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Assessing Temperature Tolerance in Ninespine Stickleback (Pungitius pungitius) in Response to Climate Change

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Assessing Temperature Tolerance in Ninespine Stickleback (*Pungitius pungitius*) in Response to Climate Change

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Abstract

Temperature is the most important environmental factor influencing fish abundance and distribution but, as temperatures rise globally, the consequences for fishes remains largely unknown. I used populations of ninespine stickleback (*Pungitius pungitius*) from the northern and southern parts of their range in a common environment to characterize thermal traits in association with varying temperatures. Cold tolerance differed between populations but heat tolerance did not. Furthermore, juveniles demonstrated less tolerance for temperature extremes. When incubated at elevated temperatures, populations exhibited higher plasticity in cold tolerance compared to heat tolerance. Plasticity in heat tolerance was associated with a number of trade-offs. The data suggest that cold tolerance is locally adapted and phenotypically plastic while heat tolerance is conserved and phenotypically restricted in this species. My thesis highlights the need for the inclusion of various life stages, time scales, and biotic interactions in climate change research to better predict its impacts on fishes.

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List of Abbreviations and Nomenclature

Symbol	Definition
ANOVA	Analysis of variance
ARR	Acclimation response ratio
CTmax	Critical maximum temperature
CTmin	Critical minimum temperature
F1	Offspring of a parent generation
LESARC	Life and Environmental Science and Animal Research Centre

CHAPTER 1 - GENERAL INTRODUCTION

1.1 Introduction

A major goal of evolutionary biology is to identify the genotypes and phenotypes that underlie adaptation to divergent environments and in response to environmental change (Rogers et al. 2013, Peichel and Marques 2016). Phenotypes are shaped by the interaction between an organism's genotype and environment and, as a consequence, its phenotype is strongly linked to and closely associated with its environment (Garrod 1902, Turesson 1922, Wright 1932). The tight phenotype-genotype-environment association is advantageous when the environment is stable but less so when a major climate event or regime shift occurs (Cushing 1990, Visser and Both 2005, Thackeray et al. 2016).

1.2 Climate change

Presently, global average temperatures are increasing, high and low temperature extremes are becoming more intense and more frequent (IPCC 2014) and, for many organisms, the phenotype-genotype-environment relationship is unraveling (Visser and Both 2005, Thackeray et al. 2016). Over the last quarter century lake temperatures have been warming faster than air temperatures and lakes that ice-over in the winter are warming at twice the rate of lakes that do not. The main drivers of temperature rise in these lakes include shorter ice duration and an increase in both summer air temperatures and shortwave radiation (O'Reilly et al. 2015). With the majority of Earth's lakes in the northern hemisphere (i.e. between 47 - 73 °N) (Lehner and Doll 2004) many

freshwater fishes are on the front line of change.

Climate change is warming northern regions in Canada at a rate twice that of the global average (IPCC 2014). It is expected to alter permafrost thaw patterns, snow distribution, runoff, and drainage systems. Consequently, river-lake connectivity and ecosystem structure and function will be affected (reviewed in AMAP 2017). These changes, amongst others, will have cascading effects on the species that occupy these regions. For instance, the life cycle of many Arctic freshwater fishes has been shaped by ice phenology so shifts in its timing will cause major changes in the spawning time, age and size at first reproduction, growth, and lifespan of cold-water fishes (Shuter 2012, Hovel et al. 2017). Given the disruption in many of the important cues and conditions used by northern species, selection will likely increasingly favour plasticity in traits that depend on seasonality (Hovel et al. 2017). Populations living at the southern limits of a species' range will likely experience habitat reductions due to the loss of cold water refugia (Shuter 2012) and may therefore be the first to experience local extinctions (Araujo et al. 2013). Further, with lake 'browning' from permafrost thaw (Wauthy et al. 2018) and increased turbidity from increased glacial discharge (Lehnherr et al. 2018), feeding may become more difficult for cold-water fishes and cause a reduction in their overall condition (Lehnherr et al. 2018). Some cold-water fishes in Europe and North America have already shown declines in their distribution and abundance (Eddy and Handy 2012), highlighting the fact that climate change is a current reality for northern regions.

In the southern regions of Canada, climate change is expected to inflict drought on the parries in the west and increase the amount of precipitation in the east (IPCC

2014). A rise in annual temperatures is elevating the temperature of lakes (Dobiesz and Lester 2009, O'Reilly et al. 2015) and causing declines in some temperate freshwater fishes (AESRD 2005, 2012). The warming of freshwater lakes will enable temperate species to expand their range northward, increasing species richness in the north (Shuter et al. 2012) and changing community dynamics of competition and coexistence (Buisson and Grenouillet 2009, Lancaster et al. 2016). Cold-water species will likely be at a disadvantage, losing their competitive edge and ultimately being out-competed by temperate species that can withstand a broader range of temperatures (ACIA 2004, Shuter et al. 2012, Poesch et al. 2016, AMPA 2017). Despite these changes, the nature of phenotype-environment associations is lacking for most of these species.

1.3 The consequence of warming for fishes

Environmental change in association with climate change will likely result in the mismatch between the phenotype, genotype, and environment and thus necessitate that species adapt, shift range and distribution, or suffer extinction (Davis et al. 2005). Given that temperature is considered to be the most important environmental factor influencing the distribution and abundance of fishes (Brett 1971, Powers et al 1991), and that the diversity of freshwater species faces a greater risk than terrestrial diversity (Dudgeon et al. 2006), it is paramount that the ecological and evolutionary consequences of climate change for freshwater fish communities are understood.

The biological rates of fishes and other ectotherms (animals that depend on external sources of body heat), are strongly influenced by environmental temperatures (Huey and Kingsolver 1989). An ectotherm's performance, fitness, physiology, and

scope of activity are thus strongly reliant on the temperature of its surroundings (Fry 1947, Portner and Farrell 2008, Payne et al. 2016). In Atlantic cod, for example, high temperatures are correlated with a reduction in growth rate, age of maturity, condition, and recruitment of the species (Drinkwater 2005). An ectotherm's thermal window within which it performs optimally is defined by its upper and lower temperature limits and is typically correlated with the thermal regimes of its environment (Huey and Kingsolver 1989). As global temperatures climb and temperature regimes shift, ectotherms, such as fishes, will be exposed to temperatures outside their thermal windows.

The ability to adjust the thermal window and its endpoints, however, may be limited. The degree of plasticity in thermal tolerance at elevated temperatures is remarkably low across ectotherm species, suggesting that they cannot effectively buffer themselves against warming with plastic responses (Gunderson and Stillman 2015). Terrestrial ectotherms have the advantage of inhabiting environments with high spatial variability in temperature, enabling them to thermoregulate with shifts in their behavioural use of those spaces (Hertz et al. 1993, Huey et al. 2003, Gunderson and Stillman 2015). The aquatic environment is more homogenous in temperature, limiting aquatic species in their ability to adapt behaviorally (Gunderson and Stillman 2015). These limitations are problematic for aquatic ectotherms - particularly fishes - whose rate of change in their distribution has already exceeded the rate of change in the distribution of terrestrial organisms (Parmesan and Yohe 2003).

Freshwater fishes may be particularly vulnerable since they are restricted to water bodies that are land-locked and thus more isolated than marine fishes (Comte and Olden 2017). Evidence from lake trout in Northern Labrador suggests that there is

essentially no gene flow between freshwater lakes (McCracken et al 2013) which will further compound the issue. As temperatures rise, northward shifts to cooler waters may not be feasible for freshwater populations and, as a result, *in situ* evolutionary responses to temperature are predicted to be the primary way for them to persist in a changing world (Gunderson and Stillman 2015).

When an ectotherm reaches its upper thermal limit, its respiration and metabolic rates become elevated which can increase growth and development rates during ontogeny (Atkinson 1994). Elevated metabolic rates, however, may lead to a mismatch between life history and prey availability, leading to the breakdown in the phenotype-environment association (Cushing 1990, Rogers and Bernatchez 2006). As a result, larvae survival may be reduced, food webs may be disrupted and the ability for populations to be sustained may be diminished (Cushing 1990, reviewed in Eddy and Handy 2012). In addition, enhanced respiration rates will increase an ectotherm's demand for food. It is anticipated that in response to rising temperatures, the change in rate of photosynthesis will not be as rapid as changes in the rate of respiration, suggesting that there may be a shortage in food, thus decreasing food web biomass and productivity (reviewed in Eddy and Handy 2012). Temperature may also have differential affects within a species. For example, in Atlantic cod, elevated temperature increases the rate of development and maturation in males while the rate at which females mature depends on their size, not temperature. As a result, there could be a mismatch in reproductive timing between males and females as temperatures continue to rise (Ottera et al. 2006). Ultimately, climate warming has the potential to destabilize species interactions and alter lake ecosystem function (Ohlberger 2013).

1.4 Consequences for conservation and management

Studying the effects of climate change on fishes is challenging given that 'climate' can refer to a multitude of timescales - ranging anywhere from annual variations in climate to climates that define entire epochs. At short time scales (i.e. <10 years) annual variations in climate may only be marginally effected by climate change (Brander 2010). The increase in frequency of extreme weather events (IPCC 2007), however, may have the greatest consequences for biodiversity given that the nature of their impacts is non-linear (Brander 2010). For example, in 2004 parts of British Columbia had record breaking summer temperatures that reduced the survival and recruitment of salmon but did not affect the annual mean temperature that year (Canadian Standing Committee on Fisheries and Oceans, 2005). Evidently, management and conservation strategies will require the understanding of the effects of climate change on fish populations over multiple timescales and life stages.

In Canada, the inland fishing industry is a valued economic resource. Recreational freshwater fishing and related expenses in Canada amount to approximately $5.4 billion per year and commercial freshwater fisheries average approximately $74 million per year (DFO 2017). Freshwater fisheries also have significant cultural and subsistence value to indigenous communities across Canada (Roux et al. 2011). Current and projected changes in climate regimes are threatening freshwater fishes by reducing the amount of suitable habitat in lakes (Brander 2010, Comte et al. 2013, Kelly et al. 2014), and affecting the productivity and resilience of fish stocks (Brander 2010, Ohlberger 2013), thereby threatening freshwater fishing industries and indigenous ways of life.

1.5 Importance of studying temperature tolerance

The physiology of fishes is governed largely by temperature (Fry 1947, Portner and Farrell 2008, Payne et al. 2016) so to be able to predict how temperature rise associated with climate change will directly impact fishes, and to develop strategies to mitigate its effects, requires an understanding of the underlying biological responses to temperature (Huey et al. 2009). Studying temperature tolerance under an evolutionary framework provides an opportunity to quantify the ability of a population to respond to temperature change through phenotypic plasticity or evolutionary responses (Somero 2010). Identifying if there is variability in a population for temperature tolerance provides an estimate of the population's resilience and potential to adapt (Chown and Gaston 2008). Furthermore, studying temperature tolerance across a species range is also important to identify biogeographical patterns and to assess whether differences in environments shape the evolutionary potential of thermal tolerance traits to respond to change (Hoffmann and Merila 1999). Information on the thermal tolerance limits of a species can be used in models projecting the impacts of climate change on its distribution and risk of extinction (Kearney and Porter 2009).

1.6 The ninespine stickleback as a model

The ninespine stickleback (*Pungitius pungitius*) is a small fish well suited to test questions about the effects of climate change on fish populations (Peichel and Marques 2016). It is a cold-water species (Nelson 1976) and has a circumpolar distribution, found predominantly in freshwater environments but also in small numbers along the coast

and in brackish waters (Merila 2013). They are relatively easy to sample and can be reared and crossed in the lab (Merila 2013). They are morphologically diverse throughout their range, varying in shape and size, differing in behaviour and physiology, and have a variable number of spines, lateral plates, and gill rakers (Coad and Reist 2018). They diverged from their sister species, the threespine stickleback, approximately 15.86 million years ago (Aldenhoven et al 2010). Ninespine stickleback exhibit lower genetic variability and higher population divergence compared to the threespine stickleback (Merila 2013).

Among different populations of ninespine stickleback, there is considerable genetic variability, with reduced heterozygosity in freshwater ponds compared to coastal populations and high divergence between freshwater ponds (Shikano et al. 2010). There also appears to be a negative relationship between levels of genetic variability and latitude - that is, the further north you go, the less genetic variability there is (Shikano et al. 2010). As a result, natural selection may be constrained in northern populations due to the lack of genetic variation (Barret and Schluter 2007).

In Canada, northern and southern populations of ninespine stickleback occupy distinct climatic regimes where the average ambient temperature ranges from -34°C to +7°C at the northern tip of its distribution on Baffin Island (Pond Inlet, Nunavut) to -15°C to +17°C at the southern reaches of its distribution in Alberta (Cold Lake, Alberta) (Environment Canada 2016). The ninespine stickleback reaches sexual maturity between the age of one or two and live to the age of three (males) or four (females) (Coad and Reist 2018). Both freshwater and anadromous forms spawn in fresh water between the months of May and July. The male constructs and defends a nest in the

shallows out of rocks or vegetation. After a female has deposited her eggs, the male fertilizes them and guards the nest until the young are free-swimming. They inhabit the shallows in the spring and summer to breed, then move to deeper water in the winter. If anadromous, adults migrate to the coast during the summer and return in the fall to over-winter in fresh water. Both the male and female may spawn multiple times per season (Coad and Reist 2018).

In the Canadian Arctic, ninespine stickleback are frequently prey to arctic char, lake trout, and migratory birds (Gallagher and Dick 2011) and are one of relatively few fish species that live in Arctic freshwater systems (Warren and Burr 2014). At lower latitudes, ninespine stickleback are part of larger ecological communities with many potential predators and competitors (Nelson and Paetz 1992, Warren and Burr 2014).

Northern and southern populations also differ in their evolutionary origins from glacial refugia. In Canada, ninespine stickleback are thought to have dispersed from three distinct glacial refuges following the Wisconsin glaciations: the Bering, Mississippi, and Atlantic (Aldenhoven et al. 2010). Evidence suggests that lineages of ninespine stickleback within those refuges were diverged from one another prior to the Wisconsin glaciation with no evidence of intermixing post-glacial retreat (Aldenhoven et al. 2010). Although neither Alberta nor Nunavut populations were included in the Aldenhoven et al. (2010) analysis, it is probable that Alberta was colonized from the Mississippi refugia; their dispersal facilitated by the multiple connected lakes left behind by the retreating glaciers after the last glacial maximum. Phylogeographic analysis of other postglacial fishes further support this hypothesis (Bernatchez and Wilson 1998, Mee et al. 2015). Unlike Alberta, the origin of the Baffin Island population is likely from a marine relict,

given that it is more probable that a marine relict colonized the freshwater habitats on Baffin Island as opposed to a freshwater relict from the mainland traversing the Arctic Ocean only to colonize freshwater once more.

Understanding the differences in the evolutionary history of these populations, their ability to colonize new habitats, and their ability to persist in changing environments and climates can inform us about their capacity to adapt in the past and in the future (Gavin et al. 2014, O'Donnell et al. 2014). It is likely that populations will respond differently to the same selection pressures inflicted by climate change due to the differences in their genotype-phenotype-environment relationship, their genetic variability, and their evolutionary histories (Barret and Schluter 2007, Barrik and Lenski 2013).

1.7 Thesis objectives

Although there has been increasing interest in understanding the impacts of climate change on biodiversity, gaps remain in understanding how it will affect freshwater fishes. Knowledge is particularly limited for fishes from northern regions where the effects of climate change are predicted to be most pronounced (Poesch et al. 2016). In Canada, freshwater fishing industries and indigenous ways of life are threatened by climate change thus it is increasingly important to understand how fishes will respond to thermal stress. In addition to changes in ambient temperature, the effects of climate change include a number of direct and indirect stressors that may interact synergistically (Heino et al. 2009). Despite these complex interactions, experiments under controlled conditions can tell us about the direct effect of a variable

on a fishes' behaviour, survival, and physiology (Brander 2010). Since fishes are strongly influenced by temperature (Brett 1971, Powers et al 1991, Brander 2010), my thesis seeks to understand its direct effect on thermal tolerance in fish, using the ninespine stickleback as the study organism. The broad objective of my thesis is to characterize the thermal phenotypes and investigate the response of ninespine stickleback to thermal stress in association with changing temperature regimes.

My thesis is presented in the following three chapters. In Chapter 2, I quantified resting thermal tolerance for four populations of the ninespine stickleback. Given that the consequence of climate-induced thermal stress can vary between and within species as well as across geographic space due to local adaptation of populations (Somero 2010, Jensen et al. 2008, Vergeer and Kunin 2013), I sought to determine if representative southern and northern populations exhibit variation in thermal tolerance traits and if these traits differ between life stages. I measured the thermal tolerance limits of wild-caught fish acclimated in the laboratory for three weeks to a common temperature within the species' natural thermal range. In Chapter 3, I experimentally subjected ninespine stickleback populations to three different temperature treatments within the species' natural thermal range over the span of three months to quantify the effects of prolonged warming on their thermal traits. In doing so, I sought to experimentally identify if plasticity in thermal tolerance is constrained at elevated temperatures, as has been suggested (Gunderson and Stillman 2015) but not directly tested. Both experiments were conducted at the University of Calgary using fish from wild populations collected in Northern Alberta and Baffin Island, Nunavut. Lastly, Chapter 4 provides a summary and synthesis of my findings as well as recommended

next steps for future research.

CHAPTER 2 - RESTING THERMAL TOLERANCE

2.1 Introduction

Predictions of climate change indicate that global average temperatures will continue to increase while high and low temperature extremes will become both more intense and more frequent (IPCC 2014). Lake water temperatures have been rapidly increasing at a rate faster than air temperatures (O'Reilly et al. 2015). These patterns in global change have been associated with correlated patterns of mortality and a global loss of biodiversity (IPCC 2014). Some species have already shifted in range, distribution, and abundance, with notable shifts northward (IPCC 2014). Both the observations and projections for further change are coupled with uncertainty in association with how species will respond to these changes.

Temperature is considered to be the most important environmental factor influencing the distribution and abundance of fishes (Brett 1971, Powers et al 1991, Brander 2010). Fish physiology and metabolism are reliant on the temperature of their surroundings (Huey and Kingsolver 1989) and even small changes in temperature can have consequences for abundance, distribution, growth, feeding, and fitness (Fry 1947, Brett 1971, Powers et al 1991, Portner and Farrell 2008, Payne et al. 2016, Lehnherr et al. 2018). The thermal tolerance of an ectotherm is the physiological ability to maintain internal homeostasis within its thermal environment. Thermal tolerance is also a measurable phenotype that allows us to see how closely associated a genotype is with its thermal environment and can imply local adaptation by natural selection (Haughen and Vollestad 2000). Fishes tend to perform best close to the maximum temperature in

their environment (Payne et al. 2016). Outside the optimum temperature range, performance and fitness drops (Huey and Kingsolver 1993, Eddy and Handy 2012), resulting in thermal stress that induces physiological constraints that are ultimately lethal (Fry 1947). Fishes with an optimum performance temperature closer to their upper thermal tolerance limit are predominantly those that suffer the most local extinctions (Comte et al. 2014). Determining whether populations exhibit variation in thermal tolerance can help identify whether they have the potential to adapt to changing climate regimes (Jensen et al. 2008).

At the physiological level, when an ectotherm experiences elevated temperatures it undergoes an increase in metabolic rate, increasing energy expenditure and oxygen demands on the heart. Because the solubility of oxygen and the affinity of hemoglobin for oxygen is reduced at higher temperatures, a fish's heart must work much harder when its environment warms (Eddy and Handy 2012). Elevated temperatures also lower the aerobic scope of an ectotherm - that is, the difference in the rate of oxygen consumption between resting and exercise becomes smaller - which may cause mortality at high temperatures (Brownscombe et al. 2017). At high temperatures beyond an ectotherm's thermal window, structural and enzymatic proteins become denatured. Temperature also affects the fluidity of membranes and, as a result, temperature can have direct effects on functioning of membrane-related processes at the cellular level. Overall, temperature can cause a reduction in the ability of molecules to function properly (Schulte 2015).

Thermally associated traits in fishes can be quite adaptable. Cold tolerance, in particular, can be highly variable between and within species, reflecting the variability of

their environments (Araujo et al. 2013). For example, in British Columbia, freshwater threespine stickleback habitats reach lower temperatures than marine ones and, as a result, freshwater threespine stickleback populations have a higher cold tolerance compared to marine populations (Barrett et al. 2010). In the same study, a marine population was translocated from the marine environment to a freshwater one and in as little as three generations it had evolved cold tolerance traits comparable to those found in freshwater populations (Barrett et al. 2010). Evidence has shown that in fishes, cold tolerance is heritable (Gibbons et al. 2016, Barrett et al. 2010), phenotypically plastic (Morris et al 2014, Gibbons et al. 2016) and adaptive in association with changes in their environment.

The same cannot be said for heat tolerance. In fact, heat tolerance appears to be largely constrained in ectotherms (Araujo et al. 2013, Gunderson and Stillman 2015). In the threespine stickleback example above, despite freshwater environments reaching a higher maximum temperature than marine ones, translocated marine populations did not experience any increase in heat tolerance like they did for cold tolerance (Barrett et al. 2010). There is some evidence for plasticity in heat tolerance in stickleback (Metzger 2016) but it is expected to be limited enough that ectotherms will not be able to adjust to warming climates (Gunderson and Stillman 2015).

A species' biogeography and range limits are thought to be shaped by constraints in physiological tolerances to climate (Spicer and Gaston 1999, Portner and Peck 2010, Sunday et al. 2012, Khaliq et al. 2017) and the size of a species' range is considered to be a strong indicator of its vulnerability to climate change (Lee and Jetz 2011, IPCC 2014). Within a species' range, however, populations may vary significantly

in thermally associated phenotypes and genotypes. For example, two populations of an Antarctic fish from different parts of the continent exhibit significantly different thermal tolerance maxima despite being from similar temperature regimes, differing only in summer temperatures by 1.5°C (Peck et al. 2014). Furthermore, in populations adapted to cold environments, natural selection may not actively maintain heat tolerance genes if they are not used or required for survival (Lahti et al. 2009, Nokelainen et al. 2018). In spite of this inter-population variation along climatic gradients, studies that have tested physiological tolerances at populations range limits are lacking (Gaston 2009).

In the current study, I examine thermal tolerance phenotypes of distinct populations of ninespine stickleback. The ninespine stickleback is a northern species, typically inhabiting cold, deep lakes (Nelson and Paetz 1992). In Alberta, its southernmost range reaches the Beaver River watershed (Nelson and Paetz 1992), from which I sampled two populations – Moose Lake and Kehewin Lake. In Nunavut, its range reaches as far north as the tip of Baffin Island (Government of Nunavut 2009). I sampled two populations from Baffin Island – Dog Lake and Nettilling Lake. Locations of populations sampled can be found in Appendix A.

Moose and Kehewin Lakes in Alberta are dimictic with temperatures ranging from around 18°C in the summer to 1°C in the winter (Mitchell and Prepas 1990). Although there is an absence of lake temperature data for most lakes in Nunavut, generally due to the long winters and short summers, northern lakes tend to be isothermic and remain around 0°C throughout the year (Vincent and Laybourn-Parry 2008). Given the species' wide range across a variety of biomes, populations at the northern and southern parts of

the ninespine stickleback's distribution experience distinctly different selection pressures.

To understand how diverse populations of fishes will respond to climate change will depend on our understanding of the extent and nature of their local adaptation (Jensen et al. 2008, Vergeer and Kunin 2013) and their physiological constraints in tolerances to climate. This is particularly true for range-limited, diverged populations with different locally adapted thermal optimums. In this study, I test the extent to which representative northern and southern populations are adapted to their distinct thermal regimes. Additionally, given that different life stages tend to be more sensitive to temperature and that the effects of temperature change may impact those life stages differently (Portner and Peck 2010, Eddy and Handy 2012, Whitney et al. 2013), I also assess whether juveniles have unique thermal tolerance limits compared to the adults of the same population.

Given the evidence presented above, I predicted that northern populations of ninespine stickleback would be more tolerant to cold temperatures and less tolerant of warm ones. In contrast, I expected that the representative southern populations would be more tolerant to heat but similarly tolerant to cold given that both habitats reach similar minimums throughout the winter months. Further, I predicted that juveniles from both regions would be less tolerance of temperature extremes compared to conspecific adults.

2.2 Methods

2.2.1 Sample populations

I collected ninespine stickleback from lakes in Alberta and Nunavut. Alberta fish were collected from Moose Lake (54.24°N, -110.91°W) in June – August 2017 and from Kehewin Lake (54.05°N, -111.0°W) in July – August 2017. Nunavut fish were collected from Dog Lake (63.75°N, -68.49°W) in July 2017 and from Nettilling Lake (66.58°N, -71.38°W) in August 2017. Approximately 100 fish were sampled from each lake, with the exception of Kehewin Lake where only 27 fish were collected. Fish were caught using seine nets, dip nets, and minnow traps baited with dry dog food. Fish were transported in solid to the Life and Environmental Science and Animal Research Centre (LESARC) at the University of Calgary in fish bags filled with lake water treated with PRIME to control chemical waste levels and saturated with oxygen. The transportation of fish occurred by vehicle in Alberta and by air from Nunavut. At the aquatics lab fish were transplanted into 38 L glass aquaria at a density of approximately 15 fish per tank, with fish from the same population housed together. Each aquarium was equipped with one large sponge filter for oxygenation and one medium-sized aquarium plant for enrichment. The conditions in the aquaria were maintained at a temperature of 17°C, salinity of 0.5 ppt, and a photoperiod of 12 L:12 D. All protocols followed procedures approved by the animal care committee at the University of Calgary in accordance with the standards set by the Canadian Council for Animal Care.

2.2.2 Fish husbandry

Adult fish were fed approximately 6g of a mixture of adult spirulina brine shrimp and chopped blood worms once per day. Juveniles were fed approximately 1g of live brine shrimp per day. The aquaria were cleaned twice per week by using a manual siphon to remove food and debris from the bottom of the tank and by replacing 1/3 of the water to maintain healthy ammonia and nitrate levels.

2.2.3 Crosses

To test for differences in thermal tolerance between life stages, I attempted to generate offspring from the adult fish that were collected. To produce a cross, I gently squeezed the eggs from a gravid female into a petri dish and mixed them with the sperm from a euthanized male, and added water to activate the sperm. After approximately ten minutes, I transferred the eggs to an aquarium treated with methylene blue which is used to prevent fungal infections.

2.2.4 Thermal tolerance

Once the adult fish acclimated to lab conditions for a minimum of three weeks (following Barrett et al. 2010), I assessed their thermal tolerance. Thermal tolerance is defined as the range between an organism's critical minimum temperature (CTmin) and critical maximum temperature (CTmax). The CTmin and CTmax are the lower and upper limits of the thermal window past which fish are no longer capable of escaping harmful conditions (Beitinger et al 2000). In the lab, the CTmin and CTmax are

evaluated by gradually cooling or heating the water to the point where the fish loses equilibrium (Fangue et al 2006).

To determine these physiological limits, following Barrett et al. (2010), I used an experimental setup that consisted of a water bath, a holding tank, and individual 1000mL beakers where the fish were held for testing. The water bath was connected by inflow and outflow pumps to the holding tank where water was either cooled or heated (for CTmin or CTmax, respectively) at a rate of 0.33°C per minute. Cooling was achieved by using water chillers supplemented with dry ice and heating was achieved using submersible aquarium heaters (see Appendix B for experimental set-up). Inside the water bath, I placed the beakers on an elevated shelf to allow water flow beneath them, ensuring the rate of cooling or heating was consistent. The beakers were filled with 200 mL of 17°C water from a water reservoir. Each beaker had an air stone bubbler for oxygen and to help prevent temperature stratification within the beaker.

Before running a thermal tolerance trial the fish were placed individually in the beakers and were given 15 minutes to acclimate before the chillers/heaters and pumps were turned on. Once a fish reached its CTmin/CTmax it was moved into a recovery tank with 17°C water aerated with an air stone. After all fish had recovered and were swimming normally (usually 20 minutes) they were returned to their original aquarium. I tested 18 randomly selected fish per population per CT trial.

2.2.5 Statistical analysis

I tested for differences in thermal tolerance between populations by conducting ANOVAs in R. Where necessary, I conducted Tukey ad hoc tests to see which groups

differed from one another. I tested for differences between adults and juveniles using t-tests conducted in R.

2.3 Results

2.3.1 Sample populations

The catch per unit effort varied between Alberta and Nunavut lakes with up to six times the number of fishing days required to catch fish in Alberta compared to Nunavut (see Appendix C for supplementary data on collection effort).

2.3.2 Crosses

The Dog lake population had a number of adults that were in condition and I was able to produce five crosses but, due to fungal infections, only one cross hatched successfully. Attempts to cross adults from the other populations were unsuccessful. From the Alberta lakes, there were a number of males that were visibly in condition with dark patches on the ventral cavity but I was not able to collect any gravid females required to produce a cross. Similarly, from the sample of Nettilling Lake adults, there was an absence of gravid females available. Instead, I collected newly hatched juveniles found in overflow ponds alongshore Nettilling Lake.

2.3.3 Thermal tolerance

Cold tolerance differed significantly between populations, $F(3, 99)=3.94$, $p=0.01$, with Nettilling Lake having the lowest CTmin (mean = -0.44°C). The cold

tolerance of Dog Lake (mean = 0.25°C), Kehewin Lake (mean = 0.18°C) and Moose Lake (mean = 0.19°C) did not differ significantly (Figure 2.1 (a)).

Heat tolerance did not differ between populations, $F(3, 119)=1.36$, $p=0.26$, with each population showing similar critical thermal maximums: Dog Lake (mean = 30.8°C), Kehewin Lake (mean = 30.5°C), Moose Lake (mean = 30.6°C) and Nettilling Lake (mean = 30.4°C) (Figure 2.1 (b)).

Juveniles from both Dog and Nettilling Lakes had significantly lower capacity for cold tolerance compared to the adults of the same population. Dog Lake juveniles had an average CTmin of 7.0°C which was a significantly lower tolerance threshold compared to the adults (mean CTmin = 0.25°C) ($p<0.001$) (Figure 2.2 (a)). Similarly, Nettilling Lake juveniles had an average CTmin of 4.0°C, whereas the adults of the same population had a mean CTmin of -0.44°C ($p<0.001$) (Figure 2.2 (b)).

The heat tolerance of juveniles was also much lower than that of the adults. Dog Lake juveniles had an average CTmax of 27.9°C compared to an average of 30.8°C for the adults ($p<0.001$) (Figure 2.3 (a)). Nettilling Lake juveniles had an average CTmax of 28.9°C which was also significantly lower than that of the adults' (CTmax = 30.4°C) ($p<0.001$) (Figure 2.3 (b)).

2.4 Discussion

This is the first study to quantify thermal tolerance phenotypes for the ninespine stickleback and to do so for different life stages and populations from geographically distinct regions in Canada.

2.4.1 Sample populations and crosses

Sample collection was challenging with variable success in catch per unit efforts between lakes. Sampling in Kehewin Lake, in particular, was complicated by green algae blooms, steep drop-offs, large debris, and limited shoreline access. As a result, I only collected a fraction of what I was able to sample for the other three lakes. I also had considerable difficulty producing crosses from any of the populations. In Alberta, I was not able to sample mature adults during their breeding season. Later in the season (when the majority of the samples were collected) many adults were no longer in condition and were heavily parasitized. In Nunavut, the majority of the crosses that I conducted from Dog Lake were unsuccessful due to fungal infections. In Nettilling Lake, the field collections were completed late in the summer so I missed the breeding season and did not collect any adults in mating condition.

2.4.2 Thermal tolerance

The thermal tolerance phenotypes found here were not as predicted. The capacity for cold tolerance was highest in the northernmost population, Nettilling Lake, but otherwise did not differ between lakes. This suggests that the temperature of the Nettilling Lake habitat may differ from that of the other three lakes and thus resulted in an evolved thermal minimum lower than that of the other populations. Temperature data from each lake would help confirm this hypothesis and help determine if the differences observed are biologically significant. Despite the four populations being from distinct climate regimes, they did not differ in heat tolerance capacity. This is similar to a study

by Bruneaux at al. (2014) that found cold-adapted populations of ninespine stickleback experienced no loss of performance at higher temperatures. My results also echo those from other studies that have found that cold tolerance can be variable while heat tolerance tends to be conserved across populations and species (e.g., Barret et al. 2010, references within Gunderson and Stillman 2015). The conserved nature of heat tolerance in these distinct populations may be explained by the biogeographic history hypothesis which follows that the majority of clades present today evolved in a period between the Palaeocene and Pliocene where the equator was warm and thermally stable. Subsequent adaptive radiations to colder, more species-poor areas would explain why most organisms – and in the present case, populations - maintain similar thermal tolerance maxima even in cold bioregions (reviewed in Araujo et al. 2013). My results may thus suggest that ninespine stickleback populations inhabiting temperatures closer to their maximum tolerance limit may be the first to experience the effects of rising temperatures (Araujo et al. 2013), resulting in local extinctions along their southern boundary.

For the juveniles studied here, the tolerance to both hot and cold temperatures was lower than that of the adults. Although my results are limited by the fact that I had few replicates and a number of uncontrolled variables (e.g., age and size of fish), they do demonstrate an enhanced sensitivity of juveniles to temperature. My results suggest that juvenile fish are more restricted in their physiological tolerances and may be particularly vulnerable to temperature highs and lows. My results support other fish research that demonstrate that the thermal window for fish eggs, larvae, and spawning adults is narrower than that of young adult and adult fish, making these groups more

vulnerable to rising temperatures (Portner and Peck 2010, Eddy and Handy 2012, Whitney et al. 2013). Elevated temperatures have also been associated with a reduction in survival of larval fish due to a compromised cellular metabolism (Madeira et al. 2016). The current study could be improved by assessing thermal tolerance of juveniles of equal age and from parents reared in the lab. Despite the shortcomings, it is evident that studies would benefit from incorporating these life stages to better understand how a species as a whole will respond to climate change.

2.5 Conclusions

The direct effect of temperature may not influence northern populations of fishes as strongly as expected given the conserved capacity for heat tolerance. In fact, based on these results, it may be southern populations that experience the direct effects of temperature first and undergo range contractions along the southern boundary. Furthermore, it is evident that temperature change may impact various life stages differently so it is imperative that future research include these and other vulnerable life stages to better predict how species will respond to rising temperatures in association with climate change.

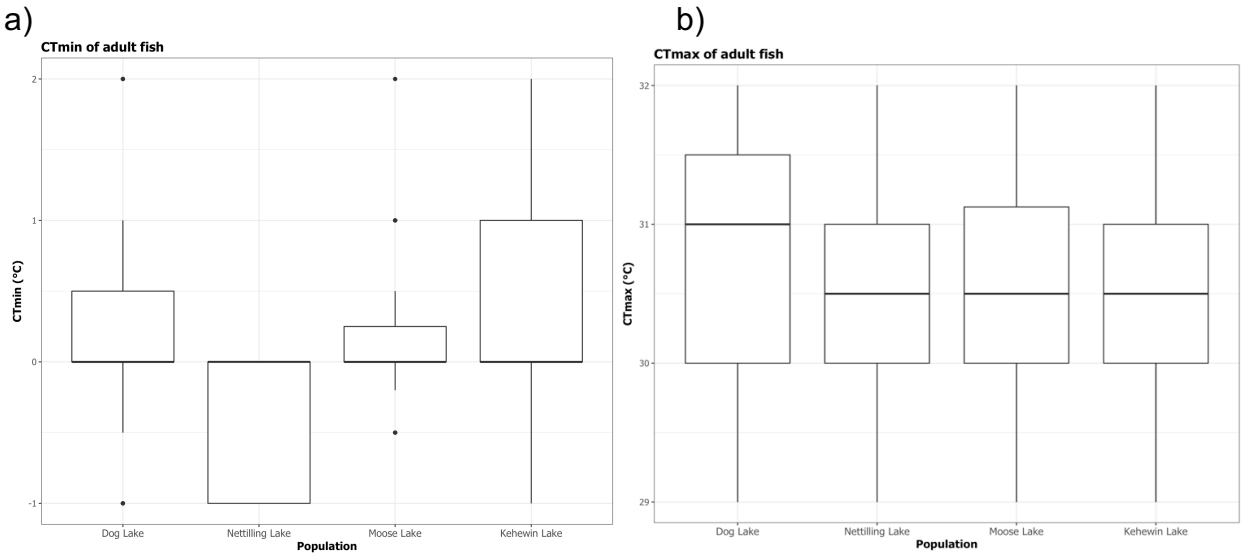

Figure 2.1. Cold tolerance (a) and heat tolerance (b) of adult ninespine stickleback. The CTmin of the Nettilling Lake population was significantly lower than the other populations F(3, 99)=3.94, p=0.01. The CTmax did not differ between populations, F(3, 119)=1.36, p=0.26.

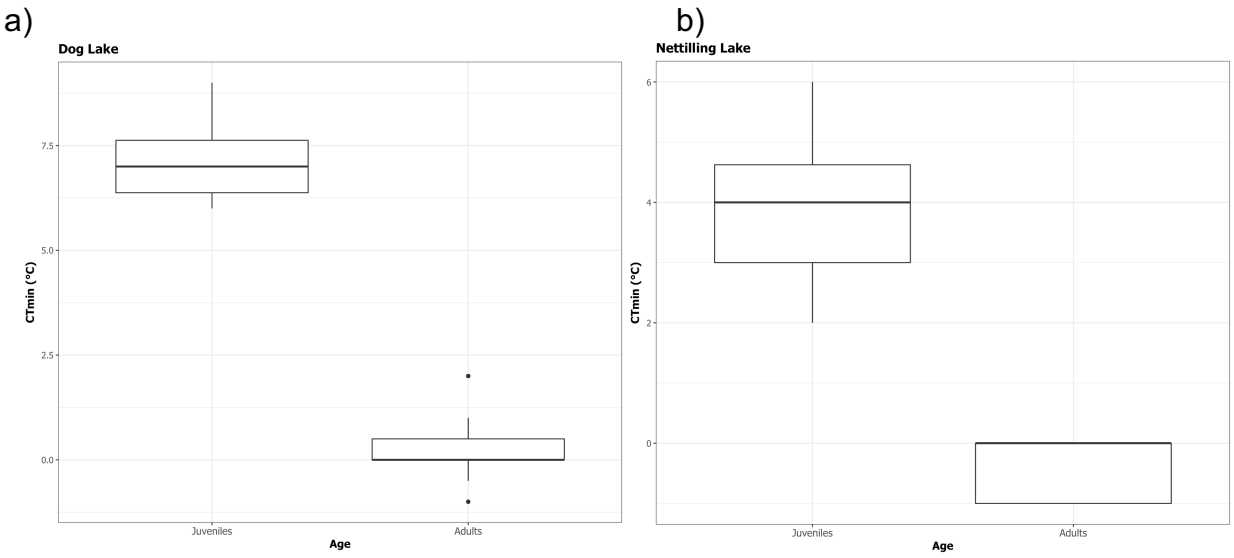

Figure 2.2. Comparison of cold tolerance limits between adult and juvenile life stages from Dog Lake (a) and Nettilling Lake (b). In both populations, cold tolerance for juveniles was significantly less than that for the adults (p<0.001).

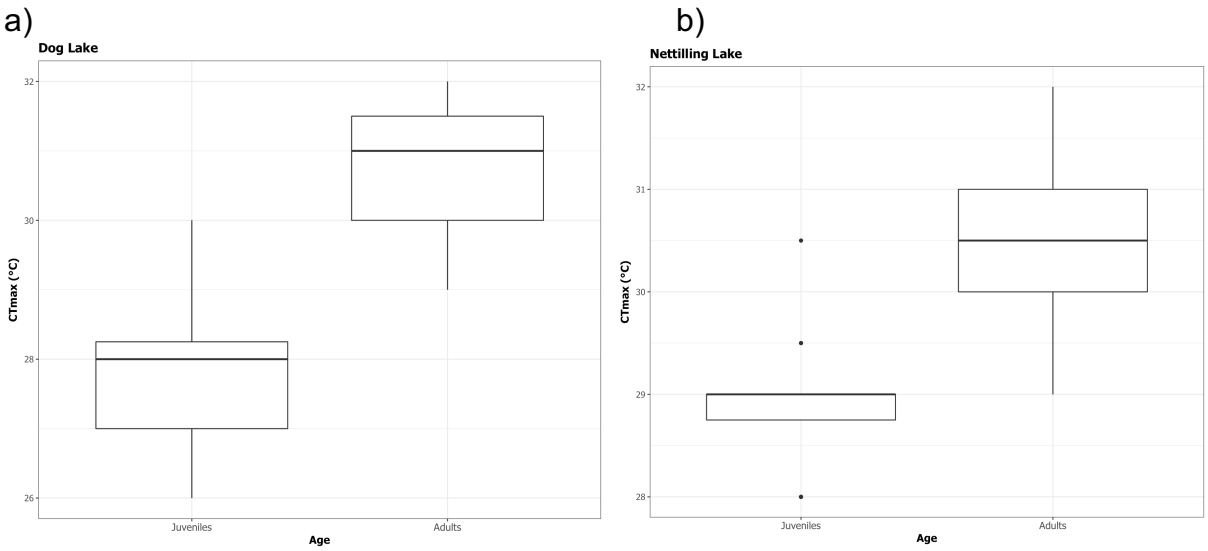

Figure 2.3. Comparison of heat tolerance limits of juvenile and adult fish from Dog Lake (a) and Nettilling Lake (b). In both populations, heat tolerance was significantly less for the juveniles than that for the adults ($p<0.001$).

CHAPTER 3 - THERMAL TOLERANCE PLASTICITY

3.1 Introduction

Since the onset of anthropogenic climate change, a plethora of environmental changes have occurred, including the rise in average global temperatures and the increased stochasticity of temperature extremes, with the Arctic being at the forefront of change; warming twice as fast as any other region on the planet (IPCC 2014). The consequences for freshwater lakes include the elongation of the ice-free season and thinner over-winter ice; both of which will contribute to warmer lake temperatures (Furgal and Prowse 2008, Shuter et al. 2012, IPCC 2014). In addition, recent data has shown that lake water temperatures have been increasing faster than air temperatures (O'Reilly et al. 2015), with the latter predicted to increase by two to six degrees in Alberta (Lemmen et al. 2008) and by four to ten degrees in Nunavut by the 2050s (Furgal and Prowse 2008). Drought is also predicted to be a severe risk in Alberta with water levels in Alberta's lakes expected to fall (Lemmen et al. 2008), further contributing to rising lake temperatures. Collectively, these changes will expose freshwater organisms to longer periods of thermal stress and introduce them to novel selection pressures. Some of the consequences of rapid environmental change for aquatic systems include changes in phenology (Shuter 2012, Hovel et al. 2017), the loss of optimal thermal habitats (Farrell 2009), range shifts (Perry at el. 2005, Shuter et al. 2012), changes in community assemblages and dynamics (Buisson and Grenouillet 2009, Lancaster et al. 2016), and shifts in trophic interactions (Eddy and Handy 2012, Ohlberger 2013); much of which are likely the direct result of rising temperatures.

These rapid changes in the aquatic environment will necessitate that species respond through adaptation to the new conditions, through phenotypic plasticity, or through a combination of both. Adaptation is the result of evolution by natural selection and may be necessary for species to persist in a warming world. This is particularly true for species that cannot shift their distribution to escape suboptimal conditions (Gunderson and Stillman 2015). Adaptation in response to environmental change involves a shift in mean trait values by changes in allele frequencies between generations and can happen in two ways: through adaptive genetic mutations or through the selection of adaptive genes from standing genetic variation. For adaptation to happen through mutations, many generations may be required thus taking some time to occur. Alternatively, if there is sufficient standing genetic variation within a population and high rates of recombination, selection will favor the most adaptive genes, causing them to spread throughout the population more rapidly (Barrik and Lenski 2013).

Evidence has shown that strong selection in a new environment or under rapidly changing conditions can lead to rapid evolution (Whitney and Gabler 2008, Hendry et al. 2008, Barrett et al. 2010, Morley et al. 2017) but the question remains whether fishes have the capacity to do so. Evidence of genetic adaptation in response to climate change is limited, likely due to difficulties in determining whether phenotypic change is genetic or plastic; if the change is indeed adaptive; and the difficulty in determining the effects of climate change in isolation of other stressors (Merila and Hendry 2012).

Organisms may also respond to changes in their environment with phenotypic plasticity. Phenotypic plasticity describes a genotype that exhibits phenotypes differentially in response to conditions within their environment (DeWitt et al. 1998). In

the short term, phenotypic plasticity can enable species to maintain performance and fitness levels under novel conditions through the acclimation of physiological traits, thus giving time for genetic adaptation to take place (Munday et al. 2013). The ability to acclimate may increase a species' chances of persistence in response to climate change (Peck 2011) and has been found to be high in organisms that have low tolerance to temperature extremes (Gunderson and Stillman 2015). Many studies that fail to attribute phenotypic differences to genetic differences typically use plasticity as the null hypothesis, however, such assumptions need to be tested. In order to adequately ascertain whether a response is plastic requires controlled experiments; ideally using animal models and common garden experiments (Merila and Hendry 2014).

Phenotypic plasticity in the thermal response of an organism to the temperature of its environment can be described by thermal reaction norms. Thermal reaction norms can have a variety of shapes and can be used to compare plasticity among traits in response to their thermal environment (West-Eberhard 2003). Phenotypes such as physiology, behaviour and morphology can be assessed under the framework of a thermal reaction norm (DeWitt and Scheiner 2004). The capacity to respond plastically to changes in temperature can also be measured in the acclimation response ratio (ARR). The ARR is the change in a thermal tolerance per degree change in environmental temperature (Morley et al. 2017). Species with higher ARR values are thought to be better able to change the physiological pathways they use when exposed to elevated temperatures. In many ectotherms, however, the degree of acclimation capacity is low, suggesting that ectotherms are limited in their ability to buffer

themselves against climate change with plastic responses (Gunderson and Stillman 2015).

Phenotypic plasticity can itself also be an adaptive response (West-Eberhard 2003). For example, plasticity in thermal traits has been shown to evolve in response to new environments to help populations persist under novel conditions (Morris et al. 2014, Akashi 2016). Transgenerational plasticity - when the environment of the previous generation(s) influences the phenotype of the new generation - can also be adaptive when the environment experienced by the previous generation(s) improves the performance of the new generation in the same environment (Herman and Sultan 2011).

The benefits of plasticity in thermal tolerance, however, may depend on how other aspects of fitness respond to temperature (Leroi et al. 1994). For instance, enhanced acclamation capacity at high temperatures may necessitate a trade-off with thermal tolerance at lower temperatures. For species whose lifecycle spans multiple seasons, such trade-offs may result in an overall reduction in fitness, especially given that weather extremes are expected to become more volatile (Glanville and Seebacher 2006). In addition, plasticity towards higher thermal maxima will likely require a re-allocation of resources away from growth and reproduction (Munday et al. 2008, Donelson et al. 2011). For example, aquatic ectotherm size is particularly sensitive to elevated temperatures, being up to ten times more sensitive than terrestrial ectotherms (Forster 2012). Further experimentation is required to assess the extent of these trade-offs.

Rising temperatures may also have ecological consequences in the freshwater

environment, such as changes in predator-prey interactions. Elevated temperatures increase an ectotherm's metabolic demand necessitating an increase in its feeding rate. Consequently, it will also increase the metabolic demand of its predators (Pink and Abrahams 2016, Allan et al. 2017, Svensson et al. 2017); increasing the need for a small fish to feed but making it riskier to do so. Further, warming has been linked to reduced prey escape rates of small fish (Allan et al. 2017). As a result, anti-predator traits will likely become more important as temperature in freshwater systems continue to rise.

In this chapter, I assess plasticity in thermal traits of the ninespine stickleback along a north to south gradient by evaluating the reaction norm of those traits in response to temperature. I determine if adult and juvenile ninespine stickleback have sufficient thermal acclimation capacity, (i.e. plasticity), to compensate for elevated temperatures. Next, I determine if plasticity towards higher heat tolerance is correlated with a loss of cold tolerance. Lastly, I examine whether other phenotypes associated with temperature, such as size and pelvic spine length, demonstrate a plastic response to elevated temperatures. To test these questions, I experimentally acclimated fish to three different temperature treatments for a period of three months. At the end of the incubation period I assessed the reaction norm of their thermal minima and maxima as well as some morphological phenotypes that may co-vary with thermal tolerance.

Given that the representative northern and southern populations of ninespine stickleback used in this study are phylogeographically distinct (Aldenhoven et al. 2010), that local adaptation can happen over short time scales (e.g. Barrett et al. 2010), and that plasticity in thermal tolerance is thought to be correlated with a species' thermal

regime (Whitney et al. 2013), I expected to see inter-population differences in response to temperature. Furthermore, given that elevated temperatures negatively affect the size of aquatic ectotherms, I expected to observe differences in size between temperature treatments. Lastly, since warmer temperatures increase predation pressure and since predation pressure selects for enlarged pelvic spines in stickleback (Lescak and Hippel 2011), I predicted that the length of the pelvic spines at elevated temperatures would be longer compared to those in cooler temperatures.

3.2 Methods

3.2.1 Sample populations

I used adult ninespine stickleback collected from Moose Lake, Dog Lake, and Nettilling Lake using methods described in Chapter 2. Unfortunately, due to low collection success, I was not able to use the Kehewin Lake population because there were too few of them for a balanced experimental design.

I used juvenile ninespine stickleback from Dog Lake that were crossed in the field and hatched in the aquatics lab. I also use juveniles from Nettilling Lake that were collected from shallow, overflow ponds adjacent to the lake. These juveniles were approximately one month old at the time of collection.

As stated in Chapter 2, all fish were housed in the Life and Environmental Science and Animal Research Centre (LESARC) at the University of Calgary and all protocols followed procedures approved by the animal care committee in accordance with the standards set by the Canadian Council for Animal Care.

3.2.2 Experimental design

To test the effect of extended warming on adult ninespine stickleback, I divided the populations in a randomized block design with three of each population represented in each experimental tank. Tanks were randomly assigned to one of three temperature treatments: 17°C as the control, and 20°C and 23°C as the heated treatments. The treatments were chosen because they were within the species' known thermal range (Bruneaux et al. 2014), so would not induce unnecessary mortality, but the differences in temperature were conceivably large enough to detect changes in thermal response (Sandblom et al. 2015). Each temperature treatment was replicated four times for a total of 12 experimental units. There were nine fish in each tank - three from each population (Moose, Dog and Nettilling Lakes). To maintain the density of nine fish per each experimental unit I kept approximately 40 fish as replacements for any mortalities that occurred in the experimental aquaria. These fish were maintained at a density of approximately 10-15 fish per aquarium at 17°C.

To achieve heating in the treatment tanks I placed a submersible heater in each aquarium, leaving the control (17°C) aquarium heaters unplugged and setting the heating treatments to their appropriate temperature (20°C and 23°C). Adult fish were incubated at the above-mentioned temperatures for a period of three months.
The juveniles from Nettilling and Dog Lakes were split into three aquaria each, keeping conspecifics together. Each population had one replicate of the three treatments described above (17°C, 20°C and 23°C) with approximately 20 fish in each replicate. There were no replacements for the juveniles. Since temperature directly effects the growth and development rates of young fish (Neuheimer and Taggart 2007), juveniles

were incubated in the treatments for the equivalent of 2380 (Nettilling Lake) and 3145 (Dog Lake) growing degree days. Dog and Nettilling Lakes did not have the same number of growing degree days because, due to logistics, the Dog Lake juveniles arrived at the aquatics lab approximately one month earlier than those from Nettilling Lake.

3.2.3 Population identifiers

The experiment with the adult fish involved having populations mixed together in a randomized block design so, to distinguish the fish from one another, I clipped each one with a unique population identifier. For Dog Lake, it was the adipose fin; for Nettilling Lake, I clipped the right pelvic spine; and for Moose Lake it was the left pelvic spine. To perform the fin clips, I first anaesthetized each fish individually using a non-lethal dose of clove oil (approximately 1 drop per 500mL of water). Once the fish was anaesthetized I snipped the fin or spine using sterilized dissection scissors. I immediately placed the fish into fresh water for recovery. Clipped fish were given at least 20 minutes to recover before being placed back in their aquaria. Fin and spine clips are a common practice and do not impede or harm the health or wellbeing of the fish. Recovered fish did not show any signs of distress following the recovery period.

3.2.4 Fish husbandry

Fish were housed in 38 L glass aquaria at a density of nine fish per tank. Each aquarium had a large sponge filter for oxygenation and two medium-sized aquarium plants for enrichment. The conditions in the aquaria were maintained at a salinity of 0.5

ppt and a photoperiod of 12 L: 12 D. Adult fish were fed approximately 6 g of a mixture of adult spirulina brine shrimp and chopped blood worms once per day. Juvenile fish were fed approximately 1 g of live brine shrimp per day. The aquarium water was cleaned up to two times per week by using a manual siphon to remove food and debris from the bottom of the tank and replacing 1/3 of the water.

Midway through the experiment an ectoparasite infection developed in several fish across multiple aquaria. Paraguard - an aldehyde-based parasite treatment - was used to treat the infection and was applied to every aquarium, regardless if infection was apparent or not, for a total of three days and up to three weeks for the most infected tanks.

3.2.5 Thermal tolerance

After the fish were incubated in the experimental treatments for three months, I tested their thermal tolerance using the same methods described in Chapter 2 (see Appendix B for experimental set-up). Briefly, for CTmin, I gradually lowered the temperature of the fish's environment by approximately 0.33°C/minute using water chillers. For CTmax, I followed the same procedure except I raised the temperature of the water using electric heaters. Once the fish reached its thermal endpoint it was moved into a recovery tank. I tested the critical thermal minimum and maximum of each fish with at least one week recovery between trials. After a number of mortalities throughout the incubation period, the final number of adult fish tested for thermal tolerance was n = 76 (for Dog Lake, Nettilling Lake, and Moose Lake, respectively: at 17°C, n = 11, 10, 11; and at 20°C, n = 11, 11, 5; at 23°C, n = 9, 8, 0). Replacement fish

used to maintain the density of each tank following a mortality were not tested for thermal tolerance. The final number of juvenile fish tested for thermal tolerance was n = 93 (for Dog Lake and Nettilling Lake, respectively: at 17°C, n = 13, 22; at 20°C, n = 13, 16; and at 23°C, n = 16, 13).

3.2.6 Other phenotypes

Upon completion of the experiment I euthanized all fish using a lethal dose of clove oil (approximately 3 drops per 500mL of water) and stored them in 70% ethanol. Later, using calipers, I measured the standard length (distance from snout to the beginning of the anal fin rays) of each fish to test if incubation temperature had an effect on size. I used alizarin red to dye the bony parts of the fish then used calipers to measure the pelvic spine under a dissecting scope. To control for body size, I standardized the length of the pelvic spine by calculating the residual of predicted spine length based on the standard length of the individual. Lastly, I examined the skin and body cavity of the mortalities that occurred throughout the incubation period to identify and quantify parasite load.

3.2.7 Statistical analysis

All statistical analyses for this study were completed in R (R Core Team 2017). Thermal tolerance data for adult fish were fit to a mixed effects model using the lme4 function (Bates et al. 2015). The model included treatment (17°C, 20°C, 23°C) as the fixed effect with population and tank as random effects. Thermal tolerance data for juvenile fish were fit to a two-way ANOVA.

The ARR - the increase in thermal tolerance per degree Celsius change in temperature – was calculated by fitting a regression to the CTmin and the CTmax data for each population (Claussen 1977). For the adults fitted to a linear mixed effects model, the ARR corresponds to the slope of the line describing the relationship between thermal tolerance and treatment temperature.

The effect of incubation temperature on the range of thermal tolerance in adult fish was tested using an ANOVA on the difference between the average CTmin and CTmax for the adult fish against temperature treatment. The effect of temperature on the standard length and the relative spine length were tested using two-way ANOVAs individually for the two juvenile populations.

3.3 Results

3.3.1 Mortalities

The mortality rate for adult fish in the experimental tanks was relatively constant across populations in the 17°C treatment (8-17%) with a rise in mortality in the 20°C for Moose Lake. The mortality rate was highest in the 23°C treatments with 25% mortality in Dog Lake, 33% in Nettilling Lake, and 100% mortality in Moose Lake fish (Figure 3.1).

Of the total number of individuals in the experiment, a number of fish had a parasite or an illness. Just 2% of Dog Lake were ill whereas 11% of Nettilling and 42% of Moose had a visible ailment. The most common parasite in Moose Lake was *Glugea anomala,* a contagious microsporidian that appears as white spores on the skin. The second-most common illness in Moose Lake mortalities was an unknown fungus that appeared on the skin of the fish. One Moose Lake mortality had *Schistocephalus*

solidus, a tapeworm found inside the body cavity. *Schistocephalus solidus* is a common parasite in small fish and has intermediate hosts in birds and copepods (Hopkins and Smyth 1951). Nettilling Lake mortalities were infected with an unknown fungus on the skin. The only visible ailment in the Dog Lake mortalities was one fish with unknown red lesions on the skin. See Appendix C for supplementary data on mortalities.

3.3.2 Thermal tolerance

Incubation temperature had a significant effect on the critical minima and maxima of adults from Dog, Moose, and Nettilling Lakes ($p<0.001$). The CTmin was higher for all populations in successively warmer treatments and was the lowest in the control treatment (Figure 3.2). The CTmin had a linear reaction norm, formulated as: CTmin = -10.18°C + 0.64xT(acclimation), where T(acclimation) is the acclimation temperature. The ARR for cold tolerance was 0.64°C.

The same trend was found for the CTmax where the control treatment had the lowest CTmax and the warmed treatments had increasingly higher thermal maxima (Figure 3.3). CTmax across populations is modelled as: CTmax = 27.64 + 0.15xT(acclimation). The ARR for heat tolerance was 0.15°C. There was no interaction between population and incubation temperature for thermal tolerance, indicating that all three populations had similar acclimation capacity over the temperature range studied. The summary of the results from the lme4 function for CTmin and CTmax can be found in Appendix C.

The incubation temperature had a significant effect on the thermal tolerance range, $F(1,1) <0.001$, $p <0.001$, with the control group having the largest range and the

heated groups experiencing reductions in thermal range (Figure 3.4).

The juveniles experienced similar patterns in thermal tolerance in response to incubation temperature. The Dog Lake juveniles had a higher CTmin than the Nettilling Lake ones, $F(1, 92)=161.26$, $p <0.001$, and the CTmin of both populations increased with temperature, $F(2, 92)=140.10$, $p <0.001$ (Figure 3.5). The average CTmin ARR values were 042°C for Dog Lake and 0.71°C for Nettilling Lake juveniles. The CTmax did not differ between populations, $F(1, 92)=2.99$, $p =0.09$, but the CTmax was positively correlated with treatment, $F(2, 92)=294.97$, $p <0.001$, as well as its interaction with population identity, $F(2, 92)=4.22$, $p =0.02$ (Figure 3.6). The average CTmax ARR values were 0.63°C for Dog Lake and 0.52°C for Nettilling Lake juveniles.

3.3.3 Other phenotypes

The size of adult fish was not effected by incubation temperature, $F(2, 75)=0.69$, $p =0.5$, but differed between populations, $F(2, 75)=8.34$, $p < 0.001$, with Moose Lake being significantly smaller (mean = 46.94 mm) than the Dog Lake and Nettilling Lake fish. The standard lengths of the Nettilling and Dog Lake fish were not significantly different from one another ($p = 0.33$) (mean = 52.89 mm and 51.23 mm, respectively) (Figure 3.7).

Similarly, there was no significant effect of temperature on the pelvic spine length in adult fish, $F(2, 40)=2.68$, $p =0.08$, but there were inter-population differences, $F(2, 40)=83.99$, $p < 0.001$, with Nettilling having the shortest and Moose Lake having the longest pelvic spine (Figure 3.8). Dog Lake had an intermediate spine length.

In terms of the juveniles, it is important to note that upon the completion of the

study I discovered that there were approximately double the number of Nettilling Lake juveniles in the 17°C tank than there were in the other treatments – an oversight made possible by the small size of the juveniles and their tendency to hide within the tank. As a consequence, these fish were very small, likely due to food limitation rather than due to the effects of the treatment. Due to these confounding variables, I have removed this group from the analysis and analyzed the data for the two juvenile populations separately. Using a one-way ANOVA, I found that incubation temperature significantly affected the length of the Dog Lake juveniles, $F(2, 41)=44.43$, $p <0.001$. Using a two-sample t-test for the Nettilling Lake juveniles using data just from the 20°C and 23°C treatments revealed that temperature had no effect on size ($p =0.8$) (Figure 3.9).

The incubation temperature had a significant effect on the relative spine length of Dog Lake juveniles, $F(2, 17)=6.09$, $p =0.01$, with a trend towards longer spines in the Dog Lake juveniles at higher temperatures. Temperature did not affect the relative spine length of the Nettilling Lake juveniles ($p = 0.78$) (Figure 3.10).

3.4 Discussion

This is the first study to assess the effects of prolonged warming on thermal tolerance and associated phenotypes in the ninespine stickleback. I found evidence for elevated heat tolerance in response to warmer temperatures in both adult and juvenile fish. My findings suggest that the ninespine stickleback responds plastically to temperature and may do so uniformly across the species' range.

3.4.1 Thermal tolerance

In this study, thermal tolerance was strongly influenced by incubation temperature. I found that the upper thermal limit (CTmax) for ninespine stickleback acclimatized to temperatures between 17 and 23°C ranged from 29.8 - 31.6°C and can be modelled by a linear reaction norm: CTmax = 27.64 + 0.15xT(acclimation). In the context of other Gasterosidae, the CTmax of brook stickleback ranges from 29 - 31°C (Brett 1994) and threespine stickleback have a CTmax of 28.74 + 0.27xT(acclimation) (Feldmuth and Baskin 1976). Interestingly, the lower thermal limit of adult ninespine stickleback demonstrated more plasticity than the upper thermal limit, as demonstrated by a steeper slope in the thermal reaction norm: CTmin = -10.18°C + 0.64xT (acclimation). In a similar study by Morris et al. (2014) that used lab-reared threespine stickleback from ancestral (marine) and derived (freshwater) populations raised under different temperature treatments, more genes were expressed plastically in cold treatments compared to heated ones. Furthermore, the derived populations had more plastically expressed genes than ancestral ones did, suggesting that plasticity evolved in response to conditions in the new environment (Morris et al. 2014). Given that freshwater fishes will likely need to adapt to changing environments *in situ,* identifying the role of temperature-induced changes in gene expression in response to new environments may be pivotal to our understanding of their potential to respond to changing thermal regimes.

In my study, there was evidence for relatively high ARR values in response to cold temperatures but low ARR values in response to high temperatures, which suggests that this species has the capacity to respond plastically to cold but less

capacity to respond to heat. Adults had particularly high cold tolerance and responded to each degree Celsius change in temperature with a 0.64°C change in cold tolerance. Heat tolerance, on the other hand, was more restricted, with adults demonstrating just a 0.15°C increase in heat tolerance for every 1°C increase in incubation temperature. The juveniles had comparably high ARR values in both hot and cold tolerance, demonstrating enhanced plasticity in thermal tolerance at this life stage.

The issue with the current study, and like other acclimation-based studies with fishes (e.g. Kelly et al. 2014, Deslauriers et al. 2016, Xia et al. 2017), is that phenotypic plasticity can happen over many time scales and studies such as these do not take into account the role of exposure to past temperatures. The effects of temperature exposure can be short-term and reversible as the result of a short period of acclimatization or acclimation (natural vs. in a controlled environment). It can also affect organisms long-term as the result of conditions experienced during development or passed on from the previous generation(s) to the new generation via transgenerational plasticity (Herman and Sultan 2011). Plasticity in response to changes in the thermal environment at all these time scales can affect thermal performance and gene expression in fish (Scott and Johnston 2012, Metzger and Schulte 2017). To parse apart the role of plasticity at different time scales would require a longer-term, multigenerational study under controlled conditions. Observing plasticity at the level of gene expression would further aid in identifying the underlying source of plasticity (Somero 2010, Metzger and Schulte 2017).

Here, I found that an increase in heat tolerance was accompanied by a reduction in cold tolerance, suggesting that this species may be vulnerable to volatile climatic

extremes and may experience reduced fitness in colder years. Furthermore, given that there was no variation in heat tolerance capacity among the populations I studied - despite originating from distinct climate regimes and glacial refugia - suggests that climate change may affect ninespine stickleback populations similarly across their geographical range.

Unfortunately, however, with the elimination of one Alberta population (Kehewin Lake) and the high mortality in the Moose Lake population, there was limited replication in the study which did not allow me to compare the results along a North-South gradient. Furthermore, the results for the juveniles have limited explanatory value given that there were few populations represented, there was an error in the control treatment for one population, there was no randomized block, and there were differences in the ages of the juveniles at the time of the experiment. The differences in age of the juveniles likely explain why their response to temperature differed. In reptiles, for example, plasticity in morphology and physiology in response to temperature differs significantly between age classes (Nobel et al. 2018). Additionally, since I was unable to follow single parent crosses I cannot assess the heritability of thermal tolerance from the current study. Consequently, these results should be interpreted with caution. A more thorough experiment using F1 fish from parents reared in the lab would provide better control for these variables. To further improve the study, F1s from parents subjected to elevated temperatures throughout maturation could be used to reveal the roles of gene expression plasticity and epigenetic effects on the subsequent generation, following similar methods described in Morris et al. (2014) and Metzger and Schulte (2017).

3.4.2 Other phenotypes

Incubation temperature had no effect on the standard lengths of adult fish, suggesting that any re-allocation of resources to increase the CTmax had no immediate effect on growth. These results may suggest that there are no size trade-offs associated with temperature in adults but could also simply be a reflection of the relatively short time scale of the experiment. Alternatively, it could reflect changes in plasticity in response to temperature throughout an individual's lifespan. For example, in reptiles, plasticity in morphological traits in response to temperature is quite high at early ages but becomes significantly reduced at later life stages (Nobel et al. 2018). Although there was no effect of temperature on size or spine length, Moose Lake adults were significantly smaller than the others and had the longest relative spine length. These differences are likely related to the nature of the Moose Lake habitat, where predation pressure is high compared to that in low-productivity Arctic lakes (Warren and Burr 2014). However, without rearing Moose Lake juveniles in the lab we cannot be sure if these differences are genetic based or a product of their rearing environment.

The size of Dog Lake juveniles was negatively associated with temperature, as is expected to be the trend with a warming climate (Chambers 1997, Forster et al. 2012). Since small body size is associated with a lack of 'boldness' in fishes, this trend may have serious consequences for their foraging and parental care abilities (Uusi-Heikkil et al. 2008), resulting in a reduction of overall fitness. After removing the 17°C group from the analysis for reasons stated above, the Nettilling Lake juveniles showed no difference in size between the 20°C and 23°C treatments. Given that these fish were

collected from Nettilling Lake after they had hatched and had begun the experiment at an older age than the Dog Lake juveniles, it is possible that they had surpassed a window of plasticity to temperature. Interestingly, although the two populations had a different number of growing degree days and were at different stages of development at the start of the experiment, their standard lengths did not differ at the end of the experiment. These results could reflect inherent differences in size between the populations, but given that the adults of the same populations did not differ in size, it is more likely that being reared from birth in the lab environment and consistently fed to satiation allowed the younger Dog Lake juveniles to grow faster than the Nettilling Lake juveniles. However, given all the uncontrolled factors in the juvenile portion of this study, analyses should be repeated with F1 juveniles to control for these variables.

Higher temperatures made Dog Lake juveniles smaller but also increased their relative spine length. The allocation of resources to the spine and not the core body may reflect an evolutionary response to a correlated increase in predation pressure at higher temperatures. However, more data is needed to test this hypothesis.

3.4.3 Mortalities

The mortality rate in the 23°C treatment was relatively high across all three populations, especially in the Moose Lake population, which suggests that 23°C is beyond the optimum temperature range for this species. A large proportion of the Moose Lake mortalities had a visible parasite or infection, suggesting that the immune system was compromised at elevated temperatures driving them to succumb to their infections. This supports observations that the immune system of a fish is repressed at

temperatures beyond its optimal temperature (Watts 2001). In an experiment with threespine stickleback, Franke et al. (2017) found that with increasing temperatures, parasite growth and fitness was elevated while that of the host was repressed. Franke et al. (2017) thus concluded that the thermal tolerance of the threespine stickleback depends on the interaction between temperature and parasite load, which is likely also true for ninespine stickleback.

Parasites are largely influenced by temperatures, with elevated temperature extending the growing season of parasites, enabling more generations per season and thus higher transmission rates (Marcogliese 2001). In the marine environment, increased temperatures have been associated with the extended range of pathogens and increases in disease-related mass mortalities (Harvell et al. 2002). Perhaps traits associated with temperature such as parasite load, rather than the direct physiological response to temperature itself, will be the biggest threat to fishes as the climate warms. The ninespine stickleback, and likely other fishes, will evidently need mechanisms to cope with these indirect effects of temperature as the climate continues to warm.

3.5 Conclusions

Whether fishes will persist in a warming world will ultimately depend not just on their capacity to acclimate to higher temperatures but on the interaction between temperature and other abiotic and biotic factors that can alter overall performance. Arguably, given that temperatures will become increasingly variable in the future (IPCC 2014), projecting how an organism will respond requires a better understanding of how temperature exposure at different time scales affects the overall thermal response

(Schulte et al. 2011). Going forward, this would be made possible by investigating plasticity in the thermal response at the genomics level (Morris et al. 2014, Metzger and Schulte 2017). By integrating knowledge of the thermal physiology, genomics, and the abiotic and biotic interactions with temperature, can allow us to better predict how species will respond to climate change and better inform conservation strategies for their long-term persistence.

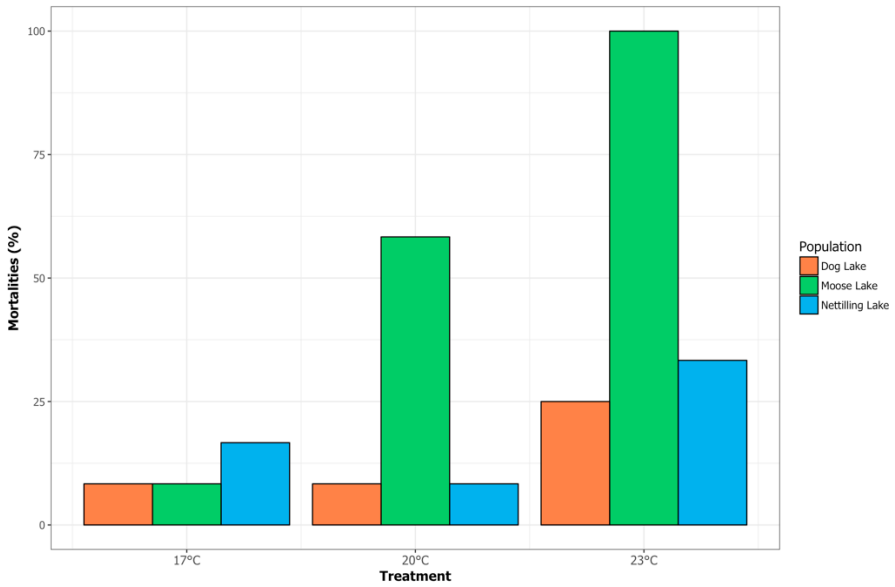

Figure 3.1. The number of mortalities throughout the experiment as a percentage of the number of fish that started the experiment in each treatment (n=12).

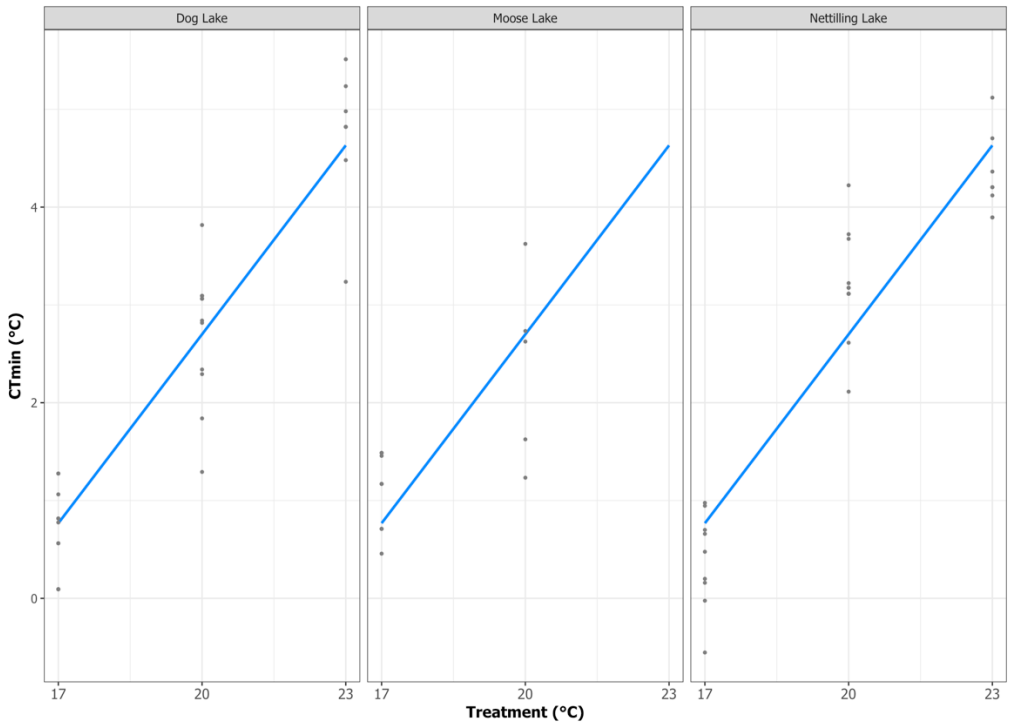

Figure 3.2. Thermal reaction norm of the critical thermal minimum (CTmin) of ninespine stickleback populations subjected to different incubation temperatures (17°C, 20°C, 23°C) for a period of three months. The reaction norm is linear with the formula CTmin = -10.18°C + 0.64xT(acclimation).

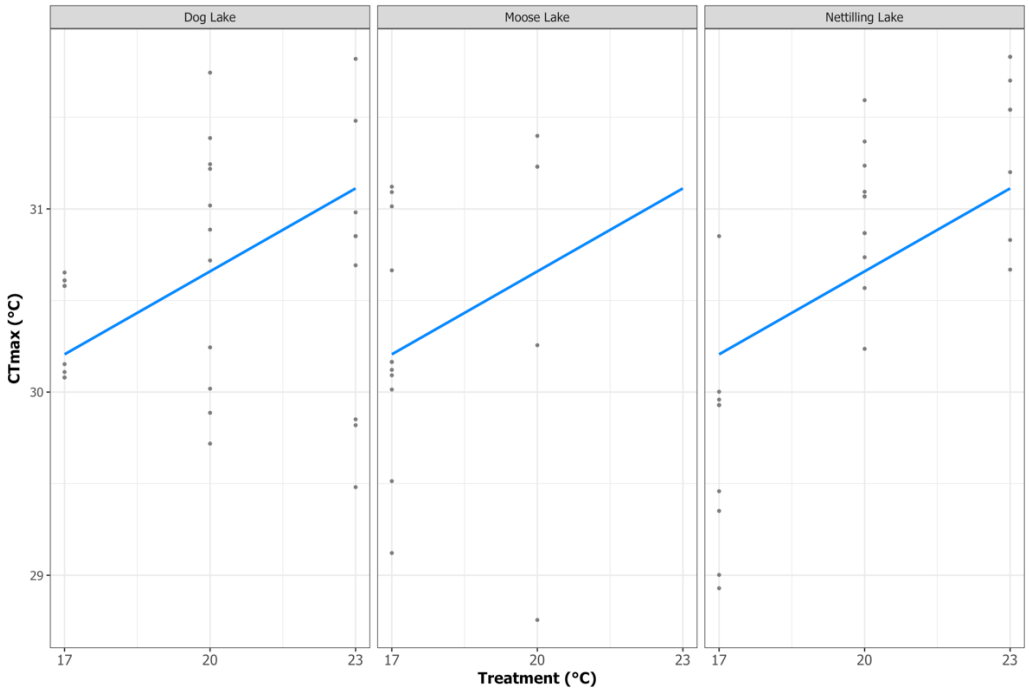

Figure 3.3. Thermal reaction norm of the critical thermal maximum (CTmax) of ninespine stickleback populations subjected to different incubation temperatures (17°C, 20°C, 23°C) for a period of three months. The reaction norm is linear with the formula CTmax = 27.64 + 0.15xT(acclimation).

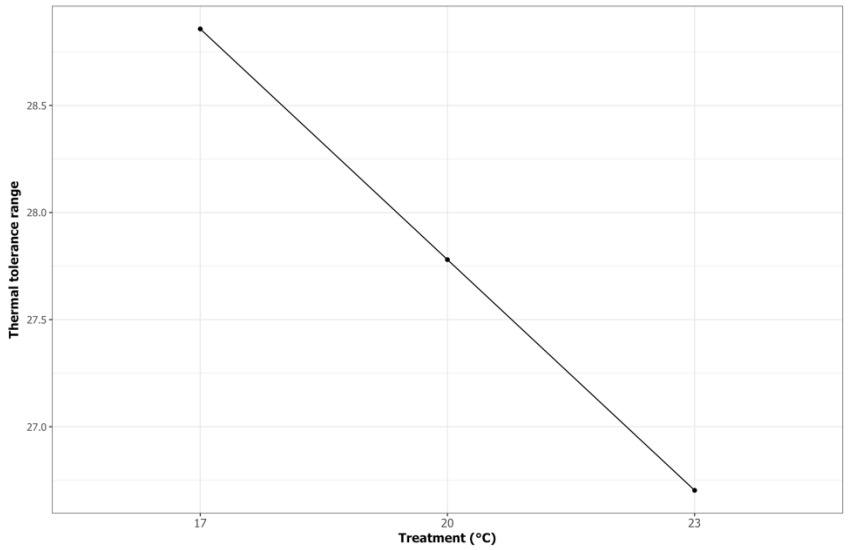

Figure 3.4. Thermal reaction norm of thermal tolerance range of adult ninespine stickleback. The range is calculated as the difference between the mean CTmax and mean CTmin. Temperature had a significant effect on the range, F(1, 1) <0.001, p <0.001.

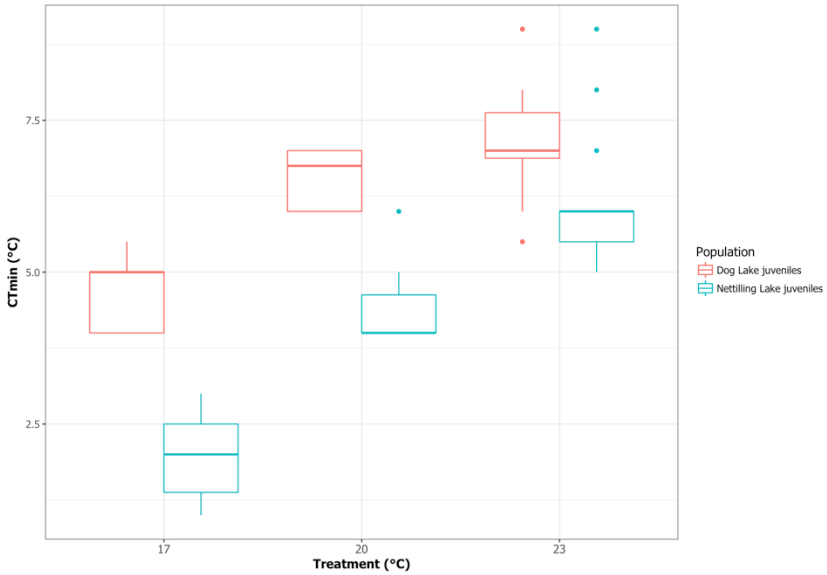

Figure 3.5. The critical thermal minimum (CTmin) of juvenile ninespine stickleback subjected to different incubation temperatures (17°C, 20°C, 23°C) for a period of three months. The identity of the population, F(1, 92)=161.26, p <0.001, and the treatment, F(2, 92)=140.10, p <0.00, had a significant effect on the CTmin.

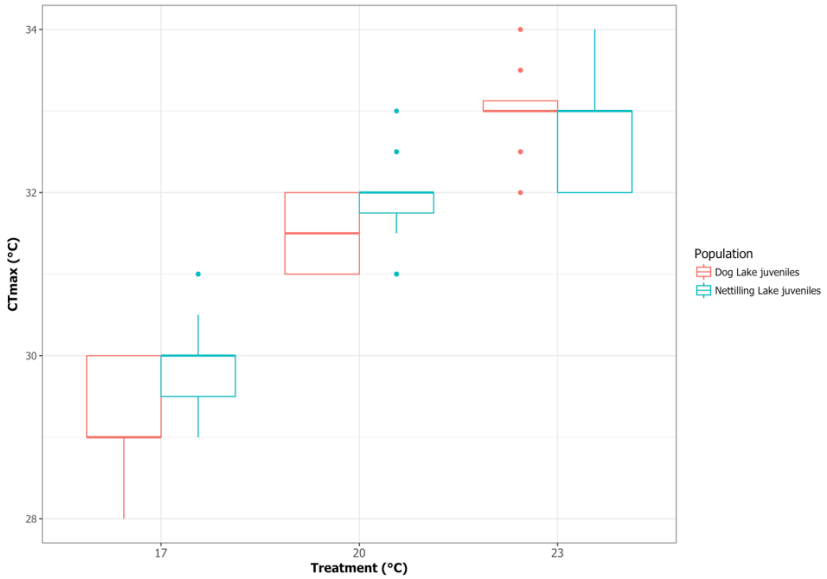

Figure 3.6. The critical thermal maximum (CTmax) of juvenile ninespine stickleback subjected to different incubation temperatures (17°C, 20°C, 23°C) for a period of three months. CTmax did not significantly differ between populations, F(1, 92)=2.99, p =0.09. However, the treatment, F(2, 92)=294.97, p <0.001 and its interaction with population identity, F(2, 92)=4.22, p =0.02, had a significant effect on CTmax.

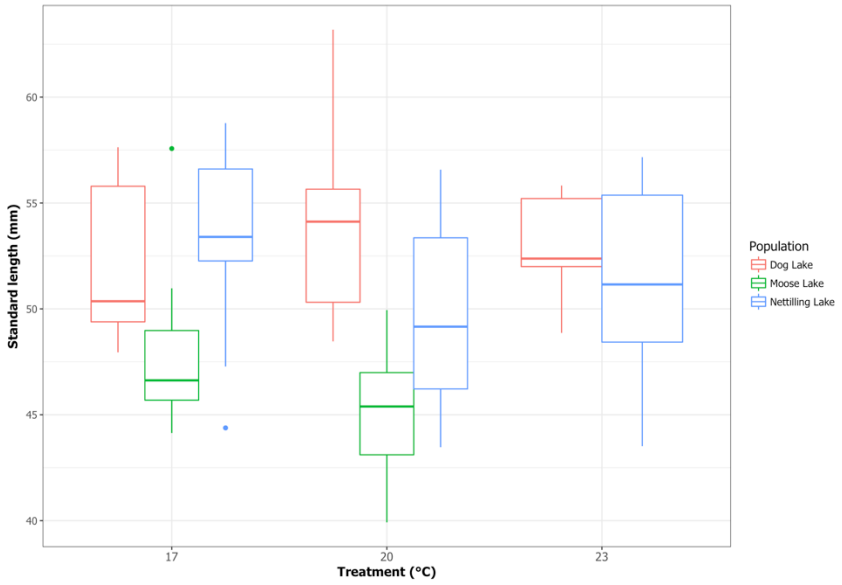

Figure 3.7. The standard length of adult ninespine stickleback exposed to incubation temperatures of 17°C, 20°C, and 23°C. Length was not effected by temperature treatment, $F(2, 75)=0.69$, $p =0.5$, but differed between populations, $F(2, 75)=8.34$, $p <0.001$, with Moose Lake being significantly smaller than the others.

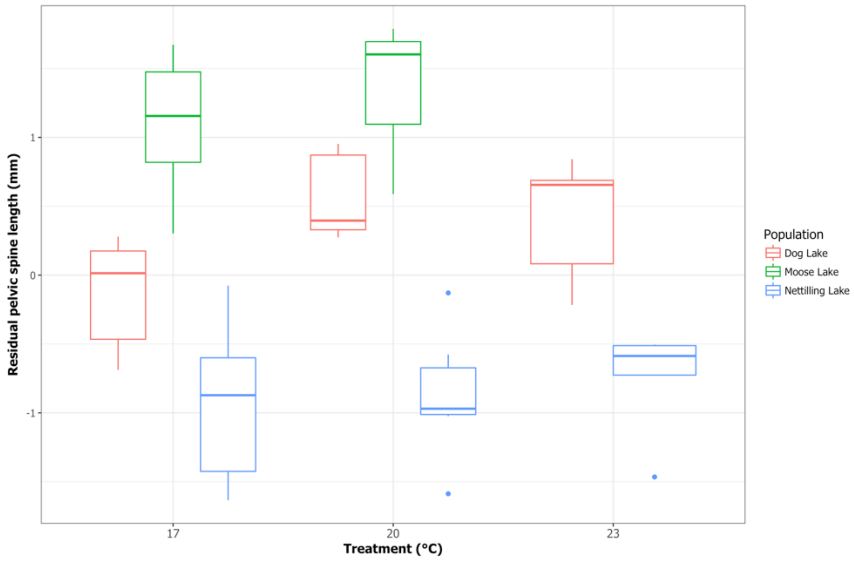

Figure 3.8. The length of the pelvic spine, corrected for size, of adult ninespine stickleback from three populations. Spine length was not significantly affected by temperature treatment, $F(2, 40)=2.68$, $p =0.08$, but differed significantly between populations, $F(2, 40)=83.99$, $p <0.001$, with Moose Lake having the longest spine compared to the others.

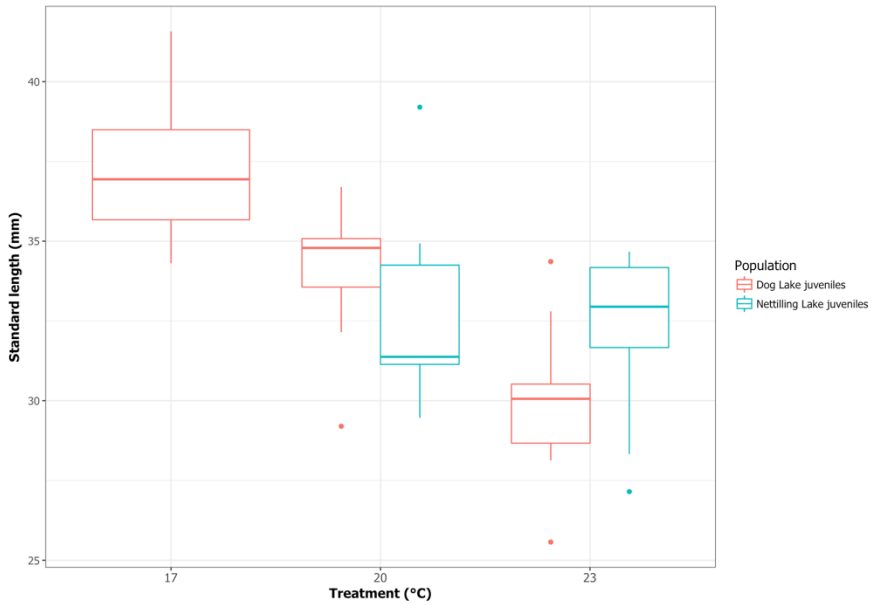

Figure 3.9. The standard length of juvenile ninespine stickleback under three temperature treatments. The size of Dog Lake individuals was significantly reduced in higher temperatures, $F(2, 41)=44.43$, $p < 0.001$, but the size of Nettilling Lake individuals was not ($p = 0.8$).

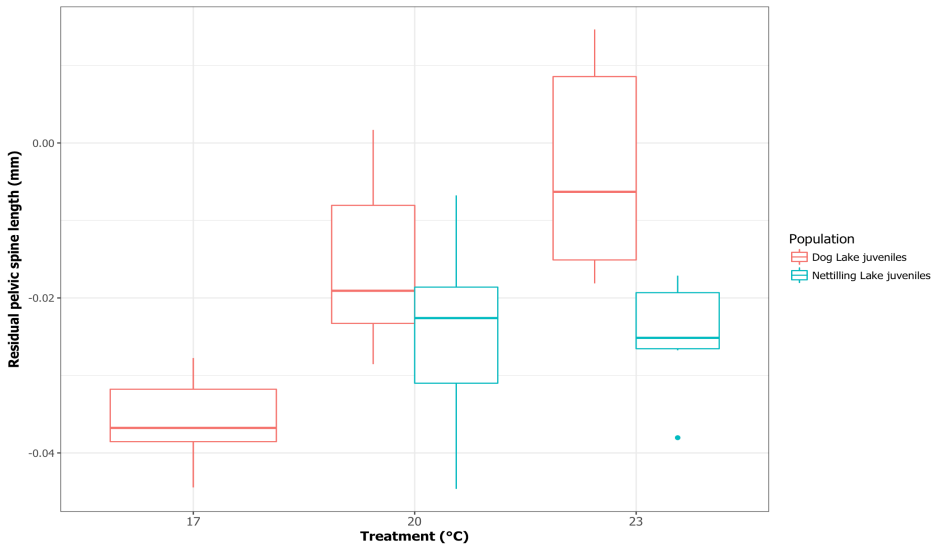

Figure 3.10. The length of the pelvic spine, corrected for size, of juvenile ninespine stickleback. Temperature had a significant effect on spine length in Dog Lake, $F(2, 17)=6.09$, $p = 0.01$, but had no significant effect on the pelvic spine length of the Nettilling Lake population ($p = 0.78$).

CHAPTER 4- GENERAL CONCLUSIONS

4.1 Summary of research

The purpose of my thesis was to investigate the direct and indirect effects of temperature on thermal tolerance traits in populations of ninespine stickleback. Against the backdrop of unique local adaptation and phylogeographic evolutionary origins, I sought to characterize thermal tolerance levels in populations from the northern and southern parts of the species' range. Furthermore, I sought to determine whether there was variation in thermal tolerance between juvenile and adult life stages and to identify whether plasticity in thermal tolerance differed at elevated temperatures.

The data presented in the preceding chapters demonstrates that there is variation in cold tolerance amongst populations, but evidently little to no variation in heat tolerance. The data also indicated that thermal tolerance phenotypes are plastic in response to acclimation temperature, but less so at elevated temperatures. I also found evidence that increased heat tolerance involved a trade-off with cold tolerance, reducing the thermal tolerance range. Juvenile stickleback demonstrated less tolerance for both hot and cold temperatures but also demonstrated relatively high plasticity in response to acclimation temperature. Additionally, lab-reared juveniles were smaller and had longer spines at higher temperatures.

Collectively, my data suggest that cold tolerance may be a locally adapted trait while heat tolerance is likely conserved in this species. Given that there were no differences in heat tolerance or plasticity between northern and southern populations, these data further suggest that the predicted responses to climate change in association

with thermal tolerance may be the same across the ninespine stickleback's range. My data also suggest that given the trade-offs in thermal tolerance identified, this species, particularly juveniles, may be more vulnerable to seasonal highs and lows and climatic extremes. These results provide important information about predicted responses to temperature changes in a region undergoing rapid environmental change for a model species with a broad distribution within the Arctic.

One unexpected outcome of the study in Chapter 3 was a steep mortality rate of adult fish that were incubated at the highest temperature. The mortalities were not caused by a lack of thermal tolerance to that temperature (see Chapter 2), but instead were likely the result of a shift in the dynamics between pathogen and host. In this case, rising temperatures may have favoured the fitness of the parasites over that of their host. These findings suggest that such indirect effects of temperature may be a bigger threat to fishes than temperature change itself. To my knowledge, this trade-off in association with thermal tolerance has not been documented experimentally or observed in the wild for this species.

The results from this study suggest that, in response to climate change, ninespine stickleback may experience stronger selection for thermal tolerance plasticity to accommodate for more frequent temperature fluctuations and extremes, shifts in breeding time or location, and stronger selection for anti-predator traits and an enhanced immune response to infection.

4.2 Next steps

Environmental change is happening at a rapid pace, requiring species to respond if they are to persist into the future. To enhance our ability to predict the consequences of these changes for aquatic biodiversity, the direction of future research should include the integration of genetic and ecological approaches. Given that species persistence may rely on their ability to adapt, understanding the genetic, evolutionary capacity of populations will help us make better predictions about their future. For instance, founder effects and population bottlenecks that reduce the genetic variability within a population may naturally negatively affect their ability to respond to novel conditions. Alternatively, populations with an evolutionary history of similar selective pressures may have the genetic architecture in place ready to respond rapidly to the selection pressures associated with climate change (O'Donnell et al. 2014). Furthermore, standing genetic variation can facilitate rapid evolution in response to novel conditions (Barrik and Lenski 2013) and will likely be the primary driver for rapid evolution in response to climate change (Barrett and Schluter 2007). However, given that this change will be measured in generations, for some long-lived species this will present further challenge. Understanding the evolutionary history and extent of standing genetic variation in a population may thus help us predict how populations will respond to change in the future.

Phenotypic plasticity is also a means for organisms to persist under rapidly changing conditions and may become increasingly important to characterize. Since plasticity can buffer populations from the negative effects of climate change allowing time for genetic response to occur (Munday et al. 2013), having estimates of plasticity in

a population may better enable us to predict how species will respond to climate change in the future (Donelson et al. 2018). Indeed, without incorporating the role of phenotypic plasticity, estimates of population susceptibility to environmental change are likely underestimated (Sandblom et al. 2016). Future studies should utilize a cross-generational, split-brood design to disentangle the individual contribution of genes and phenotypic plasticity and to characterize their potential in response to environmental change (Donelson et al. 2018), which was ultimately one short-coming of my study. Further, as discussed in Chapter 3, investigating the role of thermal plasticity over multiple time scales and at the genomics level (Morris et al. 2014, Metzger and Schulte 2017) would better enable us to characterize the overall role of plasticity in response to changing thermal regimes.

Another important next step to consider in future research is the role of biotic interactions on a species' response to environmental change. Such an endeavor is a daunting task given the vast number of species that may co-occur in any given habitat and amounting to scores of potential direct and indirect biotic interactions. Studies have characterized how species interactions have been altered by climate change - such as changes in community assemblages and dynamics (Buisson and Grenouillet 2009, Lancaster et al. 2016), and shifts in trophic interactions (Eddy and Handy 2012, Ohlberger 2013) - but few have characterized how those interactions themselves will affect a population's response to environmental change (HilleRisLambers et al. 2013). It would likely be futile to attempt to characterize the direct effect of temperature on all of these interactions because not all of them will influence how a species will respond to climate change (HilleRisLambers et al. 2013) but it may be necessary for interactions

that directly affect an individual's fitness and survival. For example, the ninespine stickleback has been associated with up to 33 species of parasites, some of which inhibit reproduction in adults (Coad and Reist 2018). As rising temperatures favor the fitness (Franke et al. 2017, Marcogliese 2008) and transmission of these parasites (Marcogliese 2008), there may be serious consequences of this interaction for the ninespine stickleback, a sample of which was demonstrated in Chapter 3.

4.3 Conservation and management

Understanding how fishes will respond both physiologically and ecologically will better enable us to predict and anticipate the response of fishes to certain aspects of climate change, allowing management bodies to make better informed conservation decisions. Given that freshwater fishes are more restricted in movement compared to terrestrial species and marine fishes (Gunderson and Stillman 2015), establishing protected areas and networks will be important for maintaining the genetic variability within a population as well as habitat connectivity in freshwater systems. In Canada, only a small portion of protected areas include freshwater bodies (Environment Canada 2015), highlighting the need for a greater commitment to ensuring the longevity of freshwater fishes. Given that northern populations may lack genetic variation (Shikano et al. 2010) and that southern populations may be at immediate risk (Chapter 3, Araujo et al. 2013), assisted migration for populations at the southern edge of a species' range to areas that are more climatically suitable within their historical range (Olden et al. 2011) may be a suitable conservation strategy. Assisted migration has been implemented fairly successfully, albeit somewhat controversially, in the forestry sector

(e.g., AMAT project, Government of BC 2013). Furthermore, selective breeding of fishes with enhanced heat tolerance and immune function may help circumvent some consequences of temperature rise and allow fish to remain within their natural range.

Species distribution models, also sometimes referred to as ecological niche models, are used to predict how species will be distributed based on environmental parameters within their current range. These models can be used in conservation decision making to predict the effects of climate change on species presence and distribution and to select priority areas for conservation (Araujo and Peterson 2012). In correlative models, climatic or environmental data is paired with a known species distribution to make predictions about a species' range in the future under a given scenario of change. Alternatively, mechanistic models incorporate the link between known physiological traits and the environment to predict species distribution. Thermal tolerance traits can be used in mechanistic species distribution models to model current or future habitat suitability within the tolerance limits of the species (Kearney and Porter 2009). To date, models developed to predict the outcomes of climate change have been based on species' response to abiotic, environmental variables (e.g., Sarmiento et al. 2005, Deslauriers et al. 2016) but the response to biotic variables and the potential to respond plastically or through genetic adaptation may be just as important. Both correlative and mechanistic models lack consideration for variables such as species interactions, evolutionary potential, and phenotypic plasticity. As discussed above, biotic interactions effecting species' response to temperature rise, the standing genetic variation and evolutionary history of a population, and the capacity to respond to change

plastically, are important variables that should be incorporated into models used to predict the outcomes of climate change.

4.4 The future of Arctic research

Doing research in Canada's Arctic has become increasingly popular, especially in light of climate change (Bolton et al. 2011). The nature of present-day research is fueled by a bias towards the new and exciting - the 'sexy science' if you will - and the increasing demand on researchers to publish in academic journals. Given that the north is notoriously data deficient (Titley et al. 2017) in terms of Western knowledge, it is an easy target for researchers seeking publishable content.

The Inuit in Nunavut, like other indigenous peoples, have a unique and rich knowledge system that is all too often ignored or seen as sub-par to Western knowledge (Armitage 2005). This colonialist attitude is frequently reflected not only in paternalistic relationships between researchers and Inuit communities (e.g., Armitage 2005, Audla and Smith 2014) but in the governing policies and management systems implemented in the north that are based on Western concepts (e.g., DFO fisheries management and the use of quotas). Since northern research is largely led by 'southerners', there is a need for the increased recognition and integration of traditional ecological knowledge (TEK) in scientific studies. TEK is holistic in nature, encompassing many environmental components and representing years of on-the-ground observations (Berkes et al. 2000). Science could benefit from the integration of this knowledge since it provides an understanding of the complexities of ecosystems

and environmental change (Bolton et al. 2011), which is often lacking in reductionist, scientific studies (the present thesis included).

For example, since Inuit rely on Arctic ecosystems for food, economic opportunity, and as a way of life, they have intimate knowledge of the environment and its biota (Government of Nunavut 2009, Bolton et al. 2011, Nickels and Knotsch 2011). Elders and active hunters and fishers from all across Nunavut interviewed through the Nunavut Coastal Resource Inventory project, conducted by the Government of Nunavut, can specifically identify species presence, abundance and distribution, life history traits, morphology, and behaviour all from their own experiences and oral history. As a nomadic society less than a century ago, Inuit oriented themselves by patterns in the snow formed by prevailing winds or by the position of the sun or stars in the sky. They could even forecast the week's weather just by looking at the clouds. In recent years, elders have noticed major shifts in wind patterns, observed shifts in the position of the sun in relation to landmarks, and noted that the weather is now largely unpredictable. Many elders have identified thinning lake ice, warmer temperatures, and freshwater fish that are becoming increasingly smaller and more parasitized. Communities have expressed concern over these changes and the desire to understand more about them (Government of Nunavut 2009).

I recommend that for science in the north to move forward more effectively, researchers must find ways to better integrate TEK and local perspectives and to reprioritize their research objectives to reflect the observations and concerns of northern communities. Ideally, we must use the strengths of different knowledge systems for the

collective good, allow communities directly affected by climate change to be the ones driving the research agenda, and use our pooled resources more effectively.

4.5 Conclusions

Climate change is predicted to involve a plethora of changes in the aquatic environment; much of which being the result of rising temperatures. My research highlights some predicted consequences of these changes for fishes, including increased susceptibility of different life stages to temperature extremes and infection along with potential consequences for body size and antipredator traits. My thesis represents the first known study to characterize thermal tolerance traits for ninespine stickleback and to do so for different life stages and populations from either end of the species' range.

Ultimately, climate change will not affect species in isolation nor will it act in isolation of other stressors. To better understand and predict how species will respond to climate change will require that we integrate knowledge of thermal physiology (Jensen et al. 2008, Araujo et al. 2013, this thesis), biotic interactions and ecology (Buisson and Grenouillet 2009, Ohlberger 2013, HilleRisLambers et al 2013, Lancaster et al. 2016), genetics (Barret and Schluter 2007, Barrik and Lenski 2013), acclimation capacity (Somero 2010), and traditional ecological knowledge (Berkes et al. 2000).

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APPENDIX A: SAMPLE LOCATIONS

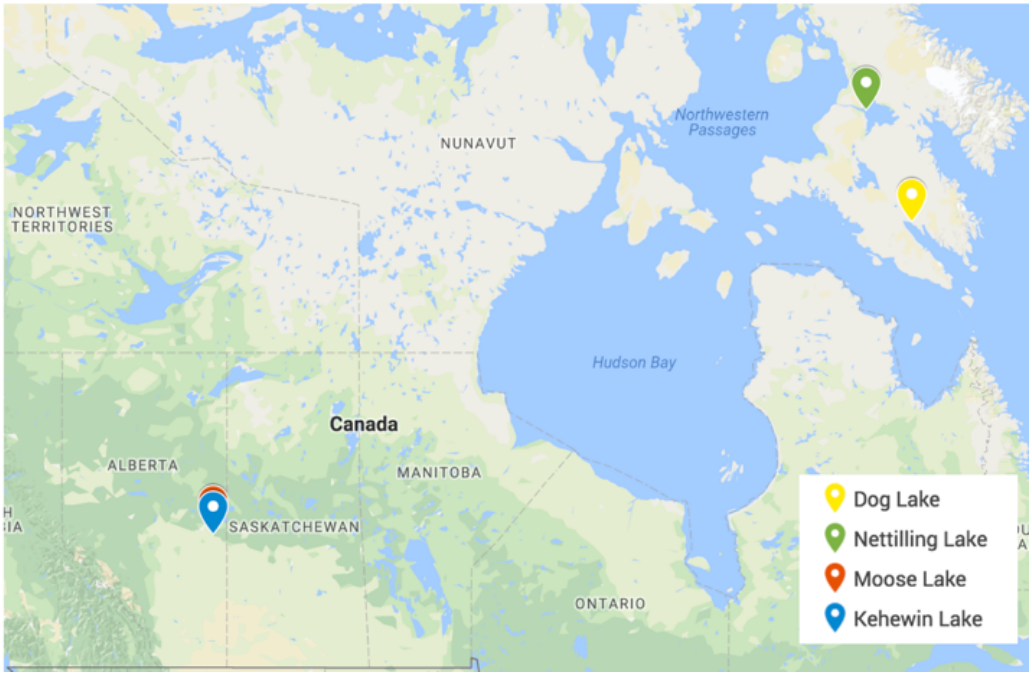

Figure A.1 Locations of the lakes from which I collected samples of ninespine stickleback. The coordinates are as follows: Dog Lake 63.75°N, -68.49°W; Nettilling Lake 66.58°N, -71.38°W; Moose Lake 54.24°N, -110.91°W; Kehewin Lake: 54.05°N, -111.0°W

APPENDIX B: THERMAL TOLERANCE TRIAL EXPERIMENTAL SET-UP

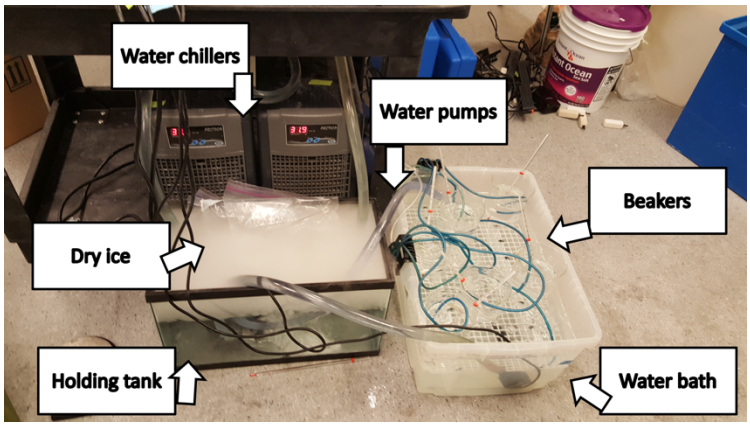

Figure B.1 Experimental set-up for cold tolerance trials

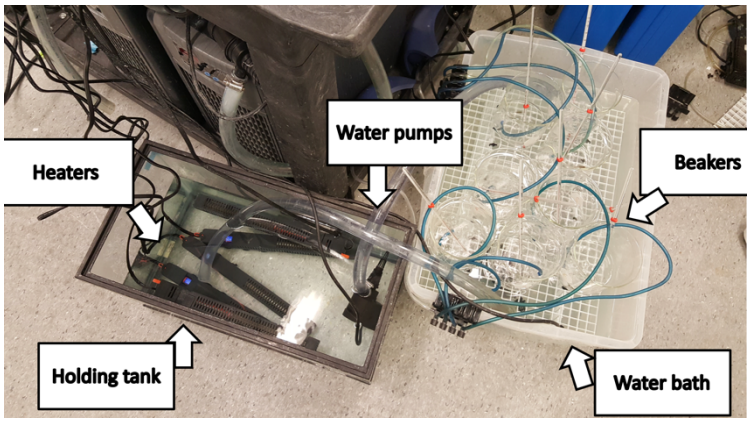

Figure B.2 Experimental set-up for heat tolerance trials

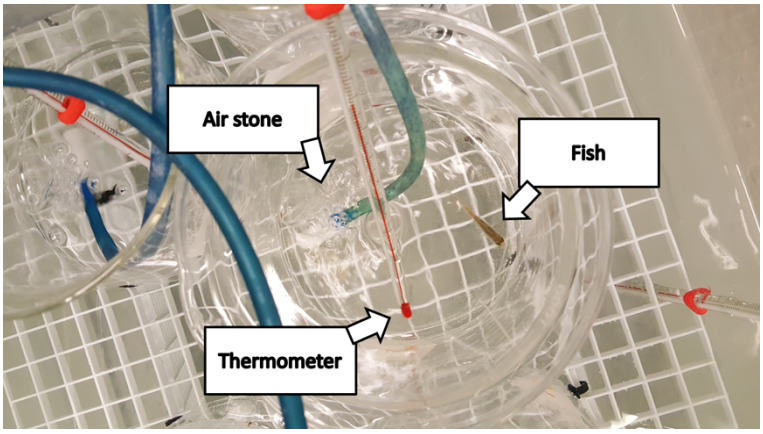

Figure B.3 Individual beakers where fish were observed for their CT limit

APPENDIX C: SUPPLEMENTARY DATA

Table C.1 Collection effort. The number of fishing days required to collect the needed sample size. At least 100 fish were collected from each population with the exception of Kehewin Lake where only 27 fish were collected.

POPULATION	# FISHING DAYS
MOOSE	18
KEHEWIN	18
DOG	4
NETTILLING	3

Table C.2 Final sample size for thermal tolerance trials in Chapter 3. After a number of mortalities throughout the incubation period, the final number of individuals sampled for thermal tolerance are listed here.

POPULATION	TREATMENT (°C)	SAMPLE SIZE
MOOSE	17	11
MOOSE	20	5
MOOSE	23	0
DOG	17	11
DOG	20	11
DOG	23	9
NETTILLING	17	10
NETTILLING	20	11
NETTILLING	23	8
DOG JUVENILE	17	13
DOG JUVENILE	20	13
DOG JUVENILE	23	16
NETTILLING JUVENILE	17	22
NETTILLING JUVENILE	20	16
NETTILLING JUVENILE	23	13

Table C.3 Mortalities incurred during the experimental incubation period in Chapter 3. This table denotes the number of mortalities in each population and treatment in the experiment.

POPULATION	TREATMENT (°C)	# MORTALITIES	% TOTAL
MOOSE	17	1	8.3
MOOSE	20	7	58.3
MOOSE	23	12	100
DOG	17	1	8.3
DOG	20	1	8.3
DOG	23	3	25
NETTILLING	17	2	16.7
NETTILLING	20	1	8.3
NETTILLING	23	4	33.3

Table C.4 Infections found in mortalities. The type of infection found either on the skin or in the abdomen of a mortality that was incurred during the experimental incubation period.

POPULATION	TYPE OF INFECTION	# WITH INFECTION
MOOSE	Glugea anomala	9
MOOSE	Unknown fungus	4
MOOSE	Schistocephalus solidus	1
DOG	Unknown skin lesions	1
NETTILLING	Unknown fungus	4

Table C.5 Summary of lme4 function for CTmin of adult ninespine stickleback.

Linear mixed model fit by REML ['lmerMod'] Formula: CTmin ~ Treatment + (1 | Tank) + (1 | Population) Data: CTmin REML criterion at convergence: 177.9
Scaled residuals: Min 1Q Median 3Q Max -2.11789 -0.61059 0.07046 0.59910 2.20026
Random effects: Groups Name Variance Std.Dev. tank (Intercept) 0.4880 0.6986 Population (Intercept) 0.3945 0.6281 Residual 0.4792 0.6923 Number of obs: 70, groups: tank, 12; Population, 3
Fixed effects: Estimate Std. Error t value (Intercept) -10.17821 1.55026 -6.565 treatment 0.64390 0.07468 8.623

Table C.6 Summary of lme4 function for CTmax of adult ninespine stickleback.

Linear mixed model fit by REML ['lmerMod'] Formula: CTmax ~ Treatment + (1 | Tank) + (1 | Population) Data: CTmax REML criterion at convergence: 181.6
Scaled residuals: Min 1Q Median 3Q Max -2.72476 -0.39991 0.08527 0.64793 1.55348
Random effects: Groups Name Variance Std.Dev. tank (Intercept) 0.2083 0.4563 Population (Intercept) 0.1348 0.3672 Residual 0.4878 0.6984 Number of obs: 75, groups: tank, 12; Population, 3
Fixed effects: Estimate Std. Error t value (Intercept) 27.63909 1.32906 20.796 treatment 0.15100 0.06596 2.289