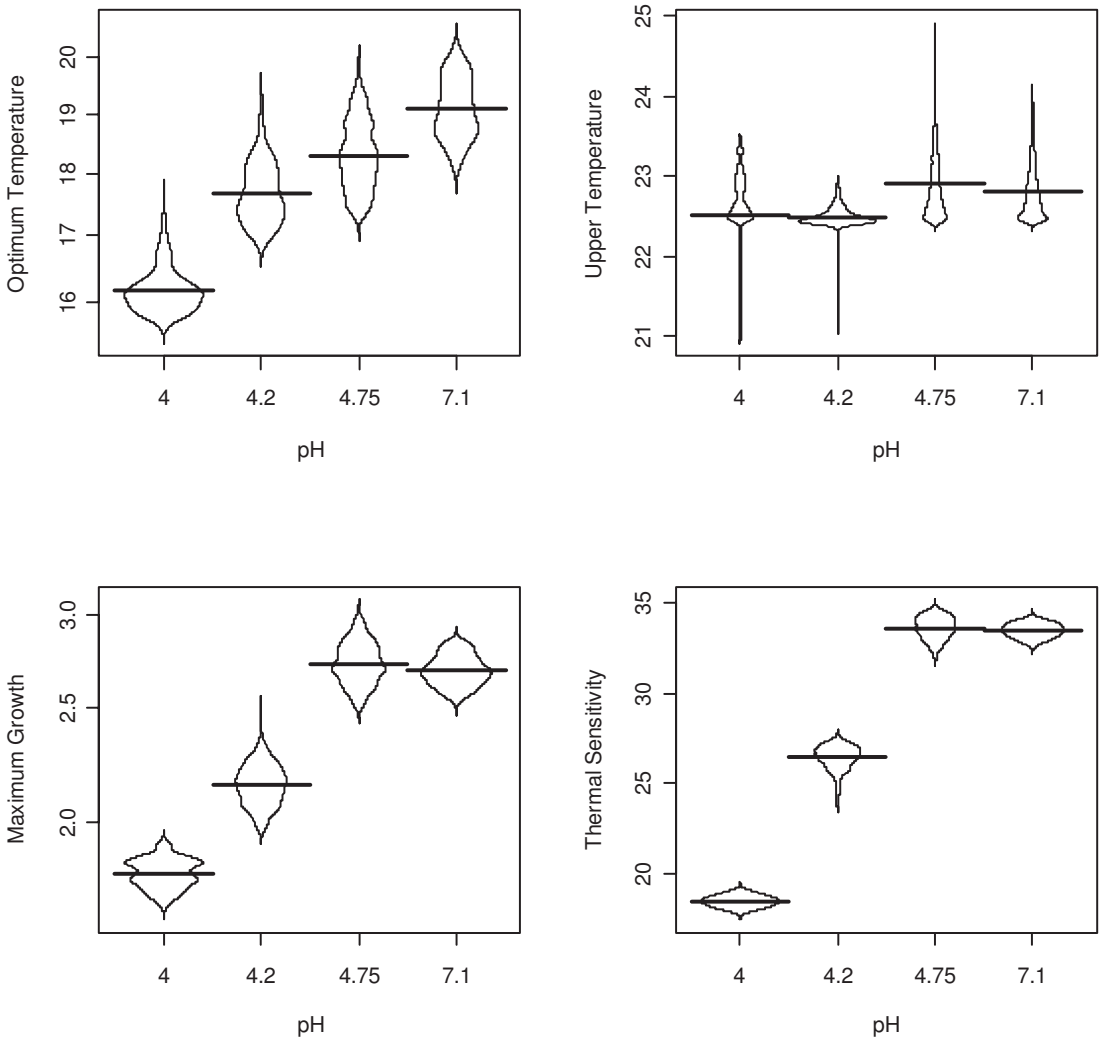

Figure 4.10: Beanplots of optimum temperature, upper growth temperature, maximum growth rate and thermal sensitivity in relation to low pH from bootstrapped regression models describing the thermal niche of juvenile Atlantic Whitefish.

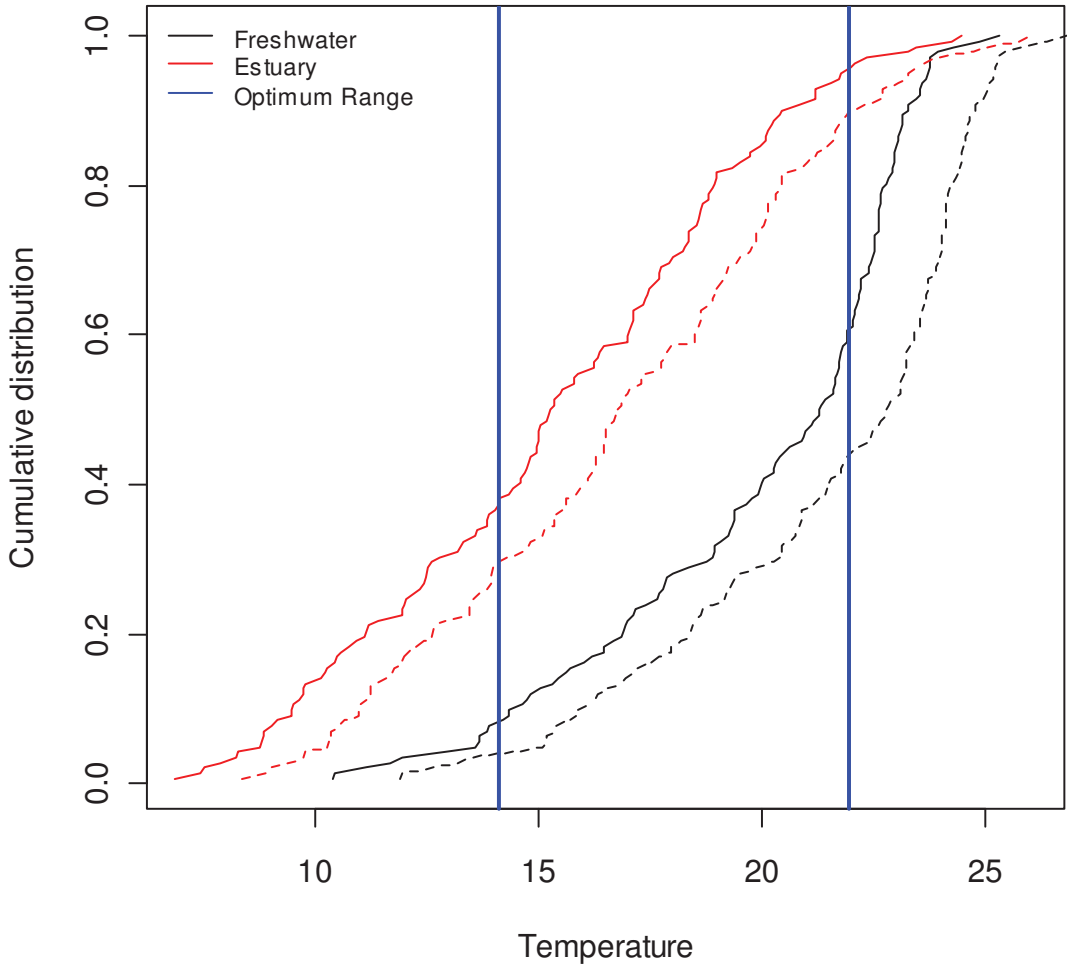

Figure 4.11: Cumulative observed (solid lines) and climate warming predicted (dashed lines) summer temperatures in fresh (black) and estuarial water (red) within Nova Scotia. Blue lines represent the optimum temperature range for growth of Atlantic Whitefish at a typical pH of 4.75 in many Nova Scotian watersheds.

CHAPTER 5: OPTIONS FOR TRANSLOCATION AND REPATRIATION OF ATLANTIC WHITEFISH AND IMPACTS OF INCORPORATING LIFE HISTORY STRATEGIES AND MULTIPLE SOURCES OF VARIABILITY INTO THE CHOICE OF OPTIMAL HABITATS

5.1 ABSTRACT

Restoration of a species to its historical ranges and population size are among the penultimate goals of any conservation program. These goals may be reached using several approaches; however, repatriating fish to their previous habitats and translocating fish to new regions have been advocated for numerous endangered and threatened species. The success of these approaches has been variable, owing partly to the understanding of the key habitat requirements of the species prior to stocking.

Here, I assessed habitat suitability for repatriation and translocation of the endangered, anadromous Atlantic Whitefish using the available information on survival in relation to several environmental conditions. Watersheds were assessed using a hierarchical modeling approach. First, the lake habitats across watersheds were compared to the physical characteristics of the lakes currently inhabited by Atlantic Whitefish. Next a series of simulation models were developed to evaluate the impact of environmental pH on habitat suitability as it is known to affect the survival of Atlantic Whitefish and to vary across the regional watersheds. These models estimated survival by incorporating the watershed-specific observed pH ranges and the relationships between low pH and stage-specific mortality examined in the laboratory. Simulation models incorporating different levels of variability in either environment or species response were examined. I further investigated the effect of anadromous out migrations on habitat suitability.

Overall, the number of optimal translocation habitats differed based on the level of model complexity and with anadromous migrations. Habitats with pH <5.0 were

generally ranked lower than those habitats with pH >5.0; however, simulations incorporating early anadromous out migrations improved survival in the low pH environments. Incorporating seasonally variable pH levels decreased the estimated survival across all habitats. More dramatic decreases in survival were estimated in watersheds with larger amounts of variability during spring months as the most sensitive hatch and early larvae stages were present. Atlantic Whitefish's current habitat was typically among the highest rated habitats. Future habitat suitability modeling should incorporate as much biological and environmental data as possible to ensure the characteristics of the species and the environment are being encompassed.

5.2 INTRODUCTION

In the conservation of endangered or threatened species moving from the study of a species' biology, life history and conservation status into implementing that knowledge and developing actions aimed at improving a species' status is an important step. Depending on the causes of a species current conservation status, different methods can be used to help restore a species to historical abundance levels. In some situations simply removing the causes of population declines (*i.e.* harvesting, habitat restoration) may be enough to allow populations to be rebuilt naturally (Rock *et al.* 2004). However, as is often the case in species requiring active conservation, abundance may be at such critically low levels that immediate action is required to preserve the species. One option that has been advocated is to extend the species range by repatriating fish into previously inhabited regions or translocating fish into new areas (Bouzat *et al.* 2009). These procedures increase range size and decrease the probability of extinction as they allow for population expansion and, with time, lead to diversification of the gene pool through local adaptation (Markert *et al.* 2010). The

perceived value of these approaches in conservation programs is evidenced by the fact that 70% of recovery plans in the United States recommend repatriation or translocation for endangered or threatened species (Tear *et al.* 1993). Moreover, the rate at which these approaches have appeared in the scientific literature has increased substantially over the past three decades from <10 publications per year in the 1980's to 60-80 publications per year in the 2000's (Seddon *et al.* 2007).

Repatriation may be best first step for range extension as habitats are known to be capable of sustaining populations of the species in question, given that the causes of the population's demise have been addressed. If repatriation is not a viable option, or to further extend a species range beyond historical regions, available habitats for translocation should be explored. Habitat suitability is a primary factor in the choice of potential translocation habitats; however other factors including species interactions, socioeconomic or political factors should be addressed (Griffith *et al.* 1989, Wolf *et al.* 1998; Roloff and Kernohan 1999). The success of translocated populations has been shown to be dependent on the understanding both habitat suitability and species response to environment prior to stocking (Forsyth *et al.* 2004).

Describing the habitat requirements of a species can be done using different types of information and has been approached several ways. A species' observed range can be used to define habitat requirements, through the correlation of current distribution with environmental variables (Harig and Fausch 2002). This approach has been broadly applied due to the accessibility of distributional and environmental data needed for these relationships. The disadvantage of this correlation approach lies in its reliance on contemporary distributions to define a species' environmental niche; whereas in fact, distributions are often influenced by other biotic and abiotic factors such as harvesting, small population sizes or barriers to dispersal (Davies *et al.* 1998; Thomas *et al.* 2001). Using correlational analyses it is also difficult to disentangle the most influential

environmental variables shaping a species distribution as habitat requirements will often vary within a species, as responses to environment change through ontogeny and in concert with other factors (Chapter 4; Davis 2008; Froeschke and Stunz 2012). Some of these issues can be addressed by using controlled studies either in the field or laboratory. By careful site selection or artificially altering environments, species responses to environmental variables can be studied across different life stages and in combination with other environmental factors. Furthermore, controlled studies provide the avenue for exploring the inherent variability in species or populations responses to environments which may prove important to the choice of optimal translocation habitat.

It is also important to consider ancillary information to inform the choices of translocation habitats. For example, considering life history strategies that a species can potentially exploit may influence a species distribution within habitats and exposure to suboptimal conditions.

As the final step in defining optimal translocation habitats, species habitat requirements need to be coupled with environmental data. In particular, it is important to assess species responses to the inherent variability in environmental conditions to gain an understanding of how not only the mean environmental conditions will influence the species survival but also the influence of the episodic or rare conditions, which have been shown to influence population persistence in some species (Lacroix and Townsend 1987).

Combining the available information to make defensible decisions about the best habitats for repatriation has been done to varying degrees using a variety of methods. Two similar approaches that rely on distributional data are the Habitat Suitability Index (United States Fish and Wildlife Service 1980) and Ecological Niche Factor Analysis (Hirzel *et al.* 2002). Both have been successful applied, however they do not easily allow for the integration of ancillary information and require substantial data inputs from

several populations. Rather than fit available data into previously developed modeling frameworks many studies have moved toward the development of study specific simulation models, which may be more difficult to implement, but allow for greater flexibility of data inputs and summary outputs (e.g. Larson *et al.* 2004).

Atlantic Whitefish are a member of the genus *Coregonus* which are distributed throughout the north temperate and polar regions of North America, Europe and Asia. Although Atlantic Whitefish were thought to be historically widespread, the species has only relatively recently been designated a distinct species (Scott 1987) at which point extant populations were only documented in two watersheds, the Petite Riviere and the Tusket River, both in Nova Scotia, Canada (Figure 5.1). The Tusket population was anadromous, and made regular upstream migrations in autumn. The extent and character of freshwater habitat used by Atlantic Whitefish on the Tusket River were never identified beyond their seasonal appearance in Lake Vaughn which is the entry point to the rest of the watershed (Edge 1987). Anadromy on the Petite Riviere was never documented. Historical data on the Petite Riviere suggest that dams with inadequate fish passage pre-date the description of the species and may have caused the demise of an anadromous component (Bradford *et al.* 2010). The surviving population of Atlantic Whitefish has been landlocked in three semi-natural oligotrophic lakes for most of the past 100 years (Bradford *et al.* 2004). Despite sampling efforts over the past decade, there have been no observations of Atlantic Whitefish in the Tusket River since 1982 (Bradford *et al.* 2004). This population is considered extirpated, with low environmental pH caused by acid precipitation, unauthorized introductions of the predatory Smallmouth Bass (*Micropterus dolomieu*) and Chain Pickerel (*Esox niger*), poaching, and inefficient fish passage around a hydroelectric dam located near the head of tide being suggested as contributing factors (Edge and Gilhen 2001). The abundance of Atlantic Whitefish in the Petite Riviere has not been satisfactorily estimated but is

considered to be generally low owing to the small (16km^2) quantity of aquatic habitat available. The Petite Riviere has not been as severely affected by acid precipitation and predatory fish were not prevalent in the system until recently. As a result of the collapse in distribution and probable low abundance, it was assessed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) as Endangered in 1984 (Edge 1984) and again in 2000. More recently, under Canadian federal legislative act, the species at risk act (*SARA*) Atlantic Whitefish has been listed as Endangered and protected from direct or indirect harmful acts (DFO 2006). The *SARA* Recovery Strategy for the Atlantic whitefish defines recovery as achieving stability in the current population, reestablish anadromous migrations and expand the species beyond its current range (DFO 2006).

Faced with paucity of data with which to identify suitable translocation habitats for Atlantic Whitefish, I used a hierarchical approach applying various levels of information and complexity to assess the habitats surrounding the historic range. In the simplest model used here (classified Level 0), I used the standard method of identifying habitats based on the physical characteristics of known Atlantic Whitefish lakes. Next, I applied the additional information describing habitat preferences and environmental tolerances obtained from laboratory based studies (Chapter 4). In Level 1 classification, watersheds were evaluated using a simulation model coupled with mean environmental characteristics and Atlantic whitefish's mean survival response to environmental conditions. Subsequent Level 2 simulation models incorporated variability in environmental conditions and species responses. As part of this section I evaluated which source of variability has the greatest impact on estimated survival potential. Finally in Level 3 simulation models I examine the role of anadromous migrations on choice of best habitat. The definition of optimal habitats differed based on the level of model complexity. In the Level 0 models, the best habitat was based on clustering analysis

with the currently known Atlantic Whitefish lakes in the Petite Riviere; whereas the ranking of habitats from the simulation models was based on the median survival estimates to the end of the first year of life.

Prior to describing the methods used in this study I provide the site description of the species current and historical habitats and the potential locations for translocations as well as some of the relevant biological information on Atlantic Whitefish.

5.2.1 Habitat information

The two known Atlantic Whitefish watersheds, Petite Riviere and Tusket River, are both part of the Southern Upland area (SU) of Nova Scotia, Canada. Throughout the 19th and early 20th century hydroelectric or mill dams were constructed within a few kilometres of the head of tide on many of the SU watersheds (Bradford *et al.* 2010). The Tusket River was one of the few watersheds free of dams for the first 15 km of flowing water (Bradford *et al.* 2010). The watersheds of the SU are some of the most acidified in the province and Canada, as they are generally characterized by shallow soils on a base of igneous and metamorphic rocks lacking in basic minerals (Watt 1986). Although, paleolimnological reports show that many of these watersheds possess naturally low pHs (Ginn *et al.* 2007), the effect of acid rain from industrial pollution reduced pHs to historic lows in the late 1970's (Clair *et al.* 2007). Since that time, pH has remained relatively stable and with the subsequent decreases in sulphur emissions, model projections indicate that under worst case scenarios pH will remain stable or begin to increase slowly over the next several decades (Clair *et al.* 2004).

Within the SU, there is both spatial and seasonal variability in pH. Spatial variability occurs as intermittent geological deposits from the last glacial retreat (*i.e.* drumlin fields) offer a degree of buffering capacity and increases the acid neutralizing

capacity (ANC) for some watersheds or portions of watersheds (Clair *et al.* 2004). The pH history on the Petite Riviere, reconstructed through paleolimnological records, suggest that the watershed has been relatively stable around an annual pH of 5.8 for more than the past century (Ginn *et al.* 2008). Similar paleolimnological studies have not been conducted on the Tusket River, however current pH data show that the main branch of the Tusket River possesses pH levels below 5.0 (Table 5.1). There is spatial variability in pH data within the Tusket River as measurements on the Annis and Carleton branches show winter pH levels between 5.2 and 5.5, however there was not a complete time series of spatial data to use in this analysis.

Seasonally, pH follows a predictable pattern remaining low through winter, dipping slightly during the spring melt, rising to its maximum in spring and summer and then decreasing again with autumn freshet events (Watt *et al.* 1983). The magnitude of the seasonal fluctuations in low pH will be influenced by a watershed's ANC, as those with lower buffering capacity will display greater seasonal variation (Walk *et al.* 1992).

5.2.2 Species information

Little is known about the natural habits of Atlantic Whitefish, as individuals have only ever been sampled intensively in freshwater lakes and very few juvenile fish have ever been captured. That said, information from exploratory studies in their native lakes suggest they require specific physical and bathymetric characteristics in lake habitat for successful life cycle completion as they are not found in all lakes within the Petite Riviere watershed (Edge 1987). Recent hydroacoustic tracking experiments have shown adult Atlantic whitefish show seasonal depth preferences within their native lakes (Cook *et al.* in review). Much of the knowledge of habitat preferences and tolerances as well as spawning and early life history has been obtained in captivity, as several adult fish were

collected from one native lake and have spawned in captivity on several occasions. Briefly, successful spawning can only occur in freshwater (Cook and Bentzen 2009; Cook *et al.* 2010a) during late November and December as temperatures decline. Eggs are semi-adhesive and negatively buoyant. Larvae swim up at hatch and have improved swimming ability over their first several days post hatch. Recent laboratory studies (Chapter 4) have assessed the ontogenetic changes in survival in low pH water with the progression most to least sensitive stages as:

hatching <early larvae< fertilization <metamorphosis <early juvenile <late juvenile

It is important to note that the more sensitive life stages occur when the seasonal cycle of pH is at its lowest.

Despite the record of Atlantic Whitefish upstream anadromous migrations in autumn on the Tusket River, there has never been an indication of timing of down stream migrations of juveniles, although post spawn adults were observed at the base of Tusket dam (RG Bradford personal communication). Laboratory studies have shown that Atlantic Whitefish can acquire tolerance to full sea water prior to metamorphosis, albeit with some survival costs, and juveniles prefer sea water over fresh or brackish water (Cook and Bentzen 2009; Cook *et al.* 2010a). The early salinity tolerance taken together with swim up at hatch and the scarcity of juvenile fish during freshwater sampling (Hasselman *et al.* 2005) implies that out-migration may occur early in the life-history, similar to some populations of the European Whitefish *Coregonus lavaretus* (Girsa *et al.* 1980; Lehtonen *et al.* 1992).

5.3 METHODS

5.3.1 Available Data

5.3.1.1 Physical and Environmental Data

Watersheds were required to meet several criteria for evaluation as potential translocation sites: first, watersheds were required to be within or adjacent to Atlantic Whitefish's native range; and second, time series of both pH and temperature were available in order to evaluate habitat suitability. Within the SU region, 16 watersheds met these criteria (Table 5.1; Figure 5.1). Across all of these watersheds pH data were collected over a 25 year period (1979 - early 2000's) at irregular intervals and variable frequency; although river-specific data sets were not equivalent in size or number of sample sites, all represent time series that covered at least 10 months of the year (Table 5.1). Monthly mean and standard deviation of pH levels were calculated for each river (see Appendix C: Chapter 5; Feinstein 1979). Missing months were estimated by combining data from adjacent months. Water temperature profiles were only available for a subset of rivers. Monthly temperature means and standard deviations were calculated across all available data as seasonal patterns and interannual variability within a watershed was greater than the observed variability among watersheds (see Appendix C: Chapter 5). Across 14 of 16 watersheds, data on the physical variables of lake drainage area, lake volume, shore length, flushing rate and maximum depth were available for 354 lakes.

5.3.1.2 Species Responses

The effect of pH or salinity (V_j) on the survival of Atlantic Whitefish through ontogeny was modeled through stage-specific (i) daily mortality rate estimates (M_{ij}) obtained from laboratory studies (Chapter 4; Cook *et al.* 2010a). Stages for the pH regressions include egg, hatch, larvae, early juvenile and juveniles. Mortality-salinity relationships were only available for larvae, early juvenile and juvenile stages as egg fertilization and hatch occurs strictly in freshwater. For the simulation modeling all pH mortality rate regression models were in the form:

$$M_{ij} = A_{ij} + \left(R_{ij} - A_{ij}\right)e^{-e^{t}\cdot V_j} \qquad \text{Equation 5.1}$$

where A_{ij} is the asymptote, R_{ij} is the y-intercept, t is the rate of change. Salinity mortality rate regressions were in the form

$$M_{ij} = a_{ij}V_j^{\,b_{ij}} \qquad \text{Equation 5.2}$$

where a_{ij} and b_{ij} are the regression parameters and V_j is the level of salinity. Variability in stage and environment specific mortality rates were assessed through bootstrapped regression parameters. These parameters were obtained by fitting the regression model through nonlinear least squares estimation to the original dataset as well as 999 datasets where the residuals from the original fitted model $\hat{\varepsilon}_j$ were resampled and added to the original growth data (y_i) such that a synthetic response variable (y_i') was created for each bootstrap iteration as, $y_i' = y_i + \hat{\varepsilon}_j$ (Wu 1986). This method was used as it maintains the information contained in the explanatory variable.

The effect of temperature on Atlantic Whitefish was evaluated on its effect on stage specific development rates, measured as a degree day metric. I did not assess the

effect of temperature on survival as a full suite of response data was not available for all stages. Distributions of degree-day development rates were estimated from data on Atlantic Whitefish development in culture across three separate spawning and rearing seasons and 14 spawning pairs. The number of degree days at each developmental period was described by a Gaussian distribution with stage-specific mean (μ) and standard deviation (σ). The specific degree-day distributions from egg fertilization to hatch, hatch to metamorphosis and metamorphosis to late juvenile stages were N(260, 5.5), N(270,10), N(400,12) respectively.

5.3.2 Habitat Suitability

5.3.2.1 Level 0- Lake Suitability

Using only the physical characteristics for lakes sustaining extant Atlantic Whitefish population as an indicator of habitat suitability an additional 354 SU lakes were compared using a suite of multivariate statistics. Lakes were grouped by complete-linkage cluster analysis on a Bray-Curtis dissimilarity matrix of the natural log of physical variables for all lakes. The strength of the node classification within the dendrogram was examined through 1000 bootstrapped iterations. Lakes that clustered with the current Atlantic Whitefish lakes (Hebb, Milipsigate and Minamkeak) with bootstrapped support >80% were chosen as the most suitable translocation habitats. The most important physical characteristics delineating Atlantic Whitefish lakes were determined through linear discriminant functional analysis.

5.3.2.2 Simulation Model

Simulation models were developed to assess the effect of watershed-specific water quality on survival of Atlantic Whitefish. These models couple the stage-specific

mortality rate models with the pH and temperature data with or without anadromous migrations. An outline of the simulation model is given in Figure 5.2, which, beginning at the top of the figure details all potential combinations of simulation model complexity used through out this paper. Each simulation iteration was performed for one year from the spawning date. The initial step was the selection of a spawning date from a uniform distribution in December U(1,31). Stage specific survival (*Sij*) was dependent on pH's or salinity's effect on stage specific mortality rates (*Mij*) and number of degree days at that particular stage (*DD*). For the simulations without variability (Level 1) the *Mij* models parameters were fixed and watershed specific pH were set to the annual mean whereas temperature followed the observed mean monthly profiles. For simulations incorporating variability (Level 2 and 3), each or all of *Mij* models, pH, temperature (*Tt*), and development time (*DD*) were randomly sampled from distributions. pH and temperature estimates were updated monthly and were censored to the range of the data. The stage specific Mij models did not change within a simulation run, but were randomly sampled with replacement from the bootstrapped parameter set between iterations.

Anadromous migrations were incorporated into Level 3 simulation models through a series of fixed dates of migration. At the end of each simulation iteration the overall proportion of young fish surviving was estimated as the product of all stage-specific survival estimates. Although the focus of these models was on the watersheds selected in Level 0 habitat choice, simulation modeling was conducted on all 16 watersheds to better describe the relationship between environmental variables and survival of Atlantic Whitefish. For each watershed, level of model complexity and/or time of anadromous migrations simulations were run 10,000 times to obtain a distribution of survival estimates. A more detailed description of the Level 2 and 3 simulation models are given below.

5.3.2.3 Level 2- Incorporation Of Variability In Water Quality And Species Response

Level 2 models incorporated variability into each of the environmental variables, Mij models and degree day developments rates independently and in all potential combinations to assess the relative effects of each on survival (Table 5.2). The optimal watershed for Atlantic Whitefish repatriation was determined through the comparison of median survival estimates within each combination of model variability where medians ±0.05 were considered equivalent. Additionally, comparisons were made across all watersheds within a combination of model variability by nonparametric rank tests. The overall effect of each combination of variability incorporated into the simulation model on choice of habitat was done by ranking the median survival estimates from each model within watershed. These ranks were combined across all watersheds and compared using a nonparametric Kruskal-Wallace test.

5.3.2.4 Level 3- Incorporation Of Anadromous Migrations

Using the Level 2 model with the full complement of variability (Table 5.2 - model 7), anadromous migrations were included into the simulation model. Anadromy resulted in the switch of *Mij* models from pH based to salinity based. Similar to the stage-specific *Mij* pH models, the *Mij* salinity models were sampled from the bootstrapped regression models. Salinity was set to 30 ppt for all stages post migration. The influence of migrations were examined at a fixed time of migration (1, 5, 10, 15, 20, 25, 30, 50, 100, 150, 200, 250 days post hatch).

From the outputs of these models I examined the change in median survival if fish leave the system either before or after day 30 post hatch using binomial generalized

linear models. Thirty days post hatch was chosen as it was visually identified as a break point in the survival – days to migration relationship across multiple watersheds.

5.4 RESULTS

5.4.1 Level 0 – Current Distribution

In total, 15 of the 354 lakes considered were assessed as possessing high physical similarities to the three lakes currently inhabited by Atlantic Whitefish. Among the 15 lakes were four from the previously occupied habitat in the Tusket River drainage. The other similar lakes were from six other watersheds including the Mersey (1 lake), Lahave (3), Mushamush (3), Jordan (1), East (1) and Medway (2) Rivers (Figure 5.3). Of the five physical characteristics used for clustering, lake volume was the most important factor in delineating the potential Atlantic Whitefish lakes, in particular, lakes with volume $>14E^9$ m^3 were most often selected. The ranking of the other physical variables were drainage area, maximum depth, shore length and flushing rate (data not shown).

5.4.2 Level 1 – Mean Response And Water Quality

Across all watersheds, survival decreased with mean annual pH, following an exponential pattern, such that pH < 5.0 resulted in rapidly decreasing survival (Figure 5.4). Of the eight watersheds identified as containing a potential Atlantic Whitefish lake, predicted survival ranged between 0.43 in the Jordan River (pH of 4.5) to 0.93 in the Mushamush River (pH of 6.3; Figure 5.4). Petite Riviere, Mushamush River and Lahave River had the highest median predicted survival at 0.95, 0.97 and 0.94 respectively. All rivers, with the exception of the Jordan River had predicted survival above that of the Tusket River (0.67; Figure 5.4; Table 5.3).

5.4.3 Level 2 – Variable Response and Water Quality

Survival with the constant pH models (models 0-3), showed similar smooth exponential declines in relation to decreasing pH (Figure 5.5). In the variable pH models (models 4 - 7), survival with pH did not follow the same smooth transitions with decreasing pH. In watersheds sharing similar mean pH levels, predicted survival was lower in those watersheds with lower and more variable pH during the critical stages of hatch and early larvae (Figure 5.5). This was particularly evident in the decrease in predicted survival between the Mersey, East and Tusket Rivers, even though mean pH's were similar at 4.98, 4.88, and 4.80 respectively, as pH was more variable through out the year in the East and Tusket systems (Table 5.1; Figure 5.5; Appendix C: Chapter 5). As with models with no variability, survival was predicted to be highest within the Petite and Mushamush Rivers, however, with variable pH levels, the Mushamush had higher survival than either the Petite Riviere or Lahave Rivers at levels of 0.96, 0.92 and 0.89 respectively. The Tusket, East and Jordan Rivers had the lowest survival regardless of the level variability incorporated into simulation models.

Models incorporating variable pH consistently showed lower survival than those without variable pH (Figure 5.5; 5.6). In the low pH watersheds (<5.0) median survival rates were higher when temperature variability was included in the simulation (models 1, 3, 5, 7), although the differences were not statistically significant ($P>0.05$; Figure 5.6 upper). Conversely, in the high pH watersheds (pH >5.0) there was no significant influence of any source of variability other than pH on predicted survival (Figure 5.6 lower).

5.4.4 Level 3 – Variable Responses, Water Quality With Anadromy

Early anadromous migrations (<30 days post hatch) resulted in markedly higher survival in watersheds with mean annual pH <5.0 as determined by the negative relationship between survival and number of days in freshwater (Figure 5.7). In contrast, watersheds with pH >5.0 had lower survival for the same early anadromous migrations (Figure 5.7). Specifically, survival rates of Atlantic Whitefish in the Tusket, Jordan and East Rivers were higher with early anadromous migrations, whereas early anadromous migrations on the Petite Riviere and Mushamush River decreased survival when compared to freshwater residents (Figure 5.7). After 30 days post hatch there was no improvement in overall survival regardless of pH as mortality rates in both environments were equal.

Incorporating anadromous migrations changed the pattern in best overall habitat choice from the variable pH models with freshwater residency (Table 5.3). In particular, the lakes on the Lahave River and Molega Lake on the Medway River were ranked alongside the Mushamush and Petite Riviere lakes, which was due to the greater decrease in survival in the latter two watersheds rather than improvements in survival in the former (Figure 5.7, Table 5.3). Overall, the greatest impact of anadromous migrations on survival occurred within rather than across watersheds as the most acidified rivers had markedly higher survival with early migrations (Figure 5.7).

5.5 DISCUSSION

Exploring increasingly complex models focused on the abiotic habitat variables to identify the best translocation habitats for Atlantic Whitefish changed the pattern of habitat choices, particularly in the moderate to low pH environments or when anadromous migrations were included. The current Petite Riviere watershed was consistently ranked among the best habitats for Atlantic Whitefish survival regardless of

modeling strategy implemented. And although there are limitations to the methods used here, which will be discussed below, the other top ranked habitats, including the Mushamush and Lahave Rivers, may provide the best options for successful translocation.

In many other studies, translocation habitats are identified by finding sites with environmental conditions similar to those found within the current species distribution (Gerber *et al.* 2003; Cook *et al.* 2010b). Here using solely the current Atlantic Whitefish habitat, 15 lakes (4% of the total lakes included in the analysis) were identified as suitable choices for translocation. Four of these lakes were from the only other previously known Atlantic Whitefish habitat, the Tusket River watershed, including Lake Vaughn where they had been captured previously (Edge 1987). None of the other 11 lakes within the Petite Riviere lakes were part of this group. This information, combined with the high bootstrapped support of the selected two branches of the dendrogram suggested that the physical variables used in the analysis, and in particular lake volume, were likely correlated with environmental features necessary for the maintenance of viable Atlantic Whitefish populations. Elsewhere, lake volume has been shown to be a strong correlate with numerous biotic and abiotic factors including food chain length (Post *et al.* 2000), fish community structure (Mehner *et al.* 2005), thermal stratification and environmental stability. The thermal refuge offered by a stratified environment may be important for Atlantic Whitefish, as recent work has shown that the optimum temperature for growth of juvenile Atlantic Whitefish, 19.1℃ (Chapter 4), is moderately high compared to that for other salmonid species, although the optimum temperature is likely lower for older fish (Bjornsson and Steinarsson 2002). Moreover, Atlantic Whitefish have recently been shown to prefer the deeper water habitats of their native lakes through out much of the year, but do show seasonal changes in their distribution moving toward shoals in late autumn, perhaps to find spawning areas (Cook *et al.* in review).

Adding environmental pH and temperature data into habitat choices decreased the number of highest ranked translocation habitats to six lakes within the Mushamush and Lahave Rivers. Not surprisingly, watersheds with mean annual pH levels at or below 5.2 were ranked lower and had lower predicted survival than those with higher pHs. Regionally, low pH has been implicated in affecting the habitat suitability for other anadromous species including Atlantic Salmon (Lacroix *et al.* 1985) and Striped Bass (*Morone saxatilis*) (Jessop 1995), but not American Eel (*Anguilla rostrata*; Reynolds 2010). Similarly, other studies have shown that species composition of lakes changes markedly with pH, as some species are more tolerant to low pH than others (Beamish and Harvey 1972; McDonald *et al.* 1991). In addition to the decreased survival in low pH, population performance suffers in low pH as production is reduced (Keinanen *et al.* 2003; Kwain and Rose 1985; Vuorinen *et al.* 2003, 2004). In the current study, the examination of the impact of low pH was restricted to survival of egg to subadult Atlantic Whitefish, which represented some of the life stages thought to be most sensitive to low pH (Chapter 4). One life stage not included in the simulation that may show strong pH effects on survival are gonad development and gonad release (Vuorinen *et al.* 2004; McCormick *et al.* 1989). Assuming low pH selection on adults parallels that in juveniles, these reproductive effects would decrease the population's productivity, as fewer individuals will spawn in a given year, however the resulting offspring may show improved survival in low pH as work from elsewhere suggests pH tolerance has a heritable component (Schom 1986).

Variable environmental conditions further altered the apparent suitability and survival in low pH habitats. Variable pH levels, reflecting the intrinsic variability in watersheds, resulted in the reduction of the number of best suited lakes to three. In particular, predicted survival was substantially decreased in watersheds with strong seasonal variation in pH, as pH minima occurred most often during the critical periods of

hatching and early larval survival. This reinforces the importance of using seasonal pH profiles and variability in pH characteristics in habitat identification, as episodic pH events have been shown to negatively affect survival in several salmonid species (Baker *et al.* 1996; McCormick *et al.* 2009). Interestingly, however, the incorporation of variable temperatures increased predicted survival in the same low pH environments. In the models where development rates were based on mean water temperature profiles the most pH sensitive stages of hatch and early larvae occurred during the period of lowest pH during spring melt. Through the incorporation of variable temperatures, the development time either increased or decreased resulting in a decoupling of these pH sensitive stages and the lowest pH levels. Under an environmental match-mismatch hypothesis, phenology of a species would evolve to match the environmental conditions which maximize fitness (Winkler *et al.* 2002; Futuyma 1998). In the case of Atlantic Whitefish, much of their recent history has been in relatively pH neutral lakes with little seasonal variability. This suggests that there would not have been selective pressures acting against those individuals that hatch during the spring melt when pH declines. It is important to note however that temperature and pH in the simulation model were decoupled, and although there may be a relationship between pH level drops from freshets and spring melt waters, these were not included here.

As a final step, incorporating anadromous migrations to simulation models increased the number of suitable translocation habitats back up to nine. Here, results show that for Atlantic Whitefish the physiologically related mortality associated with moving into marine environments was lower than that resulting from residence in freshwater environments with pHs below 5.0, such as the Tusket River. For environments with pH > 5.0, similar to the Petite Riviere, there was no physiological survival advantage to leaving freshwater habitats. There was a window between 0 and 30 days post hatch when anadromous out migration changed survival potential. In

watersheds with pH >5.0 early out migration lowered survival as mortality rates of young fish were higher in marine environments than in freshwater pHs. This pattern was reversed in pH <5.0 environments as survival rates of individuals out migrating within the first 30 days were improved. After 30 days post hatch there was no improvement in overall survival regardless of pH as mortality rates in both environments were equal. There are two reasons for the prevalence of this time window: first, mortality rates decrease in both low pH fresh water and in marine environments for fish >30 days post hatch as they are generally completing metamorphosis and are entering the more tolerant juvenile life stages. Secondly, seasonal pH levels are at their highest at the time of year when these life stages are present and even though rivers are categorized as low pH, they all generally have pHs above 5.0 during the summer months (Appendix: Chapter 5).

From these results it was suggested that the incorporation of the species life history strategies may alter the perception of optimal habitats. The improvement in survival with anadromy on the Tusket River and decreasing survival on the Petite Riviere perhaps explains why these populations displayed different life history strategies. In life history theory, species exploit characteristics and habits that increase their overall lifetime fitness (Stearns 1976). In other species, anadromous migrations have been suggested to increase fitness through several mechanisms. In northern temperate regions, anadromy has been attributed to the increased productivity in oceanic relative to freshwater habitats, implying density dependent effects will be reduced for anadromous populations or subcomponents (Gross 1987). These impacts can be seen by the increase in body size, fecundity and overall condition of fish returning to freshwater habitats for spawning (Maekawa and Nakano 2002). Opposing the fitness improvements offered by anadromy are the increased physiological mortality associated with changing habitats (Zydlewski and McCormick 1997) and broader predator fields (Dieperink *et al.*

2001). By no means does this suggest anadromy should not be a life history attribute to recover in Atlantic Whitefish, as regardless of the differences in early life stage survival, the anadromous Tusket population reached substantially larger lengths (mean adult length of 28cm on the Petite Riviere *vs*. >40cm on the Tusket River; Edge 1987) which would likely correlate with higher egg production and egg quality as has been shown in other species (Loewen *et al.* 2010).

The modeling approach used here did not explore the demographic consequences of various translocation habitats as would be typical in population viability analysis (Munzbergova and Ehrlen 2005). These factors would have implications in various low pH environments as growth and other physiological processes would be affected by sublethal pH levels during prolonged freshwater residence. That said, with anadromous migrations, there would be very little change in habitat suitability between those model results with and without sublethal responses, as it is anticipated that the short freshwater residency would have minimal impact beyond the survival changes depicted here. Furthermore other considerations such as land use surrounding lake habitats, predatory species and prey field were not included in the cluster analysis or simulation modeling, but should be considered prior to final decisions on best translocation habitats.

The survival estimates in relation to low pH found here are likely underestimates of true Atlantic Whitefish survival. This study relied on pH data collected during monitoring of Atlantic Salmon habitat (Lacroix *et al.* 1987). As such, much of the pH data were collected in moving waters surrounding Atlantic salmon spawning and early rearing habitats. Lotic environments in general may offer a greater degree of buffering from seasonally variable low pH values observed in lentic habitats as much of the pH decrease is due to the spring melt water will flow across the upper layer of a thermally stratified environment, thereby offering some degree of protection for the species

occupying regions below the thermocline (Watt 1986). Furthermore, work from elsewhere suggests that some species avoid low pH habitats (Johnson and Webster 1977; Pedder and Maly 1986; Peterson *et al.* 1989) and seek microhabitats of good pH water which can occur within watersheds thereby offering refugia during pH declines (Baker *et al.* 1996).

Feasibility of repatriation to the Tusket watershed should be among the first options explored for extending the current range of Atlantic Whitefish. Although the results presented here suggest predicted survival rates through the first year of life were among the lowest for the habitats examined, they were likely underestimates as, similar to some of the other watersheds, the pH data used were limited. The time series of data used here was from the main stem of the Tusket River and from predominantly lentic habitats, which are known to be more acidic than some of the other potential regions within the watershed. Furthermore, with the projected increases in pH as sulphur emissions decrease, pH levels on the Tusket River are likely to exceed 5.0 in the near future increasing the suitability of the habitat (Clair *et al.* 2004). As for the other identified threats on the Tusket River, poaching of Atlantic Whitefish would be likely be reduced as the species profile has been broadcast through its endangered status. Also they would garner increased monitoring and protection under *SARA*. Although a number of dams do remain on the Tusket River, upstream fish passage was used historically by Atlantic Whitefish (Bradford *et al.* 2004) and further improvements to current fish passage is being planned for the entire watershed. Perhaps the largest source of uncertainty is the interaction of Atlantic Whitefish with introduced non native predatory fish, Chain Pickerel and Smallmouth Bass. Both of these species have increased in distribution and density across southwestern Nova Scotia over the past thirty years (personal communication J. Leblanc Nova Scotia Department of Fisheries and Aquaculture). The level of overlap between Atlantic Whitefish and these predators may depend on tendencies toward

anadromy and timing of out migrations. Under early out migration scenarios Atlantic Whitefish may exit the system prior to the resumption of active feeding in the predator species after winter, effectively providing them protection. This hypothesis is untested and should be evaluated prior to restocking.

Overall, the identification of potential Atlantic Whitefish habitats for translocation varied with the choice of model complexity. Using the current habitat characteristics to identify potential translocation habitats indicated that more habitats would be suitable for translocation of Atlantic Whitefish than those that resulted from simulation modeling incorporating environmental variability and anadromous migrations. This study reinforces the importance of including available life history, physiology and environmental variability into exercises directed toward finding the most suitable habitats.

Table 5.1: Characteristics of the watershed specific data used in analyses. For each watershed the pH characteristics included overall mean pH, number of samples (N), number of sampling locations, number of locations in lentic or lotic habitats. In addition, the number of lakes used in cluster analysis was included. Watershed specific seasonal trends in pH are shown in the Appendix. Cells denoted by '-' indicate no data available.

Watershed	Number	pH					N Lakes
		Mean pH	N	Sample locations	Lentic	Lotic	
Clyde	1	4.54	23	1	1	-	-
East	2	4.88	1112	8	4	4	4
Gold	3	5.29	464	13	4	9	11
Ingram	4	5.01	354	3	3	-	-
Jordan	5	4.54	16	1	1	-	9
Lahave	6	5.63	579	6	6	-	62
Medway	7	5.23	233	81	18	63	43
Mersey	8	4.98	44	1	1	0	43
Middle	9	5.02	343	3	2	1	6
Mushamush	10	6.32	14	1	1	-	9
Petite	11	5.84	1647	38	4	34	14
Roseway	12	4.44	56	2	2	-	24
Sackville	13	5.39	338	3	3	-	19
Salmon	14	4.68	348	5	4	1	16
Sissiboo	15	4.92	25	1	1	-	12
Tusket	16	4.80	38	3	2	1	79

Table 5.2: Combinations of random variability incorporated into Level 1 (model 0) and 2 (model 1 – 7) simulation models, pluses (+) indicated the presence of the variability in that component of the simulation.

Model Distribution	Random pH N(μ,σ)	Random Temperature N(μ,σ)	Random Model Parameters U(1,1000)
0	-	-	-
1	-	+	-
2	-	-	+
3	-	+	+
4	+	-	-
5	+	+	-
6	+	-	+
7	+	+	+

Table 5.3: Relative ranks of lakes based on different levels of model complexity, with lower ranks indicating best choices. Level 0 models were the results from the multivariate analysis identifying the most similar lakes. Level 1 models incorporated the mean annual watershed specific pHs and stage-specific Atlantic Whitefish mortality in simulation models. Level 2 models incorporated variability in the environmental parameters temperature and pH as well as the mortality rate models in different combinations. Results of the Level 2 models were collapsed as relative ranks for models 1-3, which did not incorporate variability in pH and models 4-7, which did incorporate variable pH were the same within groupings. Level 3 simulation models use the Level 2 model with a complete complement of variability in parameters and variables as well as allow for anadromous migrations.

River	Lake	Distance to Estuary (km)	Rank				
			Level 0	Level 1	Level 2		Level 3
					Model		
					0-3	4-7	
Petite	Hebb	21.9	1	1	1	2	1
	Milipsigate	25.7	1	1	1	2	1
	Minamkeak	30.2	1	1	1	2	1
Mushamush	L. Mushamush	15.9	1	1	1	1	1
	Caribou	21.1	1	1	1	1	1
	B. Mushamush	21.3	1	1	1	1	1
LaHave	Seven Mile	32.9	1	1	1	2	1
	Hirtle	37.2	1	1	1	2	1
	Sherbrooke	46.2	1	1	1	2	1
Medway	Herring Cove	12.2	1	2	2	3	2
	Molega	62.7	1	2	2	3	1
Mersey East	Tobeatic	65.6	1	3	3	4	3
	Timber	18.7	1	4	4	5	4
Tusket	Vaughn	3.4	1	5	5	5	5
	Raynards	12.7	1	5	5	5	5
	Great Barren	46.8	1	5	5	5	5
Jordan	Kempt Back	47.4	1	5	5	5	5
	Jordan	51.1	1	6	6	6	6

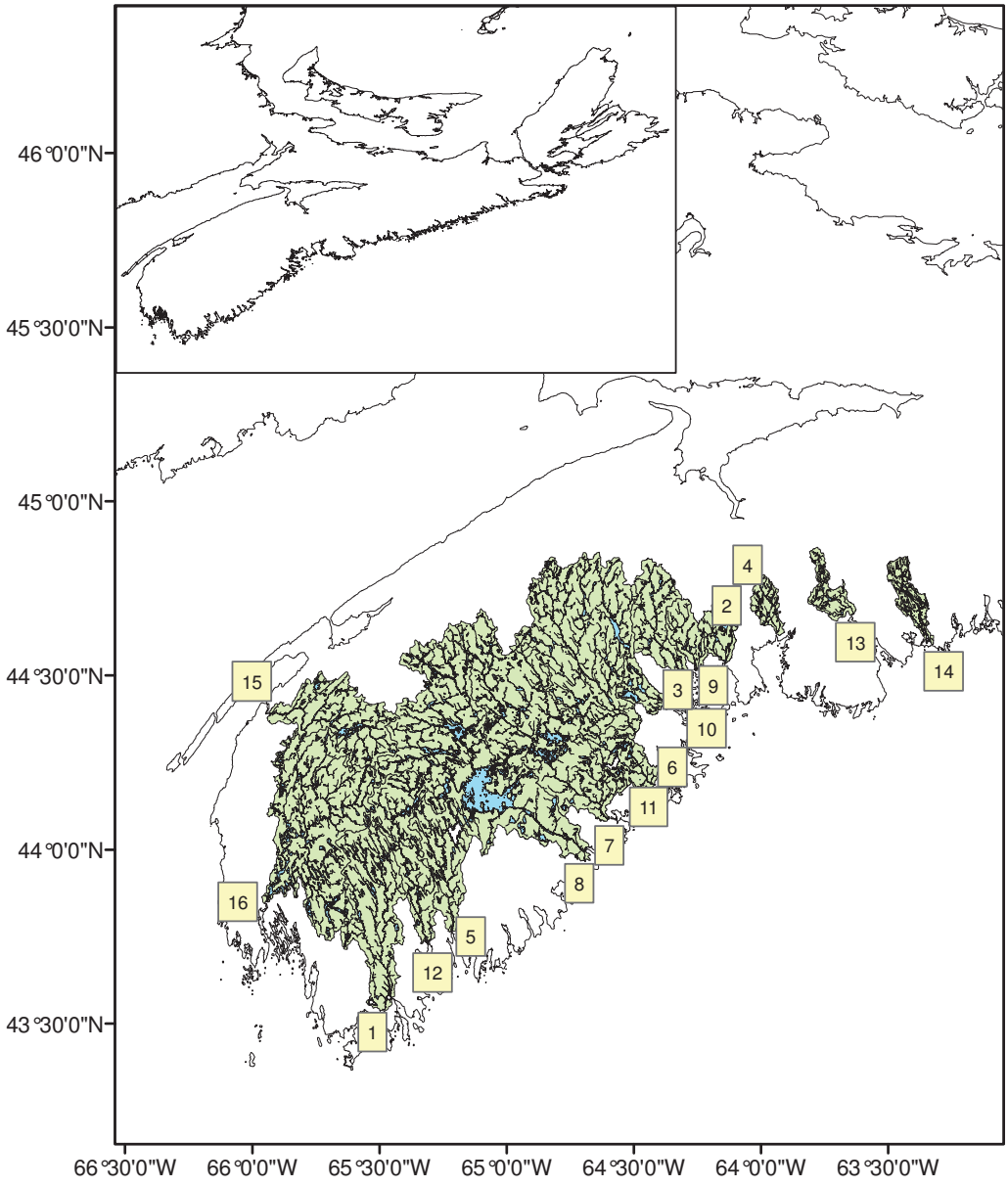

Figure 5.1: Map of the watersheds used in the analysis of potential translocation habitats for Atlantic Whitefish. Names for labeled watersheds are provided in Table 5.1.

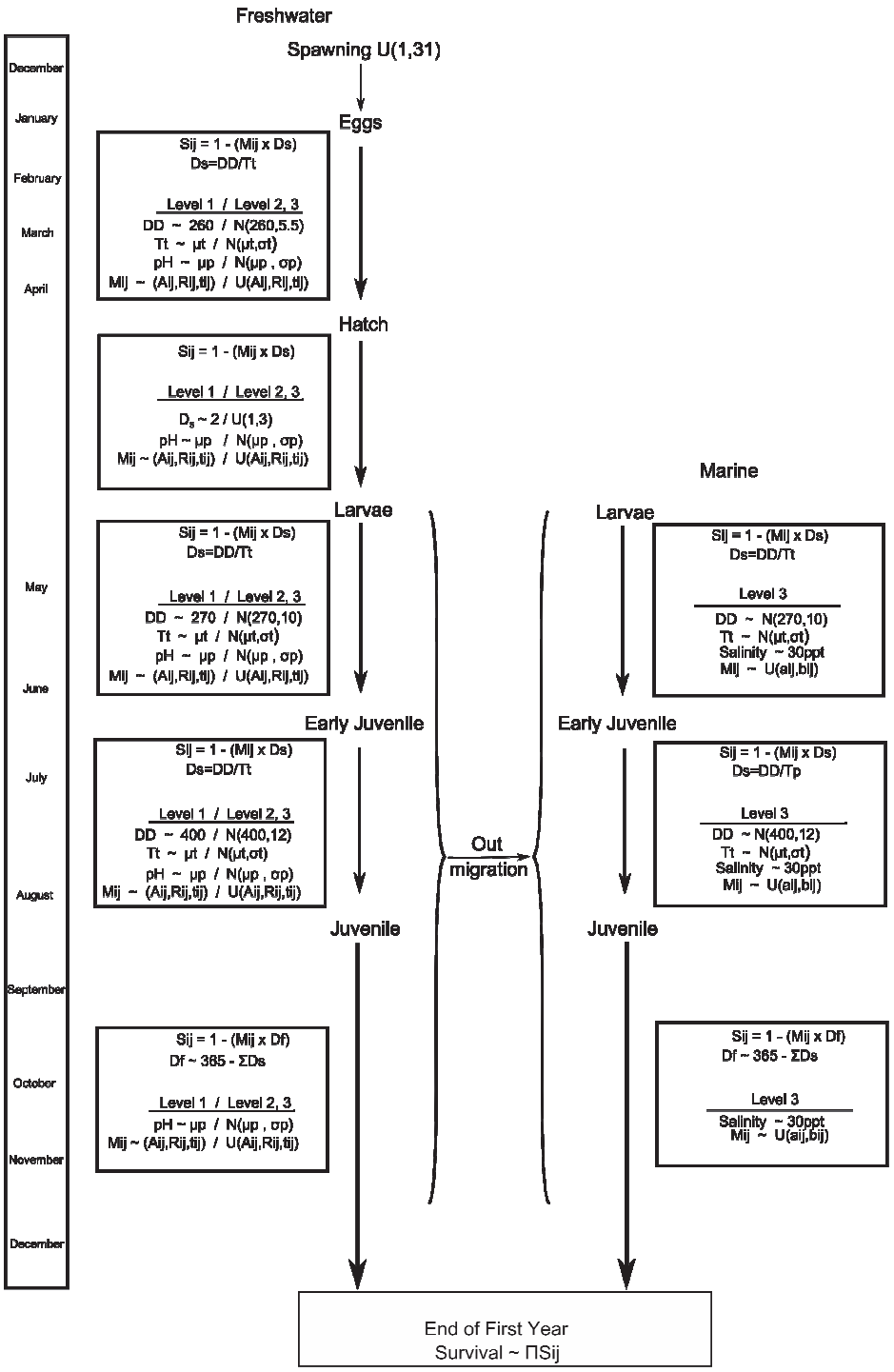

Figure 5.2: Diagram for a single iteration of the simulation model levels 1, 2 and 3 used to assess habitats for translocation and repatriation of Atlantic Whitefish. Terms are identified in text.

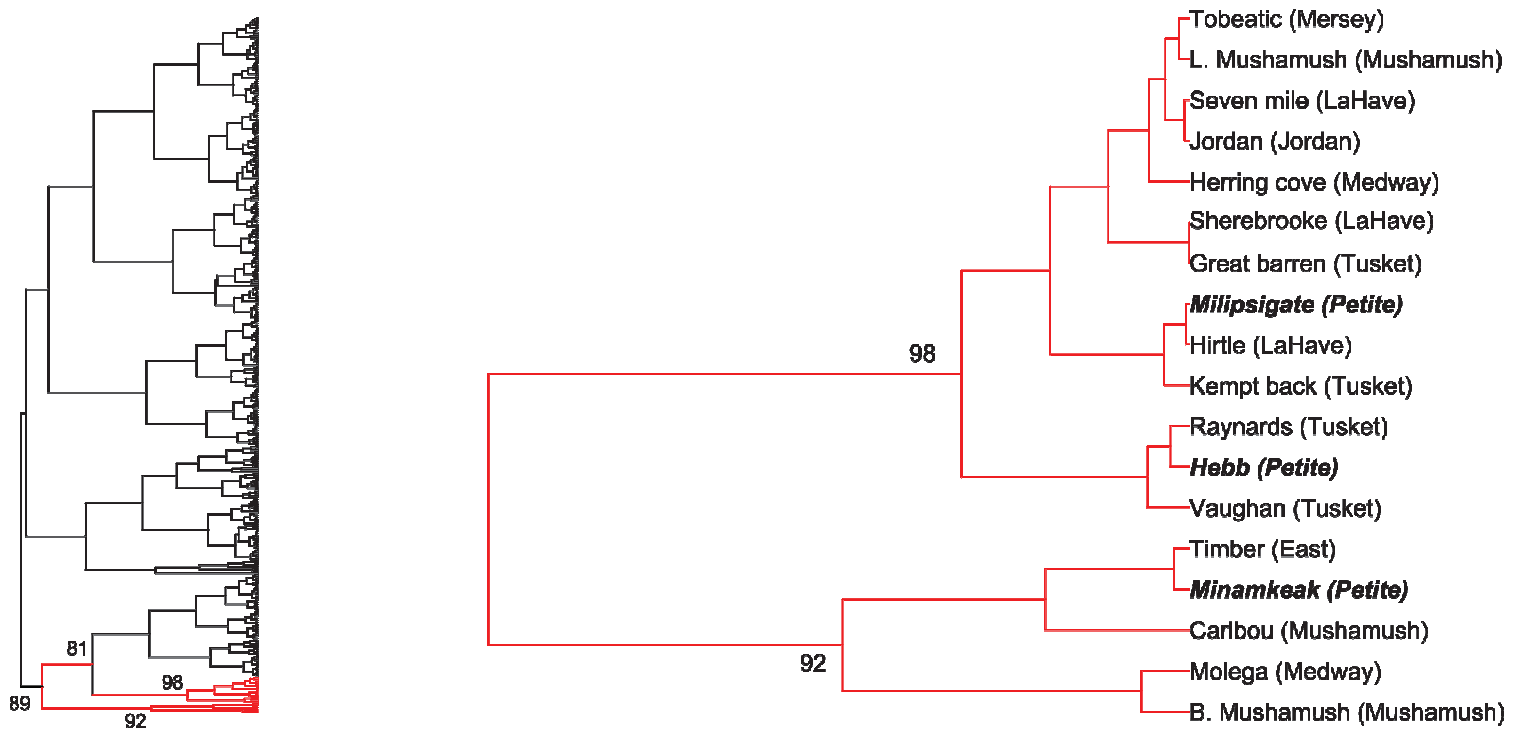

Figure 5.3: Cluster analysis of lakes to determine those most suitable for repatriation and translocation. Left plot is the entire dataset, right plot is the subset, in red, of lakes that contain the three current Atlantic whitefish lakes (bolded and italicised). Watershed names (in parentheses) are given beside lake name in plot on right. Numbers represent bootstrapped support of nodes.

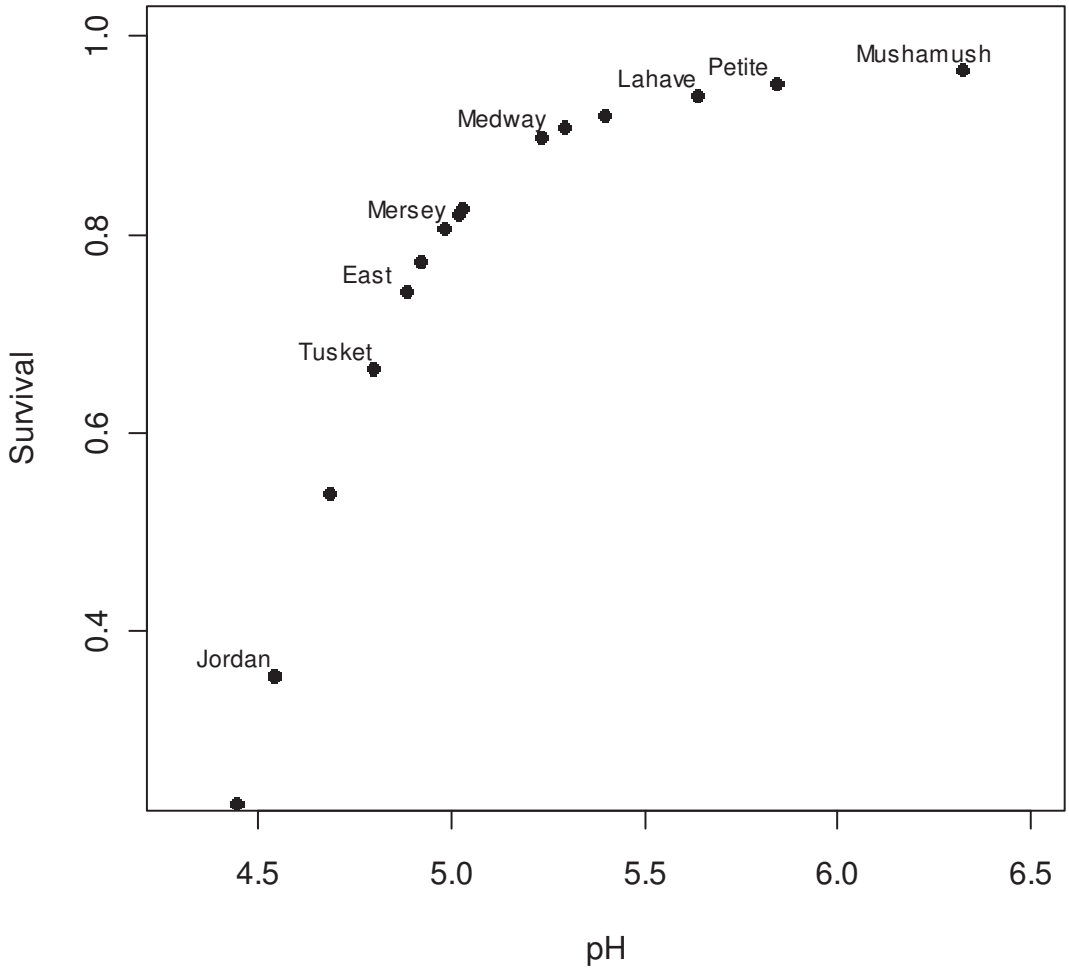

Figure 5.4: Plot of survival to the end of the first year as affected by mean observed pH (Level 1) across all watersheds in southwestern Nova Scotia. The eight watersheds identified by multivariate analysis to possess suitable Atlantic Whitefish lake habitat were labelled.

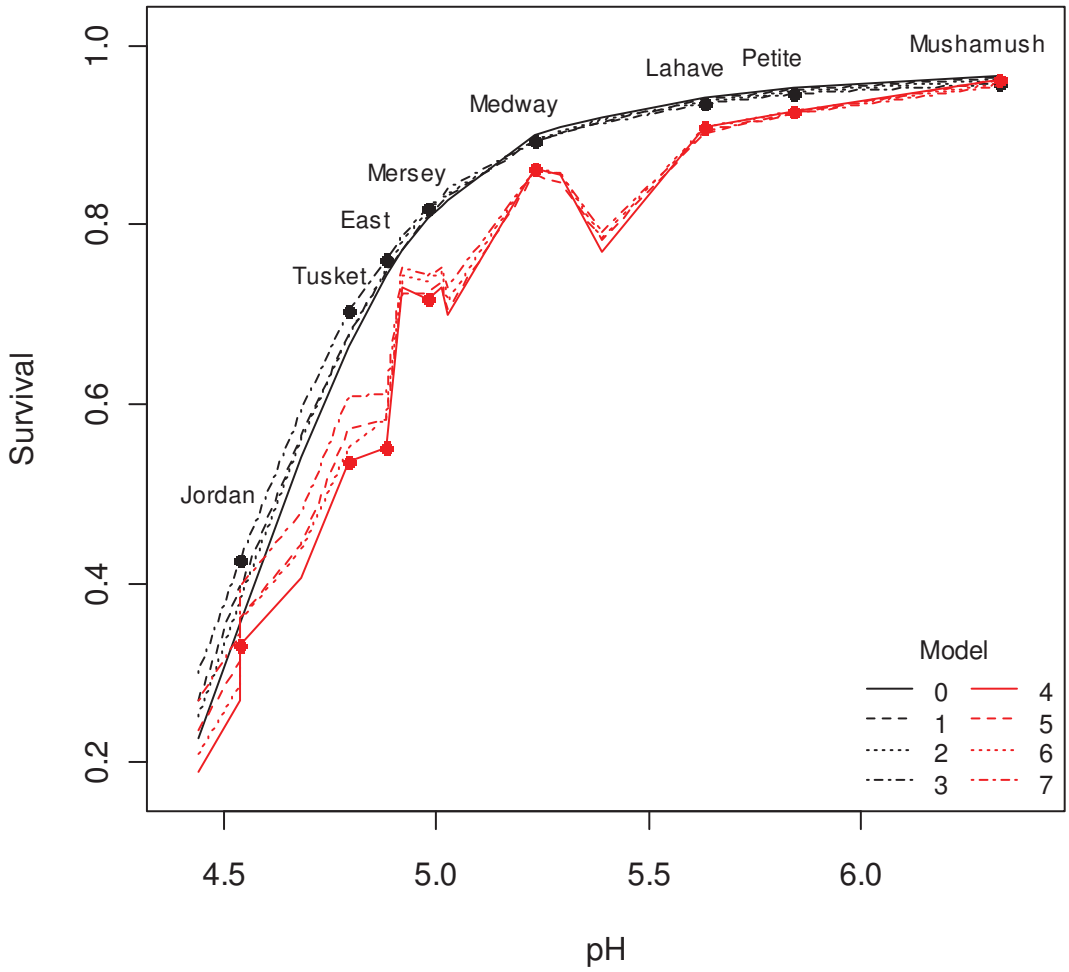

Figure 5.5: Changes in median survival with pH for the eight different model types including all watersheds. Watersheds identified as having a suitable Atlantic Whitefish lake were identified by a symbol and labels. For comparative purposes, the level 0 model, with no variability was included in this plot. The rivers were characterized based on mean annual pH.

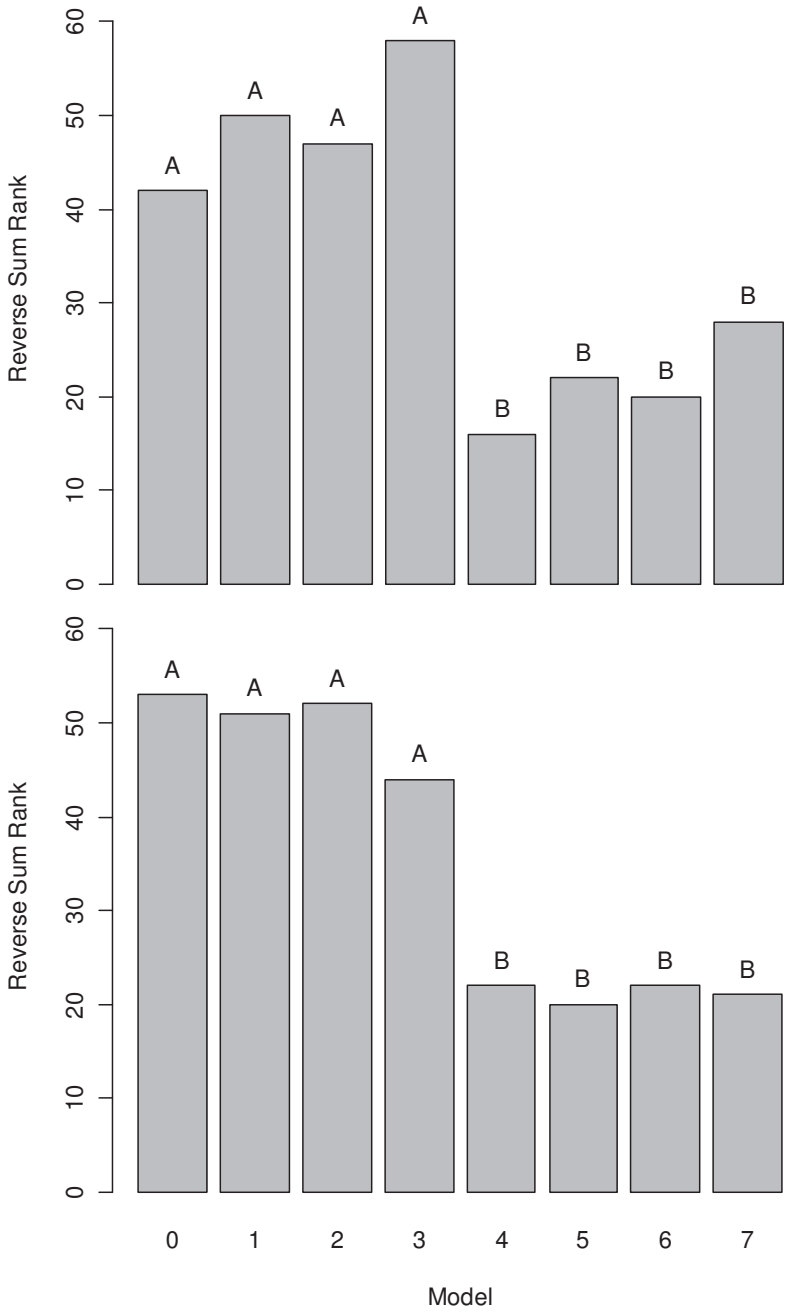

Figure 5.6: Combined reverse rank sum of survival across all modeled watersheds to show the changes in survival with model variability. Top panel for watersheds with pH<5.0. Bottom panel is for watersheds with pH >5.0. Models 4, 5, 6, 7 have variability in pH. Within a plot bars sharing the same letter are not statistically different.

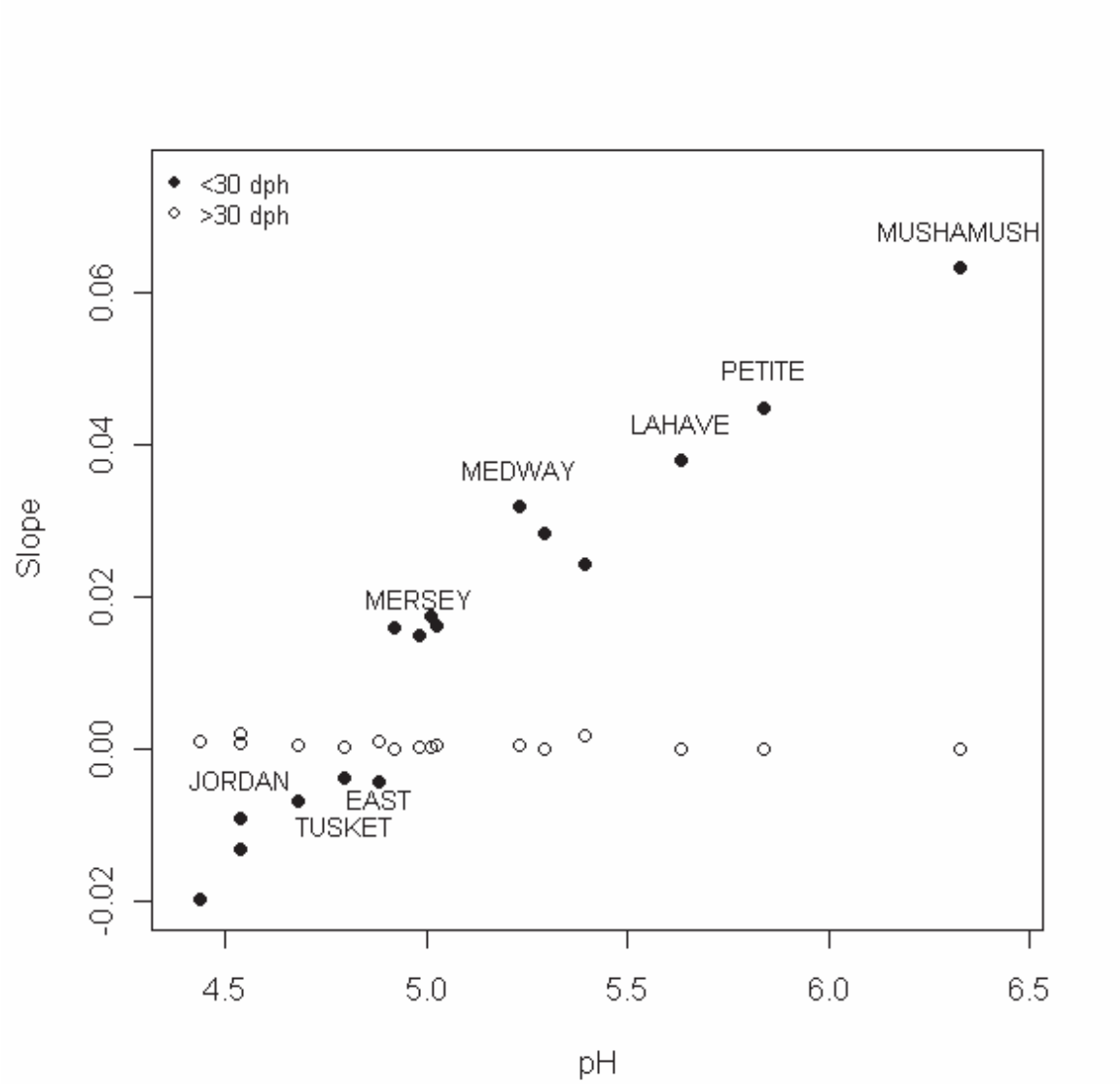

Figure 5.7: Slope of relationships between median survival to the end of first year of life and timing of anadromous migrations for fish leaving each watershed before or after day 30 post hatch.

CHAPTER 6: DISCUSSION

In conservation biology, research effort is often placed into three main categories, 1) understanding the mechanisms or threats that drive a species to low abundance, 2) the dynamics and persistence of species at low abundance and 3) the methods that can be used to rebuild abundance (Caughley 1994). Obtaining the information that can be used to address these questions often requires a long time series of data, intensive study or both. However, there is an increase in the number of 'data poor' species requiring conservation, as new species are being discovered and more species are being found in a reduced number of habitats or in reduced abundance (Hilton-Taylor *et al*. 2008). For these species, novel methods need to be applied to obtain base line information on species biology, response to threats and to inform potential recovery efforts. In this thesis, I explored some of these issues using the endangered Atlantic Whitefish, a species for which little data existed and was found only in a landlocked population in three lakes within one watershed (Bradford *et al* 2004).

In order to obtain some of the baseline information on the species demographic history and to define the distinct species status I used a suite of molecular markers. Atlantic Whitefish were clearly shown to be a distinct species based on both microsatellite data and sequencing data from a segment of the mtDNA COI. Further supporting the importance of this species, results suggested Atlantic Whitefish were a putative basal progenitor within the genus *Coregonus* found throughout the temperate and polar regions of all northern hemisphere continents.

In terms of species status and population trends these microsatellite markers suggested that the population has been at a low effective population size for most of its recent history, and has likely been influenced by a population bottleneck around the time dams were constructed restricting the species movements between habitats.

Present day genetic diversity was used to assess the status of the Atlantic Whitefish. However, it was useful to have a comparative metric to assess the level of genetic diversity present within Atlantic Whitefish, as levels of genetic diversity are known to be affected by factors external to the current population dynamics (Primmer *et al.* 1997; Bernatchez and Wilson 1998). Here, I used the same suite of genetic markers on 13 populations of regional congeners with similar life history and history of displacement by glaciation to compare against the Atlantic Whitefish, as no historical samples were available for comparison. Furthermore, to account for differences in population size, I used a surrogate, available lake habitat, to examine the relationship between genetic diversity estimates and population size (Frankham 1995a). From these analyses, Atlantic Whitefish were shown to have low genetic diversity, on the order of 2-6 times less than would be expected based on habitat size. These results suggest that Atlantic Whitefish are among some of the most genetically depleted populations examined to date. There has very likely been a long period of inbreeding which has led to purging of much of the species' genetic load (Frankham *et al.* 2001); however, a direct estimate of the quantitative genetic diversity should be performed.

Next, I examined the impacts of the perceived environmental threats of environmental acidification and the future threat of temperature variability on the persistence of Atlantic Whitefish. There was no information on the response to environmental factors outside of Cook and Bentzen (2009), which showed that Atlantic Whitefish have not lost their ability to tolerate sea water despite ~100 years of no access to the ocean. Prior to examining these threats I explored different methods and metrics to assess the relative thermal sensitivity of Atlantic Whitefish compared to other species, as there was no historic information or other outbred populations for comparison. In most previous studies, a species' temperature sensitivity was assessed using tolerable temperature ranges (*sensu* Fry 1956) or the response of specific physiological

mechanisms to temperature (*e.g.* Eliason *et al.* 2011). Here, I examined temperature - growth relationships for a suite of 25 species to determine the most appropriate model and the most informative measure of thermal sensitivity. From the outputs of these models, a series of correlative analyses were performed which showed that cold water species and those by characterized by a large asymptotic body size or maximum age were most sensitive to temperature change (Chapter 3).

Using these models and thermal sensitivity metrics, Atlantic Whitefish were found to have intermediate thermal sensitivity compared to other members of the family Salmonidae. And although I could not assess whether Atlantic Whitefish has suffered from an increased thermal sensitivity due to its current inbred and low genetic status, using the methods from Chapter 3 I was able to show that they remain within the range of species sharing similar ancestry and life history traits. Data collected within the lentic habitats of the Petite Riviere suggest that temperatures during summer will often rise above the upper temperature for growth of Atlantic Whitefish; however, there is no information on the full suite of thermal habitat available to the fish. Recent work has shown that Atlantic Whitefish preferentially utilize the deeper water regions of lake habitats, perhaps as a thermal refuge during the summer months (Cook *et al.* in review). Moving forward, this also suggests that the estimate of available lake habitat of 16 km^2 may be an overestimate of the total usable habitat as defined by the thermal bounds defined here. This habitat size may decrease in future and become a threat to the persistence of the Atlantic Whitefish within the Petite Riviere as global mean temperatures are predicted to rise 1.8 - 4.0°C over the next century (IPCC 2007). Furthermore, in watersheds with lower pHs, the potential impact of warming temperatures on growth of Atlantic Whitefish will increase because thermal sensitivity increases in low pH environments (Chapter 4). Restoration of anadromy to the Atlantic Whitefish on the Petite Riviere will allow for an escape from these small thermally

bounded habitats, as fish will be provided the opportunity to move into cooler coastal or oceanic waters (Chapter 4).

In order to assess the impact of acidification on the demise of the Tusket population, as well on the persistence of the Petite population I examined the effect of the low pH on the survival and growth of the early life stages of Atlantic Whitefish. Low pH was assessed through ontogeny as the relative sensitivity has been shown to change through development in other species (Kwain and Rose 1985). I maintained spawning groups separately for the egg and early larval stages to determine if significant plasticity in responses between family groups can be described. Results showed differences in pH effects on survival among the life stages examined and that for some responses, differences were evident between spawning groups, although they could not be detected using neutral genetic markers.

The pH levels present in the Tusket River, while lower than the Petite Riviere, likely were not the sole contributor to the loss of this Atlantic Whitefish population, although it may have been a causal factor along with the other stated threats. The current pH levels in the Petite Riviere will likely not negatively impact that population's persistence, nor should the pH for the Tusket River be detrimental to repatriating fish to this population, particularly as levels are expected to increase (Clair *et al*. 2004; Chapter 4).

In the final experimental Chapter of this thesis I assessed the freshwater habitats in south western Nova Scotia for potential repatriation and translocation of Atlantic Whitefish. I explored the impact of hierarchically incorporating different levels of information into the choice of optimal translocation habitats. At the most basic level (Level 0), I used just the available present day information of the physical characteristics of the three lakes on the Petite Riviere to define habitats. Next I developed a simulation model to evaluate the effect of watershed-specific environmental temperature and pH

with the option of anadromous migrations on the survival potential using the modeled life stage-specific responses from Chapter 4 and from Cook *et al.* (2010a). In the most basic application of the simulation models, mean species response in relation to the mean water quality variables for each of the respective watersheds were used to determine the best overall habitats (Level 1). In the Level 2 models, I incorporated the variability in environmental variables and species response in various combinations to evaluate the impact of different levels of variability on the choice of optimal habitats. In the final Level 3 models, I used the simulation model with the full suite of variability and incorporated anadromous out migrations at various times post hatch to assess the impact of anadromy on habitat choice. The results from this analysis showed that the level of model complexity used will impact the number of watersheds selected as the best, but the top watersheds will likely be chosen regardless of the level of variability included. Incorporating the full suite of life history attributes, including anadromous migrations opened up the potential for more habitats to be used for the translocation of Atlantic Whitefish (Chapter 5).

Throughout this thesis I was able to begin to address some of the key issues for Atlantic Whitefish conservation through the use of non-traditional methods. The approaches applied here may prove fruitful in future studies informing the conservation processes in other data poor species. The use of genetic markers provided information on the historical and contemporary population size and the current levels of genetic diversity. Environmental threats were assessed using a combination of laboratory studies and field collected environmental data. And the options for population restoration were explored using a suite of simulation models with varying levels of complexity.

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APPENDIX A: CHAPTER 2

A2.1 Life Table Methods

Ecological life tables describe the age or stage specific survivorship and fecundity of a species and are central to the study of population dynamics. The construction of life tables requires either tracking a cohort over its entire life span and describing the changes in abundance and fecundity with age or collecting a random sample of the entire population in one sample, assume population dynamics are static and determine the survivorship and fecundity from this sample. In the present life table, survival was reported as l_x, which is the proportion of age 1 surviving to age x and fecundity is b_x, or the relative contribution of each individual of that age class to the population.

Data were unavailable to complete an Atlantic Whitefish life table using either approach. I therefore took a theoretical approach to constructing the life table using von Bertalanffy growth parameters, Pauly's empirical M equation (Pauly 1980) and a regression equation describing the length-fecundity relationship. Von Bertalanffy's growth model (VBGM) describes the relationship between age (T) and length at age (L_T) as:

$$L_T = L\infty\left(1 - e^{-k(T-t_o)}\right)$$

where $L\infty$ is asymptotic body length, K describes the rate of increase to $L\infty$ and t_o is length at time 0. This model was fit to 258 Atlantic whitefish samples where length and age information were available using optim function with a Gaussian negative log likelihood objective function in R (version 2.11). Resultant VBGM parameter estimates were $L\infty$=29.6, K=0.57 and t0=-0.04. Using these VBGM parameters and ambient water temperature (annual mean water temperature 10.7℃- T.Hiltz Town of Bridgewater

pers.comm), natural mortality was estimated as 0.79 from the empirical relationship (Pauly 1980):

$$\log_{10}(M) = 0.6543 \cdot \log_{10}(K) + 0.4634 \cdot \log_{10} T - 0.279 \cdot \log_{10}(L\infty) - 0.066$$

Fecundity at age (F_x) was estimated using the length specific fecundity relationship from Bradford *et al.* (2010) of,

$$\log(Eggs) = 0.362 \cdot \log(L) - 12.97$$

a knife-edge age at maturity of 3, maximum age of 8 and mean length at age data from the VBGM. To obtain estimates of l_x I simulated a stable adult population size with numbers at ages 3 - 8 such that

$$\sum_{x=3}^{8} N_x = 2000 \quad and \quad N_{3+T} = N_3 e^{-MT}$$

From those numbers at age the annual egg production (N_1) was calculated as

$$N_1 = \sum_{x=3}^{8} N_x \cdot F_x$$

Assuming a type III survivorship curve I estimated N_2 allowing 99% of the mortality between N_1 and N_3 to occur in the first year. From the numbers at age, l_x were calculated as

$$l_x = \frac{N_x}{N_1}$$

and were independent of the actual simulated population size. The fecundity at age (F_x) values were used to estimate b_x but were scaled such that $\sum l_x \cdot b_x = 1$ as I assumed a stable population size. P_x indicated that proportion of l_1 provided by each age class and was calculated as $l_x \cdot b_x$.

Table A2.1:Life table data for Atlantic Whitefish.

Age	l_x	b_x	P_x
1	1.0	0.0	0
2	0.01	0.0	0
3	0.000452	974.2	0.44
4	0.000204	1369.4	0.28
5	0.000092	1638.3	0.15
6	0.000042	1806.3	0.08
7	0.000019	1906.6	0.04
8	0.000009	1965.1	0.01
9	0	0.0	0

Table A2.2 Genbank Accession Numbers for species used in CO1 phylogeny.

Species - Sample #	Accession Number	Species - Sample #	Accession Number
Coregonus artedi-1	EU523940.1	*Coregonus laurettae-5*	EU523969.1
Coregonus artedi-2	EU523941.1	*Coregonus laurettae-6*	EU523971.1
Coregonus artedi-3	EU523942.1	*Coregonus laurettae-7*	EU523972.1
Coregonus artedi-4	EU523943.1	*Coregonus lavaretus-1*	AB034824.1
Coregonus artedi-5	EU523944.1	*Coregonus nasus-1*	EU202652.1
Coregonus artedi-6	EU523945.1	*Coregonus nasus-2*	EU523973.1
Coregonus autumnalis-1	EU202649.1	*Coregonus nasus-3*	EU523974.1
Coregonus autumnalis-2	EU523946.1	*Coregonus nasus-4*	EU523975.1
Coregonus autumnalis-3	EU523947.1	*Coregonus nasus-5*	EU523976.1
Coregonus autumnalis-4	EU523948.1	*Coregonus nasus-6*	EU523977.1
Coregonus autumnalis-5	EU523949.1	*Coregonus nasus-7*	EU523978.1
Coregonus autumnalis-6	EU523950.1	*Coregonus nasus-8*	EU523979.1
Coregonus autumnalis-7	EU523951.1	*Coregonus nigripinnis-1*	EU523980.1
Coregonus clupeaformis-1	EU523952.1	*Coregonus nigripinnis-2*	EU523981.1
Coregonus clupeaformis-2	EU523953.1	*Coregonus pidschian-1*	EU202651.1
Coregonus clupeaformis-3	EU523954.1	*Coregonus pidschian-2*	EU427541.1
Coregonus clupeaformis-4	EU523955.1	*Coregonus sardinella-1*	EU202653.1
Coregonus clupeaformis-5	EU523956.1	*Coregonus sardinella-2*	EU523982.1
Coregonus clupeaformis-6	EU523957.1	*Coregonus sardinella-3*	EU523983.1
Coregonus clupeaformis-7	EU523958.1	*Coregonus sardinella-4*	EU523985.1
Coregonus clupeaformis-8	EU523959.1	*Coregonus sardinella-5*	EU523986.1
Coregonus hoyi-1	EU523960.1	*Coregonus sardinella-6*	EU523987.1
Coregonus hoyi-2	EU523961.1	*Coregonus zenithicus-1*	EU523988.1
Coregonus hoyi-3	EU523962.1	*Coregonus zenithicus-2*	EU523989.1
Coregonus hoyi-4	EU523963.1	*Coregonus zenithicus-3*	EU523990.1
Coregonus hoyi-5	EU523964.1	*Prosopium coulterii-1*	EU202654.1
Coregonus huntsmani-1	EU524489.1	*Prosopium coulterii-2*	EU202655.1
Coregonus kiyi-1	EU523965.1	*Prosopium cylindraceum-1*	EU202656.1
Coregonus laurettae-1	EU202650.1	*Prosopium cylindraceum-2*	EU202657.1
Coregonus laurettae-2	EU523966.1	*Stenodus leucichthys-1*	EU202658.1
Coregonus laurettae-3	EU523967.1	*Salmo salar-1*	HM007799.1
Coregonus laurettae-4	EU523968.1		

APPENDIX B: CHAPTER 3

Table B3.1: Life history and biological information on species used in thermal sensitivity analysis.

Common Name	Order	Species	Habitat	K	L∞ (cm)	Genbank Accession Number	Maximum Age (y)	Temperature Range (°C)	Latitudinal Range (°Lat)
European eel	Anguilliformes	*Anguilla anguilla*	Dia.	0.2	76	EU523918	88	16	67
Emerald shiner	Cypriniformes	*Notropis atherinoides*	FW	0.9	11	EU524169	4	29	34
Leatherside chub	Cypriniformes	*Snyderichthys copei*	FW	.	20	.	.	.	.
Atlantic cod	Gadiformes	*Gadus morhua*	Mar.	0.1	167	CAA68108.1	25	15	45
Burbot	Gadiformes	*Lota lota*	FW	0.1	104	EU524746	20	14	38
Walleye pollock	Gadiformes	*Theragra chalcogrammus*	Mar.	0.1	94	NC_008943	15	.	34
Spotted wolffish	Perciformes	*Anarhichas minor*	Mar.	0.1	154	ABQ08621.1	.	.	39
Snakehead	Perciformes	*Channus argus*	FW	0.2	73	.	.	.	.
Ruffe	Perciformes	*Gymnocephalus cernua*	FW	0.3	18	EU524643	10	10	31
Striped bass	Perciformes	*Morone saxatilis*	Dia.	0.2	102	EU524145	30	17	27
Blue tilapia	Perciformes	*Oreochromis aureus*	FW	0.8	20	.	.	.	.
Yellow perch	Perciformes	*Perca flavescens*	FW	0.5	33	EU524239	11	28	31
Bluefish	Perciformes	*Pomatomus saltatrix*	Mar.	0.2	94	ABK06044.1	9	.	91
Rabbitfish	Perciformes	*Siganus rivulatus*	Mar.	0.2	32	.	.	.	.
Mandarinfish	Perciformes	*Siniperca roulei*	FW	.	20	.	.	.	.
Atlantic halibut	Pleuronectiformes	*Hippoglossus hippoglossus*	Mar.	0.1	280	NC_009709	50	.	43
Rock sole	Pleuronectiformes	*Lepidopsetta bilineata*	Mar.	0.2	55	ACD40107	22	.	33
Japanese flounder	Pleuronectiformes	*Paralichthys olivaceus*	Mar.	0.2	117	BAA89035.1	.	.	61
Turbot	Pleuronectiformes	*Psetta maxima*	Mar.	0.3	82	ACD40155.1	25	.	40
Atlantic whitefish	Salmoniformes	*Coregonus huntsmani*	Dia.	0.6	29	EU524489	8	20	1
Sockeye salmon	Salmoniformes	*Oncorhynchus nerka*	Dia.	0.4	74	EU524223	8	23	30
Atlantic salmon	Salmoniformes	*Salmo salar*	Dia.	0.4	138	EU524349	13	18	30
Arctic charr	Salmoniformes	*Salvelinus alpinus*	Dia.	0	140	EU524357	40	12	42
Brook trout	Salmoniformes	*Salvelinus fontinalis*	FW	0.28	34	EU522409	24	23	35
Wels catfish	Siluriformes	*Silurus glanis*	FW	0	296	.	.	.	.

Table B3.2: Model statistics and thermal sensitivity estimates for species used in analysis.

Common name	Body Size (cm)	Treatment	Level	N	Model	Tu	Tl	Tm	AT	UAT	OAT	OLT	ULT	LT	AIC	AICC	Reference
Arctic charr	4	Population	Blasjon	6	EM	22	0.4	17.7	10.8	2.1	4.7	5.4	4.2	21.6	2.7	33	Larsson *et al.* 2005
Arctic charr	4	Population	Dunsjon	6	PKM	20.8	.	15.5	12.5	4.2	8.2	9	5.3	.	3.7	43.9	Larsson *et al.* 2005
Arctic charr	12	Population	Hornaven	6	PKM	20.2	.	17.5	10.8	2.4	6.7	7.3	2.6	.	3.5	42.1	Larsson *et al.* 2005
Arctic charr	4	Population	Hornaven	5	PKM	18.1	.	16.2	10.3	1.7	6.3	6.9	1.9	.	2.7	64.2	Larsson *et al.* 2005
Arctic charr	4	Population	Rastojaure	6	PKM	20.5	.	15.9	12.6	3.8	8.4	9.2	4.5	.	3	36	Larsson *et al.* 2005
Arctic charr	13	Population	Sommen	5	PKM	18.2	.	16.9	8.9	1.3	5	5.5	1.3	.	2.2	53.4	Larsson *et al.* 2005
Arctic charr	4	Population	Torron	7	PKM	20.9	.	15.4	12.6	4.4	8.3	9.1	5.5	.	4	32.1	Larsson *et al.* 2005
Arctic charr	14	Population	Vattern	6	PKM	26.5	.	15.9	13.4	6.1	8.3	9.1	10.6	.	3.6	43.1	Larsson *et al.* 2005
Arctic charr	12	Population	Windimere	5	PKM	18	.	16.4	10.3	1.6	6.3	6.9	1.6	.	2.3	56.1	Larsson *et al.* 2005
Arctic charr	5	Population	Hals	25	RM	22	4.4	15.5	11.4	4.6	7.6	8.3	6.5	17.6	34.1	68.3	Larsson *et al.* 2005
Arctic charr	5	Population	Litaven	27	RM	22.2	4.6	14.6	11.6	5.2	7.9	4.7	7.6	17.6	24.3	44.2	Larsson *et al.* 2005
Arctic charr	13	Population	Nackten	6	RM	28.8	3.1	17.4	17	7.8	11.7	12.3	11.4	25.7	1.2	46.3	Larsson *et al.* 2005
Atlantic cod	4	body size	0.02	6	PKM	21.1	.	14.4	11.6	4.6	7.4	8.1	6.6	.	3.5	42	Bjornsson *et al.* 2007
Atlantic cod	7	body size	0.04	6	PKM	20.5	.	14	11.9	4.7	7.7	8.4	6.6	.	3.5	41.9	Bjornsson *et al.* 2007
Atlantic cod	10	body size	0.06	6	PKM	20.6	.	13.6	11.5	4.7	7.3	8	7	.	2.6	30.7	Bjornsson *et al.* 2007
Atlantic cod	15	body size	0.09	5	PKM	19.8	.	12.3	13	5.5	9	9.8	7.5	.	2	49	Bjornsson *et al.* 2007
Atlantic cod	21	body size	0.13	5	PKM	16.2	.	12.6	12.5	3.3	9.7	10.5	3.6	.	1.8	43.2	Bjornsson *et al.* 2007
Atlantic cod	31	body size	0.19	5	PKM	16.4	.	11.9	12.5	4	9.6	10.4	4.5	.	1.8	44.3	Bjornsson *et al.* 2007
Atlantic cod	1.2	body size	0.01	8	PKM	17.4	.	13.9	9.2	2.8	5.8	6.4	3.4	.	5.3	32.1	Steirnssen and Bjornssen 1999
Atlantic cod	14	body size	0.08	8	QM	22.6	2.7	12.6	13.2	6.6	9.1	9.9	9.9	19.9	14.8	35.6	Imsland *et al.* 2005
Atlantic cod	43	body size	0.26	5	RM	19.5	-0.7	10.3	14.6	6.4	9.9	11.4	9.2	22.2	0	-10.1	Bjornsson *et al.* 2007
Atlantic cod	12	body size	0.07	8	RM	20	0	14.1	12.5	4.3	8	9.1	5.9	20	3.2	43.1	Imsland *et al.* 2005
Atlantic cod	10	body size	0.06	8	RM	21	1.2	14.4	12.6	4.7	8.3	8.7	6.7	19.8	3.8	50.9	Imsland *et al.* 2005
Atlantic cod	16	body size	0.1	8	RM	19.8	1.3	12.3	12.1	5.2	8.2	8.5	7.5	18.5	2.8	37.9	Imsland *et al.* 2005
Atlantic cod	0.5	body size	0	10	RM	23	-1.7	10.3	18.5	8.6	12.6	12.6	12.7	27.7	5.2	41.6	Steirnssen and Bjornssen 1999
Atlantic cod	0.7	body size	0	10	RM	18.7	0	13	11.7	4.1	7.5	8	5.6	18.6	5.2	41.5	Steinarsson and Bjornssen 1999
Atlantic cod	0.9	body size	0.01	10	RM	17.9	1.5	13.4	10.1	3.3	6.4	6.7	4.5	16.4	6	47.8	Steinarsson and Bjornssen 1999
Atlantic halibut	22.5	body size	0.08	20	RM	17.1	2.5	13.9	8.7	2.4	5.3	5.8	3.3	14.7	16.2	43.2	Jonassen *et al.* 1999

Common name	Body Size	Treatment	Level	N	Model	Tu	Tl	Tm	AT	UAT	OAT	OLT	ULT	LT	AIC	AICC	Reference
Atlantic halibut	45	body size	0.16	16	RM	16.4	0.7	15	10.2	1.1	4.3	4.9	1.4	15.7	6.1	22	Jonassen *et al.* 1999
Atlantic halibut	15	Population	Canada	12	RM	18.8	2.5	15.7	9.5	2.3	5.6	6.1	3.1	16.3	5.7	32.5	Jonassen *et al.* 2000
Atlantic halibut	15	Population	Iceland	12	RM	20.1	3.4	14.7	10.6	3.8	6.9	5	5.4	16.7	5.6	32.1	Jonassen *et al.* 2000
Atlantic halibut	15	Population	Norway	12	RM	19.2	3.3	14.5	9.9	3.4	6.3	5.1	4.7	15.8	6.3	35.9	Jonassen *et al.* 2000
Atlantic salmon	6	Population	Alta	39	PKM	25	.	20.8	10.6	3.2	6.3	6.9	4.2	.	101.8	69.8	Jonsson *et al.* 2001
Atlantic salmon	7	Population	Imsa	32	PKM	25.2	.	20.1	11.7	3.8	7.2	7.9	5.1	.	86.3	74	Jonsson *et al.* 2001
Atlantic salmon	5	Population	Stryn	35	PKM	26.1	.	18.8	11.7	4.6	7	7.7	7.3	.	94.7	73.3	Jonsson *et al.* 2001
Atlantic salmon	10.5	Population	Leven	12	RM	24.1	6.5	16.1	11.7	5.4	8	8.2	8	17.6	10.4	59.2	Elliott and Hurley (1997)
Atlantic salmon	10.5	Population	Lune	17	RM	22.5	5.3	15.7	11.3	4.7	7.6	7.5	6.8	17.2	22.3	74.3	Elliott and Hurley (1997)
Atlantic whitefish	10	pH-4.75	4.75	7	PKM	22.6	.	19.3	12.1	2.9	7.5	8.5	3.3	.	4.7	37.9	Chapter 4
Atlantic whitefish	10	pH-4.2	4.2	7	PKM	22	.	17.5	11.5	3.7	7.2	7.9	4.4	.	4	32.4	Chapter 4
Atlantic whitefish	10	pH-7	7	7	PKM	22.9	.	18.2	11.5	3.7	7.1	7.8	4.7	.	4	32.2	Chapter 4
Atlantic whitefish	10	pH-4	4	7	PKM	21	.	16.1	10.3	3.5	6.2	6.8	4.6	.	5.1	40.9	Chapter 4
Blue tilapia	20	.	.	8	RM	39.1	20	31.5	12.6	5.3	8.5	9.1	7.7	19.2	4.3	57.7	Baras *et al.* 2002
Bluefish	14	body size	0.15	15	EM	32.2	14.1	29.8	9	1.2	3.9	4.5	2.4	18.1	7.9	17.2	Buckel *et al.* 1995
Bluefish	11	body size	0.12	15	PKM	36	.	33.1	13.9	2.6	7.8	8.5	2.9	.	14.8	32.4	Buckel *et al.* 1995
Bluefish	4	body size	0.04	15	RM	36.4	13.9	26	15.1	7.1	10.3	11.5	10.5	22.5	14.9	59.6	Buckel *et al.* 1995
Brook trout	2	.	.	6	PKM	23.1	.	14.4	13.7	5.9	9	9.8	8.7	.	3.9	46.5	McCormick *et al.* 1972
Burbot	8	.	.	10	Ells	28.2	-0.9	19.5	15.9	4.3	7	8	8.7	32	6.4	25.6	Hofmann and Fischer 2003
Emerald shiner	2	.	.	9	PKM	37.1	.	26.6	17.4	6.8	10.7	11.7	10.5	.	7.6	36.3	McCormick and Kleiner 1977
European eel	4	.	.	16	RM	35	2.9	21	21.2	9.6	14.5	15.1	14	32.1	19.8	72.1	Seymour 1989
Japanese flounder	15	body size	0.13	5	PKM	31.4	.	21.9	13.3	5.5	7.9	8.7	9.5	.	1.9	45.2	Iwata *et al.* 1994
Japanese flounder	20	body size	0.17	5	PKM	30.1	.	24.1	15	4.7	9.3	10.2	6	.	1.7	41.1	Iwata *et al.* 1994

Common name	Body Size	Treatment	Level	N	Model	Tu	TI	Tm	AT	UAT	OAT	OLT	ULT	LT	AIC	AICC	Reference
Japanese flounder	8	body size	0.07	6	RM	31.9	8.9	25.3	14.3	4.8	9.1	10.4	6.6	23	0.8	32.4	Iwata *et al.* 1994
Japanese flounder	12	body size	0.1	6	RM	33.6	8.5	22.9	16.6	7.4	11.3	12	10.7	25.2	0.8	32.7	Iwata *et al.* 1994
Leatherside chub	2	.	.	14	RM	32.2	14	23.7	12.2	5.8	8.3	8.4	8.5	18.2	12.9	57.5	Billman *et al.* 2008
Mandarinfish	13	.	.	12	PM	39.8	.	29.9	.	5.5	4.5	5.8	9.9	.	6.8	38.9	Liu *et al.* 1998
Mandarinfish	15	.	.	17	RM	36.4	9.3	31.7	15.5	3.5	8.9	6.7	4.7	27.1	10.2	33.9	Liu *et al.* 1998
Mandarinfish	20	.	.	20	RM	42.9	4.8	29.9	24.4	9.2	16.1	17.5	13	38.1	16	42.6	Liu *et al.* 1998
Rabbitfish	8	.	.	16	RM	35.9	13.4	27.6	14.6	5.8	9.8	9	8.3	22.6	13.8	50.3	Saoud *et al.* 2008
Rock sole	6.1	.	.	12	RM	23.6	-0.6	13.2	16.1	7.2	11	10.7	10.5	24.3	7.4	42.2	Hurst and Abookire 2006
Ruffe	4	.	.	6	PM	29.1	.	20.5	.	5.9	9.7	10.7	8.6	.	0.6	33.3	Edsall *et al.* 1993
Snakehead	40	body size	0.55	5	Ells	36	7	30	14.5	3	6.4	7.3	6	29	1	23	Liu *et al.* 1998
Snakehead	16	body size	0.22	6	RM	40.5	10.8	31.5	18.6	6.4	12	14.6	8.9	29.7	1.5	60.2	Liu *et al.* 1998
Snakehead	18	body size	0.25	6	RM	39.1	10.8	30.8	17.6	6	11.3	12.3	8.3	28.3	1.5	58	Liu *et al.* 1998
Snakehead	21	body size	0.29	6	RM	37.6	10.5	31.4	16.1	4.6	9.8	11.3	6.2	27.1	1.6	63	Liu *et al.* 1998
Snakehead	25	body size	0.34	6	RM	42.2	5.7	31.1	22.9	8	14.8	16.4	11.1	36.6	1.6	63.5	Liu *et al.* 1998
Snakehead	28	body size	0.38	6	RM	43.4	9.5	32.1	21.7	8	14.2	16.1	11.3	34	1.5	60.2	Liu *et al.* 1998
Snakehead	33	body size	0.45	6	RM	42.3	8	31.7	22.8	10.4	15.6	18.8	18	41.8	1.4	57.1	Liu *et al.* 1998
Snakehead	36	body size	0.49	6	RM	35.8	1	33.8	22.3	1.6	8.9	11.3	2	34.8	1.7	67.8	Liu *et al.* 1998
Sockeye salmon	3.2	body size	0.04	15	RM	18.9	1.9	16.5	9.5	1.8	5.3	6	2.4	17	23.5	94	Brett 1974
Sockeye salmon	3.5	body size	0.05	15	RM	18.7	1.8	16.6	9.4	1.7	5.1	5.8	2.2	16.9	17.2	68.8	Brett 1974
Sockeye salmon	3.8	body size	0.05	15	RM	18.9	1.9	16.4	9.5	1.9	5.3	5.9	2.5	17	25.4	101.6	Brett 1974
Sockeye salmon	4	body size	0.05	15	RM	19.1	2	16.4	9.7	2	5.4	6.1	2.7	17.1	24.6	98.5	Brett 1974
Sockeye salmon	4.2	body size	0.06	15	RM	19.9	2	16.5	10.4	2.5	6.1	6.7	3.4	17.9	23.2	93	Brett 1974
Sockeye salmon	4.4	body size	0.06	15	RM	19.1	1.9	16.4	9.7	2	5.5	6.1	2.7	17.2	25.4	101.4	Brett 1974
Sockeye salmon	4.6	body size	0.06	15	RM	19	2.1	16.4	9.5	1.9	5.3	6	2.6	16.9	24.3	97.3	Brett 1974
Sockeye salmon	4.8	body size	0.06	15	RM	19.2	2.1	16.4	9.7	2.1	5.5	6.2	2.7	17.1	23.1	92.4	Brett 1974
Sockeye salmon	6	body size	0.08	15	RM	19.2	1.9	16.4	9.8	2.1	5.6	6.3	2.8	17.2	24.5	97.8	Brett 1974

Common name	Body Size	Treatment	Level	N	Model	Tu	Tl	Tm	AT	UAT	OAT	OLT	ULT	LT	AIC	AICC	Reference
Sockeye salmon	6.9	body size	0.09	15	RM	19.5	2	16.3	10.2	2.4	5.9	6.7	3.3	17.5	24.6	98.3	Brett 1974
Sockeye salmon	7.6	body size	0.1	15	RM	19.4	2	16.3	10	2.3	5.7	6.5	3.1	17.3	23.2	92.8	Brett 1974
Sockeye salmon	8.2	body size	0.11	15	RM	19.7	2.1	16.3	10.3	2.5	6	6.8	3.4	17.6	22	88.2	Brett 1974
Sockeye salmon	8.7	body size	0.12	15	RM	19.8	2.2	16.3	10.3	2.6	6.1	6.8	3.5	17.6	22.7	91	Brett 1974
Sockeye salmon	9.1	body size	0.12	15	RM	20.2	2.1	16.2	10.7	3	6.5	7.3	4	18.1	23.4	93.6	Brett 1974
Sockeye salmon	9.5	body size	0.13	15	RM	20.1	2.2	16.2	10.6	2.9	6.4	7.1	3.9	17.9	22.8	91.1	Brett 1974
Sockeye salmon	9.9	body size	0.13	15	RM	19.8	2.2	16.3	10.3	2.6	6.1	6.9	3.5	17.6	22.6	90.5	Brett 1974
Sockeye salmon	10.3	body size	0.14	15	RM	19.6	2.1	16.3	10.2	2.5	6	6.7	3.4	17.5	24	96.1	Brett 1974
Sockeye salmon	13	body size	0.18	15	RM	19.8	2.4	16.2	10.2	2.7	6.1	6.9	3.6	17.4	21.9	87.7	Brett 1974
Sockeye salmon	14.8	body size	0.2	15	RM	19.5	2.1	16.3	10.2	2.4	5.9	6.5	3.3	17.5	22.4	89.7	Brett 1974
Sockeye salmon	16.3	body size	0.22	15	RM	20.3	2.3	16.4	10.7	2.9	6.5	7.5	4	18	20.6	82.4	Brett 1974
Sockeye salmon	17.6	body size	0.24	15	RM	18.8	2.4	16.1	9.3	2.1	5.3	5.5	2.7	16.4	20.1	80.4	Brett 1974
Sockeye salmon	22.2	body size	0.3	15	RM	20	2.4	15.9	10.5	3	6.4	7.1	4	17.6	20.6	82.6	Brett 1974
Sockeye salmon	27.9	body size	0.38	15	RM	19	2.6	16.2	9.3	2.1	5.3	5.8	2.8	16.4	18.8	75.1	Brett 1974
Sockeye salmon	37.9	body size	0.51	15	RM	18.9	3	16.2	9.1	2	5.2	5.6	2.7	15.9	16.5	66	Brett 1974
Spotted wolfish	20	.	.	25	RM	13.5	2.2	8.1	7.5	3.6	5.2	5.6	5.4	11.3	12.4	24.8	Imsland *et al.* 2006
Striped bass	10	.	.	8	PKM	34.5	.	28.3	13.1	4.4	7.7	8.5	6.2	.	5.2	31.2	Kellogg and Gift 1983
Turbot	7	Population	Norway	12	QM	28.8	9.9	19.3	12.6	6.3	8.7	9.5	9.5	18.9	-1.7	-2.2	Imsland *et al.* 2000
Turbot	7	Population	Scotland	12	QM	26.1	9	17.6	11.4	5.7	7.8	8.5	8.5	17	1.4	1.8	Imsland *et al.* 2000
Turbot	12	.	.	12	RM	25.3	6	16.9	12.8	5.8	8.7	7.2	8.5	19.3	6	34.3	Imsland *et al.* 1996
Turbot	7	Population	France	12	RM	24.8	4.4	21.1	11.8	2.8	6.8	2.1	3.7	20.4	3.9	22.2	Imsland *et al.* 2000
Walleye Pollock	12	.	.	12	RM	17.4	-0.8	12.4	14.2	3.7	8.5	8.9	5	24.3	10	57.3	Kooka *et al.* 2007
Wels catfish	14	.	.	5	PKM	31.4	.	26	14.3	4.3	8.7	9.5	5.4	.	1.7	41.1	Hilge 1985
Yellow perch	20	.	.	6	PKM	33.7	.	24.8	14.9	5.8	9	9.8	8.9	.	4.2	50.1	Tidwell *et al.* 1999

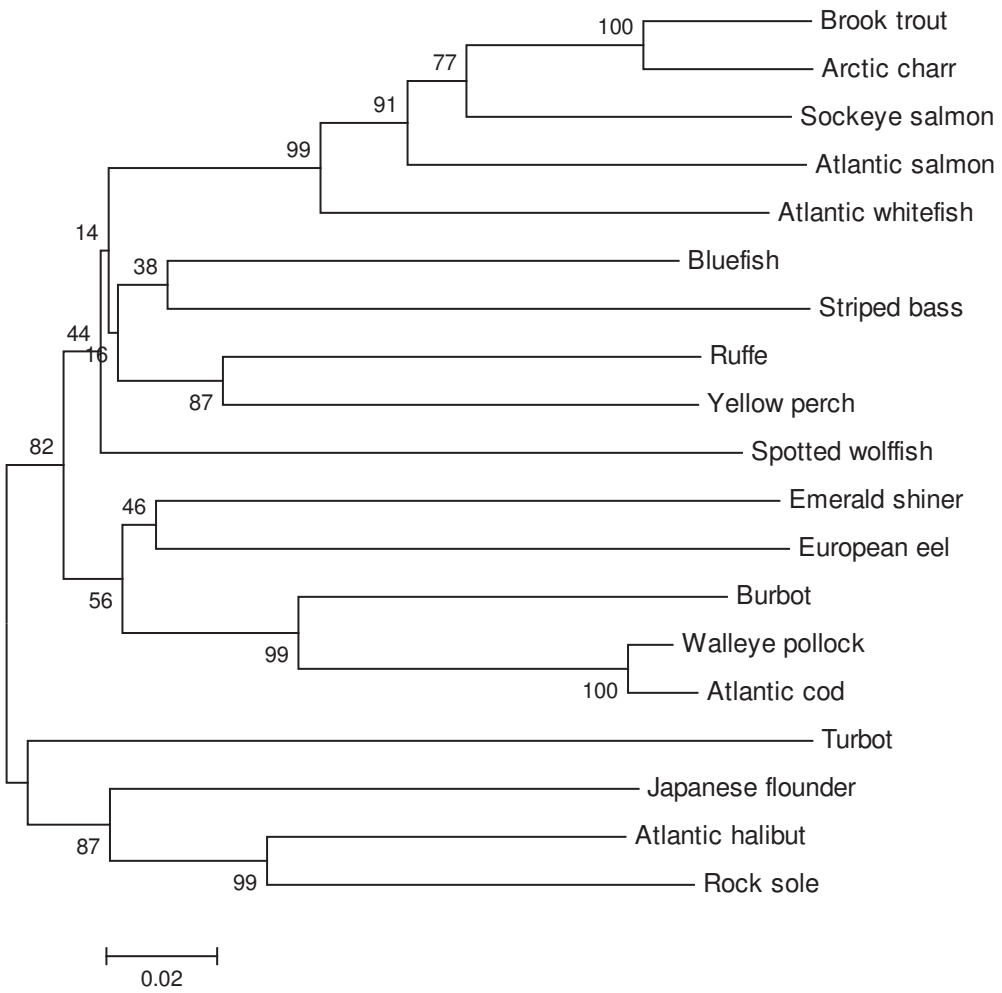

Figure B3.1. Evolutionary relationships of 19 taxa used for phylogenetically generalized least squares regression. Numbers represent percent bootstrap support for clustered taxa.

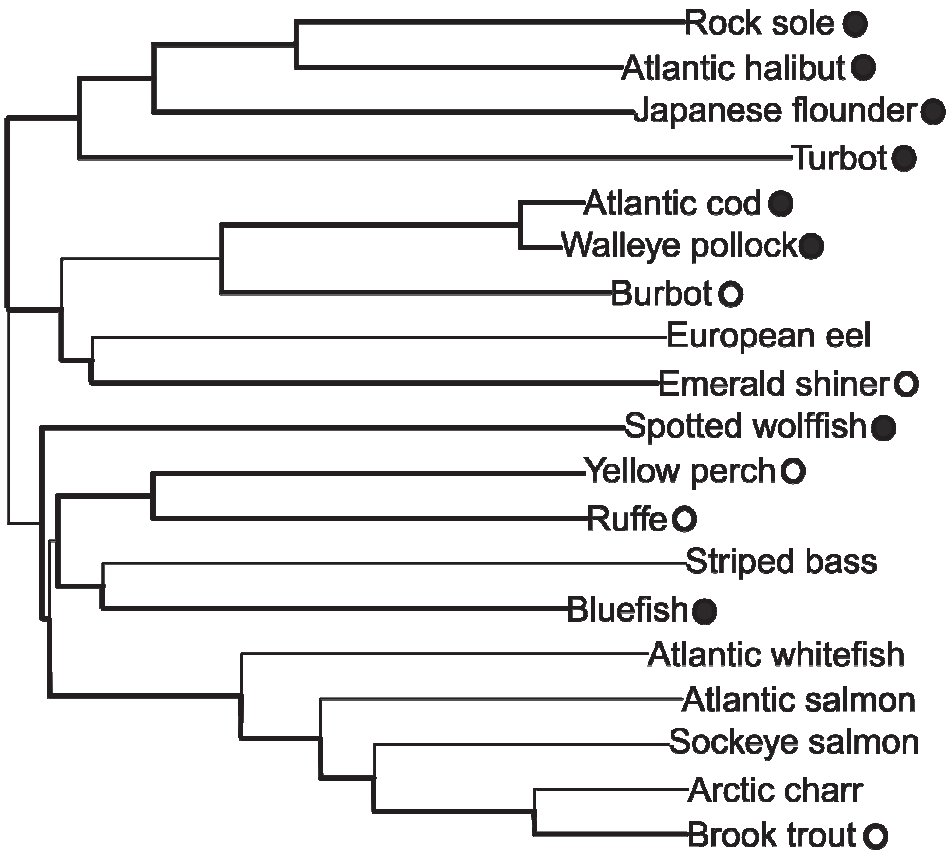

Figure B3.2: Example of independent contrasts used in the phylogenetic comparison of categorical variables. Shown by thicker connections are the groups used for assessing thermal sensitivity in marine species (solid symbols) versus with freshwater species (open symbols).

APPENDIX C: CHAPTER 5

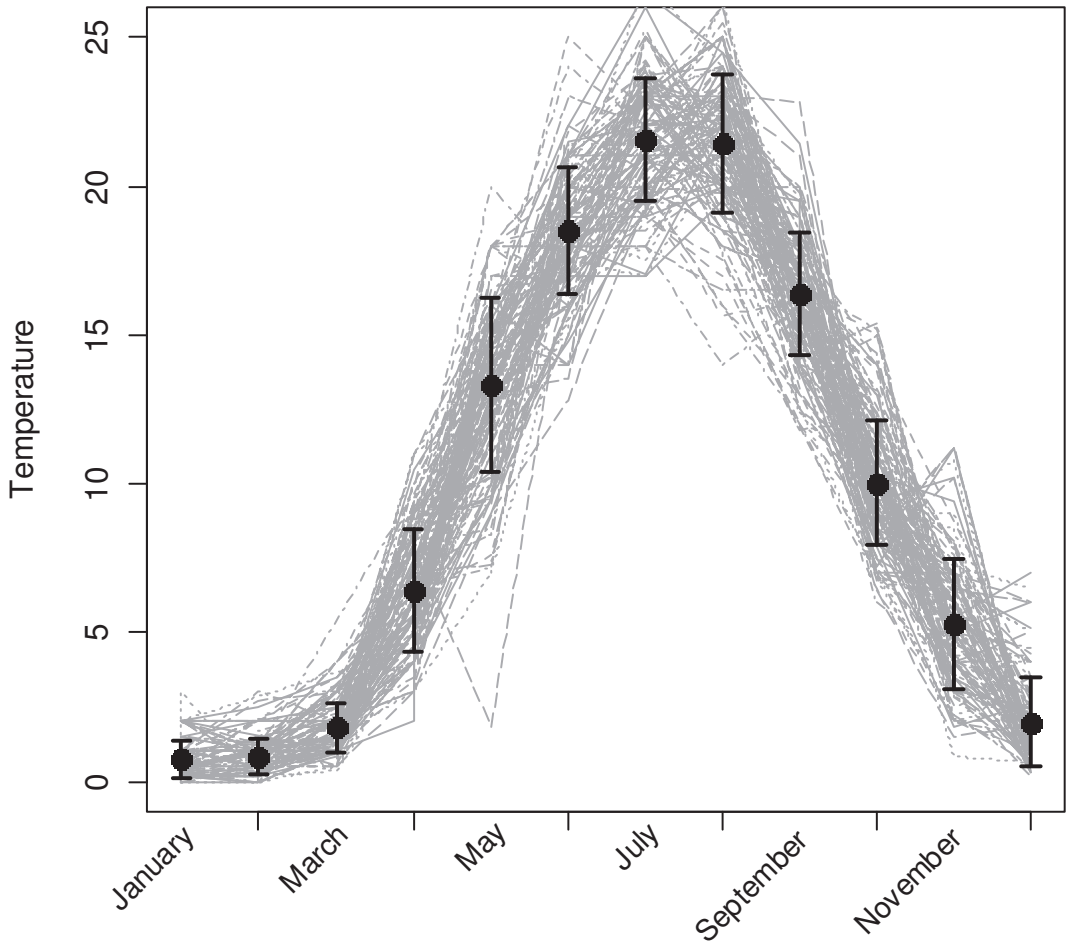

Figure C5.1: Plot of seasonal temperature profiles from watersheds within the Southern Upland region of Nova Scotia. Included in this plot are the temperature profiles from nine watersheds across 14 years. Symbols with error bars represent the mean and standard deviations used in simulation models.

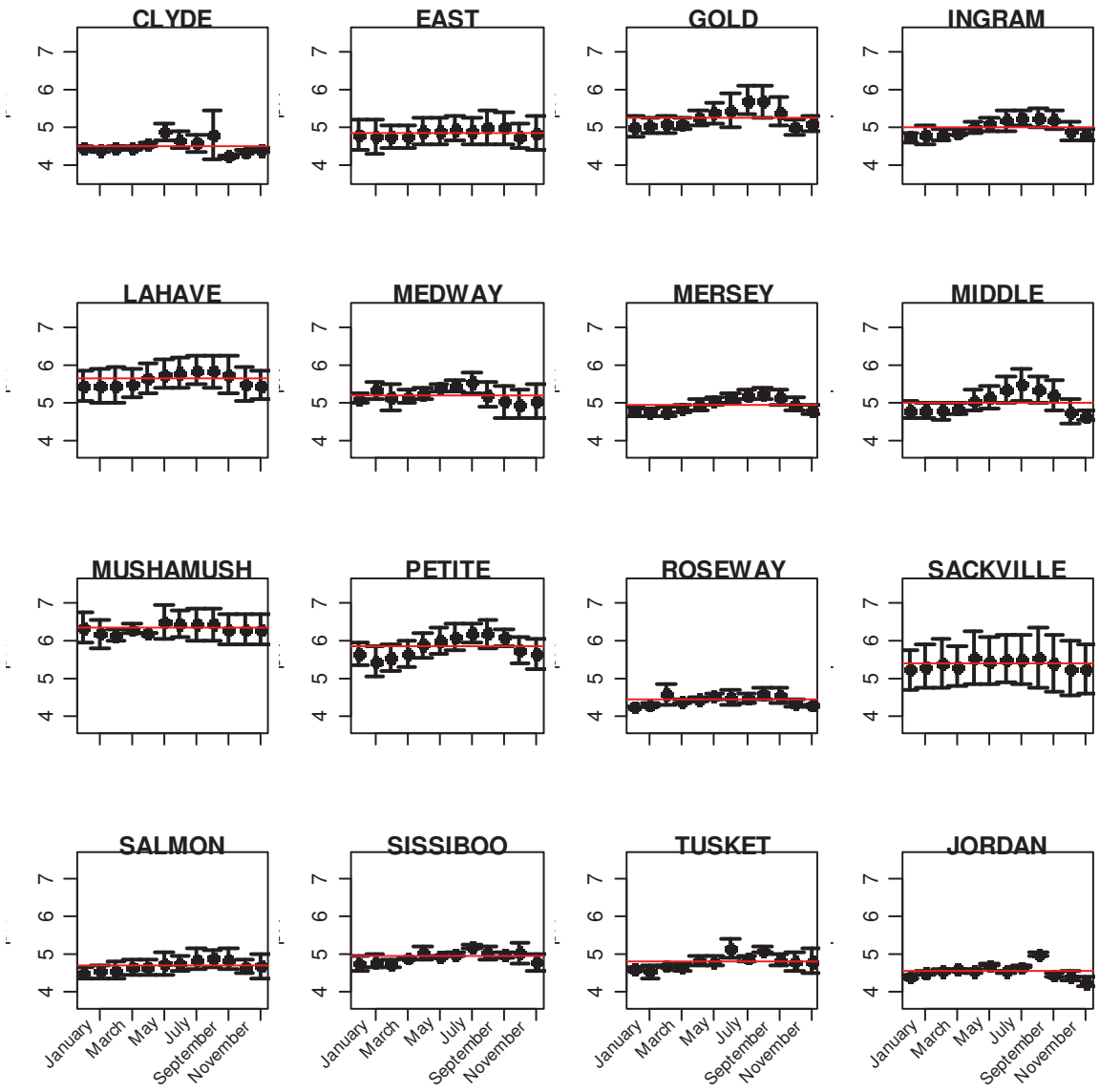

Figure C5.2: pH profiles from each of the evaluated watersheds in the Southern Upland region of Nova Scotia. Symbols represent the monthly mean values with standard deviations used in Level 2 and 3 simulations. Red line represents the overall watershed-specific pH used in the Level 1 model simulations.