Biodiversity in Arctic lake trout *Salvelinus namaycush*: assessment of factors influencing and

maintaining within species diversity

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Abstract

Biodiversity within species is influenced by both adaptation and acclimatization in order to exploit a range of environments. Taxa within the genus *Salvelinus* are considered some of the most diverse vertebrate species on earth particularly Arctic char, *Salvelinus alpinus,* and lake trout, *Salvelinus namaycush*, due to various morphotypes, ecotypes, and life history strategies documented. The goal of this thesis was to describe factors influencing the formation and maintenance of biodiversity within species, using lake trout within the brackish waters of Husky Lakes, NT. To accomplish the goal I 1) determined life history types present within the Husky Lakes drainage basin (HLDB); 2) assessed how differences in rearing environment influenced physiology; 3) assessed differences in growth rates and longevity among life history types; and 4) assessed genetic structure among life history types and sampling locations. My data indicate that three life history types are present within the HLDB, freshwater resident, semi-anadromous, and brackish-water resident, suggesting two discrete early rearing environments are used (fresh and brackish water). Assessment of rearing in fresh (0 psu) or brackish water (5 psu) indicates that lake trout reared in brackish water out performed those raised in fresh water when transferred to 20 psu salt water. Additionally, brackish-water residents grew faster and lived longer than did semi-anadromous and freshwater resident lake trout in the HLDB. Also, brackish-water residents were genetically differentiated from sympatric semi-anadromous life history types suggesting segregation in spawning habitat. These findings are the first documentation of a brackish-water resident life history type within lake trout and one of only a few within salmonids. This novel life history type appears to be influenced by both phenotypic plasticity and local adaptation to brackish-water environments allowing for faster growth rates, increased longevity, and a larger abundance in Husky Lakes. Within this thesis I expanded the

spectrum of known life history diversity within lake trout and *Salvelinus*, demonstrated that lake trout are more saline tolerant that originally documented, identified mechanisms that aid in forming and maintaining biodiversity, and contributed to the belief that lake trout are one of the most diverse vertebrates on earth.

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Chapter 1 - Introduction

"The lake trout stockings carried out in the bays and inlets and off the coast of the Gulf of

Finland have given such poor results that the species should be considered unsuitable for Finnish

marine conditions."

- p. 388 Mutenia et al. 1984, *Results of Lake Trout stocking in Finland 1957-81*

Biodiversity is "a state or attribute of a site or area and specifically refers to the variety

within and among living organisms, assemblages of living organisms, biotic communities, and

biotic processes, whether naturally occurring or modified by humans" (p. 745, DeLong 1996).

Within this definition multiple levels are observed ranging from the variation among ecosystems

to variation within species (DeLong 1996). While scientists have been fascinated by biodiversity

for centuries, the importance of classification, quantification, and preservation of biodiversity is

highly debated (Tilman and Downing 1994, Tilman 1996, Reist et al. 2013). Increased

biodiversity within species and ecosystems has been documented to reduce annual variability in

productivity, thereby increasing resistance to perturbation (Tilman et al. 1998). Assessments of

grassland plant species richness and biomass suggest that regions with greater species richness

are less prone and more resistant to drought (Tilman and Downing 1994). Similarly, high

biodiversity within Arctic char *Salvelinus alpinus* has allowed for circumpolar colonization in

regions of low and variable productivity (Klemetsen 2013). While biodiversity can increase

species resilience, extreme impacts can result in the irreversible loss of biodiversity causing both

ecosystem and economic damage (e.g., loss of Great Lakes lake trout Muir et al. 2013). Thus,

documentation and maintenance of biodiversity at multiple levels is important for species and

ecosystems (Tilman and Downing 1994, Reist et al. 2013).

Biodiversity is shaped by both biotic and abiotic factors (Jackson et al. 2001). In part, differences in the abiotic environment (e.g., temperature and salinity) influence community composition, as physiological differences and genetic precursors within and among species influence species distribution and subsequent interactions (Jackson et al. 2001). Additionally, interactions (e.g., niche partitioning) between and within species can influence morphology (Chavarie et al. 2013), life history (Swanson et al. 2010), growth rates, and longevity (Loewen et al. 2010). While biodiversity is shaped by the biotic and abiotic environment it is ultimately the product of acclimatization (phenotypic plasticity) and/or adaptation to local environments (Dodson et al. 2013).

The foundational aspect of biodiversity is speciation, the formation of distinct species via evolutionary processes (Bird et al. 2012). Speciation can occur through multiple modes but is ultimately the result of decreased gene flow among populations within a species, eventually resulting in reproductive incompatibility of new species or sterility in hybrid offspring (Nosil et al. 2009). While the creation of new species is the end point of speciation, the process is not instantaneous and much of the biodiversity observed within species represents different steps along this transition (Nosil et al. 2009). Within the process of speciation, a spectrum of stages is observed starting at: 1) a state of panmixia; 2) panmixia influenced by some disruptive factor; 3) creation of partial reproductive isolation; and 4) complete reproductive isolation (Bird et al. 2012). While this is described in a linear fashion, changes in interactions among populations (i.e., changes in gene flow) can reverse, slow, or stop the process of speciation. Gene flow can be influenced by changes in the abiotic (i.e., removal of a dam or changes in water levels) and/or biotic environments (i.e., changes in food web dynamics , Baillie et al. 2016), thus the

contemporary biodiversity within species represents some point along this spectrum that may or may not result in a new species (Nosil et al. 2009).

One genus noted for its impressive sympatric and allopatric within species biodiversity is *Salvelinus* as some members, Arctic char and lake trout *Salvelinus namaycush* (Klemetsen 2013, Muir et al. 2015), have been considered as the most diverse vertebrate species on earth (Muir et al. 2015). Within these species, multiple morphotypes, ecotypes, and life history types are observed within and among ecosystems, which are the products of physiological plasticity and/or genetic differences influencing growth rates, longevity, and ultimately fitness (Klemetsen 2013, Muir et al. 2015). Due to resource differentials in ecosystems used, adaptive traits have evolved to aid in exploitation of resources (i.e., body morphology and fat content) and may indicate the initial stages of speciation (Baillie et al. 2016).

Within Arctic char, sympatric and allopatric biodiversity is observed (Reist et al. 2013). For example, multiple life history types have been documented in Arctic char in coastal regions, specifically freshwater resident and anadromous individuals, but the presence of each life history type is dictated in part by the environment (Finstad and Hein 2012). Due to differences in biotic and abiotic factors, the presence and maintenance of biodiversity, specifically anadromy, differs across species distributions (Finstad and Hein 2012). Finstad and Hein (2012) showed that the presence of anadromy increased in Arctic char at more northern latitudes and was influenced primarily by productivity gradients. While allopatric differences in biodiversity are observed, Arctic char populations possess impressive sympatric biodiversity, most notably partial anadromy.

Partial anadromy is a form of partial migration where both resident (non-migratory) and anadromous (migratory) life history types exist in sympatry (Chapman et al. 2012a). Anadromy

is defined as a fish that spawns in fresh water but migrates to the sea as a juvenile and adult, whereas resident fish complete entire life cycles in a single environment, typically fresh water (Chapman et al. 2012a). The presence and maintenance of both life history types within one population results from costs and benefits associated with each life history type, ultimately impacting individual fitness (Chapman et al. 2012a). While anadromous fish typically benefit from migrations to more productive marine environments through faster growth rates, many costs are incurred including: increased predation risk, energy expenditure associated with migration distance and rigour, and physiological changes required to acclimatize to saltwater (Chapman et al. 2012a). It is then understandable that the retention of multiple life history types in harsh climates such as the Arctic would benefit population viability.

Though morphological differences between life histories can be dramatic, genetic differentiation of neutral markers is often subtle or non-existent, due to interbreeding between life history types (Moore et al. 2014, Harris et al. 2015a). The ability of similar genotypes to produce more than one life history strategy is often considered conditional mating tactics, where an individual's life history is influenced by the individual's status (i.e., size, Gross 1996). Though a lack of genetic differentiation in neutral markers is commonly observed, assessments of genomic differences among resident and anadromous rainbow trout *Oncorhynchus mykiss* suggest multi-genic differences may also influence life history type (Hecht et al. 2013). In combination these studies suggest that biodiversity in partially anadromous populations is influenced by both genetic precursors and some level of phenotypic plasticity (Dodson et al. 2013).

Similarly, allopatric and sympatric biodiversity is observed in lake trout. A notable example of this is polymorphism within and among locations. Across lake trout distribution,

sympatric morphological diversity is common, but differences in the number and type occur among locations (Muir et al. 2015). For example, sympatric polymorphism in lake trout is documented within Great Bear Lake, NT where there are 3 to 4 shallow water morphotypes (Chavarie et al. 2013). The assessment of diet and capture data using stable isotope and stomach content analysis suggest high degrees of habitat overlap, yet differences in morphology and trophic position persist (Chavarie et al. 2016a). Differences in morphology and trophic position subsequently influence growth rates, longevity, and maturation among some morphs, ultimately influencing fitness (Chavarie et al. 2016b). Assessment of neutral genetic markers among sympatric morphotypes suggests differentiation is present among some morphs influencing the biodiversity observed and indicates some amount of reproductive isolation (Harris et al. 2015b). Though genetic differentiation is present among some sympatric morphotypes, a lack of differentiation among others indicates significant phenotypic plasticity and/or subtle genetic differences in key genes may be present similar to differences observed in partial anadromous populations (Harris et al. 2015b).

Due to adaptations and phenotypic plasticity observed, Arctic char and lake trout are widely distributed (Scott and Crossman 1973). In saying this, the biology of Arctic char has allowed for its colonization of the entire Arctic, whereas lake trout are restricted to North America with the exception of recent introductions into Europe (Scott and Crossman 1973). One important adaptation observed in Arctic char that has aided in its larger distribution, is its common use of marine environments.

While many salmonids are known for their physiological plasticities associated with marine environment use, lake trout are commonly considered one of the least saline tolerant species within this family based on laboratory assessment (Hiroi and McCormick 2007) and

distribution (Scott and Crossman 1973). Lake trout, as their common name implies, are typified by a predominantly lacustrine biology though some river use is documented (Scott and Crossman 1973). They most often inhabit cold oligotrophic lakes and as a result are slow growing but long lived and can reach lengths > 1 m (Scott and Crossman 1973). While commonly considered a stenohaline freshwater species, recent assessments of lake trout in the Arctic have revealed that lake trout use brackish-water environments at the mouths of rivers in a semi-anadromous fashion (Swanson et al. 2010). Semi-anadromy is defined as hatching in fresh water with migration(s) to brackish water during some point in life followed by return migration(s) to fresh water to overwinter and/or spawn (Chapman et al. 2012a). Interestingly, while lake trout are documented near numerous coastal regions and are present on islands within the Arctic Ocean (Lindsey 1964), brackish water use appears to be restricted to specific Arctic regions of North America (Swanson et al. 2010; Roux et al. 2014).

One such region where brackish water use has been documented is the Husky Lakes estuary, NT (Roux et al. 2014). Husky Lakes are a series of five interconnected lake basins that drain into the Beaufort Sea. Additionally, numerous freshwater lakes are connected by small streams and as a result of fresh and marine water inputs a salinity gradient is created transitioning from 1 practical salinity units (psu) in the southernmost basin to ~17 psu in the most northerly basin closest to the Beaufort Sea (Carmack and Macdonald 2008; Roux et al. 2014; Roux et al. 2016). Based on water monitoring across numerous years and seasons, it appears that the salinity gradient is relatively stable within each lake basin though annual variability increases near Liverpool Bay (Carmack and Macdonald 2008; Roux et al. 2014). As a result of the stable salinity gradient and based in part upon capture data for lake trout, it was suggested that lake trout reside year round in brackish water (Husky Lakes) for at least part of their life (Roux et al.

2014). However, while lake trout reside in brackish water, it is unknown if they can complete entire life cycles in brackish water (termed a brackish-water resident life history) or if they use this environment in a semi-anadromous fashion similar to that documented by Swanson et al. (2010). The only example to date of an attempt to establish a brackish-water resident lake trout population occurred through stocking of Lake Superior strain lake trout into the Baltic Sea (Mutenia et al. 1984). Poor survival and subsequent recapture were observed and the project was ultimately considered a failure (Mutenia et al. 1984). Additionally, few examples of brackish-water resident life history types exist within Salmonidae and it appears that both local adaptation and the environment shape where this biodiversity occurs (Helle et al. 1964; Scott and Crossman 1973; Himberg and Lehtonen 1995).

There is a high likelihood for diverse forms to exist within lake trout from the Husky Lakes drainage basin because of the biotic and abiotic structure of the system. Within the Husky Lakes drainage basin, there appears to be at minimum two life history types including: a semi-anadromous and/or brackish-water resident life history type within Husky Lakes, with the more common freshwater resident life history type found in connected lakes. Due to biotic and abiotic differences between fresh and brackish water ecosystems, one would predict that these differences could significantly influence physiology, genetic structure, gene expression, growth rates, and subsequent longevities as has been observed in other populations exhibiting multiple life history types (Wood and Foote 1996, Loewen et al. 2010, Chapman et al. 2012b).

Chapter outline

The overall goal of this research was to use the uncommon documentation of brackish water use by lake trout in the Husky Lakes drainage basin as a means of assessing mechanisms associated with the formation and maintenance of biodiversity within species. In Chapter 2 I

describe lake trout life history type(s) present in HLBD using otolith strontium microchemistry profiles. I tested the hypothesis that more than one life history type is present in the HLDB. In Chapter 3 I assessed how differences in rearing environment impact survival and phenotype. Here I fertilized and reared lake trout in discrete environments (0, 5, 10, 15, 20 psu) and transferred them into experimental environments of 0, 5, or 20 psu. Specifically, I tested the hypotheses that, 1) lake trout can survive fertilization and early development in salinities $\geq$ 5 psu; and 2) differences in rearing environment will differentially impact physiology later in life (environment-phenotype interactions). I then built on these observations in Chapter 4 by assessing how differences in life history and environment influenced growth rates and longevities within the HLDB. Here I tested the hypothesis that growth rates and longevities significantly differ among life history types. Lastly in Chapter 5 I compared genetic structure among life history types within and surrounding the Husky Lakes drainage basin. I test the hypotheses that 1) genetic structure is present between Husky Lakes and surrounding locations; 2) genetic structure is present within Husky Lakes among locations and life history types. The synthesis of these findings in Chapter 6 will advance our understanding of the biodiversity present in lake trout within the HLDB as well as the mechanisms that effect diversification processes in species.

Chapter 2 - Brackish-water residency and semi-anadromy in Arctic lake trout (*Salvelinus namaycush*) inferred from otolith microchemistry

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Contributions of Authors: I assisted in the organization of field logistics and collection of nearly all samples within this chapter (exception those collected by L. Harwood 2000-2004 and N. Gantner in 2009). All data presented were collected and analyzed by myself and I wrote and submitted initial and final drafts of the manuscript. All co-authors assisted with revisions of drafts of the manuscript. Assistance with sample collection and logistics was provided by N. Gantner, W. G. Anderson and J. Reist. Laboratory preparation and data collection was assisted by N. Halden and data analysis by D. Gillis.

Abstract

Lake trout, *Salvelinus namaycush*, are considered a freshwater species but have been documented using brackish and marine water environments in the Arctic in a semi-anadromous manner. The objective of this study was to describe lake trout life histories present in the brackish waters of Husky Lakes, Northwest Territories (NT) using otolith Strontium (Sr) profiles obtained by Laser Ablation Inductively-Coupled Plasma Mass Spectrometry (LA-ICP-MS). Lake trout from Husky Lakes and Noell Lake (freshwater), NT were sampled by spring hook-and-line angling and open water gillnetting in 2000-2004, 2009 and 2012 by local subsistence fishers as part of a larger ecological assessment. Harvested fish were sampled for biological data and tissue samples including otoliths. The otoliths were prepared for line scan analysis of Sr from the core region to the outer edge by LA-ICP-MS. Sr profiles were evaluated visually suggesting the presence of two life histories within Husky Lakes, semi-anadromy (14%, n=8) and brackish-water residency (86%, n=50). The visual classification of life histories was supported using generalized linear mixed effects modelling indicating that a minimum of two distinct ecosystems (fresh and brackish water) were used during early life by lake trout from Husky Lakes. Otolith Sr profiles also show that the majority of Husky Lakes lake trout sampled (86%), spawn and spend their entire lives in the brackish waters of Husky Lakes. These are the first data to support an entirely brackish-water resident life history for lake trout.

Introduction

Use of brackish and marine waters is observed in most salmonids with access to marine environments (Hendry et al., 2004). True anadromy within the salmonid family is most often associated with the genera *Oncorhynchus* and *Salmo*, where an individual hatches in fresh water, develops into a smolt, migrates to and resides solely in marine waters for 1-8 years, then returns to freshwater to spawn (Hendry et al. 2004). The degree of anadromy varies within the salmonids, where some (e.g., lake trout *Salvelinus namaycush,* Walbaum, 1792) exhibit semi-anadromy defined as migrations from fresh water to brackish water, > 1 practical salinity unit (psu), principally for feeding in the summer (Swanson et al., 2010). However, due to the presence of sea ice, hyper-salinities, and water temperatures below $0^{o}C$ in preferred marine habitats, Arctic salmonids (e.g., chars and whitefishes) return to fresh water in the autumn to either overwinter or spawn (Spares et al. 2012). These spring and fall migrations are termed seasonal anadromy (Chapman et al. 2012b).

Though the use of marine habitats varies, nearly all salmonids spawn exclusively in fresh water, defined as < 1 psu (Scott and Crossman 1973), however, exceptions do occur. Some European lake whitefish, *Coregonus lavaretus* (Linnaeus, 1758), live entirely within the brackish waters of the Baltic Sea (Himberg and Lehtonen 1995). Pink salmon, *Oncorhynchus gorbuscha* (Walbaum, 1792), and chum salmon, *Oncorhynchus keta* (Walbaum, 1792), are known to spawn in the intertidal zone of Prince William Sound, Alaska (Helle et al. 1964, Scott and Crossman 1973) demonstrating exclusive marine residency. Though nearly all salmonid species with access to marine environments possess life histories that use marine or brackish-water ecosystems at some point in life, many individuals within the same anadromous population also exhibit an entirely freshwater resident life history (Babaluk et al., 1997, Swanson et al., 2010, Skov et al.,

2010, Reist et al., 2013, Chapman et al., 2012a). A resident life history is defined as an

individual that remains within one ecosystem (e.g., fresh water) throughout the entirety of life

and is referred to as non-migratory (Babaluk et al. 1997, Swanson et al. 2010). Populations with

both freshwater resident and anadromous life histories are termed 'partially anadromous' and is

observed throughout the Salmonidae (Chapman et al. 2012b). Individuals within partially

anadromous populations exhibiting anadromous life histories are considered 'optionally

anadromous'; as the ability to complete their entire life cycle in fresh water is present yet some

migrate to and from the sea (Rounsefell 1958).

Lake trout are considered a freshwater species (Scott and Crossman 1973) but have been

documented in marine-influenced environments as juveniles and adults in the Western Arctic of

North America (Harwood et al., 2008; Roux et al., 2014; Swanson et al., 2010). The use of

marine environments by lake trout in the West Kitikmeot region of Nunavut, Canada was

described as semi-anadromy (Swanson et al. 2010). These Arctic populations likely originated

from Beringia and followed a coastal colonization pathway eastward after the last glaciation

(Wilson and Hebert 1998); therefore likely would have experienced saline waters when

establishing island and coastal populations in Northern Canada (Lindsey 1964). Lake trout have

shown the ability to survive short durations (up to 7 days) in saline waters (up to 30 psu) but had

lower survival and a reduced ability to ionoregulate compared to brook trout, *Salvelinus*

fontinalis (Mitchell, 1814), and Atlantic salmon, *Salmo salar* Linnaeus, 1758 (Hiroi and

McCormick, 2007), suggesting that while lake trout have the ability to survive for short durations

in marine waters their adaptive capacity to higher salinities may be limited. This interpretation is

supported with reports of unsuccessful lake trout stocking efforts in the Baltic Sea from Lake

Superior (Mutenia et al., 1984). However, it is important to note that Lake Superior lake trout

likely re-established in Lake Superior from a Mississippian refugium after the last glaciation and are thus very unlikely to have experienced saline environments during recent evolutionary history (Wilson and Hebert, 1998). Thus, lake trout in the southern and central extent of their geographic range may not have the adaptive capacity for life in brackish and marine waters compared to conspecifics in coastal Arctic waters.

Lake trout are distributed throughout North America ranging from the Laurentian Great Lakes into the North American Arctic and are a common freshwater species in most Canadian Lakes (Scott and Crossman 1973). The distribution of lake trout includes Husky Lakes, Northwest Territories (NT) (Roux et al., 2014), which are a series of five interconnected lake basins that drain into the Beaufort Sea (Figure 2.1). These lakes receive both fresh and marine water inputs, creating a salinity gradient transitioning from 1 psu in the southern basin to 17 psu nearing Liverpool Bay and the Beaufort Sea (Carmack, 2008; Grainger and Evans, 1982; Roux et al., 2014). Although lake trout in Husky Lakes have been documented in salinities up to 11 psu (Roux et al., 2014), the duration of residency in these brackish-water environments is unknown.

Inference of fish movement and life history through analysis of otolith microchemistry has become common for fishes inhabiting fresh and marine water environments (Limburg, 1995; Zimmerman, 2005; Swanson et al., 2010; Harris et al., 2012). Concentrations of Strontium ([Sr]) in water are positively correlated with increasing salinity (Secor et al. 1995, Secor and Rooker 2000, Zimmerman 2005, Elsdon et al. 2008) and Sr has been documented to deposit in calcified structures such as the otolith of fish (Walther and Thorrold 2006) proportionally reflecting [Sr] in the ambient water (Zimmerman 2005). Due to this relationship, [Sr] in the otoliths of wild-caught fish are frequently used to determine fresh or marine water residency and movement (Babaluk et al. 1997, Harris et al. 2012) and have been successfully used to differentiate

freshwater resident and semi-anadromous life history types in lake trout populations (Swanson et al. 2010).

Although lake trout have the physiological ability to tolerate saline environments (Hiroi and McCormick 2007; Swanson et al., 2010) and are commonly caught in the brackish waters of Husky Lakes by subsistence fishers (ILA, 2011; Roux et al., 2014), it is unknown if lake trout have the ability to reside in brackish water for an entire life cycle. In the present study we test the hypothesis that more than one life history type is present in the Husky Lakes lake trout population. To achieve this we 1) describe the relationship between salinity and water [Sr] for Husky Lakes and connected freshwater lakes; 2) identify the life history(s) for lake trout sampled from Husky Lakes drainage basin, NT, inferred from otolith Sr profiles; and 3) infer ecosystem use (fresh or brackish water) throughout life by associations of salinity at capture locations, the relationship of water Sr and salinity, and otolith Sr microchemistry profiles.

Materials and Methods

Study site

Sampling occurred within the Husky Lakes drainage basin including Noell Lake, Parson Lake, Sitidgi Lake and Husky Lakes (Figure 2.1). The Husky Lakes drainage basin is located east of the Mackenzie Delta between the communities of Inuvik and Tuktoyaktuk (Figure 2.1). Husky Lakes extend approximately 130 km northeast to Liverpool Bay and the Beaufort Sea (Figure 2.1). The drainage basin is located between 68.0^o and 69.0^oN and 134.0^o and 131.0^o W covering 9,543 km^2 (Roux et al., 2014). Husky Lakes have a surface area of 1,933 km^2, shoreline of 2,269 km (Roux et al., 2014) and are a series of five interconnected lake basins (henceforth basins 1-5) separated by narrow channels (Figure 2.1). Mean depth varies within the five basins between 5.7-22.7m with a maximum depth of 99.7m. Salinity within Husky Lakes ranges

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between 1 and 17psu, increasing in salinity nearing the Beaufort Sea (Grainger and Evans, 1982; Macdonald et al., 1999; Roux et al., 2014). Noell, Parsons and Sitidgi lakes are freshwater lakes located within the Husky Lakes drainage connected by 40 km, 20 km and 5 km long streams respectively (Figure 2.1).

Field sampling

A total of 58 lake trout from Husky Lakes were analyzed from samples collected in 2000-2004 and 2012 in basins 1 (n= 12), basin 2 (n=24) and basin 3 (n=22) and from Noell Lake, NT in 2009 (n=10). Samples were obtained from local subsistence fishers during spring hook-and-line ice fishing fishery (2000-2004 and 2012) and during summer open water gillnet and hook-and-line surveys in 2009 and in 2012 as part of larger ecological assessments (J. Knopp, Trent University, personal communication 2014; Gantner and Gareis, 2013; Roux et al., 2014). The locations of capture for these samples are provided in Figure 2.1. Sagittal otoliths from the dead fish were removed and allowed to air dry at room temperature in sample envelopes. Since a large percentage of the samples used were from fish captured by subsistence hook-and-line angling sample ages and fish lengths are biased toward older and larger individuals. By using otolith microchemistry and otolith annuli we are able to infer life history patterns during early life, providing information on ecosystem residency where data are lacking. Further interpretations of these data were made with this in mind.

Water samples and salinity measures were taken at locations of fish capture and in basins 1-5 of Husky Lakes, Parsons Lake and Sitidgi Lake in 2012 and 2014 to describe the salinity and Sr gradient present, (n=10, Husky Lakes; n=1, Noell Lake; n=1, Parsons Lake; n=1, Sitidgi Lake). Water samples and salinity measures from 2012 and 2014 were taken at 1 m below the ice using a water pump. All water samples were preserved with nitric acid following the sample

preservation methods of Analytical Laboratory Services (ALS) Laboratory Group trace metals

package (Winnipeg, MB, Canada). Water salinity was measured using a hand held YSI multi

reader calibrated to measure salinity ± 0.1 psu (Yellow Springs Instruments, Yellow Springs,

Ohio, USA).

Laboratory analyses

Water samples collected in 2012 and 2014 were analyzed using ALS Laboratories Total

Metals Package for Sr via Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Sample

and data QA/ QC measures followed ALS Quality Control Protocols and included a method

blank, laboratory sample duplicates, laboratory control samples, reference materials and

calibration verification. These water samples were supplemented by data (n=21) collected and

analyzed in Roux et al., (2014) in 2000-2004 within Husky Lakes. Concentrations of Sr from

Roux et al. (2014) and those collected in 2012 and 2014 were compared with salinity measures

taken at corresponding water sampling locations. These data were combined as [Sr] and salinity

measures in each basin did not differ significantly between sampling years and location. Water

Sr data were not used for locations where salinity measurements were absent.

The otoliths were inspected under a dissecting microscope (Leica M125, Concord, ON

Canada) to determine quality. Otoliths that were crystalline or had vaterite inclusions were

excluded from further analyses as the crystalline structure differs influencing trace element

deposition. The dried otolith was then embedded in epoxy (ColdCure, Auburn, Washington,

USA) with the sulcus facing up and allowed to harden. After hardening, otolith section planes

were marked under a dissection microscope to ensure the transverse section passed through the

nucleus, thus exposing all annuli. The otolith was then positioned in the chuck of a low speed

saw (Buehler Isomet, Buehler Ltd., Lake Buff, Illinois, USA) while viewing the transect plane

through a dissecting microscope. Thin sections (approximately 4 mm) were taken and polished,

exposing the nucleus and annuli. The otolith section was then re-embedded in a 25 mm diameter

acrylic ring and allowed to harden. Following hardening, the acrylic rings were then prepared

using 30 µm and 3 µm polishing paper, which evened the disk surfaces. The rings were then

polished at 400 rpm on a soft polishing cloth using a Buehler polishing machine with 0.05 µm

diamond slurry. The disks were rinsed and photographed to provide a pre-ablation reference.

Finally, each disc was ultrasonically cleaned in deionized water, dried and stored in sterilized

sample bags wrapped in a Kimtech® wipes (Kimberly-Clark, Irving, Texas, USA) prior to

measurement.

Due to the slow growth of lake trout and small otolith size, the larger dorsal lobe of each

otolith was analyzed to maximize sensitivity and resolution of the annual patterns of Sr for each

lake trout. Following the protocol described in Swanson et al., (2010), the otolith was ablated

with a 12 µm diameter spot size at 2 $\mu m.s^{-1}$ speed, at a repetition rate of 20 Hz using a Laser

Ablation-Inductively Coupled Mass Spectrometer (LA-ICP-MS) (LUV 213 laser and Thermo

Finnigan Element 2 ICP-MS). Continuous transects were ablated across each otolith from the

outside edge of the core on the ventral side to the edge of the outer dorsal lobe (Figure 2.2). The

ablation path was selected to perpendicularly cross annuli providing annual patterns in [Sr].

Calcium (Ca) was used as an internal standard and background-subtracted counts of Sr were

adjusted to Ca and related to a glass standard reference, National Institute of Standards and

Testing 610 (NIST 610). Scans of NIST 610 were performed after every hour during LA-ICP-

MS analyses to account for changes in background levels. Using Igor Pro software by Iolite

(Paton et al. 2011), intensities of Sr, counts per second (cps), were converted to concentrations,

parts per million (ppm) by correcting for the background, adjusting for an internal Ca standard, and using NIST 610 as an external reference standard.

Following the ablation, age estimations were made by two independent readers along both the dorsal and ventral lobes of the otolith analyzed under a dissecting microscope using reflected light. Annuli were identified as dark bands representing decreased winter growth (Casselman and Gunn, 1992). Age estimations that could not be agreed upon by the readers were removed from further analysis.

Data analysis

The relationship between water [Sr] and salinity (psu) was assessed using linear regression modeling, with significance $\alpha = 0.05$. Otolith Sr profiles were visually compared to determine life history type. Individuals captured in Husky Lakes that had profiles that were initially flat, followed by a large increase (> 1000 ppm) in [Sr] were considered semi-anadromous (Figure 2.2a). For lake trout captured in Husky Lakes that maintained [Sr] profiles > 1000 ppm throughout life were classified as brackish-water residents (Figure 2.2b). Flat profiles with no large increases in [Sr] (<1000 ppm) were considered to be resident fish in Noell Lake, indicating no significant movements to higher salinities occurred throughout life (Figure 2.2c). These classifications are consistent with previous otolith microchemistry analyses conducted on lake trout (Swanson et al. 2010) and Arctic char, *Salvelinus alpinus* (Linnaeus, 1758) (Babaluk et al. 1997, Swanson et al. 2010). Age-at-first-migration was determined by overlaying Sr profiles onto the ablated otolith photographs and using the otolith annuli to estimate age.

Following visual life history identification, mean [Sr] were calculated for each classification. Lake trout were qualitatively classified (resident or semi-anadromous) based on the methods described prior and separated by the location of capture (Husky Lakes or Noell

Lake). Since semi-anadromous individuals appeared to use two distinct ecosystems [Sr] were calculated for both pre- and post-migration phases of life history. Pre-migratory (low [Sr]) and post-migratory (high [Sr]) time periods were calculated by averaging the Sr measures prior to or following the transition point and analyzed as separate classifications. A transition point was identified as a steep increase in Sr from low concentrations (<1000ppm) to measures >1000ppm (Figure 2.2a).

To test the hypothesis that mean [Sr] differed between life history classifications a linear mixed model was used (Zuur et al., 2009). Mixed effects model allows for unequal sample size among life history classifications, and for quantification of variance attributed to random effects. To correct for normality and heteroscedasticity in the residuals the mean [Sr] was $\log_{10}$ transformed. Individual fish were treated as random intercepts, so that the main effects of life history could be modelled (equation 1; Zuur et al., 2009).

$$y_{ij} = \beta_o + \beta_j \times life\ history + a_i + \varepsilon_{ij} \qquad (1)$$

$$a_i = N(0, \sigma_a^2)$$

Where y_{ij} is $\log_{10}$ mean [Sr] for lake trout i of j life history classification. Treatment contrasts were employed, where β_o refers to the reference life history category (brackish water resident, semi-anadromous pre-migration, semi-anadromous post-migration and brackish water resident) and β_j parameters are deviations from the reference value plus the random intercept a_i and an error term ε_{ij}. The random intercept a_i is assumed to be normally distributed with mean 0 and variance σ_a^2. Following modeling, a conditional F-test with Tukey post-hoc test was used to determine if mean [Sr] differed among life history classifications. Significance was set at $\alpha=0.05$.

Results

Salinity and water Sr relationship

Linear regression modeling of water [Sr] and salinity from water samples identified a significant positive relationship p <0.001, R^2= 0.96 (Figure 2.3). All freshwater lakes (Noell, Sitidgi and Parsons lakes) were below 0.05 ppm Sr. The lowest observed [Sr] in Husky Lakes was 0.62 ppm with a maximum of 4.55ppm in basin 5. The highest [Sr] lake trout were captured in was 2.09ppm (basin 3).

Otolith microchemistry

Two distinct life history types were apparent in lake trout sampled from Husky Lakes (Figure 2.2). A total of eight lake trout appeared to be semi-anadromous (Figure 2.2a) and 50 were brackish-water residents (Figure 2.2b). All individuals within Noell Lake (n=10) had freshwater resident profiles (Figure 2.2c). Of the fish classified as semi-anadromous four were captured in basin 2 and four in basin 1. The highest percentage of semi-anadromous fish from our sample were observed in basin 1 (basin 1 =33.3%, n=12; basin 2 =16.6%, n=24; and basin 3=0%, n=22).

The [Sr] of lake trout differed among life history classifications (F test: $F_{3, 73}$ = 618.5, p<0.001). Tukey post-hoc analysis indicated that mean [Sr] significantly differed between all life history classifications (p < 0.001) except when comparing Noell Lake (fresh water) and the pre-migratory phase of semi-anadromous lake trout (p= 0.747; Table 2.1 and 2.2). The lowest observed mean [Sr] was in Noell Lake (462.5 ppm, SE ±10.3 ppm; Table 2.2) and pre-anadromous phase (435.3 ppm, SE ± 9.53 ppm; Table 2.2) and the highest was observed in Husky Lakes residents (2063.1 ppm, SE ± 35.4 ppm; Table 2.2). Semi-anadromous post-migration [Sr] (1553.3 ppm, SE ± 121.0 ppm; Table 2.2) were between those of Noell Lake and Husky Lakes residents.

Age analysis

The earliest inferred migration among the semi-anadromous lake trout from our sample was age eight, and the latest was age 14. Semi- anadromous lake trout had a mean age of 16 years (SE ± 2.0, n=8) at capture with a maximum age of 24 years. Of these 8 individuals, 4 were females, 2 were males, one was unknown and one was immature. Brackish-water resident fish had a mean age of 22 years (SE= ±1.35, n=45) at capture with a maximum age of 52 years. Noell Lake fish had a mean age of 16 years and a maximum age of 24 (SE= ±1.9, n=10).

Discussion

Life history classifications

This study of otolith microchemistry in lake trout populations from within the Husky Lakes drainage basin, NT, indicates the presence of three lake trout life histories including; 1) migratory semi-anadromous fish collected in Husky Lakes, 2) resident fish collected in Husky Lakes (brackish water), and 3) freshwater resident fish present in Noell Lake. The observation that lake trout were identified to reside in Husky Lakes for the entirety of life delineates a new life history form observed for North American lake trout and will be termed a brackish-water resident life history hence forth. The identification of a resident brackish-water life history within Husky Lakes basins 1-3 is supported by water chemistry data from multiple years, locations and all seasons indicating that Husky Lakes is constantly above a minimum of 1 psu (Grainger and Evans, 1982; Macdonald et al., 1999; Roux et al., 2014). Based on our data and the literature present, slight annual changes in salinity are observed in but are most prevalent basin 4 and 5 where lake trout were not captured and marine input is greatest, indicating salinity in basins 1, 2, and 3 of Husky Lakes are relatively stable annually (Grainger and Evans, 1982; Macdonald et al., 1999; Roux et al., 2014). Furthermore, water [Sr] from within the Husky Lakes drainage basin has a significant positive relationship with increasing salinity and thus increases in otolith

[Sr] can be interpreted as movement to higher salinity environments. The flat Sr profiles and high [Sr] (>1000 ppm) for brackish water resident lake trout further indicates no significant movement to environments of salinity lower (fresh water) than capture locations. The combination of water chemistry, capture location, and otolith microchemistry suggest that the majority (86%) of lake trout sampled from the brackish waters of Husky Lakes resided in Husky Lakes throughout their lives (brackish-water residents). Findings also support the presence of semi-anadromy in Arctic lake trout populations (Swanson et al. 2010) for 14% of Husky Lakes fish sampled. Significant differences in pre-migration mean [Sr] of semi-anadromous lake trout and mean [Sr] brackish-water resident lake trout captured in Husky Lakes suggests that though these fish were all captured in Husky Lakes two distinct habitats were being used during early life. The data indicate that brackish-water residents spawn and develop in the brackish water of Husky Lakes and semi-anadromous lake trout spawn and develop in surrounding freshwater lakes similar to Noell Lake.

Drivers of a partially anadromous population in Husky Lakes

The debate as to what drives anadromy is most associated with partially anadromous populations, investigating why fish stay in fresh water or migrate to marine environments (Chapman et al. 2012a, Finstad and Hein 2012). Unlike typical partially anadromous populations where residents stay in fresh water while anadromous individuals migrate to marine environments (Chapman et al. 2012b), the Husky Lakes population is different. The data suggest that Husky Lakes' brackish-water resident lake trout remain in brackish water and are joined by semi-anadromous conspecifics migrating from connected freshwater lakes later in life (> 8 years). In partially anadromous populations the choice to reside or to migrate is an adaptive response to temporally (seasonally and/or daily) fluctuations in resource availability or predator

avoidance, theoretically in an attempt to maximize individual fitness. Numerous factors have been linked and suggested as drivers of anadromous migrations and several hypotheses have been developed (Holekamp 1986, Chapman et al. 2012b). Commonly discussed hypotheses include: physiological constraints (McCormick 1994, 2009, Swanson et al. 2010), differences in resource densities (Finstad and Hein, 2012; Reist et al., 2013), predator avoidance (Holekamp 1986, Skov et al. 2011, Chapman et al. 2012a), and sexual differences in energy allocation to reproduction (Holekamp 1986, Jonsson and Jonsson 1993, Chapman et al. 2012b). Though all hypotheses have support, it is difficult to determine if one factor solely drives anadromy as its presence is widespread and the potential influencing factors vary both spatially and temporally (Chapman et al. 2012a). The following discusses potential influences specific to the Husky Lakes partially anadromous population.

Physiological constraints

The presence of brackish-water residents suggests that the physiological capacity exists during early life for survival in brackish water. Hypothetically if brackish-water residents and semi-anadromous lake trout are genetically similar, residency in brackish water from early development indicates that genetic precursors for physiological mechanisms are present to allow survival. This suggests that size thresholds would not limit migration to increased salinities as suggested in other studies (Chapman et al., 2012a; McCormick, 1994; Swanson et al., 2010). Alternatively, environmental effects during early development in brackish-water resident and semi-anadromous life history types may be sufficient to drive subtle differences in phenotype that prepare the fish for life in fresh or brackish water. Papakostas et al., (2012) observed drastically different phenotypic responses related to osmoregulation in European whitefish from fresh and brackish-water populations in the Baltic Sea, supporting the notion that rearing

environment affects later life processes (e.g., osmoregulation, growth and reproduction) and survival.

Though the prior suggests that physiological mechanisms are present for lake trout to reside in brackish water for an entire life cycle, the observation that the earliest a lake trout migrated to brackish water was age 8 suggests that size thresholds may be limiting migration for semi-anadromous lake trout. Further, Swanson et al. (2010) found that nearly all semi-anadromous lake trout sampled, migrated later in life with the earliest migration observed at age 10 or later in Havaktok, Nauyuk, and Roberts Lakes, NU and age 3 in Glenn Lake, NU. Laboratory studies indicate that while the rate of Na^+, K^+-ATPase did not change with size of brook trout, the scaling of osmoregulatory organs (primarily the gills) increased in relation to body size allowing for greater regulation of ions (McCormick and Naiman, 1984). This suggests that body size is likely the largest factor influencing migrations and the ability to osmoregulate in *Salvelinus* spp. (McCormick and Naiman, 1984; McCormick, 1994, 2009; McCormick et al., 1985) and may be influencing migrations in semi-anadromous fish captured in Husky Lakes.

Predator avoidance

Predator avoidance in fishes is often associated with a critical period, representing a time in a fish's life when they are most vulnerable to predation (Chapman et al. 2012a). Common bream *Abramis brama* (Linnaeus 1758) in shallow Danish Lakes had an increased probability to migrate into connected streams when the probability of predation was high (Skov et al. 2011). In this example the probability of predation was based on gape limitations in predatory northern pike, *Esox lucius* (Linnaeus 1758), indicating that once fish exceeded the gape size of the northern pike, predation decreased significantly as did migration of the larger common bream (Skov et al. 2011). In the present study first migration in the semi-anadromous life history type

occurred at a mean age of 10 suggesting that predator avoidance is likely not the primary driver of migrations out of freshwater lakes as most lake trout at age 10 would not be vulnerable to predation due to their large size (approximately 550 mm fork length, Roux et al. 2014). Similarly, a large size at first migration has been observed in other semi-anadromous lake trout populations (approximately 400 mm, Swanson et al., 2010) indicating predation is not a likely influence of anadromy for Arctic lake trout. If predation had a strong influence on migration, similar to the example of common bream (Skov et al., 2011), an early age at first migration would be expected as younger and smaller fish would likely be most vulnerable.

Differences in resource densities

Resources can be thought of as both food (abundance and energy content) and space (foraging and spawning habitats) (Holekamp 1986, Chapman et al. 2012a). With our current data and the available literature it is difficult to determine if space, be it foraging or spawning locations, are limiting in Husky Lakes and freshwater lakes within the drainage basin and will not be discussed further. When considering prey abundance, greater relative fish abundance (catch per unit effort of all species) was observed for Husky Lakes (8.57, SD ± 17.09 fish / h) when compared with Sitidigi Lake (2.06, SD ± 3.27 fish / h) (Roux et al., 2014), supporting the notion that a resource gradient may drive anadromy as seen in other salmonids populations throughout northern North America (Gross 1987). In addition, species diversity is observed to be greater in Husky Lakes providing a larger variety of forage items of differing energy content (Roux et al., 2016; Roux et al., 2014). If resource differentials among habitats drive anadromy, the observation that migration occurs at a mean age of 10 would indicate that lake trout may have increased resource needs associated with reproduction and maturation, as lake trout typically become sexually mature by age 10 (Scott and Crossman 1973, Trippel 1993). In other

semi-anadromous lake trout populations a later average age at first migration was observed (between 10 and 17 years, Swanson et al., 2010) but may be related to differences in growth and age at maturity between populations as these parameters can vary spatially (Scott and Crossman, 1973; Trippel, 1993). The occurrence of migrations near or after the age of maturation and higher resource densities in Husky Lakes would support the potential that resource differentials drive semi-anadromy in the Husky Lakes drainage.

Sex (male or female)

In many partially anadromous salmonid populations, the proportion of anadromy is often highest in females (Jonsson and Jonsson 1993, Chapman et al. 2012b). It is likely a more dominant life history in females because there is a positive relationship between body size and fecundity in female fishes, but not males, hence migrating to highly productive marine environments can significantly increase reproductive capacity (Chapman et al. 2012b). In lake trout sexual dimorphism is less pronounced compared to other salmonids (*Oncorhynchus* and *Salmo)* during spawning but females do produce significantly larger gonads than males (e.g., male 3.1% and female 15.1% of total body weight, Madenjian et al. 2010). Due to our small sample size of mature semi-anadromous lake trout (n = 6) it is not valid to determine the significance of the sex ratio. In other studies of lake trout semi-anadromy, no differences were observed in the sex ratio between males and females but it was suggested that the sample size may have been too low to observe significant biases (Swanson et al. 2010). Though sex bias has been observed in other salmonids (Jonsson and Jonsson 1993, Chapman et al. 2012b), based on current information definitive statements cannot be made regarding the influence of sex on lake trout semi-anadromy.

Implications of a partially anadromous population

If a high degree of natal homing is present in both life history types, which has been observed in some freshwater lake trout populations (Scott and Crossman 1973, Horrall 1981), there is the potential for genetic isolation between freshwater hatched semi-anadromous lake trout and those hatched in the brackish-waters of Husky Lakes as two distinct spawning environments are observed in early life. Genetic differences in fine-scale population structure have been documented in Atlin and Tagish lakes, BC, Canada for lake trout, indicating lakes connected by rivers can contain distinct sub-populations (Northrup et al., 2010). If isolated, the pressures of natural selection could favor adaptations in processes associated with salinity tolerance for Husky Lakes' brackish-water residents, perpetuating physiological differences and a potential advantage over semi-anadromous lake trout born in freshwater.

Further support for physiological differences between life histories is the greater presence of semi-anadromous lake trout in low salinities within Husky Lakes. Although limited in sample size capture locations suggest that semi-anadromous lake trout reside only in basins 1 and 2 indicating that anadromous individuals stay in regions of lower salinity (1-5 psu) when compared to brackish water residents (1-11 psu) and is supported by significantly lower mean [Sr] in post-migration data (Figure 2.4). Rationale for a greater presence of semi-anadromous lake trout in basin 1 and 2 may be that semi-anadromous fish do not have the adaptive capacity to reside in higher salinities. Alternatively, a higher proportion of lake trout residing in basin 1 and 2 may be due to their closer proximity to known freshwater lake trout populations (Noell, Sitidgi, Jimmy and Parsons lakes). Though the potential source populations are closer to basins 1 and 2, migrations to basin 3 are not far (~30 km from basin 1) when compared to migrations of other salmonids. This suggests physiological limitations may play a greater role and that distribution of semi-anadromous lake trout is not even throughout Husky Lakes.

The observation that brackish water lake trout have the ability to reside in brackish waters for an entire life cycle and the potential that migrations in semi-anadromous lake trout may be size limited suggest that underling genetic differences or environmental influences to physiology in early development are present. Potential physiological differences associated with these life history types may then allow brackish-water resident lake trout to have a competitive advantage over freshwater born semi-anadromous lake trout in Husky Lakes. Though not testable due to sample size the data suggest that semi-anadromous lake trout are younger on average than brackish-water residents in Husky Lakes. It has been demonstrated that costs (increased Na^+ K^+-ATPase activity) are associated with movement between salinities and acclimation is not instantaneous (Howland et al. 2001, Hiroi and McCormick 2007). These costs suggest transition between salinities may make fish moving from fresh water more vulnerable to predation or competition, requiring increased energy for osmoregulation hence lower survival.

The standard perspective of lake trout life history diversity indicates freshwater ecosystem use as the norm (Scott and Crossman 1973), though considerable diversity is found within that, for example, resident and semi-anadromous forms; eco-morphotypes within lakes (Zimmerman et al. 2006, Chavarie et al. 2013); riverine and lacustrine forms (Scott and Crossman 1973). Use of marine environments by lake trout populations with access to the sea is thought to be atypical likely due to osmoregulatory limitations (Hiroi and McCormick 2007). In Arctic populations however, seasonal migrations to marine-influenced environments have been documented (Roux et al., 2014; Swanson et al., 2010). Thus, lake trout appear to exhibit a greater diversity than originally thought. Moreover, this study provides additional evidence of diversity, including an entirely brackish-water resident life history, within the gradient of marine water use by lake trout and salmonids. Considering the breadth of diversity in lake trout life histories

(freshwater resident, semi-anadromous, and brackish-water resident) found in coastal Arctic populations, significant questions then arise: 1) what is the origin of this diversity in the overall context of lake trout post-glacial history? 2) at a local scale, how is the diversity maintained? 3) what is the significance of this diversity at various spatial scales? These observations suggest the potential of regional adaptation and diversity that may be linked with refugial origins, for this population potentially Beringian and Nahanni sources (Wilson and Hebert 1998). Questions associated with the importance of maintaining and identifying local diversity become of greater importance as the possible impacts of anthropogenic activity and climate-change increase in Arctic regions. Examples like this underscore the need to research Arctic populations as they expand our understanding of species physiological capacity.

Conclusions

These data are the first to confirm a brackish-water life history is present for lake trout in North America and also supports the earlier observation of a semi-anadromous life history in Arctic lake trout populations. The presence of two distinct spawning and early life environments for semi-anadromous (fresh water) and brackish-water residents (Husky Lakes brackish water) indicate that these two life histories may be genetically linked or isolated. The observation that semi-anadromous lake trout hatch and develop in freshwater and then migrate to Husky Lakes, identifies connections are used between fresh and brackish ecosystems. The drivers of semi-anadromy are likely a combination of multiple factors. Our findings suggest that physiological constraints do not limit the ability of lake trout to complete entire life cycles in brackish water. However, the physiological mechanisms may be genetically distinct to brackish-water residents or associated with environmental influences on phenotypic development. Further, a size threshold may play a role in semi-anadromous migrations of freshwater hatched lake trout from

connected lakes. The inferred larger size of lake trout at first migration suggests that predation in fresh water is likely not influencing semi-anadromous migrations. These findings suggest that increased resource demands due to growth and maturation may be driving semi-anadromous migrations as resource densities are higher in Husky Lakes than connected freshwater lakes (Roux et al., 2014) and first migration typically occurs near or after maturation. Maintenance of the Husky Lakes population is important to the people of this region and an increased understanding of population connectivity and diversity of life histories is essential to sustain the Husky Lakes lake trout fishery in the future.

Acknowledgments

Table 2.1. Parameter estimates from a generalized linear mixed effects model predicting log10 mean [Sr] from fixed factor life history (LH); Noell Lake resident (1), Husky Lakes pre-migratory semi-anadromous (2), Husky Lakes post-anadromous (3), and Husky Lakes residents (4). Variation in the intercepts for individual fish was estimated as a normal distributed random effect with SD= 0.0523.

LH	Est.	SE	df	t	p
1	2.664	0.018	73	150.81	<0.001
2-1	-0.026	0.026	73	-0.989	0.326
3-1	0.518	0.026	73	19.531	<0.001
4-1	0.647	0.019	73	33.497	<0.001

Table 2.2. Tukey post-hoc pairwise comparisons of fixed parameters, life history classifications, estimated by the generalized mixed effects model; Noell Lake (1), Husky Lakes pre-migratory semi-anadromous (2), Husky Lakes post-anadromous (3), and Husky Lakes residents (4). F-test for the overall effect of life history conducted prior to Tukey post-hoc was significant $F = 618.49, p < 0.001$.

Comparison	Est.	SE	Z	p
1 – 2	-0.03	0.03	-0.989	0.747
1 – 3	0.518	0.03	19.53	< 0.001
1 – 4	0.647	0.02	33.5	< 0.001
2 – 3	0.544	0.03	19.47	< 0.001
2 – 4	0.673	0.02	31.7	< 0.001
3 – 4	0.13	0.02	6.102	< 0.001
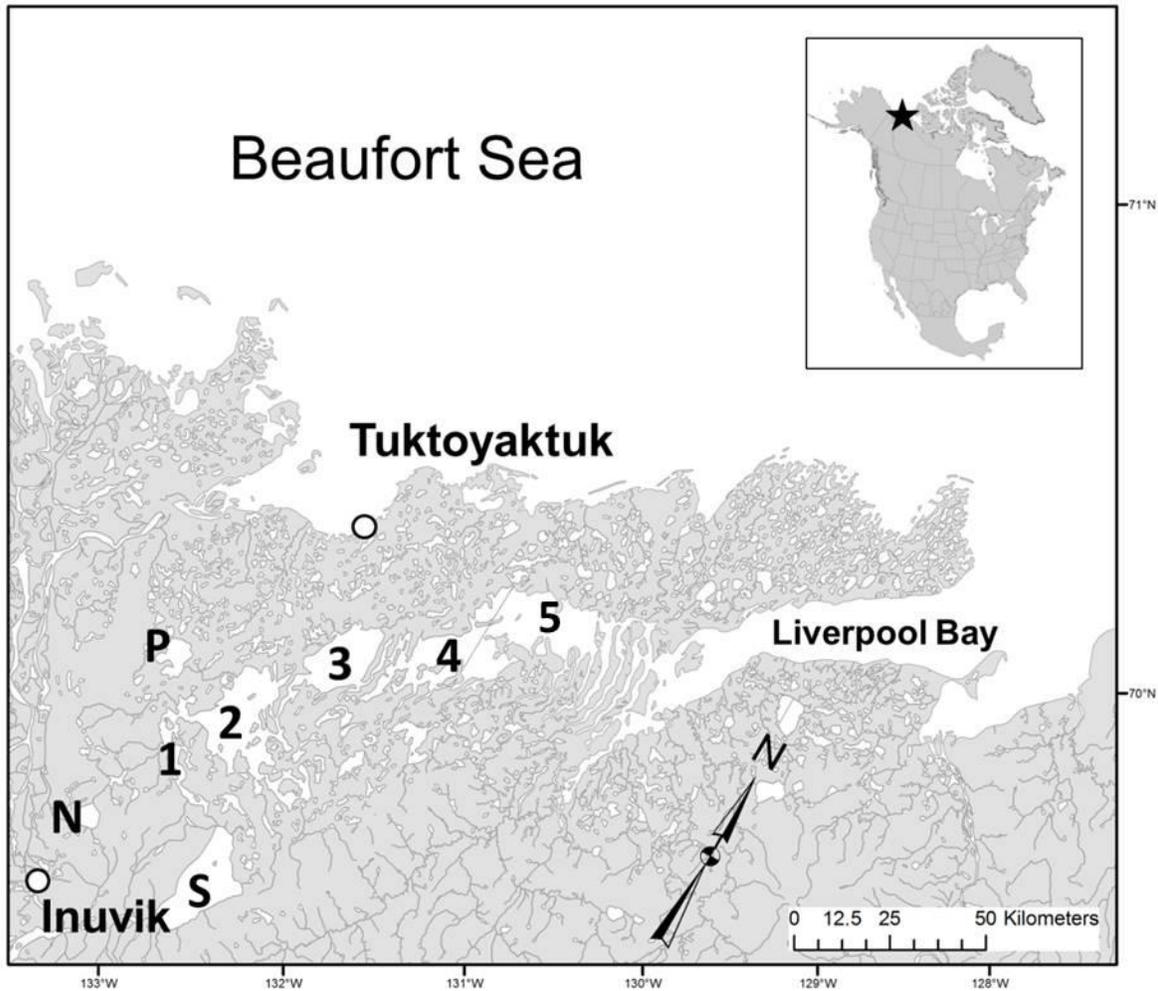
Figure 2.1. Sample locations within the Husky Lakes drainage including Noell (N, 0 = psu),
Sitidgi (S, 0 = psu), Parsons (P, 0 = psu) and Husky Lakes Basin (1, 1 - 3 psu, 2, 3 - 5 psu, 3, 5 -
11 psu, 4, 11 – 15 psu and 5, 13 – 17 psu). Basin in which fish were captured within Husky
Lakes via hook-and-line and gillnetting in 2000 - 2004 and 2012 are 1(n = 12), 2 (n = 24) and 3
(n = 22).
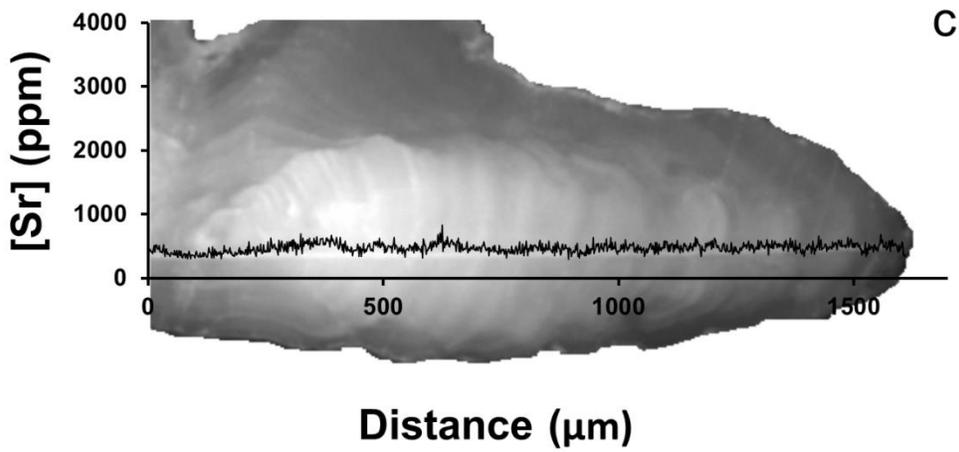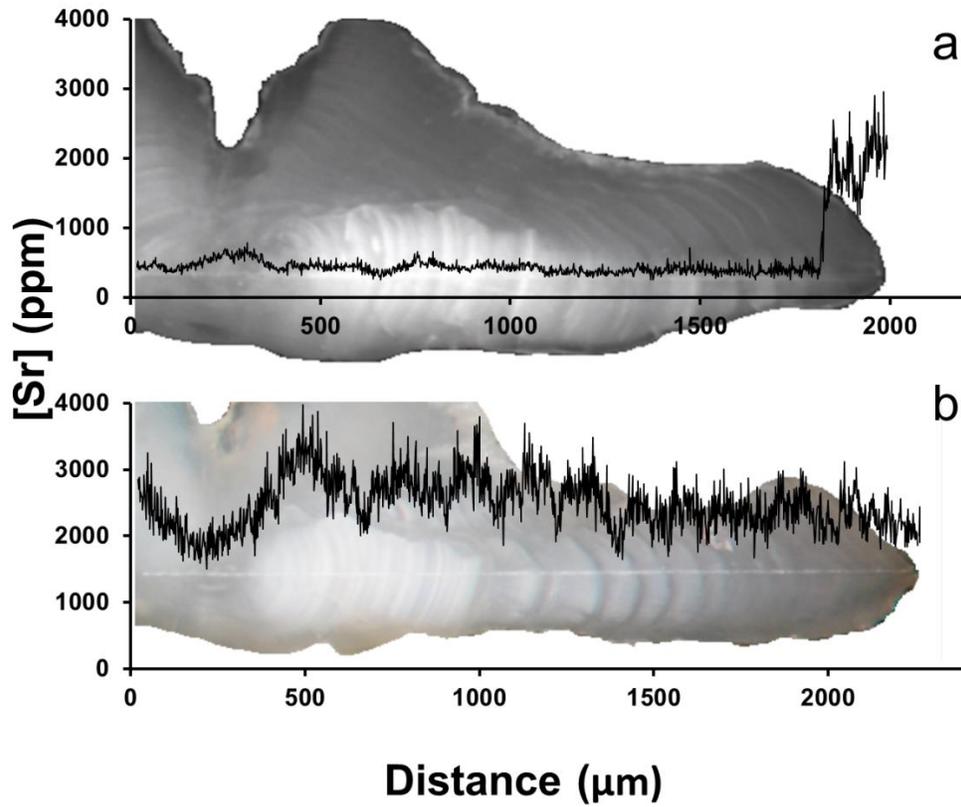
Figure 2.2. Strontium (Sr) line scans for representative lake trout captured in Husky Lakes (a and b) and Noell Lake (c), NT. The life histories present were visually classified as semi-anadromous (a) Husky Lakes (brackish water) resident (b) and Noell Lake (freshwater) resident (c).
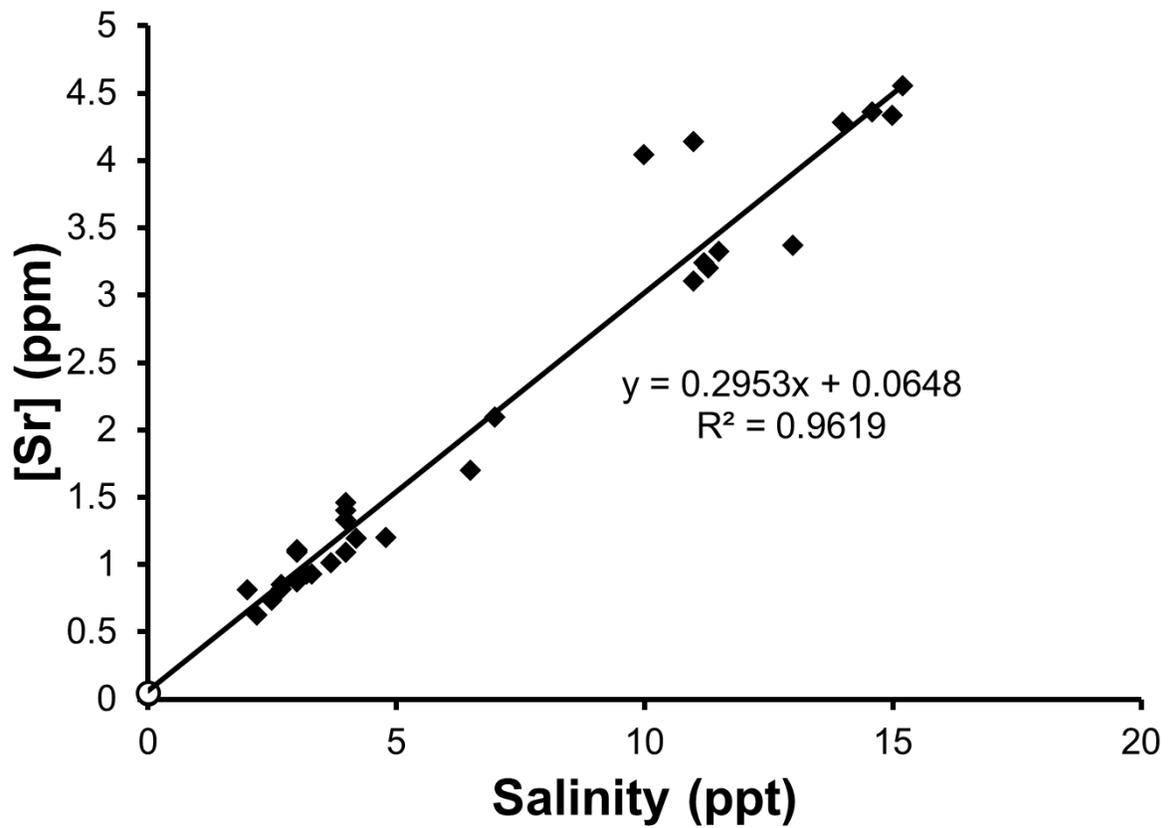
Figure 2.3. Comparison of salinity (psu) and [Sr] (ppm) for water samples collected in Husky

Lakes (♦, n = 31) and freshwater lakes Noell (○, n = 1), Parsons (○, n = 1) and Sitidgi (○, n = 1),

NT 2004, 2012 and 2014.
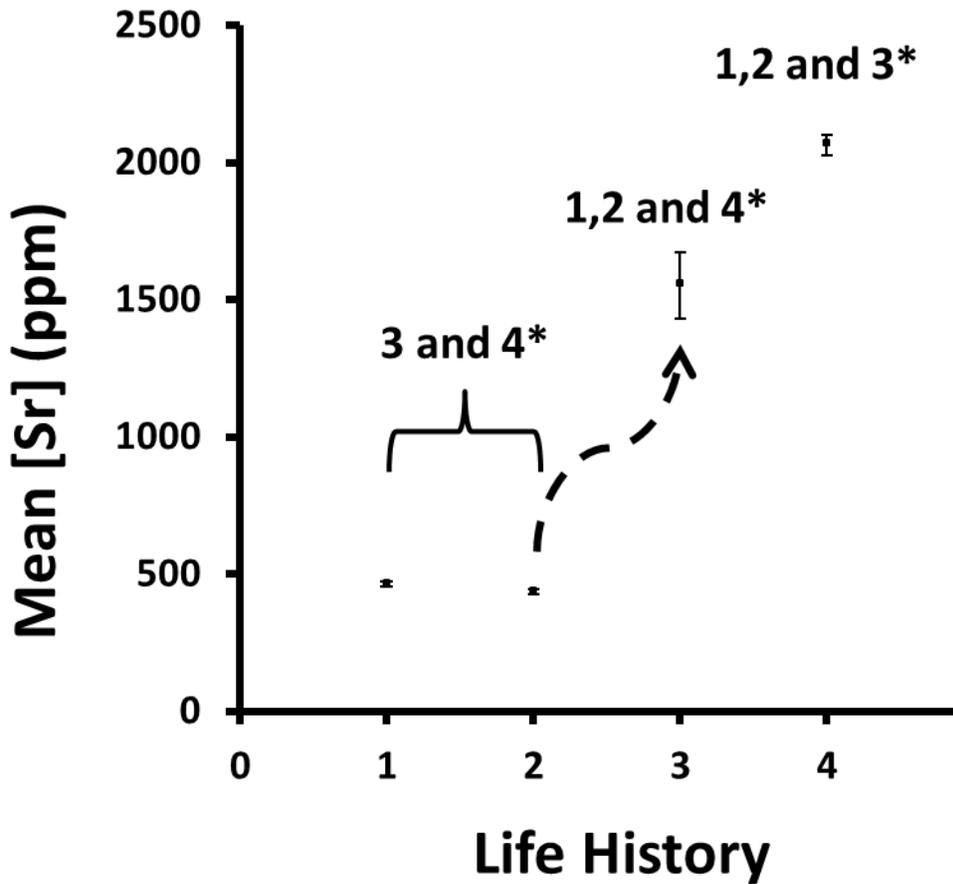
Figure. 2.4. Lake trout otolith strontium concentration [Sr] expressed as a mean parts per million (ppm) ± SE across the entire scan for Noell Lake freshwater resident (1); Husky Lakes pre-migratory semi-anadromous (2); Husky Lakes post-migratory semi- anadromous (3); and Husky Lakes brackish-water resident (4). Significant differences in mean [Sr] between life history types were assessed using a Tukey post-hoc test of pairwise comparisons. * denotes significances ($p <$ 0.001) and the corresponding number (1-4) are the pairwise comparison. The arrow represents the transition from (2) pre-migration mean [Sr] to (3) post-migration mean [Sr] by semi-anadromous lake trout.

Chapter 3 - Environment-phenotype interactions: influences of brackish-water rearing on lake trout (*Salvelinus namaycush*) physiology

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Contributions of Authors: I collected gametes from adult lake trout, fertilized, and reared all fish used in these experiments. I also designed and executed all direct transfer experiments, with the exception of the drinking rate transfer experiment, where I supervised and assisted N. Czehryn. I collected all data from tissue samples with the exception of gill Na^+ K^+-ATPase α1a and α1b gene expression which was conducted by E. Whitmore and overseen by J. Bystriansky. Laboratory equipment was provided by J. Treberg, E. Enders, and W. G. Anderson. I conducted all the data analysis and wrote the initial drafts. Revisions and guidance were provided by all co-authors.

Abstract

Fertilization and development in salmonids occurs almost exclusively within freshwater environments (< 1 ppt). A less common life history strategy in this group of fishes is the brackish-water resident life history, where entire life cycles occur in brackish water (> 1 ppt). In the present study, we tested the hypothesis that differences in rearing environment (fresh or brackish water) results in significant differences in the ability of lake trout to ionoregulate when faced with a salinity challenge later in life. To test this, genetically similar lake trout were fertilized and raised at either 0 or 5 ppt saltwater. At approximately 240 days post hatch, lake trout from both rearing environments were acutely transferred to 20 ppt salt water or their respective rearing environments as a control. Individuals were sampled at time 0, 1, 7, and 14 days post transfer. Fish raised in 5 ppt transferred to 20 ppt saltwater had significantly higher gill Na+ K+-ATPase activity, gill Na+ K+-ATPase α1b expression, and lower plasma osmolality when compared to freshwater reared lake trout transferred to 20 ppt across various time points. Additionally, the 5 ppt control treatment had greater overall aerobic scope than 0 ppt control fish and those transferred from 0 ppt to 20 ppt. These data imply that populations exhibiting a brackish-water resident life history, as has been observed in Arctic Canada, may have an advantage over freshwater reared conspecifics when foraging in marine influenced environments and colonizing new locations in coastal regions.

Introduction

Within the Salmonidae, varying degrees of marine and brackish water use have been observed (Stearns 1995; Hendry et al. 2004). The most common life histories include anadromy (most notably seen in *Oncorhynchus*), where spawning occurs in fresh water followed by migrations to the sea as juveniles (Chapman et al. 2012a); semi-anadromy (common in *Salvelinus* and *Coregonus*), where spawning occurs in fresh water followed by migration(s) from fresh water to brackish water (e.g., estuarine) habitats later in life (Chapman et al. 2012a); brackish-water resident (e.g., European whitefish *Coregonus lavaretus* (Linnaeus 1758); Himberg and Lehtonen 1995; Albert et al. 2004), where complete life cycles occur solely within brackish-water (i.e., estuarine) habitats; and freshwater residency (e.g., grayling *Thymallus thymallus* (Linnaeus 1758)) where complete life cycles occur solely within freshwater habitats (Scott and Crossman 1973). Though categorical terminology is used to define these life history types, variability is observed among and within species resulting in a spectrum of life histories rather than distinct classifications, thus, exemplifying the plasticity within populations and among species in this family (Hendry et al. 2004).

Populations possessing both resident and anadromous or semi-anadromous life histories are defined as partially anadromous (Chapman et al. 2012a). In most occurrences, these life histories interbreed and are not genetically distinct, as spawning frequently occurs in similar locations and at similar times (Hendry et al. 2004). Though often indistinguishable genetically, phenotypic differences are often dramatic (Jonsson and Johnsson 2001). Anadromous and semi-anadromous individuals commonly obtain greater maximum body lengths as a result of faster growth, hypothesized as a product of migrations to more productive marine environments (Chapman et al. 2012b). However, while benefits to growth are evident, many risks and energetic

costs are incurred through the migratory process including the potential of increased predation, physiological challenges associated with ionoregulation, and increased energy expenditure associated with migration distance (Hendry et al. 2004). The analysis of costs and benefits associated with life history selection within partially anadromous populations has been the source of much discussion resulting in an abundance of literature and the generation of numerous hypotheses (Chapman et al. 2012b). Though ubiquitous rationale for partially anadromous populations would be ideal, differences in the biotic and abiotic environments influence local drivers of anadromy within a given population (Finstad and Hein 2012).

The most common definition of a partially anadromous population suggests spawning and early rearing environments are shared by conspecifics (typically freshwater), however, a slightly different observation has been documented in lake trout from Husky Lakes, Northwest Territories (NT), Canada (Kissinger et al. 2016). While use of brackish water by lake trout in a semi-anadromous fashion has been documented in a few additional regions of the Arctic (Swanson et al. 2010), brackish-water residency has only been observed in Husky Lakes for this species (Kissinger et al. 2016). The relatively recent observation of these two life histories within this species brings into question the general consensus that lake trout is a stenohaline freshwater-dwelling species (Scott and Crossman 1973; Crespi and Fulton 2004; Hiroi and McCormick 2007; Alexandrou et al. 2013). Though lake trout are most often observed in freshwater lakes (Scott and Crossman 1973) and are documented to have reduced ionoregulatory capacity relative to other salmonids (Hiroi and McCormick 2007), Arctic populations may well benefit from the use of marine-influenced environments (Swanson et al. 2010; Kissinger et al. 2016). Numerous studies have demonstrated the ability of freshwater raised lake trout and other salmonids to acutely acclimatize to marine environments as juveniles or adults (McCormick and Saunders

1987; McCormick 1996; Hiroi and McCormick 2007), however, few have examined how rearing in different salinities impact physiology later in life.

When transitioning between different salinities, numerous changes occur within fish. Specifically, differences in osmotic and ionic gradients cause the flow of ions and water across passive barriers stimulating a chain of physiological responses (McCormick and Saunders 1987). Some key physiological changes include up and down regulation of genes (i.e., $Na^+ K^+$-ATPase $\alpha1a$ and $\alpha1b$, Bystriansky et al. 2006) and subsequent production of enzymes and proteins necessary for signaling (e.g., cortisol, McCormick 1995) and active ion transport (e.g., $Na^+ K^+$-ATPase, McCormick 1995). Additionally, behavioral responses are observed, for example, increased drinking rates in teleosts as a means to compensate for water loss within the body at salinities greater than isosmotic (Perrott et al. 1992). In combination, these physiological responses have the potential to impact whole body energetics through changes to metabolism, which can ultimately impact survival and fitness (Morgan and Iwama 1991).

Through a series of three acute transfer experiments, I tested the hypothesis that rearing environment significantly impacts the physiological ability of the fish to appropriately acclimate to salinities later in life. I predict that genetically similar lake trout spawned and reared in brackish water for the first year of life are better prepared for life in increased saline environments (20 ppt) than those spawned and reared in fresh water.

Methods

Lake Trout Collection and Husbandry

Eggs and sperm were collected from gravid lake trout captured in Clearwater Lake, MB, Canada (~10 km NE of The Pas, MB) on September 30th, 2013 in collaboration with the Manitoba Fisheries Branch. Adult lake trout were captured using short set gillnets (~1 h) on

known spawning shoals. Following capture, lake trout were transferred to mesh holding pens overnight. The following morning, eggs and sperm were stripped from three females and five males and transported in an ice-cooled cooler from Clearwater Lake to the University of Manitoba. During this process, eggs were kept in 2 l plastic bags supersaturated with oxygen and sperm was kept in 15 ml Falcon tubes. Eggs were fertilized by combining 25 μl of sperm (a mixture of all 5 males) and 2 ml of water representative of each treatment (0, 5, 10, 15, and 20 ppt salinity). Saltwater concentrations used in all experiments were mixed using Marine Salt™ (Seachem Laboratories Inc., Madison, GA, USA) and assessed using an YSI multi-meter ± 0.1 ppt (Yellow Springs Instruments, Yellow Springs, OH, USA). Fertilized eggs were equally distributed throughout the five treatments in an effort to eliminate any potential family effect on the resulting data. Approximately 16 fertilized eggs were placed in each well of a 6 well plate (CELLSTAR® Greiner Bio-One, Monroe, NC, USA) with a total of 1,150 eggs for each 40 l tank. Water movement and aeration in each tank was facilitated by recirculation filters and air stones.

Water changes (50% of tank), removal of dead eggs, and assessment of ammonium and ammonia occurred daily throughout development. Lake trout eggs were kept in the 40 l tanks throughout embryonic development. Water temperatures were adjusted to mirror temperatures at a remote water monitoring station on Clearwater Lake (www.hydro.mb.ca) in an attempt to mimic their natural environment (annual range 12-3.5 °C). The majority (~95%) of lake trout eggs hatched within two days of January 1st, 2014 and no major differences in hatch date were observed among the treatments.

Following hatch, lake trout were monitored for growth and feeding and were transferred into larger tanks to accommodate changes in density and fish size. Commercial trout feed

(EWOS Canada Ltd., Surrey, BC, CA) was supplemented to lake trout fry daily as reductions in the yolk sack were observed. During all stages of development and growth, water salinity was maintained for each treatment.

Though initial fertilization and cell division were observed in all treatments, no lake trout survival to hatch was observed in 10, 15, and 20 ppt water treatments. Interestingly, the effect of salinity on mortality was incremental in that embryonic development in 20 ppt treatment proceeded until 4 days post fertilization (dpf), in 15 ppt treatment until 10 dpf, and in 10 ppt treatment until 17 dpf. Additionally, reduced survival to hatch was observed in the 5 ppt (19%) treatment in comparison to the 0 ppt (37%) treatment. Thus, all subsequent experiments were conducted on fish raised in either 0 ppt (freshwater reared (FWR)) or 5 ppt (brackish-water reared (BWR)). In total three salinity transfer experiments were conducted; one at 240 dph to assess changes in osmolality, muscle water content, and $Na^+ K^+$-ATPase activity and mRNA expression; one at 480 dph to estimate changes in whole body metabolic rate as assessed indirectly through oxygen consumption; and one at 570 dph to analyze drinking rate. The initial experiments at 240 dph were selected to allow fish to obtain an adequate size for sample collection. Differences in experimental time points are based mainly on laboratory constraints and resources to effectively conduct the whole body metabolic and drinking rate trials. It is important to note that as natural temperature cycles were maintained throughout the rearing period, all experiments were completed in what would be the summer months for lake trout eliminating the influence of temperature among experiments and produce comparable results with previous studies.

Osmolality, water content and $Na^+ K^+$-ATPase measurement

Lake trout, 240 dph, were transferred to 20 l holding tanks at 10 °C and allowed to

acclimate to the new tanks for 7 d (3 replicates per experimental treatment). Fish were on

average 84.9 mm ± 0.3 mm SE in length and 5.9 g ± 0.1 g SE in body mass (n=11

fish/experimental treatment). Feeding, 50% water changes, and tank cleaning of excess food and

waste occurred daily. Following the 7 d acclimation, lake trout from each treatment (FWR and

BWR) were then transferred into 0, 5 or 20 ppt water treatments. 20 ppt was used as the highest

salinity in an attempt to eliminate unintended mortalities, as fish mortality over the course of 48

h was observed in FWR lake trout during an initial pilot study following acute transfer to 25 and

30 ppt water. These findings corroborate those observed in a similar direct transfer experiment

(Hiroi and McCormick 2007).

Sampling

At zero, 1, 7, and 14 days post transfer, 11 fish were terminally sampled per treatment

using a lethal dose of MS-222 (250 mg$\cdot$l^{-1}). Day zero samples were only taken from the two

control groups. Lake trout treatments were separated into replicate tanks each holding 11 fish

and at each time point fish were sampled at random from all replicate tanks to eliminate potential

for tank effect. MS-222 was mixed in water representative of each treatment. Fork length (mm)

and body mass (g) were taken for each fish prior to blood sampling and dissection. Following

measurements, blood was immediately sampled from the caudal vein using a heparinized

capillary tube, transferred to a 1.5 ml snap cap vial and centrifuged (15000 X *g* for 5 min).

Plasma was separated from the red cell layer and transferred to a new snap cap vial for

subsequent analysis and stored at -80 °C. All gill tissue was removed, separated (right and left)

and stored in two separate vials for analysis of Na$^+$ K$^+$-ATPase activity and gene expression. In

addition, whole kidney and skeletal muscle tissues were sampled. Skeletal muscle was taken

from above the lateral line starting at the pectoral fin and ending just prior to the adipose fin using muscle from one side of the fish. The muscle tissue was then removed from the skin and weighed to obtain an initial wet mass. All gill and kidney tissues were snap frozen in liquid nitrogen and stored at -80 °C for later analysis.

Plasma osmolality

Plasma osmolality (mmol·kg^{-1}) was measured on a Vapro$^{®}$ osmometer (Wescor Inc., Logan, UT, USA) for each treatment (n=5/treatment). Calibration protocols were followed prior to each run using three standards (100 mmol·kg^{-1}, 290 mmol·kg^{-1}, and 1000 mmol·kg^{-1}) with recalibration conducted following every six samples.

Skeletal Muscle Water Content

Following wet mass measurements, muscle samples (n=11/ treatment, 0.2-0.8 g wet mass) were transferred to an oven and dried at 60 °C until no further mass loss was measurable (48 h). Muscle water content was determined according to the equation:

$$Muscle\ water\% = \left((M_w - M_D) \cdot M_W^{-1}\right) \cdot 100$$

where M_W = wet mass and M_D = dry mass (g).

Na$^+$ K$^+$-ATPase Activity

Frozen gill filaments, from the first gill arch severed just above the septum, and kidney tissue (n=8/treatment) were added to 300 μl of SEID homogenization buffer containing 150 mM Sucrose, 10 mM EDTA 0.1% Na$^+$ deoxycholate and 50 mM Imidazole. The tissue and homogenization buffer were then placed in chilled (~0 °C) holding blocks and homogenized using a TissueLyser II ($^{®}$Qiagen, Valencia, CA, USA) at 15 Hz for 20 s. Following homogenization the samples were kept on ice until analyzed (within an hour). Homogenates were centrifuged at 5000 × g for 1 min at 4 °C and the supernatant was removed and further

diluted with SEID for subsequent assays based on sample activity levels. Enzyme activity of Na^+ K^+-ATPase was determined spectrophotometrically following a modified (Gibbs and Somero 1989; McCormick 1993) assay as described in Cruz et al. (2013). All assays were conducted at 10 °C to match the temperature used in the transfer experiment and each sample was run in duplicate. Na^+ K^+-ATPase activity was determined as the difference in the rate of absorbance change at 340 nm in the absence (total ATPase activity) and the presence of 1 mmol l^{-1} ouabain in parallel cuvettes using a mM extinction coefficient of 6.2 for NADH. As tissue volume varied among samples, enzyme activity was standardized to the protein concentration present in the sample. The protein concentrations of the diluted supernatants were determined using a Pierce^{TM} Biuret assay with bovine serum albumin as a standard (Thermo Fisher Scientific Inc. Waltham, MA, USA).

Relative gill Na^+ K^+-ATPase α-subunit mRNA levels

Relative gill Na^+ K^+-ATPase α-subunit mRNA levels were determined in each experimental treatment (n=9) following similar methods as those used in Bystriansky and Schulte (2011). Total RNA was extracted from gill samples using TriZol reagent (Invitrogen, Carlsbad, CA, USA) following the guanidine thiocyanate method (Chomczynski and Sacchi 1987). Isolated total RNA was quantified spectrophotometrically and electrophoresed (2 µg) on an agarose gel (1%) to check for RNA quality and integrity. First strand cDNA was synthesized from 2 µg of total RNA using a high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA). Quantitative RT-PCR (qRT-PCR) was performed on an ABI Prism 7000 sequence analysis system (Applied Biosystems). PCR reactions contained 1 µl of cDNA, 4 pmol of each primer and Universal SYBR green master mix (Applied Biosystems). Forward and reverse primers used for each gene were isoform specific and tested to ensure that

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they amplified only a single target gene. Primer sequences used were as follows: elongation factor 1α (EF1α) forward 5_ GAG ACC CAT TGA AAA GTT CGA GAAG 3_, EF1αreverse 5_ GCA CCC AGG CAT ACT TGA AAG 3_; $Na^+ K^+$-ATPase α1a forward 5_ GGC CGG CGA GTC CAA T 3_, $Na^+ K^+$-ATPase α1a reverse 5_ GAG CAG CTG TCC AGG ATCCT 3_; $Na^+ K^+$-ATPase α1b forward 5_ CTG CTA CAT CTC AACCAA CAA CAT T 3_, $Na^+ K^+$-ATPase α1b reverse 5_ CAC CATCAC AGT GTT CAT TGG AT 3_. Negative control reactions for qRT-PCR were performed with original total RNA from several representative samples to determine potential genomic DNA contamination. For all genes monitored, genomic contamination was found to be negligible, for example consisting of a maximum of 1:1048 starting copies for the $Na^+ K^+$-ATPase α1a gene. The relative quantity of each gene's mRNA in gill samples was normalized to an endogenous reference (EF1α).

Drinking Rate assessment

FWR and BWR juvenile lake trout (570 dph) were assessed for changes in drinking rate through a transfer experiment into 20 ppt salt water. Lake trout from FWR and BWR treatments were transferred into 20 ppt salt water and sampling (n=8/treatment) occurred at 1 day post transfer and 21 days post transfer, in addition to sampling of controls. Feeding was ceased 1 d prior to sampling and fish were transferred into two 8 l experimental tanks (4 fish in each tank) representative of their treatment. ^{51}Cr labelled EDTA was added to each 8 l tank for a final concentration of 10μ Ci^-l^{-1} and fish were sampled 6 h later.

Drinking rate sampling

Following the 6 h experimental period, each lake trout was terminally sampled using a lethal dose of MS-222 (250 mg$^-l^{-1}$). Measurements of length and body mass were recorded for each fish prior to dissection. The abdominal cavity was opened and a tight ligature was placed

around the esophagus and anus and the gastrointestinal tract was carefully removed. A 1 ml

sample of the ^{51}Cr treated water from the holding tanks was also taken from each experimental

tank to provide background radioactivity levels required for calculating drinking rate (Perrott et

al. 1992). In addition, blood samples were taken to assess plasma osmolality as previously

described and to confirm the absence of radioactivity in the blood of experimental animals.

^{51}Cr Assay

Radioactivity of gastrointestinal tract and water samples was determined using a

PerkinElmer® 2480 Automatic Gamma Counter (PerkinElmer® Inc., Waltham, MA, USA).

Counts were used to calculate drinking rate (ml·kg^{-1}·h^{-1}) following Perrott et al. (1992):

$$\text{Drinking rate} = \frac{C_t}{C_m \cdot T \cdot M}$$

where C_t is total counts present in the gastrointestinal tract; C_m the counts per ml of the bathing

medium; T is the time each fish spent in the bathing medium in hours; and M is body mass of

each fish in kilograms.

Whole body metabolic rate experiment

For all metabolic rate measurements, whole body oxygen consumption by fish was used

as an indirect measure of metabolic rate. Whole body oxygen consumption was assessed for

juvenile lake trout (480 dph; body mass 26.3 ± 1.8 g SE) using an intermittent-flow respirometry

system as described in the AutoResp^{TM} 2.2.0 user manual (Loligo® Systems, Tjele, Denmark).

Lake trout from two treatments, FWR and BWR, were used. During each trial, four lake trout

were placed into individual cylindrical chambers (62 mm x 160 mm, V = 483 ml, Loligo®

Systems) submerged in a temperature (recirculating cooling coil) and oxygen (air stones)

controlled water bath. Temperature was maintained at 10 ± 0.2 °C and monitored using a one

channel temperature regulator system (TMP-Reg, Loligo® Systems). Oxygen within the

chambers was recorded using an OXY-4 mini with fiber optic oxygen probes (PreSens, Regensburg, Germany) connected to DAQ-M data acquisition system (Loligo® Systems). System control measures of oxygen concentrations and calculations of consumption were accomplished using AutoResp^{TM} software (Loligo® Systems).

One day prior to each trial, the entire system was cleaned with a bleach solution to minimize microbial interference of the measurement of oxygen consumption ($\dot{M}O_2$). Following each cleaning and every trial, biological oxygen demand (BOD) was assessed to ensure biological activity was not impacting $\dot{M}O_2$ measurements. Following BOD measures, four lake trout per trial were placed in independent chambers and allowed to acclimate for 24 h. During the 24 h acclimation period, $\dot{M}O_2$ was recorded to ensure the fish were acclimating and that the equipment was working but these data were not used in analyses to follow. Dividers were placed between each chamber to eliminate any visual stimuli from nearby fish and a curtain was placed around the experimental area to ensure researcher's movement surrounding the experiment did not influence $\dot{M}O_2$.

Standard metabolic rate (SMR)

Following the acclimation period, standard metabolic rates (SMR) were determined from fish in each environment (0, 5 or 20 ppt). For this experiment there were four experimental treatments: FWR-0 ppt (FWR-control), BWR-5 ppt (BWR-control), FWR-20 ppt, and BWR-20 ppt. $\dot{M}O_2$ was measured immediately following the transfer of fish from one salinity to another, or the same salinity as a control, (day 1 time point) and 14 d following transfer, during each time point MO_2 was assessed for 24 h. Two time points were selected to assess acute and long term responses of transfer to 20 ppt and to allow for comparisons with the previous two experiments. To facilitate transfer between salinities, water was pumped into the holding tank containing the

fish within the respirometry chambers. Salinity within the water bath was monitored using a

TetraCon® 325 salinity probe (WTW, Weilheim, Germany) until the desired salinity was

achieved and maintained constant for a minimum of 2 min. Each water change lasted for 20 to 25

min. For FWR-control fish and BWR-control fish, the water changes were conducted in a similar

fashion with the exception that the water transferred was the same salinity as the initial

acclimation water. During both, the acclimation period and water change, all movement outside

the experimental area was blocked from the fish. Following the water change, a new

measurement period was started and $\dot{M}O_2$ was assessed over the next 24 h. During both, the

acclimation period and measurement period, the lights were set for 12 h of light and 12 h

darkness. Measures of $\dot{M}O_2$ were programed to control the pumps using the following flush (180

s), mixing (20 s), and measure (120 s) sequence. During this sequence, the flush would bring

new water into the system to replenish the oxygen used for 180 s. Following the flush, the flush

pump was turned off and a 20 s mixing period occurred to ensure that water was properly mixed

in the chamber. Following the 20 s mixing period, a 120 s measurement period would occur

during which $\dot{M}O_2$ was calculated based on the slope created by oxygen being consumed. During

the entire sequence, the recirculation pump maintained a constant flow of water past the oxygen

probe.

Forced Maximum Metabolic Rate (MMR$_f$)

Forced maximum metabolic rate (MMR$_f$) was measured using an exhaustive chase

protocol in flowing water similar to that previously described in Norin and Malte (2011). After

the final 24 h of $\dot{M}O_2$ measurements at 1 and 14 days post transfer, individual fish were placed in

a circular tank with flowing water (representative of their treatment environment 0, 5 or 20 ppt

salinity) at 10 °C, and chased using a small net until exhaustion (duration ~10 min). Fish were

encouraged to burst forward into flowing water through repeated tapping on the side of the tank with the net, which resulted in multiple burst swim bouts, followed by slower swimming to maintain their position, then exhaustion where fish struggled to maintain their position and equilibrium and could be easily netted three consecutive times, during each netting the fish were held briefly out of the water (5 s). Following three consecutive nettings, each fish was quickly placed back into the respirometry chamber for three $\dot{M}O_2$ measurements. The flush, mixing, and closed time intervals were as described for the SMR experiments. Since the chamber was already open and filled with fully oxygenated water the very first flush phase was omitted to ensure the first measure was taken immediately following signs of exhaustion. Following the initial measure, all subsequent cycles included a 180 s flush to ensure low water oxygen concentrations were not reached. Following the first day of SMR and MMR_f experiments, fish were moved to a 20 l holding tank (4 fish/tank) and kept at their experimental environment (0, 5 or 20 ppt) until the experiments were repeated 14 d later. Though the same fish were used, repeated measures of individuals are not reported here, as we did not mark individual fish in an attempt to minimize stress to the fish.

Statistical analysis

All data were assessed for normality and log(10) transformations were conducted to improve normality in some cases, though all figures depict the means $\pm$ SE of non-transformed data. Each dependent variable was assessed using a three-way ANOVA comparing independent variables: rearing environments (FWR or BWR), experimental treatments (0, 5 or 20 ppt), and time points (0, 1, 7, 14 days post transfer). When significant ($p < 0.05$), the interactions were assessed with a Tukey post-hoc test.

Comparisons of drinking rate and osmolality for each treatment (FWR-0 ppt, FWR-20 ppt, BWR-5 ppt or BWR-20 ppt) and time point (1 or 21 days post transfer) were assessed using a two-way ANOVA. When significant differences were found ($p < 0.05$), subsequent Tukey post-hoc test were conducted.

To standardize data collected for each individual, only $\dot{M}O_2$ measurements where $r^2 > 0.50$ for both SMR and MMR$_f$ were used in further analysis. While this threshold may be considered low, the r^2 values for most trials were lower than anticipated, however, the calculated $\dot{M}O_2$ values are comparable to previous studies examining metabolic rate in lake trout (Evans 2007). For each fish, SMR was quantified using the 10th percentile method (Herrmann and Enders 2000; Chabot et al. 2016). MMR$_f$ was determined as the highest $\dot{M}O_2$ value of the three measured (Svendsen et al. 2012). Aerobic scope (AS) was determined as the difference between MMR$_f$ and SMR. AS, SMR, and MMR$_f$ were each individually assessed using a two-way ANOVA to determine significance ($p < 0.05$) among experimental treatments (FWR-0 ppt, FWR-20 ppt, BWR-5 ppt or BWR-20 ppt) and time points (1 or 14 days post transfer). If significant, subsequent Tukey post-hoc tests were conducted. All statistics for the above experiments were conducted using R [©]3.1.3.

Results

Transfer experiment

Morphometric measures

Length was observed to be significantly higher in BWR (BWR = 86 mm SE ± 0.6 vs. FWR = 84 mm SE ± 0.4) lake trout overall but significant differences were not observed among transfer environments, time points or their interaction (Table 3.1, Supplementary Appendix 1). Similarly, BWR lake trout were observed to weigh more on average across all treatments (BWR

= 6.3 g SE ± 0.1 g vs. FWR = 5.6 g SE ± 0.1 g). However, comparisons among the interaction of rearing environments, transfer environment, and time points revealed no significant differences. Although not quantified in the present study, transfer to 20 ppt, in both BWR and FWR lake trout, demonstrated reduced to nonexistent feeding for the first day post-transfer. Interestingly, within three days post transfer, feeding increased to normal levels (i.e., all food was consumed in the tank) in the BWR-20 ppt treatment but never returned to normal in the FWR-20 ppt fish (i.e., substantial amounts of uneaten food remained) and may be linked to the significantly lower body mass observed in FWR-20 ppt fish.

Plasma osmolality and skeletal muscle water content

Plasma osmolality and muscle water content were not observed to be significantly different among rearing environments, but significant differences were observed among the interactions of rearing environment, transfer environment, and time points (Table 3.1, Supplementary Appendix 1). Plasma osmolality increased significantly FWR-20 ppt at 1 and 7 days post transfer in comparison to FWR-control and FWR-5 ppt treatments and significantly decrease between 7 and 14 days post transfer resulting in similar osmolality among all treatments at 14 days post transfer (Figure 3.1A). Plasma osmolality was observed to be significantly higher in BWR-20 ppt than BWR-0 ppt at all time points and BWR-control treatment at the 7 day time point (Figure 3.1B). When comparing between BWR and FWR fish transferred to 20 ppt, FWR-20 ppt fish were observed to have significantly higher plasma osmolality at 7 days post transfer (Figure 3.1C, Table 3.2).

FWR-20 ppt treatment had significantly lower muscle water content than FWR-control fish at 1 and 7 days post transfer (Figure 3.1D). No significant differences were observed within BWR treatments (Figure 3.1E) and no significant differences were observed between FWR-20

ppt and BWR-20 ppt water, though FWR-20 ppt fish had lower mean water content on average at 1 and 7 days post transfer (Figure 3.1C, Table 3.2).

Na$^+$ K$^+$-ATPase Activity

Significant differences in Na+ K+-ATPase activity were not observed between rearing environments but were observed among the interaction of rearing environment, transfer environment and time points (Table 3.1, Supplementary Appendix 1). Post-hoc tests revealed no significant differences within any FWR lake trout experimental treatment (Figure 3.2A). Significant increases in gill Na$^+$ K$^+$-ATPase activity in BWR-20 ppt lake trout were observed between 7 and 14 days post transfer; this resulted in significantly higher activity levels at 14 days post transfer in comparison to BWR-0 ppt and BWR-control treatments (Figure 3.2B). Gill Na$^+$ K$^+$-ATPase activity was significantly higher in BWR-20 ppt compared to FWR-20 ppt at 14 days post transfer (Figure 3.2C, Table 3.2).

Kidney Na$^+$ K$^+$-ATPase was observed to be significantly higher in FWR lake trout in this experiment and differences were also observed among the interaction of rearing environment, transfer environment and time points. Kidney Na$^+$ K$^+$-ATPase activity was significantly lower 1 day post transfer in the FWR-5 ppt treatment when compared to FWR-control treatment (Figure 3.2D). At 14 days post transfer FWR-20 ppt had significantly higher kidney Na$^+$ K$^+$-ATPase activity than did FWR-control lake trout (Figure 3.2D). BWR-0 ppt had significantly elevated kidney Na$^+$ K$^+$-ATPase activity at 7 days post transfer compared to both BWR-control and BWR- 20 ppt treatments (Figure 3.2E). No significant differences were observed in kidney Na$^+$ K$^+$-ATPase activity between FWR-20 ppt and BWR-20 ppt at any time point, though FWR-20 ppt had higher average levels at all time points (Figure 3.2F, Table 3.2).

Na$^+$ K$^+$-ATPase expression

Overall gill Na^+K^+-ATPase α1a did not differ between rearing environments but significant differences were observed among the interaction of rearing environment, transfer environment and time points (Table 3.1, Supplementary Appendix 1). Gill Na^+K^+-ATPase α1a expression in FWR-5 ppt was significantly lower than FWR-control lake trout at 7 days post transfer (Figure 3.3A). FWR-20 ppt lake trout had significantly lower gill Na^+K^+-ATPase α1a expression when compared with FWR-control and FWR-5 ppt treatments 1 day post transfer, and had significantly lower expression than FWR-control treatment 7 days post transfer (Figure 3.3A). Gill Na^+K^+-ATPase α1a expression in BWR-0 ppt treatment increased significantly between 1 and 7 days post transfer resulting in significantly higher expression than BWR-control (Figure 3.3B). BWR-20 ppt had significantly lower expression at 7 days post transfer than the BWR-control treatment. No differences in Na^+K^+-ATPase α1a expression were observed between FWR-20 ppt and BWR-20 ppt treatments over the 14 d (Figure 3.3C, Table 3.2).

Overall gill Na^+K^+-ATPase α1b was observed to be significantly lower in FWR lake trout and significant differences were observed among the interaction of rearing environment, transfer environment and time points (Table 3.1, Supplementary Appendix 1). Na^+K^+-ATPase α1b expression was significantly higher than FWR-control and FWR-5 ppt treatments at 7 and 14 days post transfer (Figure 3.3D). Additionally, FWR-20 ppt treatment was significantly lower than FWR-control treatment at 1 day post transfer. A significant increase in gill Na^+K^+-ATPase α1b expression was observed between 1 and 7 days post transfer for BWR-20 ppt treatment resulting in a significantly higher Na^+K^+-ATPase α1b expression at 7 and 14 days post transfer when compared to BWR-control and BWR-0 ppt treatments (Figure 3.3E). Gill Na^+K^+-ATPase α1b expression was significantly higher in BWR-20 ppt in comparison to FWR-20 ppt at 14 days post transfer (Figure 3.3F, Table 3.2).

Drinking rate experiment

Two-way ANOVA indicated significant differences both within and between FWR and BWR treatments and time points for both drinking rate and osmolality ($p < 0.05$). Subsequent post-hoc tests revealed significantly higher drinking rate in FWR-20 ppt compared to FWR-control at all time points (Figure 3.4A). Drinking rates were also greater in BWR-20 ppt fish compared to BWR-control fish at all time points (Figure 3.4B). When comparing between FWR-20 ppt and BWR-20 ppt treatments, no differences in drinking rates were observed at any time points (Figure 3.4C, Table 3.2). Plasma osmolality was significantly higher in FWR-20 ppt compared to FWR-control at all time points (Figure 3.4A). Plasma osmolality was significantly higher in BWR-20 ppt compared to BWR-control at all time points (Figure 3.4A). When comparing plasma osmolality between treatments and across time points, significantly higher plasma osmolality was observed at 1 day post transfer in FWR-20 ppt (Figure 3.4A, Table 3.2).

Metabolism experiment

Two-way ANOVA of SMR, MMR_f, and AS indicated significant differences among experimental treatments and the interaction of experimental treatment and time point for MMR_f and AS (Table 3.3). When compared among treatments, BWR-control lake trout had significantly higher MMR_f and AS when compared with both FWR-20 ppt and FWR-control (Table 3.3). Additional post-hoc tests indicate significantly higher MMR_f in BWR-control fish compared to BWR-20 ppt fish at 14 days post transfer (Figure 3.5E). Thus, a significantly higher AS was observed at 14 days post transfer in the BWR-control fish compared to BWR-20 ppt fish (Figure 3.5H). Although significant differences were not observed in AS between FWR-20 ppt and BWR-20 ppt fish when comparing within the same time points, BWR-20 ppt fish had on average higher AS at both 1 and 14 days post transfer (Figure 3.5I).

Discussion

This study is the first to empirically demonstrate survival and growth of lake trout in 5 ppt water. Furthermore, despite BWR and FWR fish both surviving acute transfer to 20 ppt, my results demonstrate a linkage between the rearing environment and the physiological phenotype suggesting fish raised in 5 ppt were better equipped to deal with a salinity challenge.

Brackish-water spawning in Salmonidae

The observed survival to hatch in 5 ppt support field observations by Kissinger et al. (2016) suggesting that lake trout within Husky Lakes, NT can complete their entire life cycle in salinities ranging between 1 to at least 5 ppt. Though survival was observed in 5 ppt, development and survival to hatch were not observed in higher salinities in the present study. This indicates salinity thresholds for development may be present and could restrict available spawning habitat for this species. It is important to note that the lake trout used in this study were collected from Clearwater Lake in central Manitoba, which are highly unlikely to have experienced water conditions > 1 ppt in recent evolutionary history (~10,000 years, Wilson and Hebert 1998). If local adaptations are present in the Husky Lakes brackish-water resident population, the ability to spawn and develop in elevated salinities may be present. Similar to the observations in this study, other brackish-water spawning Salmonidae including, European whitefish (Himberg and Lehtonen 1995), pink salmon *Oncorhynchus gorbuscha* (Walbaum 1792), and chum salmon *Oncorhynchus keta* (Walbaum 1792) (Helle et al. 1964; Scott and Crossman 1973), appear to be restricted to spawning in low salinity brackish water regions. Interestingly, comparison of egg survival in European whitefish from brackish-water resident and freshwater resident populations shows the effect of salinity on survival to be much less in brackish-water populations, indicating local adaptation was likely present (Papakostas et al.

2012). Although parents from the present study came from the same freshwater ecosystem, a study by Atse et al. (2002) using Arctic char *Salvelinus alpinus* (Linnaeus 1758) from fresh or marine water environments observed significant differences in the size, concentration, and components within the gametes of parents from fresh or marine environments. Thus, parental type (BWR or FWR) may impact gamete physiology and morphology in wild brackish-water resident populations, potentially influencing fertilization and development success in habitats that exceed 5 ppt salinity. The observation of survival thresholds within Salmonidae indicates that the environment (i.e., stability of salinity and salinity level, Albert et al. 2004; Papakostas et al. 2012) and ecology (i.e., timing of spawning and duration of embryonic development) of the brackish-water spawning salmonids highly influence where brackish-water spawning can occur, as brackish-water spawning has been documented in only select areas within the distribution of these species.

Environment-phenotype interactions

To my knowledge, this is the first study to assess the effects of rearing salinity on environment-phenotype interactions in *Salvelinus* from fertilization to at least the first year and a half of life. Indeed, few studies (e.g., Wertheimer 1984; Albert et al. 2004; Papakostas et al. 2012) have examined the influence of salt water on fertilization within the Salmonidae, likely due to the predominant freshwater spawning behavior observed within this family. That said, numerous studies have reported environment-phenotype interactions for juveniles and adults associated with migratory patterns between fresh water and seawater environments later in life (McCormick et al. 1985; Gross 1987; Chapman et al. 2012b; Finstad and Hein 2012). Overall my findings indicate that genetically similar lake trout spawned in different salinities retain significantly different physiological responses for multiple measured parameters following acute

transfer to an increased salinity. Significant differences among treatments indicate the ability to ionoregulate, presumably through differential effects on gene expression, drinking rates, and enzyme activity subsequently influencing whole body aerobic scope. These findings corroborate those made by others indicating that environment-phenotype interactions are associated with different salinity rearing environments (Wertheimer 1984; Papakostas et al. 2012). Specifically, within the current experiments, I observed significantly lower plasma osmolality and a higher average muscle water content in BWR-20 ppt individuals, which was likely affected by the combination of significantly higher gill $Na^+ K^+$- ATPase expression and activity in comparison to FWR-20 ppt. This result is not surprising given the central role that the gills play in homeostatic regulation of sodium and chloride in teleost fish which are in direct contact with the ambient environment (Marshall and Bryson 1998; McCormick 2001).

The observation that BWR-control lake trout had significantly higher AS than did both FWR-control and FWR-20 ppt fish suggests that lake trout raised in an environment closer to isosmotic may have reduced energetic costs. Similar observations have been documented in coho salmon *Oncorhynchus kisutch* (Walbaum 1792), rainbow trout *Oncorhynchus mykiss* (Walbaum 1792), Chinook salmon *Oncorhynchus tshawytscha* (Walbaum 1792) fry, but while oxygen consumption was observed to be greater, growth was reduced in all species assessed with increasing salinity (Morgan and Iwama 1991). However, different results were found when similar tests were conducted on coho salmon smolts showing no significant difference in oxygen consumption across a range (0 to 28 ppt) of salinities even though multiple plasma components and important ionoregulatory enzymes increased significantly with water salinity (Morgan and Iwama 1998). The combination of these two studies suggests that the effects of salinity on salmonid metabolism differ with life stage as preparatory changes are observed to occur prior to

marine migrations in anadromous salmonids (Mccormick 1995; Morgan and Iwama 1998). Lake trout are not documented to complete preparatory changes to their ionoregulatory system prior to marine movements and thus could be more prone to metabolic changes among salinities similar to the fry described Morgan and Iwama (1991). An additional study looking at specific growth rate, feed conversion, and energy adsorption efficiency in stenohaline channel catfish *Ictalurus punctatus* (Rafinesque 1818) and gold fish *Carassius auratus* (Linnaeus 1758); and euryhaline brown trout *Salmo trutta* (Linnaeus 1758), striped bass *Morone saxatilis* (Walbaum 1792), and Gulf sturgeon *Acipenser oxyrhynchus desotoi* (Vladykov 1955) found that stenohaline fish had optimal growth in environments ≤ 1 ppt, whereas all euryhaline fish had optimal growth in environments closer to isosmotic (3 to 9 ppt; Altinok and Grizzle 2001). Though lake trout are typically considered a stenohaline species, it appears that lake trout reared in 5 ppt, benefit (significantly higher AS) from the reduced ionic gradient.

Comparisons within salmonids

Within salmonids, the genus *Salvelinus* is considered one of the least saline tolerant, and previous studies have identified lake trout as a stenohaline freshwater species and perhaps the least saline tolerant species within this family (Alexandrou et al. 2013; Hiroi and McCormick 2007). However, similar physiological trends were observed in Atlantic salmon *Salmo salar* (Linnaeus 1758), brook trout *Salvelinus fontinalis* (Mitchill 1814), rainbow trout, and Arctic char direct transfer experiments (Bystriansky et al. 2006; Bystriansky and Schulte 2011; Hiroi and McCormick 2007). That said, in comparison to land-locked Arctic char, both FWR and BWR lake trout in this study were able to significantly down regulate $Na^+ K^+$-ATPase α1a and up-regulate gill $Na^+ K^+$-ATPase α1b within 7 days post transfer when compared to control fish whereas land-locked Arctic char could not (Bystriansky et al. 2007). Additionally, comparison of

α1b expression among species over similar time spans, shows that lake trout up-regulate α1b similar to anadromous Arctic char (Bystriansky et al. 2006) or above other species such as land-locked Arctic char and Atlantic salmon (Bystriansky et al. 2006; Bystriansky et al. 2007). While similar trends are observed within this family, it appears that local adaptation may influence environment-phenotype interactions within species.

When considering the evolutionary history of salmoniforms, the contemporary understanding is that the family and subgroups most likely evolved and radiated within freshwater ecosystems, as spawning in fresh water is the norm and freshwater resident life history types are present in nearly all species (Dodson et al. 2009; Alexandrou et al. 2013). The closest relative and more ancestral, Osmeridae, are thought to have a marine ancestral origin (Dodson et al. 2009). Due to the close evolutionary relationship with marine fishes, it is not surprising that physiological mechanisms for life in marine water environments would be retained even in land-locked populations of lake trout (Dodson et al. 2009; Alexandrou et al. 2013) as much of the cellular machinery is necessary to ionoregulate in both marine and freshwater environments. It appears that retention of these traits, especially in harsh climates such as low productive Arctic freshwater ecosystems, may be beneficial to these species because the ability to access resources over larger areas through migrations may result in improved survival, fitness, and ultimately colonization of new habitats.

Anadromous and semi-anadromous migrations within salmonids are commonly assessed and debated as to why, when, and where they occur (Gross 1987; Jonsson and Jonsson 1993; McCormick 1994; McCormick 2009; Chapman et al. 2012b). A common topic associated with this discussion is fish size (Chapman et al. 2012a). A typical observation in migratory fishes is that a certain body size is required for migrations to sea, presumed to be associated with

energetic cost (i.e., migration distance) and physiology (Gross 1987; McCormick 1994; Chapman et al. 2012b). Within the present study, I demonstrated that physiological mechanisms are present within lake trout to successfully fertilize and develop in brackish waters of up to 5 ppt, thus, within environments used by semi-anadromous fishes (i.e., 5 ppt brackish-water), physiological precursors to life in brackish water would not restrict their migrations. Similarly, observations of a brackish-water resident life history in European whitefish (Himberg and Lehtonen 1995; Albert et al. 2004), pink salmon and chum salmon (Helle et al. 1964), northern pike *Esox lucius* (Linnaeus 1758) (Engstedt et al. 2010), and lake trout (Kissinger et al. 2016) corroborate my findings, that traits are present and can be expressed during early development allowing for survival in brackish water.

Brackish-water residents in Husky Lakes, NT

Based on my findings, we predict that the brackish-water resident populations of lake trout present in Husky Lakes should have a physiological advantage over freshwater hatched semi-anadromous lake trout when accessing increased salinities. In addition, the observation of an increased AS in BWR-control fish and no significant differences in drinking rate or osmolality with FWR-control fish suggest that life in brackish water likely reduces overall energetic cost and could lead to increased growth. Indications of a competitive advantage by brackish-water residents were observed in the wild as 86% of lake trout captured throughout Husky Lakes were classified as brackish-water residents and those that were classified as semi-anadromous were more often found in areas of Husky Lakes with lower salinities of 1 to 2 ppt (Kissinger et al. 2016). In addition, the average age at capture and growth rates of semi-anadromous lake trout were observed to be lower when compared to brackish-water residents,

suggesting longevity and growth rate may be reduced in the freshwater hatched semi-anadromous lake trout within this system (Kissinger et al. unpublished).

Recent findings based on neutral genetic markers indicate that brackish-water residents of Husky Lakes are also genetically differentiated from all connected freshwater ecosystems and that freshwater hatched semi-anadromous lake trout captured in Husky Lakes are genetically similar to connected freshwater populations (Kissinger et al. unpublished). Thus, I predict that adaptive traits associated with life in brackish water are preferentially selected in brackish-water resident populations, thereby increasing physiological superiority in brackish and marine water environments compared to freshwater-hatched conspecifics. Under the principles of natural selection, I would also predict that these differences would eventually result in local adaptation. Based on these findings, it would be of particular interest to examine the phenotypic variation in fish raised from gametes collected from Husky Lakes Lake trout under similar conditions. It would be reasonable to assume that gametes collected from brackish-water resident fish may be adapted to the local saline environment, surpassing the phenotypical plasticity observed in this study.

Acknowledgements

Table 3.1. Comparisons of freshwater reared and brackish-water reared lake trout (rearing environment) transferred to environment 0, 5 or 20 ppt (transfer environment) assessed at 0, 1, 7, and 14 days post transfer (time point) using a three-way analysis of variance conducted for each dependent variable. Bold text with underlines represents significant differences at $p < 0.05$.

Three-Way ANOVA Comparison	df	Length			Weight*			Osmolality			% Water Content		
		Sum Sq.	F	p	Sum Sq.	F	p	Sum Sq.	F	p	Sum Sq.	F	p
Transfer Env. (TE)	2	0.6	1.2	3.1E-01	3.4E-02	1.9	1.6E-01	35239.0	76.0	**2.2E-16**	83.8	21.9	**2.6E-09**
Time Point (TP)	3	0.3	0.4	7.2E-01	3.9E-03	0.1	9.3E-01	4964.0	7.1	**2.5E-04**	18.3	3.2	**2.4E-02**
Rearing Env. (RE)	1	2.1	8.3	**4.4E-03**	0.1	14.9	**1.5E-04**	58.0	0.2	6.2E-01	0.0E+00	1.0E-04	9.9E-01
TE·TP	5	1.2	1.0	4.4E-01	3.3E-02	0.7	6.1E-01	4519.0	3.9	**3.2E-03**	22.6	2.4	**4.1E-02**
TE·RE	2	0.7	1.3	2.8E-01	0.1	4.6	**1.1E-02**	2798.0	6.0	**3.6E-03**	12.5	3.3	**4.0E-02**
TP·RE	2	0.2	0.4	6.9E-01	2.1E-02	1.1	3.2E-01	4852.0	10.5	**8.9E-05**	0.5	0.1	8.8E-01
TE·TP·RE	4	0.5	0.5	7.7E-01	2.1E-02	0.6	6.8E-01	2541.0	2.7	**3.4E-02**	31.0	4.1	**3.5E-03**

Table 3.1 continued…

Three-Way ANOVA Comparison	df	Gill NaK ATPase*			Kidney NaK ATPase*			α1a*			α1b*		
		Sum Sq.	F	p	Sum Sq.	F	p	Sum Sq.	F	p	Sum Sq.	F	p
Transfer Env. (TE)	2	0.4	8.6	**3.0E-04**	0.5	12.9	**7.2E-06**	5.8	121.7	**2.2E-16**	2.8	39.4	**1.7E-14**
Time Point (TP)	3	4.3E-02	0.6	6.0E-01	0.4	8.3	**4.3E-05**	0.6	9.1	**1.4E-05**	0.8	7.7	**8.4E-05**
Rearing Env. (RE)	1	8.7E-03	0.4	5.4E-01	0.1	4.1	**4.5E-02**	0.1	3.5	6.2E-02	0.5	13.6	**3.1E-04**
TE·TP	5	0.8	7.0	**6.7E-06**	0.9	9.5	**8.7E-08**	1.4	11.4	**2.3E-09**	3.2	18.1	**4.3E-14**
TE·RE	2	7.9E-03	0.2	8.4E-01	0.2	4.7	**1.1E-02**	9.0E-04	0.0	9.8E-01	0.1	1.2	2.9E-01
TP·RE	2	1.4E-02	0.3	7.4E-01	3.3E-02	0.9	4.0E-01	1.6	32.9	**1.2E-12**	0.1	1.0	3.7E-01
TE·TP·RE	4	0.4	4.9	**1.0E-03**	0.2	2.7	**3.6E-02**	0.2	2.6	**3.8E-02**	1.7	12.2	**1.2E-08**

*indicate Log_{10} transformation

Table 3.2. Significant differences observed in measured physiological parameters between freshwater reared lake trout (FWR) and brackish-water reared lake trout (BWR) transferred to 20 ppt salt water throughout the experiment. An ↑ indicates a significantly higher measure and "No" indicates no differences observed over the course of the experiment.

	Transfer Experiment						Drinking Rate Experiment		Metabolism Experiment		
	$Na^+ K^+$-ATPase Activity (Gill)	$Na^+ K^+$-ATPase Activity (Kidney)	α1a Exp. (Gill)	α1b Exp. (Gill)	Water Content	Osmo.	Drinking Rate	Osmo.	SMR	MMR_f	AS
FWR-20		No	No		No	↑	No	↑	No	No	No
BWR-20	↑	No	No	↑	No		No		No	No	No

Table 3.3. Comparisons of standard metabolic rate (SMR), forced maximum metabolic rate (MMR$_f$), and aerobic scope (AS) among experimental treatments (FWR-0 ppt, FWR-20 ppt, BWR-5 ppt or BWR- 20ppt) at 1 and 14 days post transfer time points using a two-way analysis of variance conducted for each dependent variable. Bold text with underlines represents significant differences at $p < 0.05$.

		SMR			MMR$_f$			AS		
	df	Sum Sq.	F	p	Sum Sq.	F	p	Sum Sq.	F	p
Experimental treatment (ET)	3	1846	1.3	0.3	35453	5.205	**3.1E-03**	47524	6.0E+00	**1.3E-03**
Time point (TP)	1	174	0.4	0.5	282	0.124	7.3E-01	13	5.0E-03	9.4E-01
ET:TP	3	1638	1.2	0.3	18939	2.78	**4.9E-02**	26303	3.3E+00	**2.6E-02**

Figures
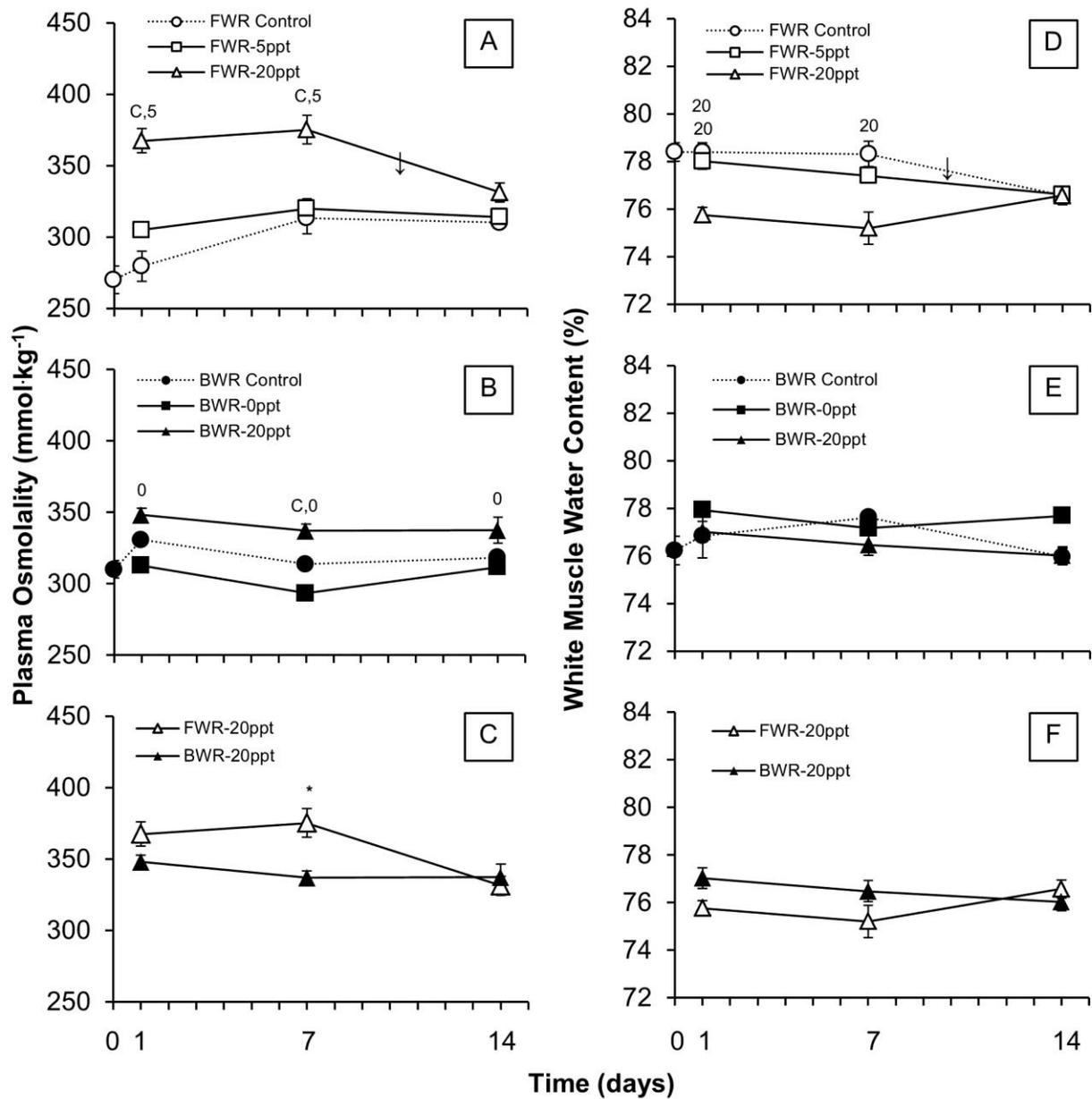
Figure 3.1. Plasma osmolality (A, B, and C; n = 5) and white muscle water content (D, E, and F; n = 11) in: A and D) freshwater reared (FWR) lake trout transferred to fresh water (control – open circles); 5 parts per thousand (ppt) seawater (open squares) and 20 ppt seawater (open triangles). B and E) brackish-water reared (BWR) lake trout transferred to fresh water (closed squares); 5 ppt seawater (control – closed circles) and 20 ppt seawater (closed triangles). C and

F) FWR lake trout transferred to 20 ppt seawater (open triangles) and BWR lake trout transferred to 20 ppt seawater (closed triangles). All treatments were sampled at 0, 1 day, 7 days, and 14 days post transfer and all fish used were ~240 days post hatch. Data are expressed as a mean ± SE. Differences among treatments at each time-point are represented by C (control), 5 (FWR-5 ppt), 0 (BWR-0 ppt), 20 (FWR-20 ppt or BWR-20 ppt), and * (comparisons between FWR-20 ppt and BWR-20ppt). Significances within treatments are represented by ↓ (decrease) ↑ (increase). Significance was accepted when $p < 0.05$.
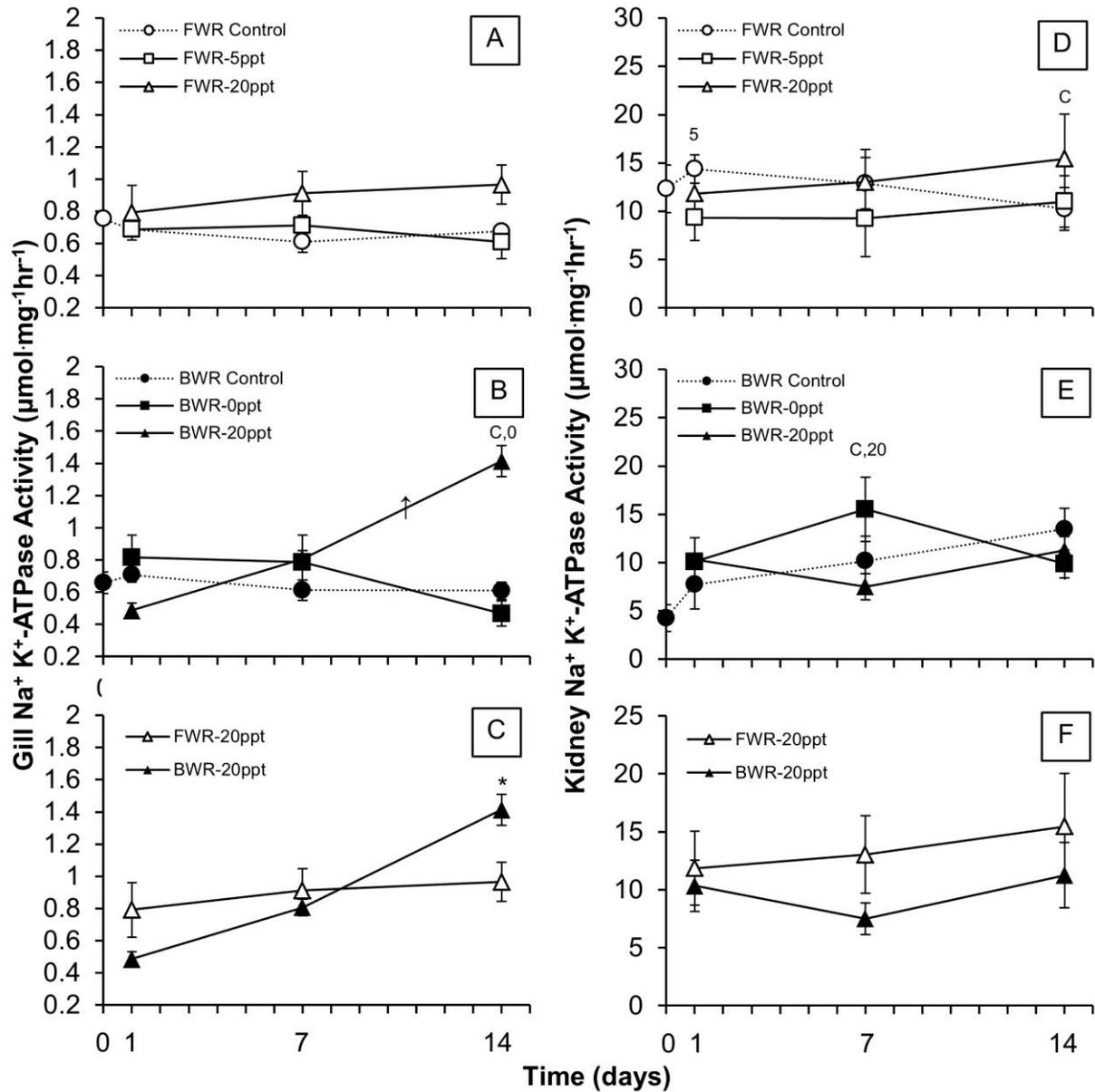
Figure 3.2. Gill (A, B, and C) and kidney (D, E, and F) Na^+ K^+-ATPase activity assessed for lake trout reared in 0 ppt (FWR) or 5 ppt (BWR) saltwater, transferred to either 0, 5 or 20 ppt saltwater and assessed at 0, 1, 7, 14 days post transfer. Data are expressed as a mean ± SE (n=8). See Figure. 3.1 for description of symbology used.
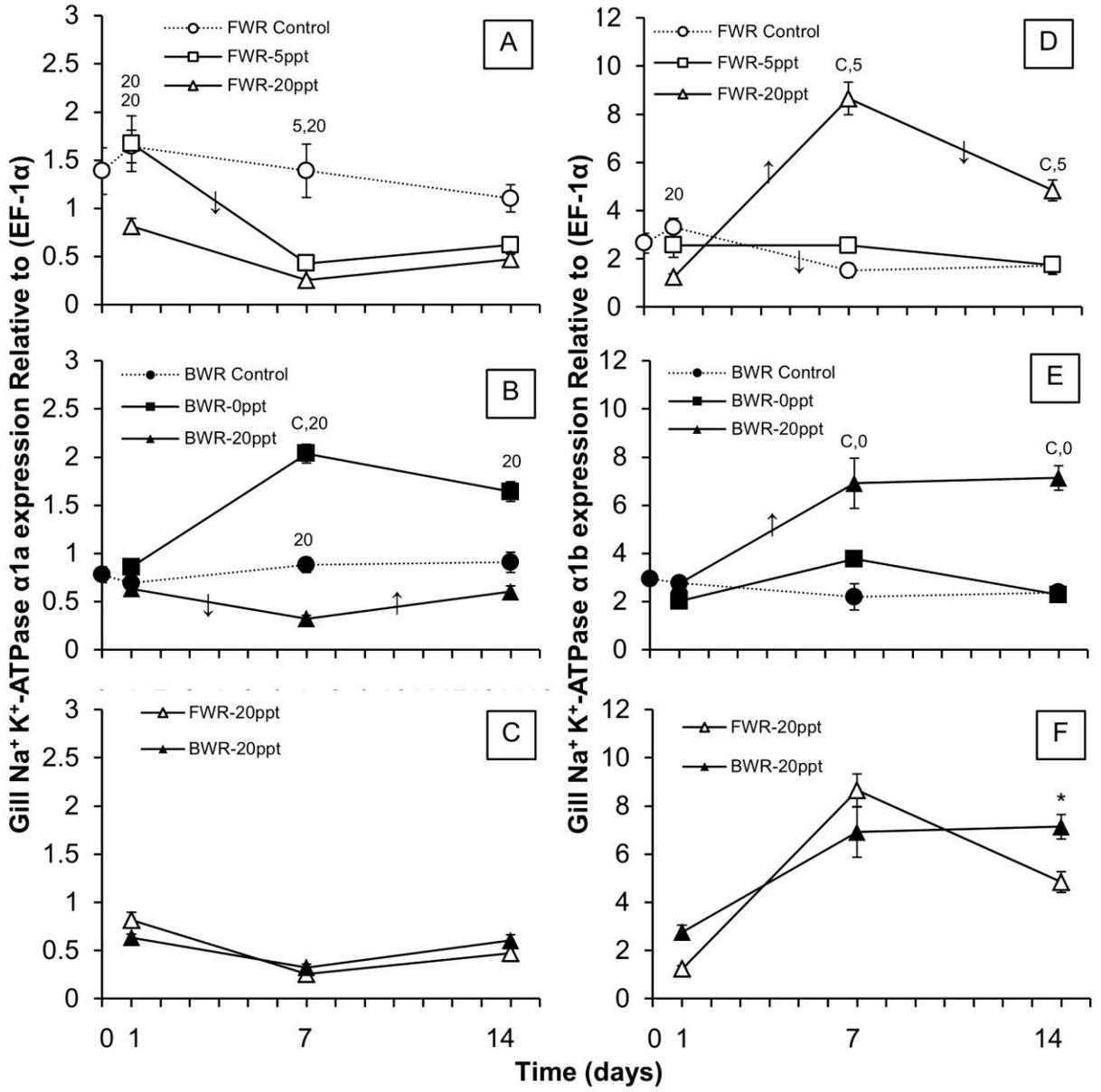
Figure 3.3. Gill Na+ K+-ATPase α1a (A,B, and C) and Gill Na+ K+-ATPase α1b (D, E, and F)

expression assessed for lake trout reared in 0 ppt (FWR) or 5 ppt (BWR) saltwater, transferred to

either 0, 5 or 20 ppt saltwater and assessed at 0, 1, 7, 14 days post transfer. Data are expressed as

a mean ± SE (n=9). See Figure 3.1 for description of symbology used.
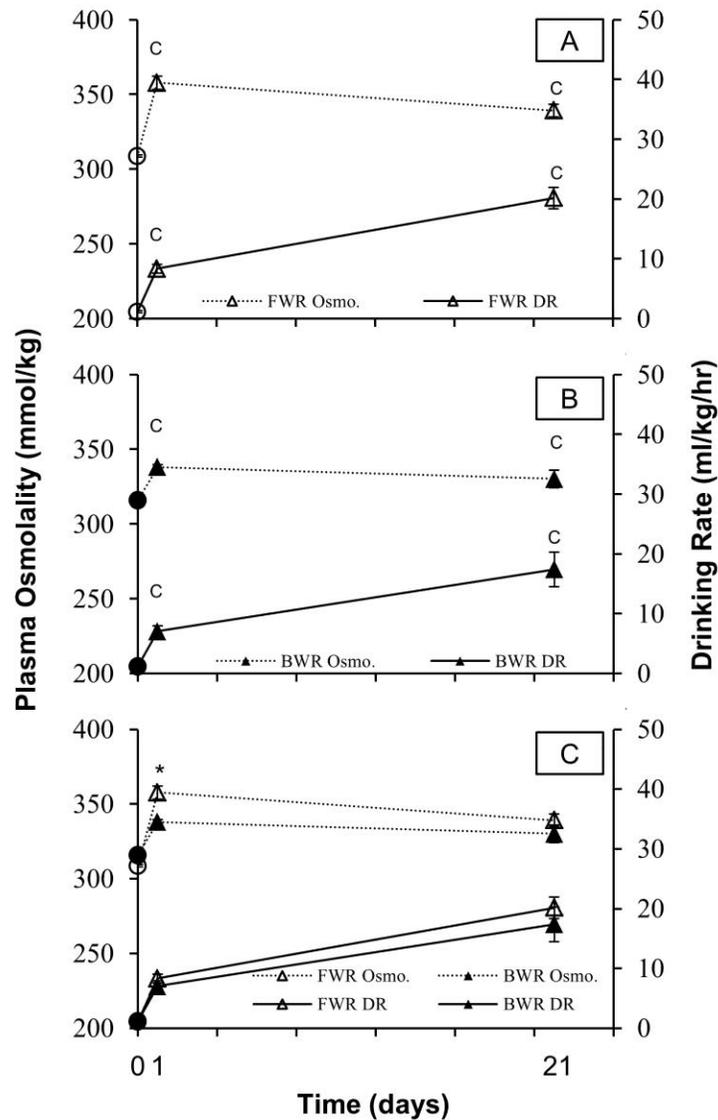
Figure 3.4. Measures of drinking rate (DR) and plasma osmolality (Osmo.) in: A) comparisons of freshwater reared lake trout (FWR) transferred to 20 ppt brackish water, (FWR-control (open circle) and FWR-20 ppt (open triangle)); B) comparisons of brackish-water reared lake trout (BWR) transferred to 20 ppt brackish water (BWR-control (closed circle) and BWR-20 ppt (closed triangle)); C) comparisons between FWR and BWR lake trout transferred to 20 ppt saltwater. Significance within treatments are represented by a C = control and among treatments with an * ($p < 0.05$, n=8).
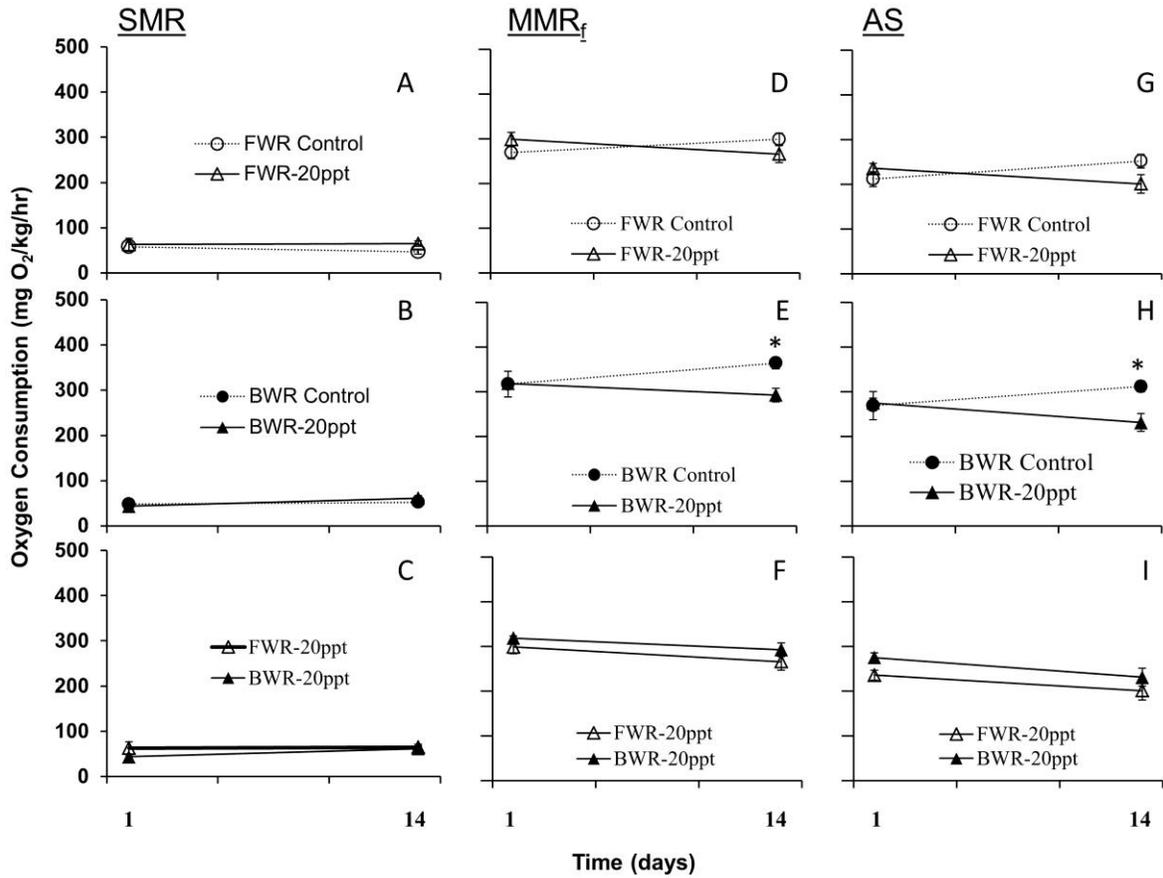
Figure 3.5. Whole body oxygen consumption ($\dot{M}O_2$) of lake trout reared in fresh water (FWR) transferred to fresh water (FWR-control), brackish-water reared (BWR) transferred to brackish-water (BWR-control), FWR transferred to 20 ppt seawater (FWR-20 ppt), and BWR transferred to 20 ppt seawater (BWR-20 ppt) at one day post transfer and fourteen days post transfer. $\dot{M}O_2$ measurements were taken at day one and day 14 post transfer for standard metabolic rate (SMR, A-C), forced max metabolic rate (MMR$_f$, D-F), and aerobic scope (AS, G-H). Significant differences are represented by an * for comparison among measures at the same time point.

Chapter 4 – Influences of life history type on lake trout (*Salvelinus namaycush*) growth and survival in the Western Canadian Arctic.

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Contributions of Authors: I organized logistics and collected all samples within this manuscript with the help of J. Reist and W. G. Anderson. I collected data through laser ablation inductively coupled plasma mass spectrometry with the guidance of N. Halden. All otolith increment widths and strontium profiles were assessed by C. Killeen and me. I conducted all statistical analysis with the guidance of D. Gillis. I wrote all drafts of this manuscript and all co-authors contributed comments.

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Abstract

The interaction between genotype and environment is perhaps the primary determinant of an individual's growth rate, longevity and ultimately fitness. Such interactions and the resultant variation in growth rates are particularly prevalent in salmonids where a rich diversity of life history types and morphotypes can often be observed within a single population. Typically lake trout, *Salvelinus namaycush*, are considered freshwater resident salmonids that rarely, if ever, experience a marine environment. However, I have recently documented two life history types within the Husky Lakes estuary, NT; semi-anadromous and brackish-water resident in addition to freshwater residents in connected lakes. In this study I compare otolith increment widths, as a proxy for annual fish growth, and age-at-capture among life history types. My results indicate that brackish-water residents grow significantly faster ($p < 0.001$) and live longer (4 years) than do semi-anadromous and freshwater resident life history types. Additionally, brackish-water residents were the dominant life history type within Husky Lakes (82%) and there was no evidence indicating that brackish-water resident fish leave this ecosystem suggesting a distinct benefit toward growth and longevity from living in Husky Lakes. This was further supported by the faster growth rate in semi-anadromous life history types compared to freshwater resident fish ($p = 0.04$), although there was no difference in longevity between these two groups. Specific factors resulting in increased growth and longevity in the brackish-water resident fish are uncertain but are most likely a combination of environment (increased productivity and near isosmotic conditions) and ecology.

Introduction

Growth rate and longevity are two important factors impacting an individual's fitness and are largely determined by biotic and abiotic environmental parameters by which an animal is affected (Jonsson et al. 1991). Heterogeneity in the biotic and abiotic environment influences where species can reside (i.e., species distribution) and the evolution of traits and tactics used to exploit resources; subsequently influencing growth rates and longevity (Finstad and Hein 2012). Variations in growth rates and longevities within an ecosystem can also be observed, in part due to heterogeneity of biotic and abiotic factors and/or variations in life history strategies (Jonsson and Jonsson 1993, Chapman et al. 2012a, Finstad and Hein 2012).

In salmonids, differences in growth rates and longevities between resident and anadromous life history types have been well documented (Jonsson and Jonsson 1993, Finstad and Hein 2012), and in many locations populations possess both resident and anadromous life history types, termed partial anadromy (Chapman et al. 2012b). Within partially anadromous populations, differences in growth rates between life history types are often linked to higher productivity in marine-influenced environments resulting in larger length at age in anadromous fish (Gross 1987). While marine environments are often more productive than connected freshwater ecosystems, numerous costs and benefits are incurred by each life history type (Chapman et al. 2011, 2012a) influencing where and the degree to which partial anadromy occurs (Finstad and Hein 2012).

A slight variation on the common definition of partial anadromy is observed in the Husky Lakes drainage basin (HLDB) NT. Husky Lakes are a series of five interconnected lake basins that drain into the Beaufort Sea (Roux et al. 2014). Numerous freshwater lakes are connected to Husky Lakes by small streams, and as a result of marine and freshwater inputs a salinity gradient

is formed transitioning from 1 practical salinity unit (psu) in the southern basin to 17 psu in the most northern basin (Roux et al. 2014). Within the HLDB, three lake trout life history types have been documented including: (1) freshwater resident, defined as a fish that is non-migratory and completes its entire life in fresh water ($<$ 1 psu); (2) semi-anadromous, defined as a fish that is hatched in fresh water but migrates to brackish water (Husky Lakes) later in life, returning to fresh water to overwinter and/or spawn; and (3) brackish-water resident, defined as a non-migratory life history that completes its entire life cycle in brackish water (Husky lakes, Kissinger et al. 2016). Within the HLDB semi-anadromous lake trout are hatch and spend early life (minimum of 8 years) in fresh water with freshwater resident conspecifics but then migrate to brackish water as juveniles and adults interacting with brackish-water resident conspecifics later in life (Kissinger et al. 2016). The presence of multiple life history strategies and significantly different biotic and abiotic environments provide potential for differences in growth rates and longevities.

Assessment of growth rates and estimates of fish age are commonly done using fish otoliths (Campana 1990). The relationship between otolith size and fish length is commonly accepted and used to compare growth rates (Campana 1990; Hansen et al. 2012), and differences in annual increment widths have been used as a proxy for comparing annual fish growth throughout life (Weisberg et al. 2010). Additionally, some trace elements have been documented to incorporate into the otolith mirroring concentrations in the ambient environment (e.g., strontium (Sr) Zimmerman 2005). When microchemistry profiles are combined with age data, individual patterns of fish movement throughout life can be determined and subsequent classifications of life history type obtained (Swanson et al. 2010). In the present study I tested the hypotheses that differences in both growth rate and longevity are present among life history

types. In addition, I compared differences in age-at-first migration between sexes to further our understanding of why semi-anadromous lake trout migrate to brackish water.

Methods

Study site

Husky Lakes are a series of five interconnected lake basins that drain northeasterly into the Beaufort Sea, located to the northeast of Inuvik, NT (Figure 4.1). A salinity gradient is present and transitions from ~1 psu in basin 1 to ~17 psu in basin 5 (Kissinger et al. 2016; Roux et al. 2014). Numerous freshwater lakes in the surrounding drainage basin are connected to Husky Lakes but only a few have been observed to contain lake trout. Known connected lake trout lakes include, Sitidgi (~5 river km), Jimmy (~20 river km), and Noell (~34 river km) lakes (Figure 4.1).

Field sampling

Lake trout from Husky Lakes were collected through a local monitoring program in 2014 and 2015. Local community members were selected by the Inuvik and Tuktoyaktuk Hunters and Trappers Committees to sample catches from the spring hook-and-line subsistence fisheries. Tissue samples (otoliths and genetics) and additional biological information (e.g., sex, length and mass) were collected from each fish sampled. An additional 68 samples were referenced from Kissinger et al. (2016) when computing mean ages and life history counts. To expand my study area, additional lakes connected to Husky Lakes were sampled during the late summer to fall period in 2014 and 2015 (Table 4.1). These included Noell, Jimmy and Sitidgi lakes (Figure 4.1). During this sampling lake trout were captured using experimental gillnets and hook-and-line angling.

These samples were part of a project aimed at collecting baseline information on lakes near and around the Inuvik to Tuktoyaktuk Highway as reference for future management. Permits issued for these surveys include: the Department of Fisheries and Oceans Canada (DFO) License to Fish for Scientific Purposes (S-14-15-3004 and S-15-16-3007) and DFO Animal Use Protocol (2014-015 and 2015-010). Furthermore, I sought and received project approvals from local Hunters and Trappers Committees (Inuvik, Aklavik and Tuktoyaktuk) in addition to the Fisheries Joint Management Committee and Gwich'in Renewable Resource Board.

Otolith microchemistry

Analysis of otolith strontium concentrations [Sr] and preparation followed the methods described in Kissinger et al. (2016). Briefly, otoliths were embedded in epoxy (ColdCure, Auburn, Washington, USA) and were sectioned with a low speed saw (Buehler Isomet, Buehler Ltd., Lake Buff, Illinois, USA). The thin sections produced (approximately 4 mm) were polished, exposing the nucleus and annuli. The otolith section was then re-embedded in a 25 mm diameter acrylic ring and allowed to harden. Following hardening, the acrylic rings were then prepared using multiple sanding and polishing steps which evened the disk surface. The disks were rinsed and photographed to provide a pre-ablation reference image and then ultrasonically cleaned in deionized water, dried and stored in sterilized sample bags wrapped in a Kimtech® wipe (Kimberly-Clark, Irving, Texas, USA) prior to measurement.

Due to the slow growth of lake trout and small otolith size, the larger dorsal lobe of each otolith was analysed to maximize sensitivity and resolution of the annual patterns of Sr for each lake trout. Following the protocols described in Swanson et al. (2010) and Kissinger et al. (2016), the otolith was ablated with a 12 μm diameter spot size at 2 $\mu m^{.} s^{-1}$ speed and a repetition rate of 20 Hz using a Laser Ablation-Inductively Coupled Mass Spectrometer (LA-ICP-MS,

LUV 213 laser and Thermo Finnigan Element 2 ICP-MS). Continuous transects were ablated across each otolith from the outside edge of the otolith core on the ventral side to the edge of the outer dorsal lobe (Figure 4.2). The ablation path was selected to perpendicularly cross annuli providing annual patterns in Sr. Calcium (Ca) was used as an internal standard and background-subtracted counts of Sr were adjusted to Ca and related to a glass standard reference, National Institute of Standards and Testing 610 (NIST 610). Scans of NIST 610 were performed after every hour during LA-ICP-MS analysis to account for changes in background levels. Using Igor Pro software by Iolite (Paton et al. 2011), intensities of Sr, counts per second (cps), were converted to concentrations, parts per million (ppm) by correcting for the background, adjusting for an internal Ca standard, and using NIST 610 as an external reference. In addition, distances in µm were calculated by dividing the collection time points by the ablation speed (2 µm·s^{-1}). Analysis of growth increment widths and annual [Sr]

Following otolith ablation, the ablated otoliths were re-photographed to visualize otolith increments and the ablation path. Lake trout ages and increment widths were then estimated along the dorsal lobe from these photographs by two independent readers. Annuli were identified as dark bands representing decreased growth in winter (Casselman and Gunn 1992). When disagreement was present between the two readers, age estimates were reassessed. If agreement could not be reached the otolith was removed from further analysis. Following the conversion of Sr counts per second to [Sr] ppm and age estimation, Sr profiles were visualized in Microsoft Excel 2010 and these line graphs were then overlaid on the post ablation images (Figure 4.2). By doing this I was able to determine [Sr] for each increment by measuring the distance from the start of the ablation transect to the start of each annulus. Distance measures started at the beginning of the laser transect and were made to each annulus along the laser transect (Figure

4.2). All distance measures were completed in Image-Pro® (version 6.0). [Sr] and increment distances along the ablation transect were entered into R version 3.3.1. Custom data manipulations were coded in R to create data boundaries using increment distance information representing an individual year of growth and Sr incorporation. From this I quantified median [Sr] for each year of life. Following the methods of Kissinger et al. (2016) I used annual median [Sr] of 1000 ppm as a separation between life in brackish water (Husky Lakes) and fresh water (e.g., Noell, Sitidgi or Jimmy lakes). Median [Sr] was used to classify which ecosystem the fish spent the majority of their time in during that year of growth as the median represents the environment where the majority of annual growth occurred. This was particularly relevant when one considered that migrations between fresh and brackish water likely occur within a single growth year. For samples classified as semi-anadromous, the first transition from [Sr] < 1000 ppm to > 1000 ppm was considered the age of first migration.

Since LA-ICP-MS started at the outer edge of the core and did not always intersect the annuli perfectly at their widest point, second readings using the same otolith were conducted for improved accuracy in the assessment of annual growth. Second readings began at the otolith primordium and were made perpendicular to each annulus along the dorsal lobe using the same methods for annuli delineation as those described above. These distance measures were then incorporated into growth models to assess potential differences among life history types.

Statistical Growth Models

To determine if growth differed among life history types, annual increment widths were compared using a mixed-effects model based on principles described in Weisberg et al. (2010). A mixed-effects model allows for unbalanced sample sizes among life history classifications, while accounting for repeated measures on the same individual through the random effects (Zuur

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et al. 2009). To correct for normality and heteroscedasticity in the residuals, increment width was log$_{10}$ transformed. Samples from Noell Lake were excluded from further analyses as no semi-anadromous lake trout were captured; thus freshwater residents represent only individuals captured in Jimmy and Sitidgi lakes. Due to the small sample sizes and similarities in increment widths, lake trout classified as freshwater residents from Jimmy and Sitidgi lakes were combined for subsequent analyses. A total of 36 semi-anadromous, 36 brackish-water resident, and 33 freshwater resident lake trout were analyzed within the mixed-effects model. To minimize differences in the age-at-capture among life history types, samples were selected from brackish-water resident and semi-anadromous life history types so that mean age at capture did not significantly differ from freshwater residents (mean= 19 years old). In addition, due to low sample numbers in later years, increments were only analyzed up to age 22.

Individual fish and the year in which growth occurred were treated as random intercepts and life history and their interaction were modeled as fixed effects. In addition, polynomial contrasts of the interaction of life history and age were assessed up to the cubic level for the following model:

$$I_{ij} = \beta_0 + \beta_1 \times age_{ij} + \beta_2 \times life\ history_i + \beta_3 \times age_{ij} \times life\ history_i + \alpha_i + \delta_j + \varepsilon_{ij}$$

where I_{ij} is the log$_{10}$ of increment width for lake trout i in year j. *Life history$_i$* was a nominal factor with three levels (freshwater resident, semi-anadromous, and brackish-water resident) and *age$_{ij}$* was an ordered factor for ages 1 to 22. The interaction of β_3 X *age$_{ij}$* X *life history$_i$* was also assessed. The terms α_i and δ_j are random intercepts for fish and year and are assumed to be normally distributed with mean 0 and variance σ_{ij}^2. The residual error term ε_{ij} is also assumed to be normally distributed with mean of 0 and variance σ_{ij}^2. Random intercepts (α_i and α_j) and ε_{ij} terms are assumed to be independently distributed and independent of each other.

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Age-at-capture for each life history type was compared using a one-way ANOVA. Since no semi-anadromous lake trout were captured in Noell Lake it was excluded from this analysis. In total, 147 brackish-water resident, 33 freshwater resident, and 45 semi-anadromous lake trout were compared in this analysis. Age at capture was $\log_{10}$ transformed for normality. Following significant ANOVA, Tukey post-hoc analysis was conducted.

For lake trout identified as semi-anadromous, age at first migration was compared between sexes. Sex was determined for 36 semi-anadromous lake trout, of which 17 were female and 19 were male. Mean age at first migration was transformed to correct for normality and were assessed using a two-way t-test. All statistics were conducted in R version 3.3.1 (requiring packages: car ver. 2.1-25, lattice ver. 0.20-30, lem4 ver. 1.1-10, and lemrTest ver. 2.0-30).

Results

In total, 255 lake trout were used in the analysis of otolith microchemistry, of these 45 were classified as semi-anadromous, 150 as brackish-water resident and 60 as freshwater resident (Table 4.1). Of these fish the majority of semi-anadromous lake trout were captured in Husky Lakes, n = 33 but constituted 18% of the samples analyzed from this water body (Table 4.1). The dominant life history type in Husky Lakes was brackish-water resident representing 82% of samples analyzed, no freshwater resident life histories were found in Husky Lakes (Table 4.1). Sitidgi Lake had the second largest number of semi-anadromous individuals in its sample, but represented the highest proportion of semi-anadromous individuals captured in this study at 44% (Table 4.1). The remaining lake trout from Sitidgi Lake were classified as freshwater residents. Of the 20 lake trout analyzed from Jimmy Lake, one was classified as being semi-anadromous and the remainder were freshwater residents (Table 4.1). Only freshwater residents were identified in Noell Lake.

Significant differences in increment widths were observed among all ages when compared to age one (Table 4.2). In addition the brackish-water residents had larger increment widths overall than did semi-anadromous individuals, whereas no difference was found between semi-anadromous and freshwater residents (Table 4.2). When the interaction of age and life history was compared among polynomial contrasts, the linear relationship of increment width was observed to be significantly highest in brackish-water residents followed by semi-anadromous lake trout then finally freshwater residents (Table 4.2, Figure 4.3). Larger increment widths became most apparent starting around age 9 and were higher on average until age 22 in brackish-water residents. Interestingly this difference occurs around the age of first migration in semi-anadromous lake trout (Figure 4.3).

One-way ANOVA of age at capture among life history types indicated that significant differences were present ($p = 0.006$). Subsequent Tukey post-hoc tests revealed that brackish-water residents had a significantly older mean age at capture (mean = 21.8, SE ± 0.7) than did both semi-anadromous ($p = 0.03$, mean = 18.0, SE ± 0.9) and freshwater resident ($p = 0.04$, mean = 18.0, SE ± 1.3) life history types. Significant differences ($t = -3.0$, $df = 16$, $p = 0.008$) were observed between sexes in age at first migration for semi-anadromous lake trout, suggesting females migrated at an earlier age (mean age of females = 9.5, SE ± 0.4, males = 11.3, SE ± 0.8).

Discussion

These are the first data to compare growth rates and longevities among brackish-water resident, semi-anadromous, and freshwater resident life history types in lake trout within the same drainage basin; and suggest that significant variation is observed among life history types. These data indicate that brackish-water residents grow faster and live longer than do semi-anadromous and freshwater resident life history types. Due to differences in the ecology among

life history types, numerous factors likely influence the differences observed in growth and longevity.

Influences on growth

It is commonly reported and accepted that anadromous salmonids benefit from migration(s) to marine environments through increased growth (Chapman et al. 2012a; Finstad and Hein 2012; Gross 1987). Interestingly, in the present study I found that while semi-anadromous lake trout benefit in regard to growth rate from this life history in comparison to freshwater residents, their growth rate is not as fast as brackish-water residents. This indicates that the environment as well as the life history type influenced the observed differences.

Fish diversity and relative densities (CPUE) were documented to be highest in Husky Lakes when compared to Sitidgi Lake, and key forage fish (Arctic cisco *Coregonus autumnalis,* Pallas (1776) and Pacific herring *Clupea pallasii* Valenciennes, 1847) were in greater abundance in the outer basins of Husky Lakes (Roux et al. 2014, 2016). Transitions in species diversity to higher numbers and proportions of forage fishes would suggest fish based resources necessary for growth would be more abundant and diverse in Husky Lakes compared to Sitidgi Lake (Roux et al. 2014, 2016). These observations are compatible with our understanding for other Arctic estuarine and marine fish communities and are most often suggested as the basis for anadromous migrations (Chapman et al. 2012a; Gross 1987; Finstad and Hein 2012, Kendall et al. 2015). Additional support for Husky Lakes providing a better environment for faster growth rate is that no brackish-water residents were captured in any of the connected freshwater lakes, suggesting there is not a significant benefit to leaving Husky Lakes for those individuals.

In addition to biotic differences, water salinity varies between ecosystems assessed (Roux et al. 2014). Through a variety of physiological mechanisms teleosts maintain plasma osmolality

within a narrow range (290-340 $mOsmol^{-1} \cdot L$) (Bystriansky et al. 2006; Hiroi and McCormick 2007; McCormick and Saunders 1987; Papakostas et al. 2012; Perrott et al. 1992). To account for differences in osmolality between the internal and external environments, enzyme production and activity rates change to influence whole body metabolism and subsequently overall energy budgets (McCormick and Saunders 1987). As 10 psu is approximately isosmotic for most teleost fish, costs associated with ionoregulation should be reduced and reflected in an increase aerobic scope (Eliason and Farrell 2016). While research specific to osmoregulation and ionoregulation in lake trout is limited, numerous studies have assessed this question within salmonids. Broad trends observed indicate that acclimation to salinities differ by species (Hiroi and McCormick 2007) and by fish size and life stage (McCormick and Saunders 1987; McCormick et al. 1998). While many studies show that changes in enzyme activity, hormone levels and plasma osmolality occur across a range of salinities, assessment of oxygen consumption (a proxy for whole body metabolic rate) in salmonids are less clear (Eliason and Farrell 2016; Morgan and George 1991; Morgan and Iwama 1998). These studies suggest that oxygen consumption is most affected by changes in salinity during younger life stages and that differences are less extreme later in life (Eliason and Farrell 2016), likely as a result of scaling of surface area to volume ratios and physiological preparedness with age (McCormick and Saunders 1987). Additionally, studies examining feed conversion and energy absorption suggest that some fish grow faster in waters near isosmotic, but again results differed by species suggesting species specific adaptations are likely present (Altinok and Grizzle 2001). When assessing lake trout metabolism in genetically similar fish reared in either 0 or 5 psu and transferred to either 0, 5, or 20 psu, aerobic scope and forced maximum metabolic rate were documented to be significantly higher in

the lake trout reared in 5 psu transferred to 5 psu, suggesting that life in salinities nearing isosmoticity should benefit growth in a natural setting (Chapter 3).

Support for faster growth in the brackish water of Husky Lakes is provided by faster growth rates in both life history types that use this environment compared to that of freshwater residents. Assessment of otolith increment widths during early life indicate that annual growth is similar or slightly less in brackish-water residents and semi-anadromous fish when compared to freshwater residents. Reduced growth observed in brackish-water residents during early life may be influenced by reduced physiological preparation for osmoregulation in heightened salinities (McCormick and Saunders 1987), increased predator avoidance (Gross 1987), and water temperature (Roux et al. 2014). However, larger increment widths in brackish-water residents and semi-anadromous individuals become apparent at approximately age 8 and are maintained throughout nearly all subsequent years. Around this time point two major ontogenetic changes occur in lake trout, sexual maturation and dietary shifts (Scott and Crossman 1973, Zimmerman et al. 2009). Based on the data collected here and by Roux et al. (2014) the youngest sexually mature individuals were documented at age 8 for males and age 10 for females. With the onset of sexual maturation, additional energetic demands are placed on a fish which reduce its growth rate and can result in dietary shifts (Zimmerman et al. 2009). Ontogenetic shifts in lake trout diet have been documented to occur around 400 to 490 mm standard length, transitioning from an invertebrate dominated diet to a more fish based diet (Zimmerman et al. 2009). Sizes of 400 mm standard length are attained between age 7 to 36 within the Husky Lakes drainage basin based on length-at-age data (Roux et al. 2014). Thus, semi-anadromous lake trout migrations around a mean age of 10 may be linked to increased energy demands associated with maturation and the need to transition to a more fish based diet. Additionally, data suggest that semi-anadromous

females migrate at a younger age than do semi-anadromous males. Energy invested into gonad production in females is greater than that for males (Chavarie et al. 2016b) and may explain why migrations occur at a younger age in females. Thus it appears that growth is higher in brackish water and is likely influenced by differences in prey diversity and abundance in addition to a reduced ionic gradient.

Influences on longevity

Reductions in longevity are influenced by numerous factors including: early maturation (Rideout et al. 2005), a lack of familiarity with local environments (Chapman et al. 2012a), energetic costs associated with migration (Eliason and Farrell 2016; Finstad and Hein 2012), and physiological challenges associated with migration (McCormick et al. 1998). Costs associated with maturation and reproduction can be high and are observed in the most extreme example of reproduction in salmonids, i.e., semelparity in a number of genera (Hendry et al. 2004). Though lake trout are iteroparous (Scott and Crossman 1973) studies have shown that earlier maturation negatively influenced longevity (Chavarie et al. 2016; Jonsson et al. 1991). Though a lack of immature samples hindered my ability to compare age at maturation among life history types, increased longevities in brackish-water residents may be a product of delayed maturation which would allow for more energy to be put towards growth.

Additional rationale for reduced longevity in semi-anadromous lake trout may be influenced by a lack of familiarity with the local environment (i.e., foraging and predator avoidance) as the first 10 years of life are spent in different locations. Unfamiliarity with local environments has the potential to increase predation and reduce foraging efficiency (Gross 1987, Chapman et al. 2011, 2012a, Eliason and Farrell 2016). In the present study, most semi-anadromous lake trout migrated to Husky Lakes at age 10 which corresponds to a mean fork

length of approximately 475 mm (based on length-at-age data). By this size, lake trout likely

have low vulnerability to predation due to gape size limitations in predatory species present (with

the possible exception of beluga whales), thus predation effects on survival should be minimal.

When considering foraging effectiveness, differences in feeding habits, growth rates, and

morphology have been documented for lake trout occupying the same lake (Chavarie et al. 2016;

Hansen et al. 2012; Zimmerman et al. 2006) and can be linked to differences in foraging

locations (i.e., depth strata, Hansen et al., 2012; Zimmerman et al., 2006). While both brackish-

water residents and semi-anadromous lake trout were often caught at the same locations, semi-

anadromous catch rates were observed to be highest in the most southerly basin (Kissinger et al.

2016). While I only assessed growth rates in the present study the potential for differences in

foraging ability, prey availability and morphology between the life history types could also

impact longevity.

In total, these data suggest that brackish-water residents are potentially locally adapted to

Husky Lakes, as faster growth and increased longevity are observed in comparison to semi-

anadromous lake trout. Potential for local adaptation is also supported by the analysis of neutral

genetic markers indicating that brackish-water residents are genetically differentiated from those

in all connected freshwater lakes (Chapter 5). Due to the presence of differences in the

environments used and genetics among life history types it is impossible to say with certainty

that a single parameter influences growth and longevity; rather it appears that differences

observed are most likely a product of both.

Conclusion

These data are the first to show that brackish-water resident lake trout grow faster and

live longer than do other life history types in the same drainage basin. In addition these data

suggest that both brackish-water resident and semi-anadromous lake trout benefit from life in the brackish water of Husky Lakes likely influenced by higher prey availability and reduced physiological costs. Due to ecosystem differences, documentation of first migrations by semi-anadromous lake trout at a mean age of 10 is likely the product of increased energy demands associated with sexual maturation and subsequent ontogenetic shifts in diet to support the increased energy demands of maturation. The observed increased growth rate and longevity, lack of migrations and dominant life history type of brackish water resident Lake Trout in Husky Lakes are all strong indicators of local adaptation. Current work to determine genetic differentiation in this population of Lake trout is ongoing (Chapter 5) and will provide evidence to support a genetic link to the observed environmental impact on growth rate in this population of Lake Trout. In addition, it seems likely that genetic segregation of life history types in Husky Lakes would provide a means for natural selection to act on traits beneficial to survival in brackish water. Finally, knowing that growth rates and longevity differ among life history types and potentially age at maturation, managers should ensure that future regulations are developed with the knowledge that size specific harvests will impact life history types differently.

Acknowledgments

Table 4.1. Sample count and life history classification for each capture location.

| | Life History Type* | | | |
Lake	SA	BWR	FWR	n
Husky	33	150		183
Jimmy	1		19	20
Noell			27	27
Sitidgi	11		14	25
Total	**45**	**150**	**60**	**255**

*Life history types: SA= semi-anadromous, BWR=brackish-water resident, FWR= freshwater

resident.

Table 4.2. Parameter estimates from a mixed-effects model predicting $\log_{10}$ annual otolith increment widths from fixed factors age (1-22) and life history type (semi-anadromous, brackish-water resident and freshwater resident) and their interactions and random factors individual fish and sample years, for lake trout from the Husky Lakes drainage basin, NT. Variation in the intercepts for individual fish and sample years were estimated as normally distributed random effect with SD = 0.10 and 0.04 respectively. The model was fit without a common constant, thus each main effect level for age corresponds to a constant for the semi-anadromous life history at that age. Comparisons among life histories assessing the interaction of age and life history type were made up to the cubic order.

Comparison	Estimate	SE	df	t	p
Age 1	-0.95	0.046	1192	-20.91	< 0.0001
Age 2	-1.33	0.046	1196	-29.22	< 0.0001
Age 3	-1.69	0.046	1201	-37.09	< 0.0001
Age 4	-1.81	0.046	1204	-39.71	< 0.0001
Age 5	-1.96	0.046	1204	-43.10	< 0.0001
Age 6	-2.10	0.046	1202	-46.08	< 0.0001
Age 7	-2.26	0.046	1197	-49.68	< 0.0001
Age 8	-2.38	0.046	1189	-52.18	< 0.0001
Age 9	-2.56	0.046	1178	-56.15	< 0.0001
Age 10	-2.71	0.046	1163	-59.49	< 0.0001
Age 11	-2.87	0.046	1147	-62.98	< 0.0001
Age 12	-2.93	0.046	1147	-63.29	< 0.0001
Age 13	-3.06	0.048	1167	-64.38	< 0.0001
Age 14	-3.16	0.050	1208	-63.60	< 0.0001
Age 15	-3.22	0.051	1227	-62.93	< 0.0001
Age 16	-3.30	0.056	1351	-58.89	< 0.0001
Age 17	-3.43	0.061	1449	-56.21	< 0.0001
Age 18	-3.48	0.066	1522	-52.70	< 0.0001
Age 19	-3.55	0.070	1558	-50.49	< 0.0001
Age 20	-3.56	0.073	1559	-48.73	< 0.0001
Age 21	-3.68	0.079	1598	-46.59	< 0.0001
Age 22	-3.66	0.079	1575	-46.35	< 0.0001
Brackish	0.08	0.030	116	2.57	0.0115
Resident	-0.04	0.030	115	-1.40	0.1633
Age (linear) X brackish	0.41	0.085	1689	4.84	< 0.0001
Age (linear) X fresh	-0.17	0.084	1700	-2.01	0.0443
Age (quadratic) X brackish	-0.12	0.079	1640	-1.51	0.1319
Age (quadratic) X fresh	0.00	0.078	1625	0.00	0.9972
Age (cubic) X brackish	0.03	0.078	1644	0.40	0.6889
Age (cubic) X fresh	0.13	0.077	1635	1.74	0.0819

* Life history types: brackish = brackish-water resident and fresh = freshwater resident.

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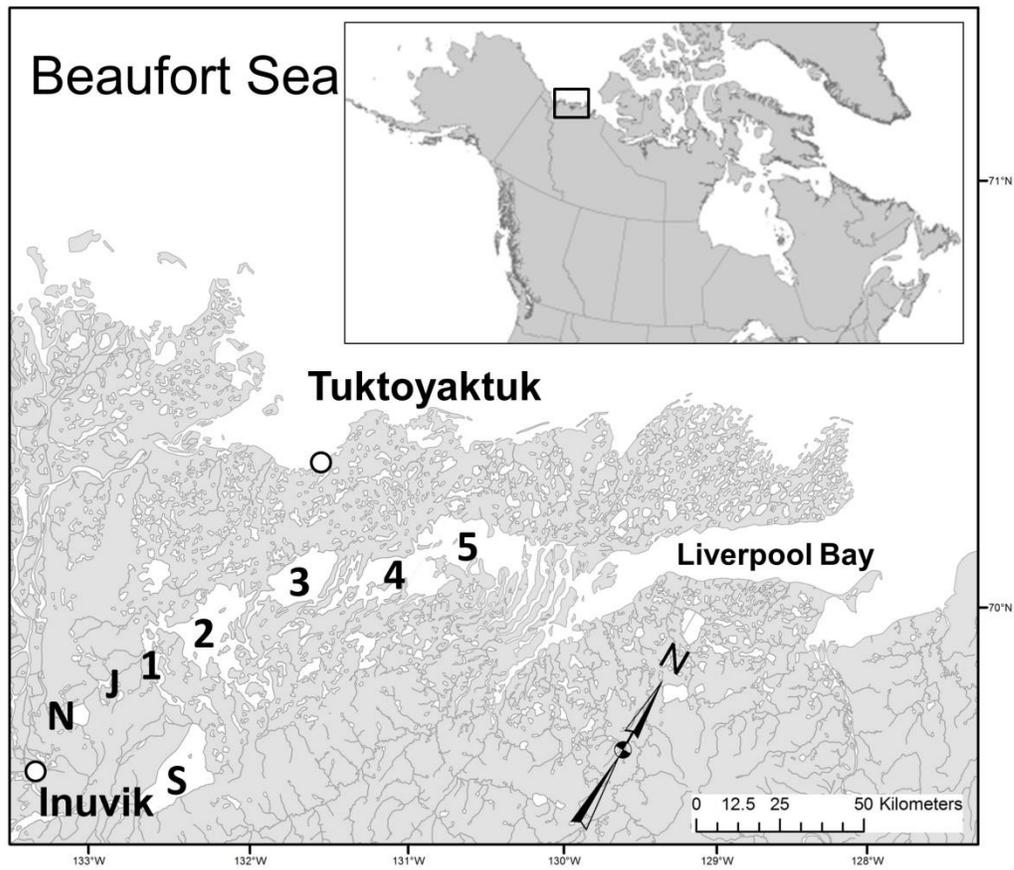
Figures

Figure 4.1. Locations of sample collections within the Husky Lakes drainage basin. N= Noell

Lake, J=Jimmy Lake, S= Sitidgi Lake and 1, 2, 3, 4, and 5 = Husky Lakes basins 1-5.
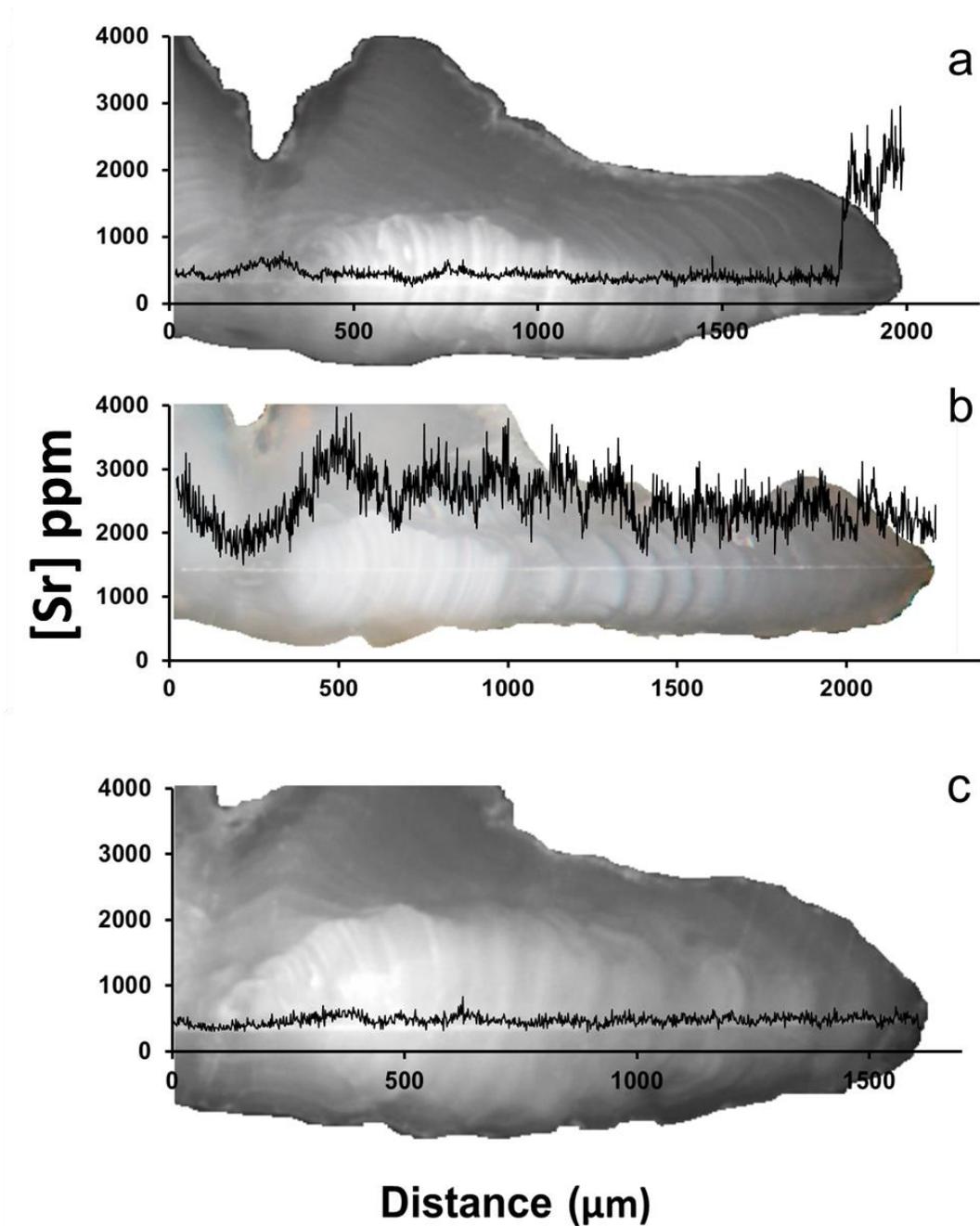
Figure 4.2. Examples of life history types based on otolith Sr microchemistry profiles identified in Kissinger et al. (2016). A. Semi-anadromous captured in Husky Lakes, NT; B. brackish-water resident captured in Husky Lakes, NT; C. freshwater resident captured in Noell Lake, NT. Concentrations of Sr > 1000 ppm represents time spent in brackish water.
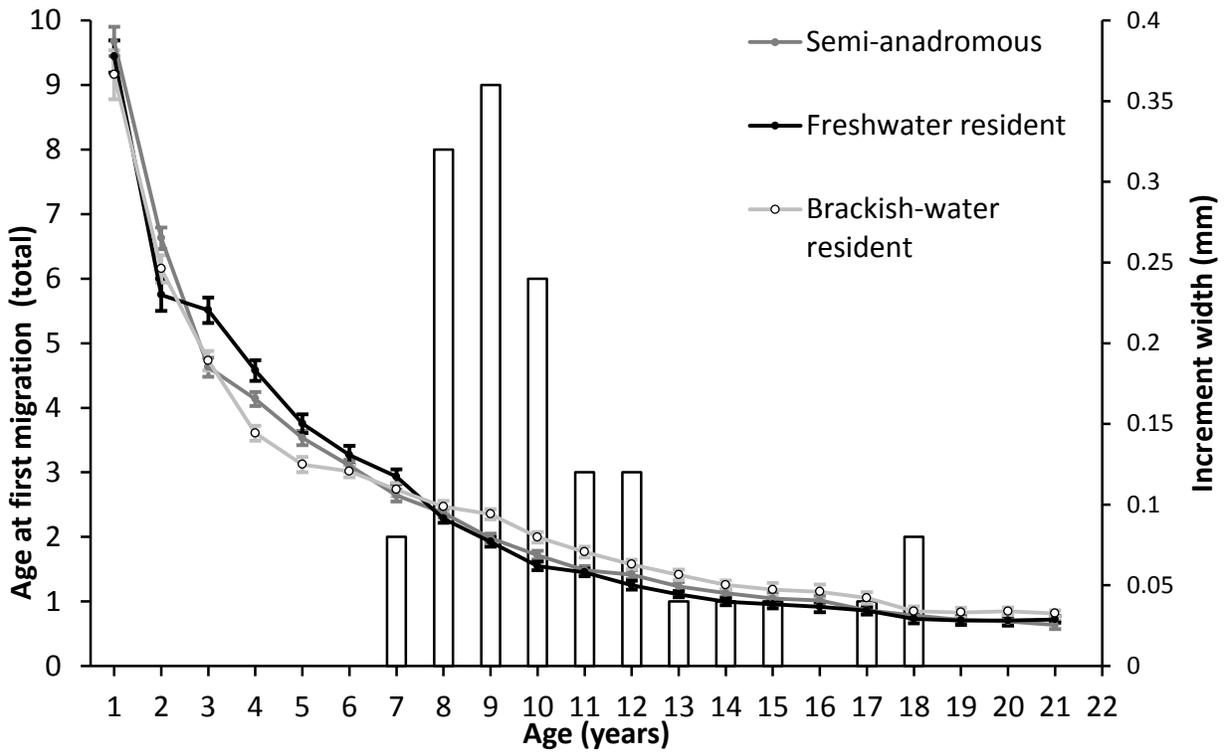
Figure 4.3. Age-at-first migration for semi-anadromous lake trout (left y-axis) and changes mean increment widths (± SE) for lake trout life history types captured in Jimmy, Sitidgi and Husky lakes, NT.

Chapter 5 - Fine-scale population structure in lake trout (*Salvelinus namaycush*) influenced by life history variation in the Husky Lakes drainage basin, NT Canada

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Contributions of Authors: I organized field logistics and collected all samples within this manuscript with the help of J. Reist, D. Swainson, and W. G. Anderson. I collected all genetics data with guidance from L. Harris. I conducted all statistical analysis with the guidance from L. Harris. I wrote all drafts of this manuscript and all co-authors contributed comments.

Abstract

Partial anadromy is a form of partial migration that is common within salmonids, where both resident and anadromous individuals in a population interbreed and overlap in habitat use during portions of their life. Variation within this ecological strategy is observed within the Husky Lakes drainage basin (HLDB), NT, where the co-occurrence of freshwater resident, semi-anadromous, and brackish-water resident lake trout life history types has been documented. While multiple life history types have been observed, nothing is known regarding the genetic population structure of lake trout in the HLDB. In the present study, I assessed microsatellite DNA variation to describe the genetic population structure of lake trout within the HLDB and surrounding area to understand how these three life histories evolved and interact. My results indicate significant structure among nearly all locations (global F_{ST} = 0.192). The brackish-water resident life history type found solely within this system is genetically differentiated from all other sampling locations and life histories, including semi-anadromous lake trout captured at the same locations. However, semi-anadromous lake trout captured in Husky Lakes were not genetically distinct from freshwater residents in connected Sitidgi Lake and some were found on spawning shoals among freshwater residents; consistent with a lack of reproductive isolation between semi-anadromous and freshwater-resident lake trout and suggests philopatry. This study provides the first evidence of breeding partial migration in salmonids using marine-influenced environments, where brackish-water residents and semi-anadromous migrants interact during the non-breeding season but semi-anadromous individuals migrate to spawn elsewhere.

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Introduction

Fishes display some of the most remarkable examples of inter- and intra-specific variations in life history and migratory strategies (Chapman et al. 2012a). For example, migration strategies range from diurnal migrations among habitats within water bodies (Reebs 2002) to vast geographic movements between freshwater and marine environments (Dodson et al. 2009). Partial anadromy, a form of partial migration involving the use of marine habitats, is a common life history strategy within salmonids, where the anadromous individuals in the population migrate from fresh water to the sea, eventually returning to fresh water to spawn whereas resident individuals complete their entire life cycle in fresh water often proximal to natal areas (Chapman et al. 2012b).

The presence of sympatric resident and semi-anadromous life history types within a population, have traditionally been thought to be maintained by conditional mating tactics (Gross 1996). Under the conditional mating tactics framework, one genotype is able to give rise to more than one alternative mating tactic (i.e., residency or anadromy) and the selection of a tactic is conditional on the individual's status (commonly its size/growth rate in salmonids; Hendry et al. 2004a) such that the tactic will maximize an individual's fitness (Gross 1996). An alternative view is that these traits are genetically-fixed strategies that are inherited and passed on through generations and thus, would result in genetically distinct phenotypes (Gross 1996). Under the latter model, differences in migratory behaviour are the result of a genetic polymorphism, and are maintained either because of frequency-dependent selection or because the two behaviours are expressed by two reproductively isolated populations (Gross 1996). Recent work suggests a complex multi-genic basis for the presence of resident and anadromous life histories in some populations of rainbow trout *Oncorhynchus mykiss* (Hale et al. 2013; Hecht et al. 2013), and

reproductively isolated life history types co-existing in sympatry are not uncommon. Genetic

differentiation among sympatric life history types have been documented in Atlantic salmon

Salmo salar (Adams et al. 2016), rainbow trout (Narum et al. 2008), and sockeye salmon

Oncorhynchus nerka (Wood and Foote 1996), with genetic differentiation between life history

types in these species being attributed to segregation of spawning habitats (Narum et al. 2008;

Adams et al. 2016). While genetic differentiation is sometimes observed, life history type in

salmonids may not be solely predetermined by a fish's genotype. In at least some populations of

rainbow trout, each life history type can give rise to the other (Pascual et al. 2001; Thrower et al.

2004), and sympatric life history types are not always genetically differentiated (Docker and

Heath 2003; Adams et al. 2016). Additional studies have documented subtle to no genetic

structure among life history types assessed across broad geographic ranges in Arctic Canada for

Arctic char *Salvelinus alpinus* (Moore et al. 2014) and Dolly Varden, *Salvelinus malma* (Harris

et al. 2015a), suggesting conditional mating tactics are more related to environmental factors

(e.g., limited spawning habitat). Results from these studies suggest when and where these life

history types spawn plays a central role in the presence or absence of genetic structure (Moore et

al. 2014 and Harris et al. 2015a).

While the common definition of partial anadromy is described above, a deviation to this

paradigm was recently documented by Kissinger et al. (2016) in lake trout *Salvelinus namaycush*

sampled from the Husky Lakes drainage basin (HLDB), Northwest Territories, Canada. Husky

Lakes are a series of five interconnected basins, defined by a salinity gradient transitioning from

1 to 17 practical salinity units (psu) (Roux et al. 2014). Within the HLDB numerous smaller

freshwater lakes are connected to Husky Lakes by streams (Roux et al. 2014). Previous

assessment of lake trout otolith strontium (Sr) profiles within the HLDB identified three distinct

lake trout life history types (Kissinger et al. 2016). These include: (1) a semi-anadromous type, defined as lake trout that are hatched in fresh water, migrate to brackish water and eventually return to fresh water for spawning and/or overwintering; (2) a brackish-water resident type, defined as a non-migratory lake trout that completes its entire life cycle in brackish water (> 1 psu; Husky Lakes); and (3) a freshwater resident type that is non-migratory and completes its entire life cycle in fresh water (< 1 psu; Kissinger et al. 2016). Though semi-anadromous and freshwater resident lake trout hatch in fresh water and exist in sympatry during early stages of their life, the former also exists in sympatry with brackish-water residents from Husky Lakes later in life as juveniles and adults. The relatively high presence (14%, Kissinger et al. 2016) of freshwater hatched semi-anadromous migrants in Husky Lakes suggests the potential for gene flow. To date, results from otolith microchemistry can only describe ecosystem use and while overlap in habitat is observed, it is unknown if these life history types are genetically differentiated populations. If genetic differentiation is not observed among life history types I would consider all life histories part of one population, that is, a single interbreeding gene pool (Figure 5.1A). In contrast, if genetic differentiation is observed among all life history types, I would predict that the origin and maintenance of genetic differentiation is the product of genetic drift and maintained through the segregation of spawning habitats among life history types and perhaps selective processes; in this case, each life history type would represent a population (Figure 5.1 B). Alternatively, the observed situation may be a combination of the two, with the three life history types representing two genetically differentiated populations (Figure 5.1C and 1D).

The goal of this study was to describe the fine-scale genetic population structure of lake trout within the HLDB and Lower Mackenzie Region. Specifically, I used neutral microsatellite

DNA variation coupled with analysis of otolith microchemistry (Sr profiles) as a determinant of life history type to assess if: (1) lake trout from the HLDB are genetically differentiated from lake trout populations in close proximity to but outside the HLDB; and (2) if life history types within the HLDB identified using Sr otolith microchemistry profiles are genetically differentiated. The latter objective will further our understanding of how these three life history types within the HLDB interact and will allow us to resolve whether they are the product of conditional mating tactics or discrete spawning areas. Additionally, results of this study will further our collective understanding of lake trout biodiversity in the region and provide baseline data for monitoring potential changes in genetic structure following the completion of a highway intended to connect Arctic communities in the region.

Materials and methods

Study system and sample collection

The HLDB is located northeast of Inuvik in Canada's Northwest Territories (Table 5.1, Figure 5.2). This system consists of a series of five interconnected lake basins transitioning in salinity from ~ 1 practical salinity unit (psu) in the southern basin to 17 psu in the northern basin (Roux et al. 2014). The HLDB shares a western boundary with the Mackenzie River drainage and covers 9 543 km^2 including numerous smaller freshwater lakes (Roux et al. 2014). For this study, lake trout were sampled from Husky Lakes and connected freshwater lakes including Sitidgi, Noell and Jimmy lakes (Table 5.1, Figure 5.2). In addition, four lakes located outside the HLDB were opportunistically sampled and used as geographically distinct sampling locations; including Wolf, Yukon 105, Sandy, and Jayko lakes (Table 5.1, Figure 5.2) to provide further insight into the spatial scale of genetic structuring among lake trout populations in the region. Of note, Yukon Lake 105 is likely land-locked as the small outflow travels through a lowland tundra

bog restricting access to other lakes and the Arctic Ocean, whereas the other lakes are connected to marine systems. In addition, contemporary access to the HLDB is limited through Liverpool Bay, thus requiring migrations through marine influenced environments by fish from all locations outside the HLDB (Figure 5.2).

Lake trout samples for genetic (adipose fin clip), age (otoliths), and biological (i.e., fork length, weight, and sex) analyses were collected from the Husky Lakes subsistence fishery primarily through a local lake trout monitoring program conducted by Inuvialuit fishermen from Inuvik and Tuktoyaktuk during the spring subsistence ice-fishing harvests of 2014 and 2015. Additional samples used for this study were obtained in 2012 in a similar fashion and are described in Kissinger et al. (2016). Lake trout in Husky Lakes were captured in the southern three basins of the lake in salinities ranging from 1-12 psu. Samples collected from Noell, Jimmy, Wolf, Yukon 105, and Sitidgi lakes were obtained in the late summer using multi-paneled experimental gill nets and angling. These samples were part of a project aimed at collecting baseline information on lakes near and around the Inuvik to Tuktoyaktuk Highway as reference for future management. Samples were also collected from Sandy Lake which is further upstream in the Mackenzie River system and Jayko Lake on Victoria Island, NU as described in Harris et al. (2015b).

Life history classification

Brackish-water resident and semi-anadromous life histories of lake trout in Husky Lakes and freshwater resident life histories in connected freshwater lakes (e.g., Noell Lake) were identified using otolith Sr concentration ([Sr]) via Laser Ablation-Inductively Coupled Mass Spectrometry (LA-ICP-MS) as described in Kissinger et al. (2016). Briefly, lake trout otoliths

were prepared for LA-ICP-MS by removing thin sections of the otolith with a slow speed saw (Buehler Isomet, Buehler Ltd., Lake Buff, Illinois, USA), exposing annuli from the otolith core to the outer edge mounted in 25 mm acrylic rings. Continuous transects were ablated from the core of the otolith to the outer edge of the dorsal lobe with a 12 μm diameter spot size at 2 μms^{-1} and repetition rate of 20 Hz. Calcium (Ca) was used as an internal standard and background-subtracted counts of Sr were adjusted to Ca and related to a glass standard reference, National Institute of Standards and Testing 610 (NIST 610). Using Igor Pro software by Iolite (Paton et al. 2011), intensities of Sr, counts per second (cps), were converted to concentrations [parts per million (ppm)] by correcting for the background, adjusting for an internal Ca standard, and using NIST 610 as an external reference standard. Using the same guidelines as Kissinger et al. (2016), [Sr] > 1000 ppm were considered as time spent in brackish water. If the entire Sr profile had [Sr] > than 1000 ppm the fish was considered a brackish-water resident (Figure 5.3A); if [Sr] never rose above 1000 ppm then the fish was considered a freshwater resident (Figure 5.3 B); and if the initial [Sr] was < 1000 ppm but then increased above 1000 ppm the lake trout was considered semi-anadromous (Figure 5.3C). Further background information can be found in Chapter 4 and counts of life history types by location can be found in Table 5.2.

Life history classification via otolith microchemistry was not completed for all samples within the HLDB as research licences restricted the number of lethal samples to minimize impacts to local populations. No life history classification via otolith microchemistry was completed for lakes outside the HLDB. Based on the geographic location of these sites I suggest Yukon Lake 105 is land locked and thus composed solely of freshwater residents. Similarly, Wolf Lake has low flows in its outlet stream and would only have access to the Mackenzie River during high flow events thus most fish would be considered freshwater residents. Sandy Lake is

located approximately 500 river km from the Beaufort Sea and based on the distances I would predict little to no levels of semi-anadromy and would consider this population predominantly composed of freshwater residents. Jayko Lake is known to have low numbers of semi-anadromous life history types based on upstream weir collection at the outflow, but the population is believed to be primarily composed of freshwater residents (L.N. Harris personal communication).

DNA extraction and microsatellite genotyping

DNA extraction and genotyping of microsatellites followed methods described in Harris et al. (2015b). Briefly, DNA was extracted using Qiagen DNeasy tissue extraction kits (Qiagen Inc., Valencia, CA, USA). Samples were assayed for variation at 23 microsatellite loci amplified in four multiplexes (Supplementary Appendix 2 in Harris et al. 2015b). An automated sequencer (ABI 3130xl Genetic Analyzer; Applied Biosystems, Foster City, CA, USA) was used for microsatellite analysis using the LIZ 600 size standard. GeneMapper^{TM} (ver. 4.0, Applied Biosystems) software was used to score all microsatellite data followed by subsequent visual assessments to check for errors.

Statistical analysis

Genetic variation, Hardy–Weinberg and linkage disequilibrium

The program MICRO-CHECKER (ver. 2.2.3; Van Oosterhout et al. 2004) was used to assess the quality of the microsatellite markers by testing for null alleles and large allele dropout. The programs FSTAT (ver. 2.9.2.3; Goudet 2002) and GENEPOP (ver. 4.4; Rousset 2008, 2015) were used to compile descriptive statistics (number of alleles (N_A), expected (H_E, Nei's unbiased gene diversity) and observed (H_O) heterozygosities, and the fixation index (F_{IS})) for each locus

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within each sampling location. Deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium were assessed using GENEPOP. The significance of simultaneous comparisons was initially compared with a nominal α of 0.05 and then to an adjusted α following the false discovery rate (FDR) procedure (Narum 2006).

Genetic population structure

Global values of F_{ST} (θ, Weir and Cockerham 1984) were generated using FSTAT and pairwise estimates of F_{ST} among all sampling locations were compared in ARLEQUIN (ver. 3.5; Excoffier and Lischer 2010). For these tests, Husky Lakes brackish-water resident samples were separated by year and life history. I chose to separate Husky Lakes brackish-water residents by year to assess the potential of year to year variability in my sample. Due to low sample size (n = 1 to 22 individuals per lake), all semi-anadromous fish collected within the HLDB were grouped together (n = 32). The significance of all pairwise estimates was assessed using 10 000 permutations, and significance was assessed at p < 0.05 and FDR corrected α.

Population structure was visualized among all samples and then among only those samples from within the HLDB using multiple methods. First, using Cavalli-Sforza and Edwards (1967) chord distance (DCE), bootstrapped neighbour-joining trees were built using PHYLIP (ver. 3.6.9; Felsenstein 2009). Second, I used Bayesian clustering implemented in the program STRUCTURE (ver. 2.3.4; Pritchard et al. 2000) to estimate the number of putative populations or clusters (K). For this analysis, I used the admixture model with independent allele frequencies while varying K from 1 to 20. I ran 10 independent runs for each value of K to assess variability of the log-likelihood values using a burn-in of 100 000 iterations followed by 100 000 Markov chain Monte Carlo (MCMC) iterations. I performed STRUCTURE analyses for two data sets: (1)

one consisting of all samples; and (2) one containing only the HLDB samples separated by life history type. The program STRUCTURE HARVESTER (ver. 06.6.92; Earl and vonHoldt 2012) was first used to visualize and compile the results based on both the posterior probability of the data (ln P[D]) and the post hoc ΔK statistic of Evanno et al. (2005). The best alignment of replicate runs was assessed using the program CLUMPP (ver. 1.1; Jakobsson and Rosenberg 2007) using 1 000 permutations and the Large K Greedy algorithm. The program DISTRUCT (ver. 1.1; Rosenberg 2004) was then used to produce plots of the best alignments for average memberships calculated using CLUMPP. For STRUCTURE analyses, I report the results of both the data ln P[D] and the post hoc ΔK statistic.

Assessment of migrants

I assessed the potential for migrants among lake trout populations within the HLDB using GENECLASS (version 2.0; Piry et al. 2004). Within this program, I first used assignment tests in order to determine the origin of semi-anadromous lake trout. I used Husky, Jimmy, Noell, or Sitidgi lakes as potential source populations as each were identified as genetically differentiated in the prior methods. I combined all brackish-water residents samples as previous tests suggest a lack of genetic differentiation among years. I assigned semi-anadromous lake trout using an assignment threshold of 0.01 and with Bayesian methods described in Rannala and Mountain (1997). Probability computation used Monte-Carlo resampling simulations with 10 000 simulated individuals and type one error rate set at 0.01 (Paetkau et al. 2004). The most likely origin for each individual was identified as the highest probability value among all locations. If the origin of a semi-anadromous lake trout was different from its capture location, I considered the individual a migrant. Secondly, I used detection tests to identify potential first generation migrants within Husky, Noell, Jimmy and Sitidgi lakes, excluding semi-anadromous lake trout

from each location as all had been assigned in the step prior. Again Husky Lakes 2012, 2014 and 2015 samples were combined for this analysis. I used the test statistic $\Lambda = L_h/L_{max}$ as the likelihood of finding a given individual in the population in which it was sampled, where L_h is the likelihood of drawing the genotype of an individual from the population in which it was sampled, and L_{max} is the maximum likelihood for the genotype across all populations (Paetkau et al. 1995). This statistic is the most appropriate when, as is likely in the present case, all source populations have been sampled (Paetkau et al. 2004). For computation, I used the Bayesian method of Rannala and Mountain (1997) and the Monte Carlo resampling method of Paetkau et al. (2004), incorporating 10 000 simulated individuals and a type I error rate of 0.01.

Results

Genetic variation and Hardy–Weinberg and linkage disequilibrium.

Loci Sco218 and Sco102 were monomorphic and the program MICRO-CHECKER consistently identified Smm21 and OtsG253b as loci containing null alleles. Removing these four loci resulted in 19 informative loci that were used in all subsequent analyses across 379 samples. Genetic variation was relatively high with the number of alleles ranging from 4 (SSOSL4) to 45 (SnaMSU10) alleles per locus and averaging 22.94 alleles across all loci (Supplementary Table 1). The highest mean number of alleles observed was in Husky Lakes 2015 samples ($N_A = 15.0$) and the lowest in Yukon Lake 105 ($N_A = 1.94$). Per locus observed heterozygosity ranged from 0.34 (SSOSL456) to 0.84 (SnaMSU1), averaging 0.67 across all loci (Supplementary Appendix 2). The highest observed heterozygosity was found in Husky Lakes 2014 samples ($H_o = 0.76$) and lowest in Yukon Lake 105 ($H_o = 0.17$). Low heterozygosity in Yukon Lake 105 is predominantly due to fixation of alleles at 11 out of 19 loci. Per locus expected heterozygosity ranged from 0.35 (SSOSL4) to 0.90 (SnaMSU6), averaging 0.71 across

all loci (Supplementary Appendix 2). Expected heterozygosity was highest in Husky Lakes 2014 samples ($H_E = 0.80$) and lowest in Yukon Lake 105 ($H_E = 0.18$). Allelic richness ranged from 2.81 (SSOSL4) to 13.10 (SnaMSU13) and averaged 8.44 across all loci (Supplementary Appendix 2). Allelic richness was highest in the semi-anadromous group ($A_R = 7.79$) and lowest in Yukon Lake 105 ($A_R = 1.71$).

When all samples were assessed, HWE was rejected in 44 of a possible 209 population–locus comparisons ($p < 0.05$), but following adjustments of α based on the FDR procedure, only 18 significant deviations were found ($p < 0.0084$). All deviations were the result of heterozygote deficiencies. Linkage disequilibrium was detected in 121 of 1 881 tests ($p < 0.05$), but after using the FDR procedure, linkage disequilibrium was detected in only 49 comparisons ($p < 0.0061$). Observations of HWE and linkage disequilibrium occurred evenly throughout sample locations and loci assessed.

Genetic population structure

Differentiation among all samples was high (global F_{ST} estimate of 0.192, 95% confidence interval (CI) 0.168–0.214), but pairwise comparisons among Husky Lakes sampling years was low as well as between Sitidgi Lake and semi-anadromous samples (Table 5.3). Across all sampling locations pairwise F_{ST} ranged from -0.001 to 0.498. The large global F_{ST} value was influenced by high F_{ST} values found in comparisons with Wolf Lake in the Mackenzie Delta (F_{ST}, 0.123–0.466) and Yukon Lake 105 (F_{ST}, 0.390–0.498) on the Yukon North Slope. The majority (92.7%) of pairwise comparisons among sampling locations were significant ($p < 0.05$, Table 5.3), even after adjusting for multiple comparisons using the FDR ($p < 0.011$, 87.2%, Table 5.3). Non-significant differences at $p < 0.05$ included comparisons between Husky Lakes

2012 and 2015 samples, Husky Lakes 2012 and semi-anadromous individuals, Husky Lakes

2012 and Sitidgi Lake, and Sitidgi Lake and semi-anadromous individuals. Following FDR

adjustments, all comparisons among Husky Lakes samples, Husky Lakes 2012 and semi-

anadromous samples, Husky Lakes 2012 and Sitidgi Lakes samples, Noell Lake and Husky

Lakes 2014 samples, and Sitidgi Lake and semi-anadromous samples, were non-significant. A

lack of differentiation among Husky Lakes 2012 and Sitidgi Lake samples is likely influenced by

the small sample size in Husky Lakes 2012 (Table 5.3) as assessment of STRUCTURE and

neighbour-joining trees suggest significant differences between brackish-water residents in all

years and samples from Sitidgi Lake (Figure 5.4, 5.5, and 5.6).

The neighbour-joining tree based on Cavalli-Sforza and Edward's chord distance also

highlighted the clear genetic distinctiveness of lake trout from Yukon and Wolf lakes, which

grouped away from all other sampling locations with high bootstrap support (Figure 5.4). The

other two lakes outside of the HLDB, Jayko and Sandy lakes, were also clearly distinct and their

separation from sampling locations within the HLDB was also highly supported (Figure 5.4).

Overall, most branches had strong bootstrap support (> 80 %, Figure 5.4), but lower support was

observed among brackish-water residents, suggesting a lack of genetic differentiation among

years (Figure 5.4). A separate comparison of samples from only within the HLDB (Figure 5.4)

indicated that brackish-water residents from Husky Lakes clearly grouped together across

multiple sampling years (with 94 % bootstrap support) and away from Jimmy and Noell lakes

(100%) and Sitidgi Lake and, importantly, the semi-anadromous fish (100 %, Figure 5.4).

Pilot runs of STRUCTURE were conducted on the entire data set which resulted in the

delineation of two distinct groups when using the approach outlined in Evanno et al. (2005).

Delineation of only two distinct groups is likely the result of extreme genetic outliers (e.g.,

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Yukon Lake 105, Wolf, Sandy and Jayko lakes). Thus, I removed all samples from outside the HLDB in order to focus on the fine-scale differences within the HLDB.

Comparison of samples from within the HLDB using STRUCTURE suggested the existence of four (ln P[D] = -17 375.6) or six ($\Delta K = 14.65$, Figure 5.4, Supplementary Appendix 3) genetic clusters. When admixture plots were visualized assuming $K = 4$, four distinct clusters were observed including: (1) a Husky Lakes brackish-water resident (all years) group; (2) a group that consisted of semi-anadromous lake trout and those from Sitidgi Lake; (3) Jimmy Lake; and (4) Noell Lake (Figure 5.4). These same genetic clusters were also clearly visualized at $K = 6$ (Figure 5.4). Support for these genetic clusters is concordant with the F_{ST} pairwise comparisons and neighbour-joining tree (Table 5.3, Figure 5.4).

Assessment of migrants

Assessment of the 32 semi-anadromous individuals indicated all were of Husky or Sitidgi lakes origin and none were assigned to Noell or Jimmy lakes. Based on my ability to assign all 32 individuals to one of the four locations suggest my assumption that all source populations were sampled was likely valid. Most of the semi-anadromous (75%) lake trout were identified as having a different natal origin than their site of capture and were thus considered migrants (Table 5.4). Of the semi-anadromous individuals identified as being migrants, the majority (n = 21) were identified as being from Sitidgi Lake (Table 5.4). Subsequent assessment using "detection" of first generation migrants (FGM) for the remaining unknown life histories (i.e., those with no microchemistry classification) and those classified as not being semi-anadromous found a low proportion to be identified as FGM (n = 8 of 218, 3.6%). FGM were detected in low numbers in Husky (n = 5, 5%), Jimmy (n = 2, 6%), and Sitidgi (n = 1, 2%) lakes. No FGM were found in

Noell Lake which is geographically upstream and furthest from any other population assessed in the HLDB (Table 5.4, Figure 5.2). Husky Lakes brackish-water residents had the largest number of FGM, with parental origins from either Sitidgi or Noell lakes (Table 5.4). Both FGM found in Jimmy Lake were of Noell Lake origin and the one FGM in Sitidgi Lake had a Husky Lakes origin (Table 5.4). These analyses indicate that the majority of movement within the HLDB occurs between Sitidgi and Husky lakes but that some degree of movement is present among all populations. Additionally, 27 of the 32 semi-anadromous lake trout were identified as having a Sitidgi Lake origin supporting the STRUCTURE, F_{ST} pairwise comparisons, and neighbour-joining tree findings prior (Table 5.4, Figure 5.4 and 5.6).

Discussion

This work represents the first assessment of genetic structure among life history types in lake trout displaying partial anadromy. In addition, it is the first to provide a comprehensive assessment of fine-scale genetic structure among lake trout in the Lower Mackenzie Region. Several important findings were revealed in this assessment. First, sympatric brackish-water resident and semi-anadromous life histories found within Husky Lakes appeared to be genetically differentiated and the vast majority of semi-anadromous lake trout were of Sitidgi Lake origin and not genetically distinct from the freshwater-resident lake trout within Sitidgi Lake. I thus provide evidence for two genetically distinct populations within the HLDB: 1) brackish-water residents and 2) semi-anadromous and freshwater residents (Figure 5.1C). Secondly, while genetic differentiation is clearly present across the HLDB, relatively high amounts of movement were resolved particularly between Husky Lakes and Sitidgi Lake and consistencies were observed between microsatellite variation and otolith microchemistry. Finally, my results suggest that populations within the HLDB are highly differentiated (global

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$F_{ST}= 0.192$) from populations in close proximity (< 60 km linear distance) but in different drainages (i.e., Wolf Lake and Sandy Lake).

Determinants of population structure

Establishment and maintenance of genetic structure within and among populations is the product of some barrier to reproduction between individuals of the same species (see Hendry and Stearns 2004). Barriers influencing genetic population structuring can be physical (e.g., dams and waterfalls, Torterotot et al. 2014), geographical (e.g., long distances, Harris et al. 2015), behavioural (e.g., assortative mating, Elmer et al. 2010), temporal (Wood and Foote 1996), and physiological (e.g., salinity tolerance, Papakostas et al. 2012), establishing over numerous generations (Hendry and Stearns 2004). The presence of population structure within the HLDB, especially within Husky Lakes, is surprising as many of the barriers described above are not observed or are less extreme and high levels of movement (44% semi-anadromous lake trout in Sitidgi Lake) are observed based on otolith microchemistry. Distances among sampling locations in the HLDB are well within the migratory capabilities of lake trout as a species (Harris et al. 2014), and the documentation of lake trout movement in this region supports this conclusion (Kissinger et al. 2016). Additionally, though lake trout are considered to have reduced ionoregulatory and osmoregulatory abilities compared to other salmonids, transitioning from fresh water to salinities of a minimum of 1-2 psu (Husky Lakes) are well within their physiological capabilities (Hiroi and McCormick 2007). Assortative mating is unlikely as lake trout do not construct redds, are not considered to be sexually dimorphic, and are not documented to show male–male agonistic behaviour or mate selection (Esteve 2005). Also, it is difficult for broadcast spawners to ensure gametes are fertilized by only one individual as females often release their gametes when surrounded by multiple males (Muir et al. 2012). The

presence of high proportions of migratory individuals (i.e., semi-anadromous life history) and a lack of obvious barriers to gene flow would suggest that genetic structure should not be observed in the HLDB, yet it is.

In other salmonids, genetic structure linked with life history types has been attributed to segregation of spawning habitat between life histories (Wood and Foote 1996; Narum et al. 2008; Adams et al. 2016) and would be a barrier to gene flow, creating and maintaining the genetic structure observed. In this study, support for segregation of spawning grounds among brackish-water residents and all other life history types is provided by: (1) genetic structure between life history types; (2) documentation (via otolith microchemistry) of brackish-water residents only within Husky Lakes (Kissinger et al. 2016); (3) semi-anadromous lake trout are documented (via otolith microchemistry) in Sitidgi Lake during the spawning period (44%); and (4) assignment of nearly all semi-anadromous lake trout captured within Husky Lakes to a Sitidgi Lake origin. While brackish-water resident spawning grounds appear to be geographically distinct, a lack of genetic differentiation between semi-anadromous lake trout and Sitidgi Lake residents suggest ongoing gene flow and shared spawning grounds between these life history types, which is potentially the product of conditional mating tactics or a lack of assessment of genes specific to migration (Hale et al. 2013; Hecht et al. 2013). In addition, it appears that semi-anadromous lake trout display philopatry as they were documented in Sitidgi Lake during the spawning season. Thus Sitidgi Lake would be termed a partially anadromous population possessing genetically similar semi-anadromous lake trout and freshwater residents (Chapman et al. 2012), whereas Husky Lakes possesses two genetically differentiated populations semi-anadromous and brackish-water residents (i.e., prediction Figure 5.1C). Under this context I would consider the interaction between genetically differentiated sympatric life histories in

Husky Lakes as a form of breeding partial migration (see Fig. 1B; Chapman et al. 2011), where residents (brackish-water residents) and migrants (semi-anadromous) share an ecosystem when not breeding (Husky Lakes) but breed in discrete areas (Husky Lakes or Sitidgi Lake). While genetically differentiated sympatric life histories are documented in numerous salmonid species and in numerous freshwater locations, this is the first documentation to my knowledge of breeding partial migration for salmonids in marine influenced systems.

The observation of population genetic structure outside the HLDB is less surprising as numerous potential barriers to gene flow are present. Physical barriers (e.g., land-locked Yukon Lake 105), distance (e.g., Jayko Lake), physiological barriers (i.e., salt water), and segregation of spawning locations (within HLDB) have been present and acting on these populations for many generations. While river use and some large scale movements (~ 50 km) are documented within this species (Harris et al. 2014), lake trout are most often typified by their relatively sedentary lacustrine biology (Scott and Crossman 1973). It is then likely that distances among sampling locations outside the HLDB influence population structure. An additional barrier is the Beaufort Sea. Only recently have studies identified lake trout as a semi-anadromous species and these observations appear to be restricted to specific regions within the Canadian Arctic (Swanson et al. 2010; Kissinger et al. 2016). While use of brackish water has been observed, capture data and laboratory studies suggest that migrations are likely restricted to regions of lower salinities (Hiroi and McCormick 2007; Swanson et al. 2010; Kissinger et al. 2016), and thus coastal regions of the Beaufort Sea act as a barrier to lake trout dispersal and gene flow between the HLDB and surrounding locations (i.e., the coastal entrance to Husky Lakes has a salinity of ~17 psu, Roux et al. 2014).

Comparisons of microchemistry and genetics

An interesting facet of this study was the general concordance between results based on otolith microchemistry and genetics. Both methods suggest Sitidgi Lake as the source of most semi-anadromous migrants and the lake with highest connectivity to Husky Lakes. Consistencies between genetics and microchemistry used in stock discrimination have been documented for a variety of other Arctic fish species in the region such as Dolly Varden (Harris et al. 2015a; Loewen et al. 2015) and broad whitefish (*Coregonus nasus*, Harris et al. 2012). Although microchemistry and genetic results were concordant, the addition of the latter has provided a clearer understanding of movement, natal origin and population structure among water bodies within the HLDB. For example, while otolith microchemistry indicated that semi-anadromous lake trout were present in the HLDB, previous studies were unable to identify with certainty their natal origin, as Sr profiles could only broadly classify fresh or brackish-water ecosystem use. Lastly, through the combination of these two approaches, I was able to determine that some freshwater resident and semi-anadromous lake trout captured in Sitidgi Lake were not natal to this system and thus these migrants clearly had a Husky Lakes brackish-water resident parental origin. Since the microchemistry profiles indicate that these fish had never been to brackish water during early life (i.e., inferred from otolith [Sr] < 1 000 ppm), it is likely that their parents were of brackish-water resident origin and had made a short-term migration into Sitidgi Lake to spawn. These particular observations highlight the benefits of combining otolith microchemistry with genetics to ascertain the origin of life history types.

Implications of genetic differentiation within HLBD

Genetic differentiation between brackish-water residents and all other life history types within the HLDB may provide the potential for local adaptation. Lake trout are thought of as one of the least saline tolerant salmonids (Scott and Crossman 1973; Hiroi and McCormick 2007;

Alexandrou et al. 2013) and brackish-water resident life history types in lake trout has to date only been documented in Husky Lakes (Kissinger et al. 2016). While salmonids are known for their phenotypic plasticity associated with anadromy (McCormick and Saunders 1987; Bystriansky and Schulte 2011; Chapman et al. 2012a) and often lack genetic differentiation in neutral markers among life history types (Docker and Heath 2003; Moore et al. 2014; Harris et al. 2015), genetic differentiation among life history types (Wood and Foote 1996; Narum et al. 2008) and local adaptation are found within this family (see review by Fraser et al. 2011).

Within the HLDB, significant differences in the abiotic environments of Husky Lakes and connected freshwater lakes, specifically salinity, likely result in physiological differences in mechanisms associated with ionoregulation and osmoregulation among life histories observed (Hiroi and McCormick 2007). Fertilization and embryonic development are critical time periods, especially for species with external fertilization and minimal parental care, like lake trout. During this stage gametes and subsequently embryo success are influenced by both biotic (e.g., egg predation) and abiotic factors (e.g., tidal changes in salinity), and due to the long embryogenesis of lake trout compared to spring spawning salmonids (Scott and Crossman 1973), these risks increase. It is accepted that ionoregulation and osmoregulation in salmonids change throughout life and that younger life stages are typically less tolerant to changes in salinity, which may impact survival when spawning in brackish-water environments (McCormick et al. 1985; McCormick and Saunders 1987; Morgan and Iwama 1998). An assessment of Arctic char gonads and gametes from fresh versus marine environments indicated significant differences in the size and concentration of glucose and lipids (Atse et al. 2002). While the differences found in that study could be linked to phenotypic plasticity, ongoing selection for subtle differences in traits associated with ionoregulation and osmoregulation could improve fertilization success and

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individual survival, eventually producing local adaptation. Additionally, significant reductions in fertilization success and changes to protein expression associated with osmoregulation were observed in freshwater resident populations of European whitefish *Coregonus lavaretus* when reared at salinities > 4 psu (Papakostas et al. 2012). In contrast, fertilization success and changes to protein expression in brackish-water resident European whitefish were only affected at salinities > 10 psu, and differences were attributed to local adaptation (Papakostas et al. 2012). Similarly, laboratory studies assessing environment-phenotype interactions in freshwater-origin lake trout reared in 20 psu, 15psu, 10 psu, 5 psu or 0 psu salt water showed no survival to hatch in 20 – 10 psu and significant reductions in survival to hatch in 5 psu (19% survival) vs. 0 psu (37% survival), suggesting thresholds are present for successful fertilization and embryogenesis in freshwater lake trout (Kissinger et al. 2017). Based on these data it would not be advantageous or as effective for lake trout to spawn in brackish water as initial reductions in survival and fertilization success would be predicted unless local adaptation is present.

How the brackish-water resident population was established in Husky Lakes is currently unknown, but my data suggest completing life in brackish water may be advantageous. No brackish-water spawned fish were documented in any connected freshwater ecosystem, while 44% of Sitidigi Lake lake trout migrated to Husky Lakes (based on microchemistry analysis). Additionally, brackish-water residents are the dominant life history type in Husky Lakes constituting 86% of samples analyzed (Kissinger et al. 2016). Further, assessment of growth rates indicates that brackish-water resident lake trout grow faster than conspecifics (Chapter 4). Lastly, to date the brackish-water resident life history type for lake trout is only documented within Husky Lakes, NT. These lines of evidence strongly suggest that local adaptation is present in this genetically differentiated life history type, and differences observed could be attributed to

some point along the spectrum of adaptive radiation within this species (Schluter 1996; Baillie et al. 2016).

Implications to local management

Lake trout fisheries within the HLDB are an important source for subsistence to local community members of Inuvik and Tuktoyaktuk, NT. Until the construction of the Inuvik to Tuktoyaktuk highway is complete (in fall 2017), access to Husky Lakes and connected lakes via land are logistically restricted to snow covered seasons via snowmobile. With the completion of this highway, Husky Lakes will be accessible year round, specifically during the fall spawning period when lake trout are highly vulnerable. Based on the susceptibility of lake trout to overharvest (Clark and Huang 1985; Muir et al. 2013), insight from findings in this study should be considered. Specifically, the documentation of fish movement among lakes highlights the need to ensure connectivity is maintained as its loss will disproportionately impact semi-anadromous life history types. Knowledge that Husky Lakes is a mixed-stock fishery and that proportions of life history types differ spatially (i.e., more semi-anadromous lake trout in basin 1, Kissinger et al. 2016) within Husky Lakes, suggest that harvest of fish in different regions of Husky Lakes will disproportionately impact life history types. Also, harvest of semi-anadromous lake trout in Husky Lakes will directly affect population levels and the spawning stock within Sitidgi Lake. Lastly, knowledge that brackish-water residents are only documented in Husky Lakes and are likely locally adapted to this environment suggests that if populations are lost or heavily reduced, restoration would be difficult. Thus, proactive measures (e.g., gear and/or harvest regulations, and season closures) should be taken to ensure these lake trout fisheries and unique biodiversity are maintained for generations to come.

Conclusions

The analysis of microsatellite DNA markers and otolith microchemistry for lake trout populations from the Lower Mackenzie region demonstrates that the lake trout from the HLDB are differentiated from populations in close proximity. Additionally, within basin comparisons revealed the brackish-water resident life history type, known only to exist in Husky Lakes, are genetically distinct from those in connected freshwater lakes and from the sympatric semi-anadromous form, suggesting the potential of local adaptation (Sitidgi Lake freshwater-resident and semi-anadromous forms were not genetically differentiated from each other). Genetic differentiation among life history types present in Husky Lakes demonstrates a unique example of breeding partial migration for salmonids (see Fig. 1, Chapman et al. 2011). The importance of the HLDB to local subsistence fishers and the unique biodiversity of lake trout life histories observed highlight the need to protect this lake trout fishery for both ecological and anthropogenic value.

Acknowledgments

Tables

Table 5.1. Lake trout sample collection information.

Lake	Latitude	Longitude	Region*	Count	Year	Month
Husky Lakes 2012	68.903114° N	133.086152° W	HLDB	16	2012	August
Husky Lakes 2014	68.903114° N	133.086152° W	HLDB	26	2014	May
Husky Lakes 2015	68.903114° N	133.086152° W	HLDB	63	2015	May
Jayko Lake	69.813043° N	103.188465° W	AEB	46	2012	September
Jimmy Lake	68.636269° N	133.531613° W	HLDB	32	2014	September
Noell Lake	68.520250° N	133.580990° W	HLDB	41	2014	September
Sandy Lake	67.799014° N	132.212055° W	MR	39	2005	August
Sitidgi Lake	68.555223° N	132.715046° W	HLDB	50	2015	September
Wolf Lake	69.216440° N	134.433241° W	MR	33	2014	September
Yukon Lake 105	69.286574° N	139.226804° W	YNS	33	2014	August

Table 5.2. Life history classifications based on the assessment of otolith Sr microchemistry profiles (Chapter 4).

	Life History Type*			
Lake	**SA**	**BWR**	**FWR**	**n**
Husky	33	150		183
Jimmy	1		19	20
Noell			27	27
Sitidgi	11		14	25
Total	**45**	**150**	**60**	**255**

*SA = semi-anadromous; BWR = brackish-water resident; and FWR = freshwater resident.

Table 5.3. Pairwise F_{ST} comparisons values based on microsatellite data among lake trout

sampling locations in the Western Canada Arctic (HL = Husky Lakes, YK = Yukon Lake, and

SA = semi-anadromous).

	HL 2012	HL 2014	HL 2015	SA	Jayko	Jimmy	Noell	Sandy	Sitidgi	Wolf
HL 2012										
HL 2014	**0.0129**									
HL 2015	0.0054	**0.0026**								
SA	0.0197	**0.0230**	**0.0196**							
Jayko	**0.0667**	**0.0646**	**0.0619**	**0.0813**						
Jimmy	**0.0652**	**0.0584**	**0.0565**	**0.0731**	**0.1138**					
Noell	**0.0493**	**0.0353**	**0.0339**	**0.0357**	**0.0906**	**0.0727**				
Sandy	**0.0707**	**0.0846**	**0.0710**	**0.0873**	**0.1038**	**0.1090**	**0.1047**			
Sitidgi	0.0285	**0.0240**	**0.0239**	-0.0009	**0.0807**	**0.0725**	**0.0348**	**0.0970**		
Wolf	**0.1256**	**0.1341**	**0.1255**	**0.1234**	**0.1473**	**0.1689**	**0.1516**	**0.1828**	**0.1293**	
YK 105	**0.4984**	**0.4580**	**0.3896**	**0.4437**	**0.4241**	**0.4843**	**0.4537**	**0.4513**	**0.4256**	**0.4662**

Bold values represent significance at $\alpha = 0.05$ and values that are underlined represent

significance following false discovery rate corrections ($p < 0.0108$).

Table 5.4. Counts of life history type for each location, and migrants and non-migrants classified with GENECLASS software, using predetermined life history classification from otolith microchemistry analysis of strontium and capture location information.

Location	Life History*	Non-Migrants	Migrants	Total	% Migrants	Origin of Migrants			
						Husky Lakes	Jimmy Lake	Noell Lake	Sitidgi Lake
Husky Lakes	SA	2	20	22	90.9				20
Husky Lakes	BWR	100	5	105	4.8			2	3
Jimmy Lake	SA	0	1	1	100.0				1
Jimmy Lake	FWR	14	2	16	12.6		2		
Jimmy Lake	UK	15	0	15	0				
Noell Lake	FWR	16	0	16	0.0				
Noell Lake	UK	25	0	25	0.0				
Sitidgi Lake	SA	6	3	9	33.3	3			
Sitidgi Lake	FWR	14	1	15	6.7	1			
Sitidgi Lake	UK	26	0	26	0.0				
					Total FGM =	4	2	3	24

* Freshwater resident (FWR), semi-anadromous (SA), and brackish-water resident (BWR). Unknown (UK) life histories are individuals where genetic data are present but microchemistry was not conducted.

Figures
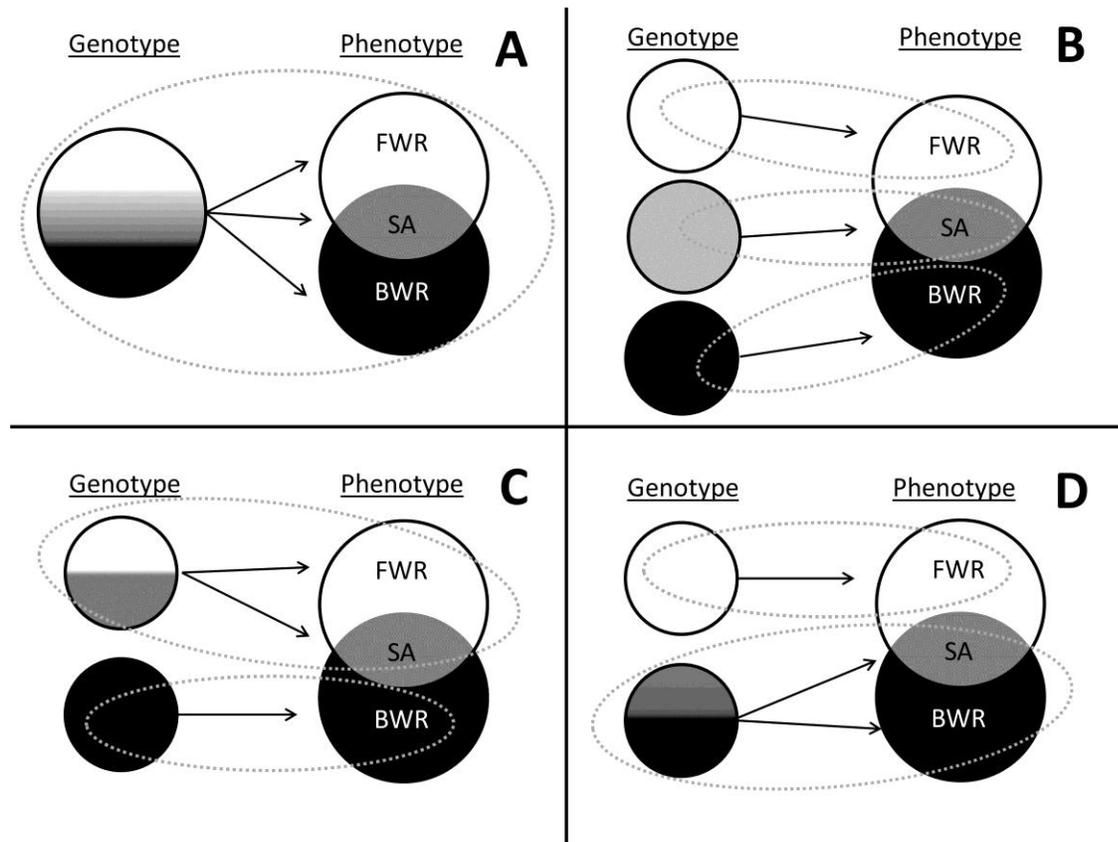
Figure 5.1. Possible lake trout genetic structuring within Husky Lakes drainage basin (HLDB) among life history types (freshwater resident (FWR), semi-anadromous (SA), brackish-water resident (BWR)) and ecosystems (solely fresh water (white), brackish water and fresh water (gray), and solely brackish water (black)). The gray dashed lines represent distinct hypothetical populations. A. All life history types are genetically similar and considered one genetic population within the HLDB. B. Each life history type is genetically differentiated and considered three genetic populations within the HLDB. C. FWR and SA are genetically similar and BWR are genetically differentiated and considered two populations within the HLDB. D. FWR are genetically differentiated and SA and BWR are genetically similar and considered two populations within the HLDB.
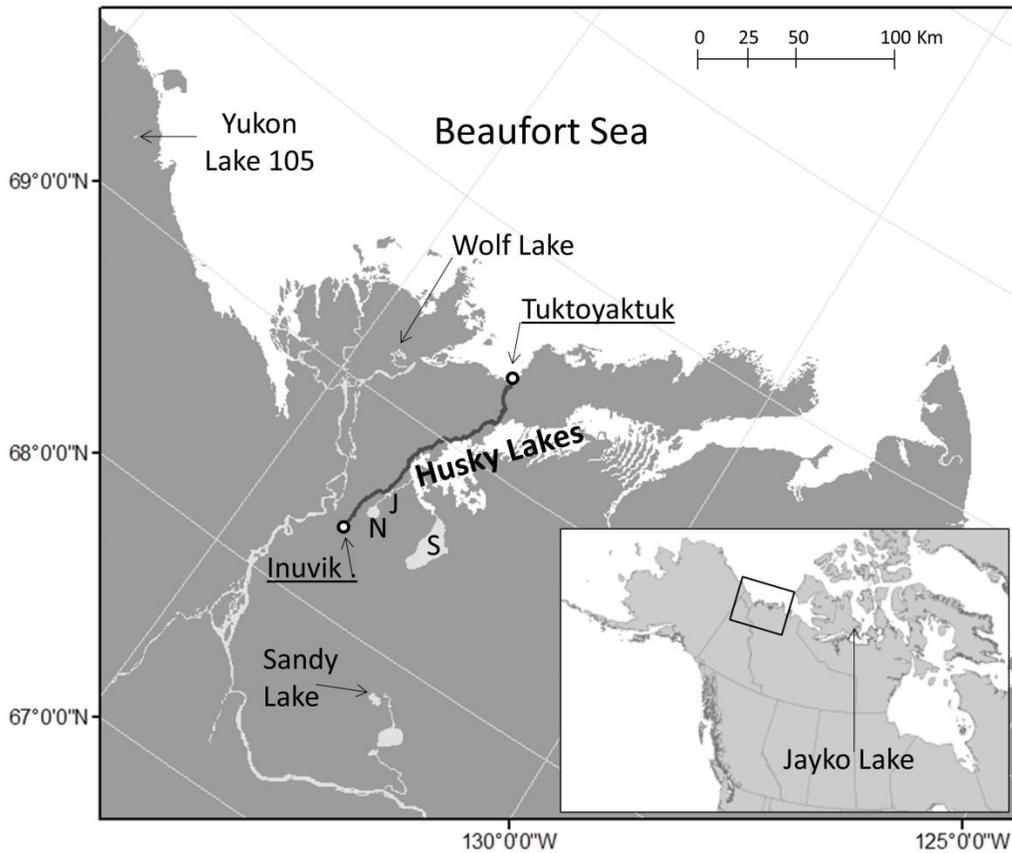
Figure 5.2. Sampling locations of lake trout assessed in the present study. The black line represents the proposed location of the Inuvik to Tuktoyaktuk Highway which is under construction. N = Noell Lake, J = Jimmy Lake and S = Sitidgi Lake.
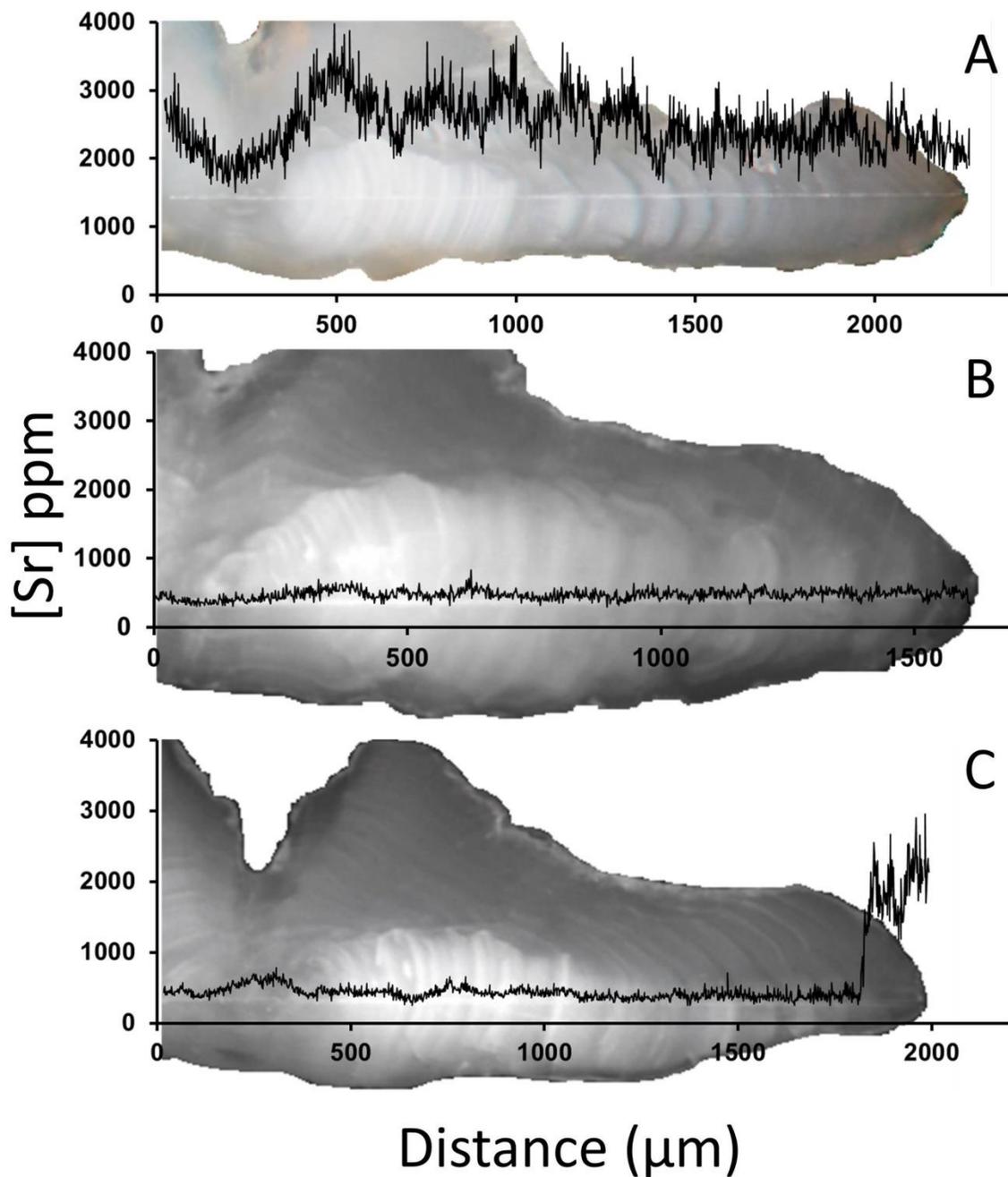
Figure 5.3. Examples of life history classifications based on otolith Sr microchemistry profiles (Kissinger et al. 2016). A. Brackish-water resident captured in Husky Lakes, NT; B. Freshwater resident captured in Noell Lake, NT; C. Semi-anadromous fish captured in Husky Lakes. Concentrations of Sr > 1000 ppm were used to infer occupancy of brackish water.
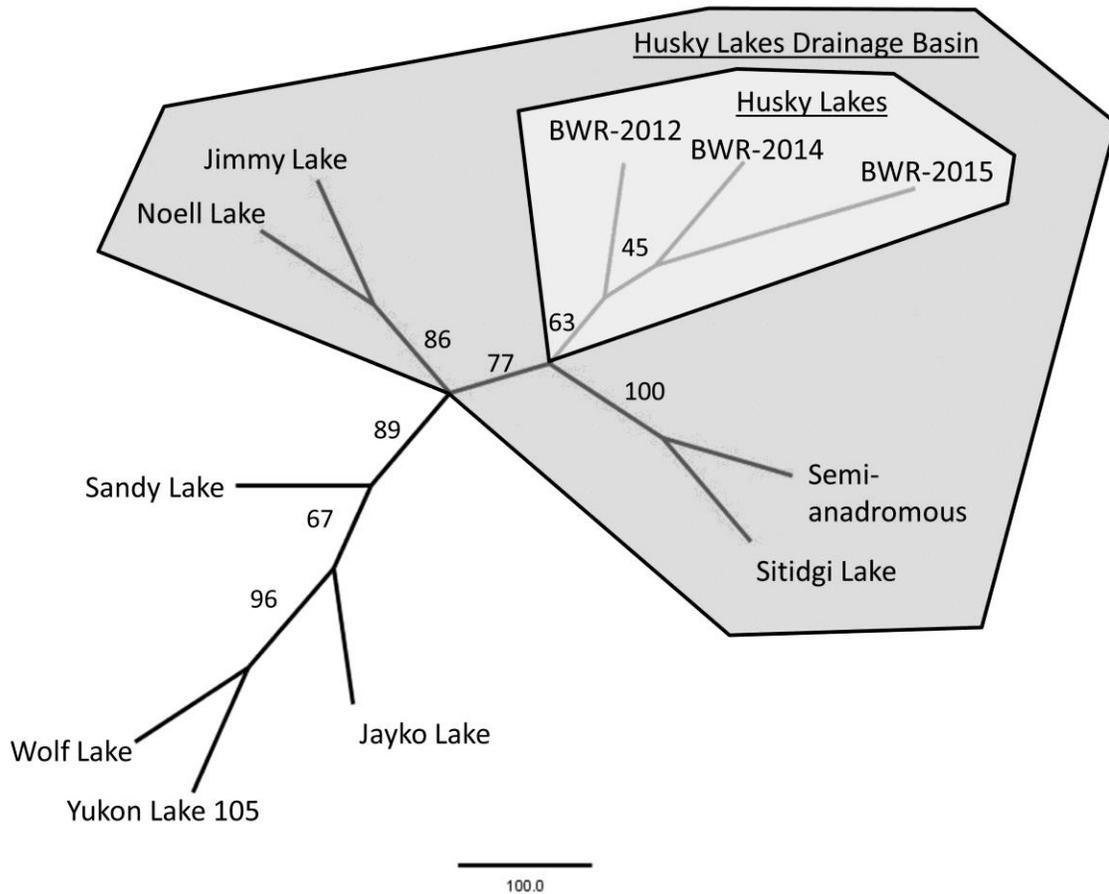
Figure 5.4. Neighbour-joining tree based on Cavalli-Sforza and Edwards' (1967) chord distance for lake trout from the Western Arctic of Canada. The darker shading includes samples from within the Husky Lakes drainage basin and the lighter shading indicates samples collected within Husky Lakes over three years. Bootstrap support is represented as a percentage along each branch; BWR = brackish-water resident.
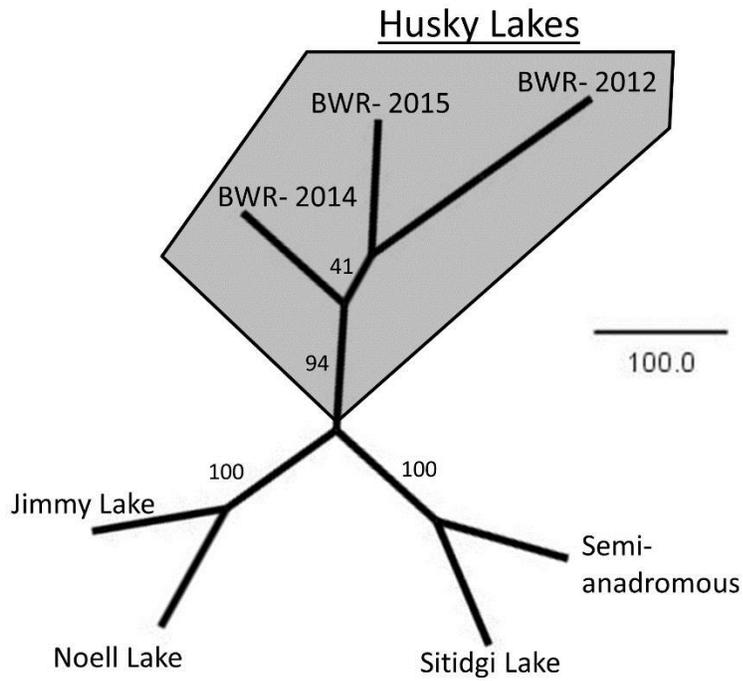
Figure 5.5. Neighbour-joining tree based on Cavalli-Sforza and Edwards' (1967) chord distance for lake trout within the Husky Lakes drainage basin. The shaded region represents samples collected within Husky Lakes over three years. Bootstrap support is indicated by numbers along each branch; BWR = brackish-water resident.
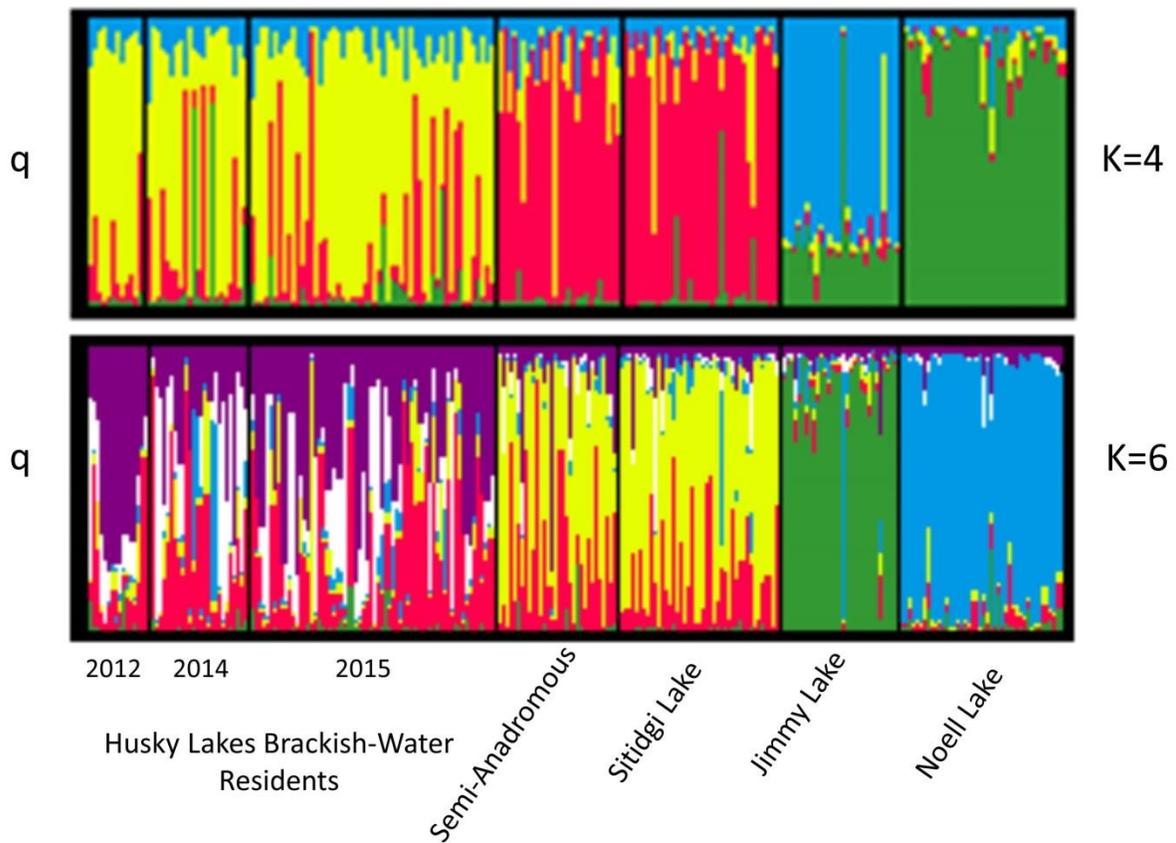
Figure 5.6. Results of the hierarchical Bayesian clustering analysis implemented in STRUCTURE inferred using the ln P[D] (K = 4) and ΔK statistic (K = 6). Individuals within each lake are represented by a single vertical bar broken into four or six colored segments. The length of each color within the bar represents the strength of the genetic assignment of that individual to a population cluster. Semi-anadromous and brackish-water resident life histories were classified using otolith microchemistry as described in Figure 5.3.

Chapter 6 - Discussion

Summary of key findings

The goal of this thesis was to examine mechanisms involved in the formation and maintenance of biodiversity within species. Through my research assessing otolith microchemistry, I documented multiple lake trout life history types within the Husky Lakes drainage basin (HLDB) including freshwater resident, semi-anadromous, and brackish-water resident (Kissinger et al. 2016). This expands the known distribution of semi-anadromy in lake trout and was the first documentation of a brackish-water resident life history type for lake trout and the genus *Salvelinus* (Kissinger et al. 2016). Through these initial findings, I suggest that spawning occurs in two different environments within the HLDB, including fresh and brackish water (Kissinger et al. 2016). Until this research, no documentation or experimentation on brackish-water spawning has been recorded for lake trout. I documented that lake trout can survive spawning and early development in brackish water up to 5 practical salinity units (psu) within the laboratory (Chapter 3). Following one to two years of development in discrete environments (0 or 5 psu), and transfer to 20 psu salt water, results indicated that genetically similar lake trout raised in 0 or 5 psu responded differently when assessed across multiple parameters (Chapter 3). Specifically, I found that lake trout reared in 5 psu water outperformed those raised in 0 psu water in their ability to ionoregulate, suggesting environment-phenotype interactions (Chapter 3). While differences were observed among laboratory treatments, both had the ability to ionoregulate in part due to the upregulation of key enzymes ($Na^+ K^+$-ATPase) and changes in behaviour (increased drinking rates in 20 psu, Chapter 3) demonstrating similar levels of phenotypic plasticity when compared to more saltwater associated relatives (Bystriansky et al. 2006, 2007). Following this I found significant differences in growth rates and longevities

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among life history types found in the HLDB, indicating that brackish-water resident lake trout grow faster and live longer than do semi-anadromous and freshwater resident life history types (Chapter 4). Differences in growth rates and longevity in brackish-water resident life history types suggest that both the environment and life history may be influencing differences observed. I also found that brackish-water resident lake trout were genetically differentiated from sympatric semi-anadromous life history types and all other locations assessed, supporting the initial otolith microchemistry data indicating segregation among spawning habitats and the potential for environment-phenotype interactions and possible local adaptation over the long-term (Kissinger et al. 2016, Chapter 5). From the research conducted, three principal factors have emerged that appear to influence the biodiversity observed, namely: 1) environmental gradients (biotic and abiotic), 2) phenotypic plasticity, and 3) adaptation.

Influence of environmental gradients

Within the HLDB, both biotic and abiotic gradients are observed (Roux et al. 2014). Assessment of species presence and within-lake diversity suggest the abiotic environment significantly influenced species distribution (Roux et al. 2016). Within this ecosystem, species presence was most strongly influenced by the salinity gradient, which is perhaps not surprising as salinity tolerance among teleosts is highly variable (Altinok and Grizzle 2001, Hiroi and McCormick 2007). While species physiology may restrict movements and residency throughout the HLDB, many common freshwater species (i.e., lake whitefish *Coregonus clupeaformis*, Arctic grayling *Thymallus arcticus*, round whitefish *Prosopium cylindraceum*, broad whitefish *Coregonus nasus*, northern pike *Esox lucius*, burbot *Lota lota*, and lake trout *Salvelinus namaycush*) were documented within Husky Lakes (Roux et al. 2014) in locations nearing or exceeding their reported distributional and physiological limits (Scott and Crossman 1973,

Hendry and Stearns 2004). These observations suggest that there is likely some benefit to inhabiting saline waters that are at the extremes of a species biological norm.

Comparisons of species diversity, species richness and catch per unit effort (CPUE) between Sitidgi Lake (freshwater) and Husky Lakes suggested that Husky Lakes had greater species diversity, CPUE, and abundance of important forage species (i.e., Arctic cisco *Coregonus autumnalis* and Pacific herring *Clupea pallasii*) (Roux et al. 2014, 2016). Thus, a greater resource base would increase growth rates observed, which was ultimately documented through faster growth rates in semi-anadromous and brackish-water resident lake trout when compared to freshwater resident conspecifics (Chapter 4). Additionally, the lack of brackish-water resident lake trout in any of the connected freshwater lakes assessed, and high proportions of freshwater hatched fish showing evidence of migration to the brackish water of Husky Lakes indicated a bias toward movement of lake trout to, or residence in, Husky Lakes (Kissinger et al. 2016 and Chapter 4 and 5). Lastly, brackish-water resident lake trout had increased longevity and dominated the demographic constituting between 84-86% of lake trout within Husky Lakes (Kissinger et al. 2016 and Chapter 4). In total these data suggest that there is a benefit to growth and longevity for lake trout migrating to or completing entire life cycles in the brackish water of Husky Lakes likely linked to the environmental gradient present.

Increased use of marine environments has been shown to be more prevalent in salmonids at higher latitudes. Finstad and Hein (2012) suggest a transition in the presence of anadromy in Arctic char along the Norwegian coast, where anadromy becomes more common north of the 65th parallel. Productivity differentials between freshwater systems and coastal regions were considered a major factor driving the presence of marine water use in Arctic char (Finstad and Hein 2012). They found that coastal marine environments had higher productivity than did

freshwater systems at more northern latitudes, and when physical barriers to movement (e.g.,

waterfall) and excessive distances were not present, marine water use in an anadromous fashion

was common (Finstad and Hein 2012). Many studies have shown that anadromous fish benefit

from migrations to marine environments as anadromous life history types typically obtain greater

sizes in comparison to freshwater resident conspecifics (see table 1 in Gross 1987). While the

observation of benefits to growth and longevity are evident for lake trout within this study and

salmonids in other locations, questions remain regarding how a typically freshwater species can

complete a semi-anadromous or brackish-water resident life history.

Phenotypic plasticity

In Chapter 3, I showed that lake trout raised from the same freshwater resident parental

cross that have likely not experienced salinities > 1 practical salinity unit (psu) in recent history

(last 10,000 years, Wilson and Hebert 1998), retained the ability to ionoregulate in salinities of

20 psu. In particular they were able to regulate key genes such as $Na^+ K^+$-ATPase α1a and α1b in

the gills, necessary for the production of $Na^+ K^+$-ATPase, an enzyme crucial to ionoregulation

(Chapter 3). Also, both treatment groups changed their behaviour by increasing drinking rates

when transferred to 20 psu salt water (Chapter 3). These data suggest that some level of

phenotypic plasticity allows lake trout to acclimatize to new environments and that mechanisms

necessary for life in brackish water are present in this predominantly fresh water species.

Interestingly unlike lake trout in my study, a more marine associated relative, Arctic char, was

unable to up-regulate gill $Na^+ K^+$-ATPase α1b when placed in a similar challenge subsequently

influencing whole body ionoregulation (Bystriansky et al. 2007). Within Bystriansky et al.

(2007), failure to up regulate gill $Na^+ K^+$-ATPase α1b and acclimatize to salt water was

considered in part due to the Arctic char being from a land locked freshwater population

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(Bystriansky et al. 2007), as further studies showed the species does possess the ability to up-regulate this gene (Bystriansky et al. 2006). I was also able to demonstrate that lake trout were able to survive fertilization and rearing in salinities of 5 psu which is not documented in any species within *Salvelinus* (Chapter 3). While this was observed, genetically similar lake trout reared in 5 psu outperformed conspecifics reared in 0 psu salinities suggesting the environment of early development influenced the capacity and rate of acclimatization in later years (environment-phenotype interactions, Chapter 3).

Similar to the portfolio effect described in ecosystems, where increased species diversity maintains greater stability (e.g., biomass) in the face of perturbation (Tilman 1999), increased physiological plasticity allows for survival in regions of heterogeneous and annually variable resources (i.e., forage and/ or spawning habitat) (Dodson et al. 2013, Klemetsen 2013). Thus, retention and maintenance of genes associated with traits specific to ecosystem use becomes beneficial (e.g., up and down regulation $Na^+ K^+$-ATPase α1a and α1b in the gills; Chapter 3). Species within the genus *Salvelinus* are recognized for their vast distribution and ability to survive in harsh climates; traits that are believed to be in part due to the high degree of phenotypic plasticity and local adaptation observed within its species (Muir et al. 2015). Within numerous populations of lake trout and Arctic char, multiple morphotypes (Jonsson and Johnsson 2001, Zimmerman et al. 2006, Chavarie et al. 2013) and life history types are observed (Swanson et al. 2010). While genetic differentiation is observed among some morphotypes and life histories, many are not genetically differentiated suggesting differences are in part influenced by phenotypic plasticity (Moore et al. 2014, Harris et al. 2015b, Baillie et al. 2016). Use of a range of salinities in an anadromous fashion is partly due to specific genes (Hecht et al. 2013), but the behaviour is complex and is also influenced by an individual's condition and interactions

with the biotic environment (Dodson et al. 2013). Based on my data I assume that part of lake trout's ability to thrive and survive in brackish water is associated with the degree of phenotypic plasticity observed within the species (Chapter 3), but documentation of genetic differentiation (Chapter 5), faster growth rates, and increased longevity (Chapter 4) indicate local adaptation may further influence the presence of a brackish water-resident life history type.

Adaptation

Documentation of significant genetic differentiation in neutral markers among sympatric lake trout life history types indicate that relatively high levels of reproductive isolation are present and have been established long enough to promote genetic differences in neutral markers (Chapter 5). Documentation of isolation in two discrete environments (fresh or brackish water) during early life (first 8 years, Kissinger et al. 2016) suggests adaptation(s) specific to the environment (brackish water) would be selected for (Chapter 5). While genes and traits associated with adaptation to increased salinities were not directly assessed in the field, the observation of faster growth rates, increased longevity (Chapter 4), higher proportions in regions of greater salinity, and higher proportions overall in Husky Lakes (84-86%, Kissinger et al. 2016) suggest local adaptation may influence differences observed. Though life history selection is complex, and is influenced by multiple genes (Hecht et al. 2013) in addition to phenotypic plasticity within traits (Dodson et al. 2013); the data presented suggest the brackish-water resident life history may be illustrative of early stages of adaptive radiation (Bernatchez 2004), ultimately leading to complete reproductive isolation and speciation (Bird et al. 2012). However, brackish-water residents are likely still far away from complete reproductive isolation and these processes can easily be influenced by changes in gene flow (Nosil et al. 2009, Bird et al. 2012).

Adaptive radiation is the evolution of ecological and phenotypic diversity within a rapidly multiplying lineage that occurs when a single ancestor diverges into a host of species that use a variety of environments (Bernatchez 2004). The process includes both speciation and phenotypic adaptation to divergent environments and subsequent species differ in morphological or physiological traits used to exploit those environments (Bernatchez 2004). Four key factors have been used to identify adaptive radiation among species including: 1) divergent species must share a common ancestor; 2) evidence of rapid speciation, relative to prior phylogeny; 3) species must use a variety of environments (phenotype-environment correlation); 4) there must be trait unity, or evidence that particular phenotypic trait(s) have enhanced performance in the environment in which they are associated with (Bernatchez 2004). Though significant genetic differentiation is observed I do not suggest brackish-water residents are a new species thus 1 and 2 do not apply, but 3 and 4 may. While brackish water use is observed in a semi-anadromous fashion in other locations, this is the only example to date where complete life cycles occur in brackish water, thus there is a phenotype-environment correlation (factor 3). As mentioned I do not know if morphological or physiological differences are observed in brackish water residents but the combination of Chapters 4 and 5 strongly suggest some adaptation may be present to complete life in brackish water, thus fulfilling factor 4. Adaptive radiation has been credited by many for the species diversity observed in salmonids and some believe this is associated with their colonization, specialization, and speciation in a variety of low productive freshwater environments (e.g., alpine streams, Arctic regions, and oligotrophic lakes, Bernatchez 2004), thus in the Husky Lakes lake trout, we may be observing the mid-stages of adaptive radiation within this diverse family of fishes.

Documentation of unique lake trout biodiversity

Lake trout are considered one of the least saline tolerant salmonid species based on their distribution, ecology and performance in laboratory experiments (Scott and Crossman 1973, Crespi and Fulton 2004, Hiroi and McCormick 2007, Alexandrou et al. 2013). Though lake trout are not known to be a highly migratory species and limited lotic use has been observed, lake trout have successfully colonized throughout North America ranging from the Laurentian Great Lakes to islands in the Arctic Archipelago (Scott and Crossman 1973, Muir et al. 2015). While the presence of lake trout on islands in the Arctic Archipelago would suggest that some marine water use would be necessary (Lindsey 1964), little documentation of brackish or marine water use have occurred (Swanson et al. 2010, Roux et al. 2014). Though most marine regions surrounding lake trout's distribution have salinities between 30-35 psu, it is important to note that during deglaciation freshwater input from glaciers and changes in currents reduced water salinity in many Arctic regions (Ślubowska et al. 2005) and would have allowed for colonization of islands in the Arctic archipelago through lower salinities than currently observed.

The lack of marine water use in many regions where access is present may be due to low numbers of assessments (i.e., difficulties sampling Arctic regions which constitute a large proportion of their coastal interface) or a lack of its presence. If use of marine water in lake trout is truly restricted to a few select locations, its absence may also be influenced by lake trout's ability to survive in extremely low productive oligotrophic environments (Scott and Crossman 1973) as lake trout are typified by their slow growth rates and impressive longevity (Roux et al. 2014; Chavarie et al. 2016). Marine water use in an anadromous or semi-anadromous fashion is only observed in locations where there is a net benefit in migrating to marine environments and is often influenced by: differences in productivity between environments, costs associated with physiological changes in order to survive in the new environment, and travel distance between

environments (Finstad and Hein 2012). Since lake trout have adapted to survive and thrive in low productivity freshwater lakes (Scott and Crossman 1973) and have reduced ionoregulatory capabilities compared to other salmonids (Hiroi and McCormick 2007), benefits of migrations to marine waters may not outweigh the costs (Finstad and Hein 2012). Interestingly, semi-anadromy and brackish-water residency were observed at the more northern extent of their distribution and would suggest productivity gradients similar to those observed in Finstad and Hein (2012) do play a role in life history distribution within lake trout.

While lake trout are arguably one of the most diverse vertebrate species on earth, due to the various morphologies, life histories, breeding methods, and colourations documented, it appears their use of marine environments is limited compared to other salmonids (Muir et al. 2015). Thus, the documentation of a brackish-water resident life history type is novel for lake trout and this life history is not widespread within the family. Observation of this unique life history type within lake trout brings into question, why is it present only in Husky Lakes or is this life history type much more prevalent than assumed but simply has not been documented due to a lack of assessment? It is extremely difficult to say with certainty, as monitoring of Arctic fisheries where we would more likely anticipate its presence and which represent a large proportion of their distribution, receives less attention than southern fisheries (Reist et al. 2016), however, it is also important to note that the extent, range of the salinity gradient, and long-term stability in Husky Lakes may play a large role in determining this behaviour and thus the resulting brackish-water resident life history.

The relatively low (1 to 17 psu) and fairly stable (change in salinity ± 2 psu, Macdonald et al. 1999) salinity gradient within Husky Lakes differs greatly from locations in the nearby Mackenzie Delta, where salinity near the river mouth was documented to change from 0 psu to

20 psu over the course of 20 days at the same location (see Figure 10 and 11, Macdonald et al. 1999). Maintenance of a relatively stable annual salinity gradient within Husky Lakes can be attributed to three major factors: 1) low and constant input of fresh and marine water; 2) small size of the drainage basin (less extreme pulses in flow); and 3) the bathymetry (numerous peninsulas and narrow channels) (Macdonald et al. 1999). Due to this relatively stable brackish-water environment, developing lake trout eggs would experience heightened but stable water salinity throughout development. Development in fall spawning salmonids takes in excess of 3 months and following hatch yolk-sac larvae are restricted in movement and are subject to changes in the abiotic environment. Since laboratory studies have shown that ionoregulatory and osmoregulatory processes are reduced in young salmonids (McCormick and Saunders 1987), I would predict extreme changes (i.e., 0 to 20 psu) to be detrimental to survival thus restricting where spawning can occur. Support for this is provided by the inability of lake trout eggs to develop and hatch in salinities > 5 psu, suggesting developmental thresholds in salinity may be present in lake trout (Chapter 3). In addition, unlike most full strength Arctic marine waters, Husky Lakes maintains regions above 0 ^{o}C, due to the maintenance of stable low salinities, warm freshwater input (relative to the Arctic Ocean), and a large lake volume (Carmack and Macdonald 2008). In salmonids, temperature is a major determinant of embryogenesis so much so that a standard metric to assess development in salmonids (and most fishes) is "degree days" (Peterson et al. 1974). In regions, such as the Arctic where water temperatures near 0 ^{o}C, development time increases and at some point temperature influences developmental rates so much that developing embryos cannot complete the process. In addition, if temperatures go sub-zero, special adaptations to prevent freezing are required for survival which are not documented in salmonids, hence why over wintering in fresh water or low salinity water is observed in all

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Arctic salmonid species (Hendry and Stearns 2004). The relatively stable salinity gradient and temperatures that are maintained above 0 °C in Husky Lakes provide an environment where completion of entire life cycles in brackish water is possible.

While many estuaries are observed throughout North America, ones that meet the criteria to support brackish-water resident lake trout populations are likely infrequent. Regions throughout the world where brackish-water resident life histories and brackish-water spawning in salmonids are documented appear only in select areas within species distributions. Documentation of brackish-water spawning by pink salmon *Oncorhynchus gorbuscha* near the mouths of rivers in Prince William Sound, Alaska indicate that spawning likely occurred in the intertidal regions of river mouths at near 0 psu salinity, but this region was exposed to intrusions of tidal salinity up to 20 psu (Helle et al. 1964). In this species the eggs are buried in the gravel through the preparation of redds (Scott and Crossman 1973) and site selection occurs in regions where ground water intrusions occur (Helle et al. 1964). Thus, in these intertidal areas changes in salinity within the gravel are buffered by ground water sources keeping eggs near 0 psu (Helle et al. 1964). In addition, spawning in pink salmon occurs in the summer where water temperatures are warmer (Helle et al. 1964), thus development and degree-days necessary for successful hatch (2-4 weeks, Scott and Crossman 1973) are lower than fall spawning fish, resulting in reduced exposure time to variable salinities during this particularly sensitive life history stage. This suggests that the presence of ground water and the time of spawning likely influence successful development in intertidal waters.

Brackish-water resident life history has also been observed in European whitefish in the Baltic Sea (Himberg and Lehtonen 1995). Similar to Husky Lakes the Baltic Sea maintains low saline environments (surface water < 11 psu, Herlemann et al. 2011) that would allow for

development to occur at slightly elevated (1-11 psu) but stable salinities. Furthermore, in a close

relative and predominantly freshwater species, northern pike *Esox lucius,* brackish-water resident

life history types have only been documented in the Baltic Sea (Engstedt et al. 2010). Based on

the documented occurrences of brackish-water residency among species, it appears that this form

of biodiversity is restricted to select regions of low (near or lower than isosmotic) but stable

salinities during the spawning and early developmental periods where temperatures are above 0

^{o}C (Helle et al. 1964, Himberg and Lehtonen 1995, Kissinger et al. 2016).

Though it appears the abiotic environment plays a major role in the presence of the

brackish-water resident life history type, local adaptation also aids in the establishment of this

life history (Chapters 2, 4, and 5; Papakostas et al. 2012). While common freshwater species like

northern pike and European whitefish are documented to complete entire life cycles in the Baltic

Sea, a stocking effort using lake trout from Lake Superior had poor results to the point that

Finnish fisheries managers stated, "the species should be considered unsuitable for Finnish

marine conditions" (Mutenia et al. 1984). This suggests that though the environmental conditions

of the Baltic Sea may be conducive to brackish-water residency in salmonids and other

freshwater species, Lake Superior strain lake trout which have not likely experienced marine

water conditions in recent history (Wilson and Hebert 1998), lack the adaptive traits to complete

life in brackish water, at least to the level found in the Baltic Sea. While lake trout from Husky

Lakes are not technically geographically restricted from the Baltic Sea, numerous physiological

barriers (e.g., salinity, temperature, and distance) likely preclude the colonization of the Baltic

Sea from Husky Lakes. This being said, a higher presence of marine water use in Arctic North

America (Swanson et al. 2010, Kissinger et al. 2016) suggest that increased adaptation to marine

water could be more common in Arctic populations, thus brackish-water resident life history types may be more widespread than previously documented.

Management implications

Lake trout within Husky Lakes are highly valued by subsistence fishers from Inuvik and Tuktoyaktuk. Currently hook-and-line angling is restricted to the winter months when access to the lakes is possible via snowmobile. With the construction of the Inuvik to Tuktoyaktuk Highway which passes near (1 km) Husky Lakes, increased access will allow for year round fishing opportunities including during the spawning period when lake trout are highly vulnerable. As lake trout are vulnerable to over harvest in part due to their slow growth rates and delayed maturation, consideration of precautionary measures should be taken with this potential increased fishing pressure (Muir et al. 2013). Documentation of slow recovery following wide spread extirpation in the mid-1900's in the Laurentian Great Lakes, historically the World's largest lake trout fishery, suggests that the loss of locally adapted populations has hampered efficient and successful restoration of this fishery (Krueger and Ihssen 1995, Muir et al. 2013). In addition, changes to food web dynamics may have reduced genetic structure present among morphotypes creating a state closer to panmixia (Baillie et al. 2016). Thus, lake trout are vulnerable to overharvest and the presence of local adaptation would make recovery of extirpated or over harvested fisheries even more challenging.

Based on my research, Husky lakes should be considered a mixed stock fishery possessing at least two sympatric populations consisting of two life history types (semi-anadromous and brackish-water residents, Chapter 5). Thus, increased harvest of fish within Husky Lakes may in fact be influencing population levels in Sitidgi Lake, as most semi-anadromous lake trout were of Sitidgi Lake origin (Chapters 4, 5). Fish movement occurs among

all lakes assessed within the Husky Lakes drainage basin (Jimmy, Noell, Sitidgi, and Husky Lakes) and loss of connectivity could impact population dynamics, disproportionately affecting semi-anadromous life history types (Chapters 2, 4, 5). Due to differences in growth rates and longevity among life history types, caution should be taken if setting length limits, as size or age at maturation will differ among life history types creating harvest bias (Chapter 4). In addition, indications that brackish-water residents are locally adapted to Husky Lakes, suggest reestablishment of this population following extirpation or significant reduction could prove difficult. Thus, great care through monitoring programs and regulations (e.g., harvest limits and/or seasonal closures) should be taken to preserve this unique lake trout biodiversity and fishery for future generations of subsistence fishers and scientists (Chapters 2, 4, 5).

Concluding statements

My research into the formation and maintenance of this biodiversity within species suggest that environmental gradients (biotic and abiotic), phenotypic plasticity, and adaptation are key factors influencing its presence. While phenotypic plasticity influences survival of brackish-water residents in Husky Lakes, the lack of this life histories documentation elsewhere strongly suggests either the environment and/or local adaptations play a larger role in its presences. In saying this, due to limited assessment of Arctic ecosystems, there is potential for brackish-water residents elsewhere as recent research into brackish-water residency and semi-anadromy in Arctic lake trout populations suggest that lake trout are more saline tolerant than previously thought. This highlights the need to expand research in Arctic regions, and demonstrates that adaptations and regional environmental differences can change our perspective on a species' physiological capabilities. Lastly, these findings expand our understanding of lake

trout and *Salvelinus* biodiversity and further support the notion that lake trout are one of the most

diverse vertebrate species on earth (Muir et al. 2015).

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Supplementary Appendix 1.

Parameter estimates from a three-way ANOVA associated with the direct transfer experiment conducted on lake trout reared two rearing environments (env.) freshwater (FW) or brackish-water (BW) and transferred to one of three transfer environments (env.) of 0, 5 or 20 ppt salinities and assessed at four time points (zero, one, seven, and fourteen).

Length

Parameter	Estimate	SE	t	Pr(>|t|)
(Intercept)	6.31	0.40	15.72	<2e-16
Rearing Env. (FW)	-0.84	0.57	-1.48	0.139
Time Point (one)	0.28	0.57	0.50	0.621
Time Point (seven)	-0.16	0.57	-0.29	0.772
Time Point (zero)	0.44	0.57	0.77	0.442
Transfer Env. (20)	0.46	0.58	0.79	0.43
Transfer Env. (0)	0.24	0.58	0.41	0.679
Rearing Env. (FW) · Time Point (one)	0.49	0.80	0.61	0.545
Rearing Env. (FW) · Time Point (seven)	0.24	0.80	0.30	0.767
Rearing Env. (FW) · Time Point (zero)	-0.30	0.80	-0.37	0.71
Rearing Env. (FW) · Transfer Env. (20)	-1.05	0.81	-1.29	0.199
Rearing Env. (FW) · Transfer Env. (0)	-0.03	0.81	-0.04	0.969
Time Point (one) · Transfer Env. (20)	-1.06	0.81	-1.30	0.195
Time Point (seven) · Transfer Env. (20)	0.05	0.81	0.06	0.949
Time Point (one) · Transfer Env. (0)	-0.85	0.81	-1.04	0.299
Time Point (seven) · Transfer Env. (0)	-0.61	0.81	-0.75	0.457
Rearing Env. (FW) · Time Point (one) · Transfer Env. (20)	0.47	1.14	0.41	0.68
Rearing Env. (FW) · Time Point (seven) · Transfer Env. (20)	-0.14	1.14	-0.12	0.903
Rearing Env. (FW) · Time Point (one) · Transfer Env. (0)	0.22	1.14	0.19	0.849
Rearing Env. (FW) · Time Point (seven) · Transfer Env. (0)	1.06	1.14	0.93	0.354

Log$_{(10)}$ Weight

Parameter	Estimate	SE	t	Pr(>|t|)
(Intercept)	0.79	0.03	27.46	<2e-16
Rearing Env. (FW)	-0.06	0.04	-1.47	0.14
Time Point (one)	0.02	0.04	0.52	0.60
Time Point (seven)	-0.01	0.04	-0.36	0.72
Time Point (zero)	0.02	0.04	0.51	0.61
Transfer Env. (20)	0.02	0.04	0.57	0.57
Transfer Env. (0)	0.01	0.04	0.26	0.79
Rearing Env. (FW) · Time Point (one)	0.03	0.06	0.58	0.57
Rearing Env. (FW) · Time Point (seven)	0.02	0.06	0.36	0.72
Rearing Env. (FW) · Time Point (zero)	-0.02	0.06	-0.31	0.76
Rearing Env. (FW) · Transfer Env. (20)	-0.07	0.06	-1.25	0.21
Rearing Env. (FW) · Transfer Env. (0)	0.01	0.06	0.13	0.90
Time Point (one) · Transfer Env. (20)	-0.08	0.06	-1.35	0.18
Time Point (seven) · Transfer Env. (20)	0.01	0.06	0.21	0.84
Time Point (one) · Transfer Env. (0)	-0.06	0.06	-1.04	0.30
Time Point (seven) · Transfer Env. (0)	-0.05	0.06	-0.88	0.38
Rearing Env. (FW) · Time Point (one) · Transfer Env. (20)	0.04	0.08	0.46	0.65
Rearing Env. (FW) · Time Point (seven) · Transfer Env. (20)	-0.02	0.08	-0.23	0.82
Rearing Env. (FW) · Time Point (one) · Transfer Env. (0)	0.01	0.08	0.12	0.91
Rearing Env. (FW) · Time Point (seven) · Transfer Env. (0)	0.07	0.08	0.82	0.41

Osmolality

Parameter	Estimate	SE	t	Pr(>|t|)
(Intercept)	318.40	6.81	46.76	<2e-16
Rearing Env. (FW)	-4.20	9.63	-0.44	0.66
Time Point (one)	12.40	9.63	1.29	0.20
Time Point (seven)	-4.60	9.63	-0.48	0.63
Time Point (zero)	-8.40	9.63	-0.87	0.39
Transfer Env. (20)	19.00	9.63	1.97	0.05
Transfer Env. (0)	-6.80	9.63	-0.71	0.48
Rearing Env. (FW) · Time Point (one)	-21.40	13.62	-1.57	0.12
Rearing Env. (FW) · Time Point (seven)	10.60	13.62	0.78	0.44
Rearing Env. (FW) · Time Point (zero)	-31.63	12.97	-2.44	0.01
Rearing Env. (FW) · Transfer Env. (20)	-1.80	13.62	-0.13	0.90
Rearing Env. (FW) · Transfer Env. (0)	3.00	13.62	0.22	0.83
Time Point (one) · Transfer Env. (20)	-1.60	13.62	-0.12	0.91
Time Point (seven) · Transfer Env. (20)	4.20	13.62	0.31	0.76
Time Point (one) · Transfer Env. (0)	-11.20	13.62	-0.82	0.41
Time Point (seven) · Transfer Env. (0)	-13.60	13.62	-1.00	0.32
Rearing Env. (FW) · Time Point (one) · Transfer Env. (20)	46.80	19.26	2.43	0.01
Rearing Env. (FW) · Time Point (seven) · Transfer Env. (20)	33.80	19.26	1.76	0.08
Rearing Env. (FW) · Time Point (one) · Transfer Env. (0)	-10.40	19.26	-0.54	0.59
Rearing Env. (FW) · Time Point (seven) · Transfer Env. (0)	10.80	19.26	0.56	0.58

% Water Content

Parameter	Estimate	SE	t	Pr(>|t|)
(Intercept)	75.98	0.46	164.95	2.00E-16
Rearing Env. (FW)	0.65	0.62	1.05	0.30
Time Point (one)	0.88	0.62	1.42	0.16
Time Point (seven)	1.66	0.62	2.67	0.01
Time Point (zero)	0.26	0.62	0.43	0.67
Transfer Env. (20)	0.05	0.63	0.08	0.93
Transfer Env. (0)	1.73	0.63	2.72	0.01
Rearing Env. (FW) · Time Point (one)	0.51	0.86	0.60	0.55
Rearing Env. (FW) · Time Point (seven)	-0.87	0.86	-1.02	0.31
Rearing Env. (FW) · Time Point (zero)	1.56	0.86	1.82	0.07
Rearing Env. (FW) · Transfer Env. (20)	-0.11	0.87	-0.12	0.90
Rearing Env. (FW) · Transfer Env. (0)	-1.77	0.87	-2.04	0.04
Time Point (one) · Transfer Env. (20)	0.12	0.87	0.14	0.89
Time Point (seven) · Transfer Env. (20)	-1.21	0.87	-1.39	0.17
Time Point (one) · Transfer Env. (0)	-0.64	0.87	-0.74	0.46
Time Point (seven) · Transfer Env. (0)	-2.18	0.87	-2.52	0.01
Rearing Env. (FW) · Time Point (one) · Transfer Env. (20)	-2.33	1.20	-1.94	0.05
Rearing Env. (FW) · Time Point (seven) · Transfer Env. (20)	-0.95	1.20	-0.79	0.43
Rearing Env. (FW) · Time Point (one) · Transfer Env. (0)	1.07	1.20	0.89	0.37
Rearing Env. (FW) · Time Point (seven) · Transfer Env. (0)	3.16	1.21	2.62	0.01

Log$_{(10)}$ Gill Na$^+$ K$^+$-ATPase activity

Parameter	Estimate	SE	t	Pr(>|t|)
(Intercept)	-0.23	0.05	-4.23	4.26E-05
Rearing Env. (FW)	-0.03	0.08	-0.39	0.69
Time Point (one)	0.07	0.08	0.91	0.37
Time Point (seven)	-0.01	0.08	-0.08	0.94
Time Point (zero)	0.02	0.08	0.31	0.76
Transfer Env. (20)	0.37	0.08	4.88	2.85E-06
Transfer Env. (0)	-0.14	0.08	-1.91	0.06
Rearing Env. (FW) · Time Point (one)	0.00	0.11	0.03	0.97
Rearing Env. (FW) · Time Point (seven)	0.11	0.11	1.03	0.30
Rearing Env. (FW) · Time Point (zero)	0.02	0.11	0.23	0.82
Rearing Env. (FW) · Transfer Env. (20)	-0.16	0.11	-1.53	0.13
Rearing Env. (FW) · Transfer Env. (0)	0.23	0.11	2.12	0.04
Time Point (one) · Transfer Env. (20)	-0.54	0.11	-5.03	1.46E-06
Time Point (seven) · Transfer Env. (20)	-0.24	0.11	-2.22	0.03
Time Point (one) · Transfer Env. (0)	0.16	0.11	1.52	0.13
Time Point (seven) · Transfer Env. (0)	0.21	0.11	1.93	0.06
Rearing Env. (FW) · Time Point (one) · Transfer Env. (20)	0.36	0.15	2.40	0.02
Rearing Env. (FW) · Time Point (seven) · Transfer Env. (20)	0.11	0.15	0.70	0.48
Rearing Env. (FW) · Time Point (one) · Transfer Env. (0)	-0.23	0.15	-1.51	0.13
Rearing Env. (FW) · Time Point (seven) · Transfer Env. (0)	-0.37	0.15	-2.44	0.02

Log$_{(10)}$ Kidney Na$^+$ K$^+$-ATPase activity				
Parameter	Estimate	SE	t	Pr(>|t|)
(Intercept)	-2.18	0.05	-45.86	< 2e-16
Rearing Env. (FW)	-0.10	0.07	-1.43	0.15
Time Point (one)	-0.26	0.07	-3.91	1.42E-04
Time Point (seven)	-0.13	0.07	-1.92	0.06
Time Point (zero)	-0.49	0.07	-7.23	2.82E-11
Transfer Env. (20)	-0.08	0.07	-1.26	0.21
Transfer Env. (0)	-0.14	0.07	-2.15	0.03
Rearing Env. (FW) · Time Point (one)	0.19	0.09	2.03	0.04
Rearing Env. (FW) · Time Point (seven)	0.03	0.09	0.32	0.75
Rearing Env. (FW) · Time Point (zero)	0.56	0.09	5.91	2.51E-08
Rearing Env. (FW) · Transfer Env. (20)	0.23	0.09	2.39	0.02
Rearing Env. (FW) · Transfer Env. (0)	0.12	0.09	1.24	0.22
Time Point (one) · Transfer Env. (20)	0.23	0.09	2.41	0.02
Time Point (seven) · Transfer Env. (20)	-0.04	0.09	-0.45	0.66
Time Point (one) · Transfer Env. (0)	0.32	0.09	3.42	8.00E-04
Time Point (seven) · Transfer Env. (0)	0.33	0.09	3.48	6.77E-04
Rearing Env. (FW) · Time Point (one) · Transfer Env. (20)	-0.27	0.13	-2.01	0.04
Rearing Env. (FW) · Time Point (seven) · Transfer Env. (20)	0.07	0.13	0.54	0.59
Rearing Env. (FW) · Time Point (one) · Transfer Env. (0)	-0.10	0.13	-0.75	0.46
Rearing Env. (FW) · Time Point (seven) · Transfer Env. (0)	-0.13	0.13	-0.97	0.33

Log$_{(10)}$ Gill Na$^+$ K$^+$-ATPase α1a expression

Parameter	Estimate	SE	t	Pr(>|t|)
(Intercept)	-0.08	0.05	-1.47	0.14
Rearing Env. (FW)	-0.14	0.07	-1.97	0.05
Time Point (one)	-0.09	0.07	-1.20	0.23
Time Point (seven)	0.01	0.07	0.08	0.94
Time Point (zero)	-0.05	0.07	-0.75	0.45
Transfer Env. (20)	-0.16	0.07	-2.25	0.03
Transfer Env. (0)	0.28	0.07	3.91	1.39E-04
Rearing Env. (FW) · Time Point (one)	0.48	0.10	4.65	7.10E-06
Rearing Env. (FW) · Time Point (seven)	-0.18	0.10	-1.71	0.10
Rearing Env. (FW) · Time Point (zero)	0.12	0.10	1.19	0.24
Rearing Env. (FW) · Transfer Env. (20)	0.03	0.10	0.29	0.77
Rearing Env. (FW) · Transfer Env. (0)	-0.05	0.10	-0.48	0.63
Time Point (one) · Transfer Env. (20)	0.13	0.10	1.20	0.23
Time Point (seven) · Transfer Env. (20)	-0.27	0.10	-2.61	0.01
Time Point (one) · Transfer Env. (0)	-0.20	0.10	-1.92	0.06
Time Point (seven) · Transfer Env. (0)	0.09	0.10	0.88	0.38
Rearing Env. (FW) · Time Point (one) · Transfer Env. (20)	-0.27	0.15	-1.82	0.07
Rearing Env. (FW) · Time Point (seven) · Transfer Env. (20)	0.19	0.15	1.31	0.19
Rearing Env. (FW) · Time Point (one) · Transfer Env. (0)	-0.01	0.15	-0.07	0.94
Rearing Env. (FW) · Time Point (seven) · Transfer Env. (0)	0.14	0.15	0.99	0.33

Log$_{(10)}$ Gill Na$^+$ K$^+$-ATPase α1b expression

Parameter	Estimate	SE	t	Pr(>|t|)
(Intercept)	0.35	0.06	5.63	8.38E-08
Rearing Env. (FW)	-0.12	0.09	-1.37	0.17
Time Point (one)	0.08	0.09	0.86	0.39
Time Point (seven)	-0.08	0.10	-0.80	0.43
Time Point (zero)	0.10	0.09	1.11	0.27
Transfer Env. (20)	0.49	0.09	5.54	1.30E-07
Transfer Env. (0)	0.00	0.09	-0.02	0.99
Rearing Env. (FW) · Time Point (one)	0.05	0.13	0.38	0.71
Rearing Env. (FW) · Time Point (seven)	0.24	0.14	1.71	0.09
Rearing Env. (FW) · Time Point (zero)	0.12	0.13	0.98	0.33
Rearing Env. (FW) · Transfer Env. (20)	-0.05	0.13	-0.42	0.68
Rearing Env. (FW) · Transfer Env. (0)	-0.08	0.13	-0.62	0.53
Time Point (one) · Transfer Env. (20)	-0.50	0.13	-3.90	1.45E-04
Time Point (seven) · Transfer Env. (20)	0.03	0.14	0.22	0.83
Time Point (one) · Transfer Env. (0)	-0.16	0.13	-1.27	0.21
Time Point (seven) · Transfer Env. (0)	0.30	0.14	2.19	0.03
Rearing Env. (FW) · Time Point (one) · Transfer Env. (20)	-0.25	0.18	-1.37	0.17
Rearing Env. (FW) · Time Point (seven) · Transfer Env. (20)	0.07	0.19	0.39	0.70
Rearing Env. (FW) · Time Point (one) · Transfer Env. (0)	0.38	0.18	2.16	0.03
Rearing Env. (FW) · Time Point (seven) · Transfer Env. (0)	-0.52	0.19	-2.79	0.01

Supplementary Appendix 2.

Basic descriptive statistics for 19 microsatellite loci for all sampling locations assessed showing the average number of alleles per locus (N_A), expected (H_E) and observed (H_O) heterozygosities, inbreeding coefficient (F_{IS}), and allelic richness (A_R).

Husky Lakes 2012	Ots G83	Sco 215	Smm 17	Sna MSU1	Sna MSU8	OMM 1105	Smm 22	Sna MSU13	Sna MSU5	SSOSL 456	Sco 19	Sco 202	Sna MSU10	Sna MSU12	Sna MSU6	Sal 38	Sco 200	Sna MSU11	Sna MSU3	Ave.
N_a	4	5	5	12	8	5	12	15	8	3	6	6	11	8	14	6	14	9	9	8.42
H_E	0.57	0.71	0.73	0.91	0.8	0.67	0.94	0.91	0.84	0.43	0.76	0.75	0.94	0.84	0.96	0.77	0.93	0.85	0.84	0.8
H_0	0.63	0.75	0.88	1	0.88	0.75	0.77	0.86	0.88	0.4	0.86	0.71	0.64	0.55	0.8	0.5	0.86	0.88	0.81	0.76
F_{is}	-0.11	-0.06	-0.2	-0.1	0.06	-0.32	0.18	0.06	-0.04	0.08	-0.12	0.04	0.32	0.36	0.17	0.35	0.08	-0.03	0.03	0.04
A_R	3.74	4.38	4.38	9.59	6.45	4.1	10.37	11.07	6.89	2.85	5.17	4.93	10.14	6.39	12.98	5.3	10.85	7.38	6.8	7.04

Husky Lakes 2014	Ots G83	Sco 215	Smm 17	Sna MSU1	Sna MSU8	OMM 1105	Smm 22	Sna MSU13	Sna MSU5	SSOSL 456	Sco 19	Sco 202	Sna MSU10	Sna MSU12	Sna MSU6	Sal 38	Sco 200	Sna MSU11	Sna MSU3	Ave.
N_a	4	6	8	15	11	5	14	24	15	3	8	9	17	9	22	10	20	11	14	11.48
H_E	0.69	0.74	0.78	0.91	0.81	0.7	0.92	0.94	0.86	0.48	0.68	0.59	0.94	0.8	0.96	0.79	0.95	0.8	0.91	0.8
H_0	0.72	0.79	0.76	0.92	0.64	0.58	0.89	0.88	0.81	0.5	0.68	0.56	0.87	0.8	0.86	0.68	0.95	0.95	0.59	0.76
F_{is}	-0.04	-0.03	0.02	-0.01	0.21	0.18	0.04	0.07	0.06	-0.04	0	0.06	0.07	0.01	0.1	0.14	0	-0.19	0.35	0.05
A_R	3.59	4.7	5.57	10.06	7.12	3.92	9.94	12.8	8.63	2.93	5.95	5.22	11.18	6.65	12.77	6.5	12.31	7.71	9.7	7.75

Husky Lakes 2015	Ots G83	Sco 215	Smm 17	Sna MSU1	Sna MSU8	OMM 1105	Smm 22	Sna MSU13	Sna MSU5	SSOSL 456	Sco 19	Sco 202	Sna MSU10	Sna MSU12	Sna MSU6	Sal 38	Sco 200	Sna MSU11	Sna MSU3	Ave.
N_a	8	6	7	20	12	6	16	33	20	3	11	11	23	11	28	15	28	11	16	15
H_E	0.59	0.76	0.71	0.92	0.79	0.66	0.92	0.96	0.84	0.34	0.7	0.71	0.93	0.79	0.96	0.8	0.95	0.83	0.85	0.79
H_0	0.6	0.72	0.74	0.89	0.7	0.69	0.92	0.9	0.76	0.32	0.76	0.68	0.77	0.66	0.71	0.84	0.81	0.85	0.71	0.74
F_{is}	-0.01	0.05	-0.04	0.02	0.11	-0.06	0	0.07	0.1	0.06	-0.1	0.04	0.17	0.16	0.25	-0.05	0.15	-0.02	0.16	0.06

	Ots G83	Sco 215	Smm 17	Sna MSU1	Sna MSU8	OMM 1105	Smm 22	Sna MSU13	Sna MSU5	SSOSL 456	Sco 19	Sco 202	Sna MSU10	Sna MSU12	Sna MSU6	Sal 38	Sco 200	Sna MSU11	Sna MSU3	Ave.
A_R	4.32	5.33	4.11	9.95	6.59	3.43	9.97	13.5	8.48	2.72	5.54	6.02	11.29	6.24	12.81	6.87	12.35	7.11	8.35	7.63

Semi-Anad.	Ots G83	Sco 215	Smm 17	Sna MSU1	Sna MSU8	OMM 1105	Smm 22	Sna MSU13	Sna MSU5	SSOSL 456	Sco 19	Sco 202	Sna MSU10	Sna MSU12	Sna MSU6	Sal 38	Sco 200	Sna MSU11	Sna MSU3	Ave.
N_a	7	5	9	18	11	6	16	21	19	3	11	10	17	13	22	10	16	10	16	12.63
H_E	0.6	0.76	0.72	0.93	0.87	0.55	0.91	0.93	0.92	0.39	0.8	0.77	0.93	0.78	0.94	0.83	0.9	0.76	0.85	0.8
H_0	0.42	0.69	0.58	0.97	0.83	0.5	0.93	0.85	0.73	0.39	0.79	0.79	0.81	0.76	0.76	0.87	0.88	0.83	0.75	0.74
F_{is}	0.3	0.09	0.19	-0.03	0.05	0.08	-0.02	0.09	0.2	-0.01	0.01	-0.04	0.12	0.03	0.19	-0.05	0.02	-0.08	0.11	0.07
A_R	4.31	4.46	5.3	11.09	7.86	4.18	9.9	11.23	10.62	2.86	7.21	6.67	10.82	7.37	12.1	6.99	9.95	6.24	8.99	7.8

Jayko Lake	Ots G83	Sco 215	Smm 17	Sna MSU1	Sna MSU8	OMM 1105	Smm 22	Sna MSU13	Sna MSU5	SSOSL 456	Sco 19	Sco 202	Sna MSU10	Sna MSU12	Sna MSU6	Sal 38	Sco 200	Sna MSU11	Sna MSU3	Ave.
N_a	7	6	4	9	9	8	16	20	9	2	9	6	23	10	28	10	18	12	16	11.68
H_E	0.74	0.7	0.53	0.85	0.76	0.73	0.9	0.93	0.66	0.18	0.76	0.72	0.94	0.81	0.95	0.54	0.93	0.85	0.9	0.76
H_0	0.79	0.65	0.51	0.91	0.72	0.8	0.78	0.93	0.67	0.16	0.7	0.72	0.93	0.76	0.93	0.56	0.95	0.92	0.91	0.75
F_{is}	-0.07	0.07	0.03	-0.07	0.05	-0.1	0.13	0	-0.02	0.15	0.09	0	0.01	0.07	0.02	-0.02	-0.02	-0.07	-0.01	0.01
A_R	5.24	5.2	3.16	7.25	6.3	4.79	9.45	11.06	5.02	1.88	5.73	4.69	11.63	6.14	12.5	5.21	10.92	7.66	9.06	6.99

Jimmy Lake	Ots G83	Sco 215	Smm 17	Sna MSU1	Sna MSU8	OMM 1105	Smm 22	Sna MSU13	Sna MSU5	SSOSL 456	Sco 19	Sco 202	Sna MSU10	Sna MSU12	Sna MSU6	Sal 38	Sco 200	Sna MSU11	Sna MSU3	Ave.
N_a	5	6	4	10	9	4	11	15	13	3	8	8	8	7	8	7	12	7	8	8.05
H_E	0.64	0.73	0.66	0.88	0.52	0.61	0.89	0.92	0.88	0.52	0.75	0.83	0.83	0.82	0.9	0.78	0.9	0.73	0.57	0.76
H_0	0.74	0.58	0.55	0.92	0.55	0.61	0.69	0.89	0.61	0.63	0.67	0.83	0.5	0.8	0.44	0.83	0.8	0.7	0.5	0.68
F_{is}	-0.16	0.21	0.17	-0.04	-0.07	0	0.23	0.03	0.31	-0.2	0.11	0	0.4	0.02	0.51	-0.06	0.11	0.04	0.12	0.09
A_R	4.57	4.82	3.86	7.95	5.1	3.21	8.31	10.32	8.62	2.97	5.85	6.41	6.45	5.83	8	5.57	9.06	5.62	5.31	6.2

Noell Lake	Ots G83	Sco 215	Smm 17	Sna MSU1	Sna MSU8	OMM 1105	Smm 22	Sna MSU13	Sna MSU5	SSOSL 456	Sco 19	Sco 202	Sna MSU10	Sna MSU12	Sna MSU6	Sal 38	Sco 200	Sna MSU11	Sna MSU3	Ave.
N_a	6	6	6	14	11	5	11	17	8	3	7	7	11	8	13	10	16	10	17	9.79
H_E	0.63	0.82	0.74	0.9	0.82	0.59	0.85	0.93	0.73	0.3	0.6	0.65	0.9	0.78	0.86	0.8	0.91	0.77	0.89	0.76
H_0	0.51	0.92	0.74	0.85	0.77	0.54	0.72	0.85	0.56	0.34	0.53	0.71	0.52	0.72	0.6	0.68	0.74	0.77	0.79	0.68

F_{is}	0.19	-0.12	0	0.06	0.07	0.09	0.15	0.09	0.23	-0.13	0.12	-0.1	0.42	-0.01	0.31	0.16	0.19	-0.01	0.1	0.09
A_R	4.12	5.73	4.7	9.08	6.89	4.33	7.71	10.51	5.18	2.69	4.32	4.97	8.37	6.27	8.82	6.21	9.52	6.52	9.81	6.62

Sandy Lake	Ots G83	Sco 215	Smm 17	Sna MSU1	Sna MSU8	OMM 1105	Smm 22	Sna MSU13	Sna MSU5	SSOSL 456	Sco 19	Sco 202	Sna MSU10	Sna MSU12	Sna MSU6	Sal 38	Sco 200	Sna MSU11	Sna MSU3	Ave.
N_a	9	4	5	8	7	5	16	21	9	2	5	7	22	6	17	9	20	9	9	10
H_E	0.6	0.39	0.68	0.76	0.76	0.54	0.85	0.94	0.51	0.17	0.64	0.74	0.9	0.57	0.89	0.81	0.91	0.84	0.85	0.7
H_0	0.51	0.3	0.54	0.68	0.81	0.49	0.85	0.95	0.49	0.18	0.51	0.49	0.82	0.51	0.82	0.66	0.87	0.79	0.82	0.63
F_{is}	-0.03	0.25	0.2	0.1	-0.07	0.1	0	-0.01	0.04	-0.09	0.2	0.34	0.09	0.11	0.09	0.19	0.05	0.06	0.04	0.09
A_R	5.16	3.35	4.34	5.7	4.98	2.69	8.34	11.67	4.85	1.85	3.97	5.05	10.33	4.53	9.95	6.77	10.42	6.68	6.79	6.18

Sitidgi Lake	Ots G83	Sco 215	Smm 17	Sna MSU1	Sna MSU8	OMM 1105	Smm 22	Sna MSU13	Sna MSU5	SSOSL 456	Sco 19	Sco 202	Sna MSU10	Sna MSU12	Sna MSU6	Sal 38	Sco 200	Sna MSU11	Sna MSU3	Ave.
N_a	6	5	7	20	13	5	15	20	18	3	10	11	16	16	21	10	19	12	18	12.89
H_E	0.65	0.76	0.72	0.94	0.86	0.4	0.86	0.93	0.9	0.49	0.79	0.72	0.93	0.85	0.95	0.81	0.9	0.79	0.84	0.79
H_0	0.66	0.88	0.73	0.95	0.78	0.27	0.69	0.91	0.84	0.31	0.78	0.7	0.7	0.82	0.67	0.85	0.82	0.75	0.83	0.73
F_{is}	-0.02	-0.16	-0.02	-0.02	0.1	0.32	0.2	0.02	0.07	0.36	0.02	0.03	0.25	0.04	0.3	-0.05	0.09	0.05	0.02	0.08
A_R	4.31	4.37	5.01	11.13	7.61	3.76	8.36	11.25	9.61	2.84	6.23	6.59	10.56	8.5	12.1	6.4	9.64	6.98	8.55	7.57

Wolf Lake	Ots G83	Sco 215	Smm 17	Sna MSU1	Sna MSU8	OMM 1105	Smm 22	Sna MSU13	Sna MSU5	SSOSL 456	Sco 19	Sco 202	Sna MSU10	Sna MSU12	Sna MSU6	Sal 38	Sco 200	Sna MSU11	Sna MSU3	Ave.
N_a	8	5	7	10	8	6	8	16	9	4	4	8	14	8	14	7	15	8	9	8.84
H_E	0.79	0.59	0.62	0.8	0.65	0.73	0.82	0.9	0.55	0.54	0.28	0.8	0.74	0.76	0.92	0.53	0.84	0.82	0.72	0.7
H_0	0.81	0.62	0.53	0.66	0.59	0.77	0.8	0.78	0.5	0.55	0.31	0.9	0.78	0.83	0.96	0.52	0.8	0.81	0.65	0.69
F_{is}	-0.04	-0.05	0.15	0.18	0.09	-0.05	0.02	0.13	0.09	-0.03	-0.1	-0.13	-0.06	-0.08	-0.04	0.02	0.05	0.01	0.09	0.01
A_R	5.95	3.74	5.17	7.17	5.24	4.55	6.41	9.6	4.96	3.7	2.99	5.64	7.27	6.13	10.01	4.64	8.42	6.1	5.87	5.98

Yukon Lake 105	Ots G83	Sco 215	Smm 17	Sna MSU1	Sna MSU8	OMM 1105	Smm 22	Sna MSU13	Sna MSU5	SSOSL 456	Sco 19	Sco 202	Sna MSU10	Sna MSU12	Sna MSU6	Sal 38	Sco 200	Sna MSU11	Sna MSU3	Ave.
N_a	1	1	1	4	1	1	2	1	1	1	1	1	3	2	4	1	4	2	5	1.947

	Ots G83	Sco 215	Smm 17	Sna MSU1	Sna MSU8	OMM 1105	Smm 22	Sna MSU13	Sna MSU5	SSOSL 456	Sco 19	Sco 202	Sna MSU10	Sna MSU12	Sna MSU6	Sal 38	Sco 200	Sna MSU11	Sna MSU3	Ave.
H_E	0	0	0	0.54	0	0	0.03	0	0	0	0	0	0.56	0.19	0.62	0	0.45	0.37	0.73	0.18
H_0	0	0	0	0.53	0	0	0.03	0	0	0	0	0	0.45	0.21	0.68	0	0.41	0.34	0.59	0.17
F_{is}	NA	NA	NA	0.02	NA	NA	0	NA	NA	NA	NA	NA	0.2	-0.1	-0.09	NA	0.08	0.06	0.19	0.04
A_R	1	1	1	2.97	1	1	1.27	1	1	1	1	1	2.86	1.91	3.31	1	2.84	2	4.25	1.71

All	Ots G83	Sco 215	Smm 17	Sna MSU1	Sna MSU8	OMM 1105	Smm 22	Sna MSU13	Sna MSU5	SSOSL 456	Sco 19	Sco 202	Sna MSU10	Sna MSU12	Sna MSU6	Sal 38	Sco 200	Sna MSU11	Sna MSU3	Ave.
N_a	11	7	13	25	22	12	24	43	26	4	16	13	45	21	43	20	32	24	35	22.95
H_E	0.59	0.63	0.63	0.85	0.69	0.56	0.81	0.85	0.7	0.35	0.61	0.66	0.87	0.73	0.9	0.68	0.87	0.76	0.81	0.71
H_0	0.58	0.63	0.6	0.84	0.66	0.55	0.73	0.8	0.62	0.34	0.6	0.64	0.71	0.68	0.75	0.63	0.81	0.78	0.72	0.67
F_{is}	0	0.03	0.05	0.01	0.06	0.02	0.09	0.06	0.1	0.01	0.02	0.02	0.18	0.07	0.19	0.06	0.07	-0.02	0.1	0.06
A_R	5.2	5.17	5.92	10.78	7.92	5.02	9.94	13.09	8.17	2.81	6.33	7.17	12.76	8.02	12.98	7.85	11.79	9.07	10.49	8.45

Supplementary Appendix 3.

Statistics from Bayesian clustering implemented in STRUCTURE (Pritchard et al. 2000) for locations within the Husky Lakes drainage basin. Statistics were calculated from 10 independent runs for each value of K employing a burn-in of 100,000 iterations followed by 100,000 MCMC iterations. Shown are the mean and standard deviations (SD) of the log-likelihood values (LnP[D]) for different hypothesized numbers of genetic populations (K). Also shown is the mean value of ΔK, the ad hoc statistic of Evanno et al. (2005) used to summarize the second-order rate of change in LnP(D). The underlined values represent the most likely number of genetic groups (clusters) for each statistic for each clustering scenario. NA = not applicable given that ΔK cannot be calculated for these values of K.

K	Reps	Mean ln P(K)	S.D. Ln P(K)	ln(K)	|ln(K)|	Delta K
1	10	-18232.2	1.616753	NA	NA	NA
2	10	-17814	73.13115	418.12	134.88	1.844358
3	10	-17530.8	30.50186	283.24	128.05	4.198105
4	10	**-17375.6**	135.8744	155.19	209.88	1.544662
5	10	-17430.3	130.3774	-54.69	77.76	0.596422
6	10	-17407.2	39.03721	23.07	572.26	**14.65935**
7	10	-17956.4	302.4212	-549.19	361.77	1.196245
8	10	-18143.8	256.6614	-187.42	107.58	0.419151
9	10	-18223.7	249.6385	-79.84	382.3	1.531414
10	10	-18685.8	408.1519	-462.14	NA	NA