The migratory life history and physiology of arctic char, *Salvelinus alpinus,* navigating change in the Canadian North

by

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Abstract

Global climate change (GCC) is most pronounced at higher latitudes; to what degree northern migratory fish species can tolerate this change remains largely unknown. Imminent effects of GCC on arctic rivers include warmer water temperatures and changes in the timing, frequency and magnitude of high and low flow events, all of which could reduce fish passage. The arctic char (*Salvelinus alpinus)* is a salmonid species of great ecological, cultural, and subsistence value, and are among the fish species likely to be impacted by these changes. Based on this, my research aim was to identify life-history and physiological characteristics that likely shape the ability of arctic char to cope with GCC. To this end, in chapter 2, I characterized alternative migratory life-history strategies that facilitate the existence of a char population in a harsh environment. The most significant of these strategies included the earliest documented return migration timing in the Canadian Arctic and a very low annual fidelity (near 0%) which together, reduced the exposure of fish to even harsher conditions than those they already faced. In the physiological components of my research I took a comparative approach and conducted experiments on rainbow trout, a well-studied temperate reference species, in addition to arctic char. In chapter 3, I used laboratory simulations to identify potential physiological constraints on the migration of arctic char through current and future thermal regimes that include large diurnal temperature fluctuations. In chapter 4, I revealed transcriptional and biochemical responses of arctic char to these thermal regimes that were indicative of only partially successful compensatory responses in addition to severe heat-stress and disruption of biochemical processes. In chapter 4, I also verified the utility of a suite of transcripts as biomarkers for thermal stress in wild arctic char. Together my research suggests that arctic char possess life history and physiological traits that may make them more tolerant, and adaptable to GCC than

previously thought but may still place them at a competitive disadvantage relative to more temperate species whose ranges are expanding northward.

Preface

Experiments and field sampling procedures were approved by the University of Alberta and the Canadian Department of Fisheries and Oceans Freshwater Institute Science Laboratories Animal Care Committees (FWI-ACC-2012-019; FWI-ACC-2013-020; FWI-ACC-2014-022; UofA AUP-022).

Dedication:

To Grandpa.

For fostering my love for the fishes of the family salmonidae at a young age.

Acknowledgements

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I am hopeful that my present and future work proves worthy of the contributions so many people have graciously made.

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Chapter 1: General Introduction

The effects of global climate change (GCC) are most pronounced at high latitudes, and the degree to which northern species can cope with this change remains largely unknown. In many northern rivers, climate-related fluctuations in flow regimes may impair the passage of fish such as anadromous salmonids (Wrona et al. 2006b). While overall total water input into these systems may or may not experience large change, the patterns of input and discharge likely will (Syvitski 2002; Wrona et al. 2006a). More rapid, earlier snow and ice melts may result in fish experiencing peak flows that are larger and earlier than they historically have been. Conversely early melts may mean that snow runoff will dissipate earlier causing a similar shift in the timing of low discharge periods as well as lower minimal discharges (Wrona et al. 2006a). Additionally, GCC has and will continue to increase the water temperatures in these systems. Such conditions may favour more temperate species whose ranges are expanding northward (Dunmall et al. 2012).

For migratory northern salmonids, their persistence and competitive ability under drastically changing conditions will be greatly influenced by an interaction between their migratory ecology and their physiology (Dowd et al. 2015). Temporal and spatial aspects of these fish's migratory life histories determine the environmental conditions they encounter on their migrations while their exercise and thermal physiology determine their ability to overcome challenging conditions. In more southerly distributed, relatively well-studied salmonids mismatches between a species' migratory life-history and its physiology have resulted in reduced migration success (Farrell 2009a). For example, in sockeye salmon, early upstream migrations caused salmon to experience temperatures above their optimal ranges for physiological performance, which is associated with reduced migratory success (Eliason et al. 2011; Farrell et al. 2008a; Hinch et al. 2012; Mathes et

al. 2010). In northern salmonids, there is a relative paucity of information regarding their migratory ecology and environmental physiology (Johnson 1980; Klemetsen et al. 2003; Penney et al. 2014; Roux et al. 2011) so the links between the two are poorly understood.

The arctic char is among the anadromous northern salmonids that will be forced to migrate though changing thermal and flow regimes. The species is distributed throughout the global arctic, and possess arguably the greatest life-history diversity of any known vertebrate (Johnson 1980; Klemetsen 2010). This broad distribution and life history diversity makes them an excellent candidate for the study of local adaptation. The arctic char is also of substantial cultural, economic, and ecological value (Jenness 1922; Roux et al. 2011) and is closely related to other northern salmonids including lake trout (*Salvelinus namaycush*) and dolly varden (*S. malma*) that move through similar environments and will therefore experience similar environmental change. This value and similarity make arctic char a suitable representative northern salmonid.

Based on the identified knowledge gaps for northern salmonids, the concerning findings in more temperate salmonids, and the ongoing environmental change my goal was identify life-history and physiological characteristics that will likely shape the ability of arctic char to cope with GCC. Within this goal I had three broad objectives to (i) characterize novel migratory life-history attributes of arctic char that allow them to persist in extreme environments, (ii) identify physiological constraints on the migration of arctic char under current and future thermal regimes, and (iii), investigate transcriptional and biochemical responses of arctic char to these thermal regimes and assess their utility as biomarkers for thermal stress. To achieve these objectives, I focused on the Nulahugyuk Creek arctic char migration in northwest Nunavut. The challenging, shallow-flow conditions and thermal variation of the creek allowed me to study life

history attributes in an extreme environment (Chapter 2), as well physiological limitations and responses to thermal variation during migration (Chapter 3 and 4). In the laboratory, I simulated diurnal temperature variations that were representative of those recorded in Nulahugyuk Creek to allow me to address objectives two and three with techniques and a level of control not practical in the field.

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Chapter 2: Alternative migratory strategies of arctic char (*Salvelinus alpinus)* in an extreme environment

Abstract

Anadromous arctic char (*Salvelinus alpinus*) employ diverse life histories, however, our understanding of the extent of this diversity is limited. Here, I detail key aspects of the physical environment and life history of a population of arctic char from Nulahugyuk Creek, Nunavut, to reveal unique traits. Over the course of their migration creek discharge declined precipitously, forcing char to migrate through shallow water with large diel temperature fluctuations (>10°C) and high temperature extremes (>21°C). The downstream migration of adults (>55 cm) began in mid-June and continued into early July, while the downstream migration of smolts (<30 cm) began in late June and continued until late July. The upstream adult migration began in late June and ended in late July; far earlier than other upstream migrations in the region. There was no appreciable upstream migration of juveniles, and char 30 to 55 cm in length were absent from the migratory population. The average age at first migration was four years and the youngest returning adult char were eight and nine years old. The missing size and age classes, and the fact that most upstream migrants were near reproductive maturity, indicate that char in this system typically leave at a length of 19 cm and an age of four years and do not return for four to five years, when they are ready to reproduce. Together our data suggest that anadromous arctic char possess alternative life history strategies that limit unnecessary exposure to restrictive migratory conditions and facilitate their existence in otherwise uninhabitable systems. Understanding such population-specific migratory strategies is critical to the management of arctic fisheries that have mixed stocks, as well as to our knowledge of arctic char life history diversity that will likely

contribute to the adaptability and persistence of the species as global climate change progresses.

Introduction

There is a large and well-documented diversity in the life histories of temperate anadromous salmonids. This diversity undoubtedly contributes to species resilience to exploitation and environmental changes such as those associated with global climate change (Hilborn et al. 2003; Schindler et al. 2010). Salmonid life history can vary in spatial and temporal migratory traits such as population fidelity to natal waters (Keefer and Caudill 2013; Quinn 1993), age at first migration (Beechie et al. 2006), timing of return migrations, (Beechie et al. 2006; Hodgson and Quinn 2002; Schindler et al. 2010), and in iteroparous species, migration frequency (Narum et al. 2008). Diversity in migratory life-history traits is also prevalent in northern salmonids, however, our understanding of its extent is limited in part by their apparent complexity, by a paucity of long-term datasets, and by the large, remote geographical range of these fish (Gyselman 1994; Reist et al. 2006a; Roux et al. 2011). Our limited understanding is exemplified by recent discoveries regarding the fundamental ecology of northern salmonids. For example, northern populations of anadromous brown trout (*Salmo trutta*) and arctic char *(Salvelinus alpinus*) were first confirmed to occasionally overwinter in estuarine environments in 2012 (Jensen and Rikardsen), and although it was traditional knowledge among local Inuit and documented by early expeditionists (Walters 1953), anadromy in arctic lake trout (*Salvelinus namaycush*) was only scientifically documented in 2010 (Swanson et al. 2010). Among anadromous northern salmonids, arctic char are of particular interest because they are an important cultural, subsistence and commercial resource that has traditionally made up a primary protein source for the Inuit people (Jenness 1922; Roux et al. 2011). Furthermore, arctic char have the most northerly distribution of any freshwater fish making them vulnerable to the effects of GCC, which are most pronounced at higher latitudes (ACIA 2005; Wrona et al. 2006c).

The arctic char is a long-lived, cold water salmonid that spawns and initially grows relatively slowly in cold, oligotrophic freshwater environments. Arctic char possess among the highest levels of life-history diversity of all vertebrates (Klemetsen 2010). This diversity has been credited with facilitating their existence in a range of extreme environments throughout the global arctic (Roux et al. 2011). A key aspect of this diversity is that the arctic char has both resident and anadromous forms, which allows them to utilize more productive marine environments when resources are limited in their freshwater habitat. Anadromous arctic char typically migrate to sea in late spring or early summer and return to their natal freshwater system in late summer or early fall, after a period of four to eight weeks at sea (Gulseth and Nilssen 2000; Johnson 1980; Johnson 1989). These migrations usually begin between the ages of three and six years old but this age can vary from two to eleven (Johnson 1980; Swanson et al. 2010). Once they begin, migrations continue annually or intermittently depending on an individual's reproductive status, physical condition, and numerous other environmental factors. The maximum age of anadromous char in northern Canada ranges between populations from 11 to 28 years old (Roux et al. 2011), which can influence the number of reproductive events an individual fish may be able to carry out. The annual fidelity of arctic char to their natal system also varies greatly between populations with straying estimates as high as 53% (Gyselman 1994; Moore et al. 2013). This level of straying creates a great deal of population mixing, however, an individual's likelihood of utilizing a non-natal freshwater systems is lower in spawning years which limits genetic exchange with other population and likely allows for the persistence of local adaptation (Moore et al. 2013). Given the relatively small number of anadromous arctic char populations whose migrations have been thoroughly studied it is likely that there are many aspects of their migrations to be described.

The current study was focused on the Nulahugyuk Creek, Nunavut (NU), arctic char migration. The traditional harvest of this arctic char run by the Copper Inuit was first documented by an ethnographer, Diamond Jenness, on the 1913-1918 Canadian Arctic Expedition (Jenness 1922; Jenness and Jenness 1991). The information he collected suggested that anadromous char in this area, and in Nulahugyuk Creek in particular, have unique migratory life history traits that have not previously been studied. Recently, locals have become concerned over a putative decline in the health of the population. Given these historical observations and local concerns, our goals were to inform local management decisions and to identify novel aspects of the migration that would expand our understanding of life-history diversity in anadromous arctic char. To address these goals we conducted a field study to characterize the key life-history traits and physical environment of migrating char in Nulahugyuk Creek, NU.

Methods

Field sampling. — Fieldwork took place in the summers of 2012–2014 along Nulahugyuk Creek, NU, Canada (68.75°N 114.8°W; Fig. 2.1). Nulahugyuk Creek is approximately 10km long and connects Hingittok Lake to the Arctic Ocean at Bernard Harbour, NU. Arctic char were captured using two fyke-nets that were set up side-by-side with trap mouths facing in opposite directions in the main thalweg and wings that extended to the bank on both sides. This trap was set up approximately 300m upstream from the creek mouth from July 3–22, 2012 and from June 13–July 17, 2014. In 2013, fieldwork took place from August 7–14, however, at that time water levels were too low to permit fish passage so the trap was not installed. Captured char were enumerated, their direction of travel was recorded and they were released on the opposite side of the trap from which they were caught. In 2012, a sub-sample of char were weighed (g) and measured (mm) and adult adipose fins were clipped. Subsampling was done to limit time in

captivity, particularly under warm water conditions (> 18°C). In 2014, length and weight data were collected for all adults and up to 20 smolts each time the trap was cleared.

In 2012, a subsample of adults (n = 121) were implanted with half duplex passive integrated transponder tags (HDX PIT; Oregon RFID, Portland, Oregon) in their peritoneal cavity. In 2014, upstream migrating adults received PIT tags (N = 303) and downstream migrating adults were implanted with a numbered dorsal T-bar tag (N = 498; Orange tags series 0001-1061, Floy Tag Incorporated, Seattle, Washington). Tagging was completed as part of an ongoing study being conducted by Golder Associates (Edmonton, Alberta, Canada).

The lower three kilometers of the creek below a large pool, was regularly surveyed for arctic char mortalities. The location, length and weight data, reproductive status, and otolith and adipose fin samples were collected from any recent mortalities that were found sufficiently intact. These samples and data were also collected from fish harvested by locals when permitted. Reproductive status was determined using a sexual maturity key (McGowan 1992). In 2013, five char were harvested by locals near the mouth of the creek in Bernard Harbour using either gill nets or angling. These fish were also sampled for comparison to char captured in Nulahugyuk creek. Sampling procedures were approved by the Canadian Department of Fisheries and Oceans Freshwater Institute Science Laboratories and University of Alberta Animal Care Committees (FWI-ACC-2012-019; FWI-ACC-2013-020; FWI-ACC-2014-022; UofA AUP-022).

Physical Environment. — Water temperature was recorded at the fish trap every 15 min using HOBO Water Temperature Pro data loggers (Onset Computer Corporation, Bourne Massachusetts). In 2014, water temperature was also recorded every time the trap was cleared after July 1st 2014 . Creek discharge was measured using the velocity-area method at a site with

relatively uniform flow, 200m upstream from the trap but still below all branch's and sources of inflow. Velocity measurements were taken at 60% depth at a minimum of 20 intervals using a magnetic velocity meter (2012 and 2013: FP111, Global Water Instrumentation, College Station, Texas; 2014: Model 2100 Current Velocity Meter, Swoffer Instruments, Seattle, Washington).

Otolith Analysis. — Otolith analysis was conducted as previously described (Swanson et al. 2010). In brief, after sampling in field, otoliths were stored dry in a paper envelop until analysis. One of each otolith pair was embedded in epoxy (2012 and 2013: EpoThin, Buehler, Lake Bluff, Illinois; 2014: Cold Cure, System Three, Auburn, Washington), sectioned through the nucleus using a precision, low-speed saw (ISOMET, Buehler, Lake Bluff, Illinois), embedded in epoxy inside an acrylic ring with the surface of the cross section exposed, and then polished using a lapping paper (50 – 3μm Grit). Samples that were used in the otolith microchemisty analysis were further polished using a 0.5μm polishing paste on a Grinder-Polisher (MetaServ™ , Buelher; Lake Bluff, Illinois) and washed in an ultrasonic bath.

Laser ablation inductively-coupled mass spectrometery (LA-ICP-MS) was performed on all 2012 and 2013 samples at the Advanced Instrumentation Laboratory (University of Alaska Fairbanks, Fairbanks, Alaska) as in Swanson et al. (Swanson et al. 2010) with minor differences. For laser ablation, we ablated a single transect from the primoridia to beyond the distal edge of the otolith using a New Wave UP213 laser (Fremont, California) set to 80% power, a 25 μm spot size and a 5 μm s^{-1} scan speed. Specific element isotope concentrations were determined using an Agilent 7500ce mass spectrometer (Santa Clara, California) that was coupled to the laser and calibrated with a glass standard (SRM 610, National Institute of Standards and Technology, Gaithersburg, Maryland). ^{86}Sr and ^{66}Zn isotope concentrations were plotted over images of their corresponding otolith along the laser ablation line. In fish that had previously migrated, the age at first

migration (AFM) was determined by counting annuli up to the point of increase in [Sr^{86}] (Swanson et al. 2010). The AFM was verified by counting the number of annual cycles of Zn^{66} prior to migration.

Length at age values were back-calculated using an otolith radius vs. length, regression approach to fill in length data for the missing size class between smolts and adults. A regression approach was used because the radius-length relationship was sigmoidal and was not readily transformable into a linear relationship for use with the biological intercept model (Stevenson and Campana 1992). For each otolith cross-section, the radius was measured at the outer edge of every hyaline zone along the longest line of the ventral lobe from the primordium to the distal edge using ImageJ 1.48 (National Institute of Mental Health, Bethesda, Maryland). The four-parameter sigmoidal regression between the total ventral otolith radius and fork length at capture had the formula:

$$L = 176.5 + \frac{576.2}{1 + e^{\left(-\frac{1(R-1154.134)}{104.7}\right)}}$$

Where R is the ventral otolith radius (μm) and L is fork length (mm). The radius at each annuli after age three was put into the length-radius formula to calculate the length at age for all sampled individuals. Age three was selected as the cut off for back calculation to avoid extrapolation outside of the dataset used to generate the sigmoidal regression model.

Data analysis. — Arctic char less than 350mm in length were considered to be smolts based upon their seaward trajectory, proximity to the ocean, size (length and weight) difference from adults, and lack of prior migration to marine environments as determined by Sr isotope analysis. All arctic char greater than 500mm in length were considered adults. Only three individuals

(<0.1%) fell between the two life-history classes and were therefore omitted from the analysis. Fish recaptured less than one week after release, including all smolts captured in the upstream trap, were also not included in the statistical analysis as they likely reentered the trap accidently. The possible implications of this omission are discussed below. Length frequency distributions were calculated for smolts and adults in each year (2012 and 2014). Distributions were compared between years using separate Kolmogorov–Smirnov tests (K-S test) for smolts and adults. Differences in length, weight and condition factor between years, migratory direction and life-history stage were analyzed using an ANOVA with a tukey HSD post-hoc pairwise comparison test. Condition factor was calculated as $K = 10^5 \times W/L^3$, where W is wet weight (g) and L is fork length (mm).

Linear regression models were constructed to examine relationships between length and weight. The length-weight relationships were established using $\log_{10}$-transformed data. In addition to the conventional length-weight model, a second model was generated that included life-history stage (smolt and upstream and downstream adults). Given the sigmoidal nature of the back-calculated length at age data, growth was modeled using three and four parameter sigmoidal and Gompertz curves as well as a logistic curve. The four-parameter Gompertz curve had the highest R^2 and lowest residual standard error (RSE) so it was selected as the final growth model. In all analyses with multiple predictor variables all interactions were tested. If an interaction effect was not significant (α=0.05) it was removed from the analysis as long as no higher order interaction was significant.

Statistical analysis was completed in R Studio (Team, 2014). Data presentation was completed using Sigmaplot 11.1 (Systat Software, San Jose, California) and Adobe Illustrator CS4 (Adobe systems, San Jose, California).

Results

Creek temperature and discharge. — Discharge in Nulahugyuk creek was low and declined over the course of the arctic char migration (6.00-0.50 m^3/s; Fig. 2.2A-C). Low discharge resulted in numerous incidences of upstream migrating adults becoming stranded and being scavenged or preyed upon by gulls (*Larus spp.*) and grizzly bears *(Ursus arctos*). In August, after the run was complete, flow was sufficiently low to restrict arctic char passage altogether (0.03 m^3/s; Fig. 2.2B-C). In 2013, a single adult char was observed attempting to migrate upstream but became stranded within 300m of the mouth of the creek. In 2012 and 2013, there were large diurnal temperature fluctuations occasionally exceeding 10°C per day (Fig. 2.2E). The minimum and maximum temperatures recorded during the course of the 2012 migration were 7.2 and 21.0°C, respectively. The minimum and maximum temperatures recorded manually at trap checks in 2014 were 7.0 and 19.8°C but may not represent the true minima and maxima of the creek due to the low sampling frequency in this year.

Migration size and timing. — Seaward adult arctic char were the first to enter Nulahugyuk Creek after ice breakup. In 2014, they were first captured moving out to sea on June 16th; the migration peaked June 21st, gradually tapered off, and was near completion by July 3rd (N_{total} = 472; Fig. 2.3 B). In 2012 the downstream migration appeared to be near completion shortly after the trap was installed on July 4th as a relatively small number of downstream adults were captured (N_{total} = 84; Fig. 2.3B). The 2014 adult upstream migration began June 25th, peaked on July 3rd, and appeared to be near completion upon our departure on July 17th (N_{total} = 332 , Fig. 2.3A) . This pattern generally agrees with 2012 as a large number of adults were migrating upstream when the trap was installed and the run had dissipated by July 20th (N_{total} = 387; Fig. 2.3A). The period of low upstream migration frequency in 2012 between July 12th and 16th corresponded with the

arrival of a large amount of drift ice that may have blocked sea access to Bernard Harbour. In either year only one adult trapped on their downstream migration was captured subsequently moving upstream after a period of more than a week suggesting that nearly all individuals that left the creek did not return the same year. There was a large disparity in the seaward smolt migrations in 2012 and 2014. In 2014 only 460 smolts were captured while in 2012, 6221 smolts were captured (Fig. 2.3C). In 2014 the first smolt was captured on June 25th, and capture was irregular until the end of sampling (July 16th). In 2012 the smolt migration appeared to peak shortly after the trap was installed (July 4th) and end by July 23rd. These movement patterns indicate overlap between upstream adult and downstream adult and smolt migrations (Fig. 2.3). In 2012, three adult char were captured migrating upstream with floy tags from a previous study that consisted of tagging fish near the mouth of the Coppermine River, NU (Fig. 2.1). In 2014 two tagged adults from the same tag-series were captured moving out to sea.

Size and maturity. — Two distinct and normally distributed length-frequency groups were apparent within the population and corresponded to smolt and adult life history stages (smolt: 150 to 300 and adult: 550 to 900mm; Fig. 2.4). Smolts and adults were separated by a near complete absence of fish between 300 and 550 mm (N = 3, Fig. 2.4). In 2014 there was a rightward shift (increased length) in both the smolt and adult length-frequency distributions relative to 2012 (K-S test: Smolt: $D = 0.229$, $p < 0.001$, $N_{2012} = 168$, $N_{2014} = 302$; Adult: $D = 0.536$, $p < 0.001$, $N_{2012} = 321$, $N_{2014} = 782$). The shift in length frequency distributions agreed with comparisons of mean length and weight, which revealed that fish captured in 2014 were generally larger (length and weight) than those of the same life history stage in 2012 (Tukeys HSD: $p < 0.05$; Table 2.1). Arctic char migrating to the sea (downstream adults and smolts) had a substantially lower condition factor (i.e. were relatively thinner) than those returning from sea (-

26 to 28%; Tukeys HSD: $p < 0.05$; Table 2.1). In 2014, natural mortalities were larger than average upstream adults (length: +8.2%; weight +26.0% Tukeys HSD: $p < 0.05$; Table 2.1). Like in most salmonids, Nulahugyuk Creek arctic char exhibited an exponential length-weight relationship ($R^2 = .996$, RSE = 0.054, $F_{998} = 2.6e^5$, $p < 2.2e^{-16}$; Fig. 2.5; Table 2.3), however, this relationship varied depending on life history stage so it was better represented by a model including it as an explanatory variable (RSE = 0.042, $R^2 = 0.998$, $F_{996} = 1.38e^5$, $p < 2.2e^{-16}$; Fig. 2.5; Table 2.3).

In 2014, 16 adult char were available for assessment of their sexual maturity. Of a total of five downstream migrating adults, two females and two males had resting characteristics suggesting they had spawned upstream in the previous year, and one female had developed but had firm ovaries suggesting it had not fully matured and did not previously spawn. Of the 11 upstream migrating adults sampled, all six males and one of the females were sexually mature and in spawning condition. Three females had not fully matured but had clearly distinguishable eggs likely capable of maturation within the year, and one female was in resting condition.

Age, growth and otolith microchemistry. — The visual otolith based age estimate for smolts was almost identical to AFM obtained from adult otoliths through Sr isotope analysis (Table 2.2). The difference between smolt age/AFM and the youngest returning adults was four to five years. The measured and back-calculated length at age relationships were sigmoidal with a relatively high growth between the ages of smoltification and return to their natal system (Fig. 2.6, Table 2.3). Together, the age difference between life-history stages and the age-length relationship reveal that the gap in the length frequency distributions corresponds with an absence of individuals typically between the ages of four and nine years old.

Discussion

Arctic char possess extraordinary life history diversity that is thought to facilitate their existence in a range of extreme and variable environments (Hammar 2014; Johnson 1980; Jonsson 2001; Klemetsen 2010; Roux et al. 2011). We have identified novel migratory life history strategies within a small arctic char population that demonstrate that this diversity is even greater than previously established. Specifically, Nulahugyuk Creek arctic char typically do not return to their natal watershed for four to five years after their first migration, likely resulting in extensive mixing with surrounding populations. Furthermore, returning adult char begin their upstream migration in late June and it is complete by mid to late July, when most other return migrations have not yet begun. Given that Nulahugyuk Creek has a highly variable thermal regime, is marginally navigable at the best of times, and completely impassible by early August, these life history traits are likely adaptive and facilitate the persistence of anadromous arctic char in an otherwise uninhabitable system.

Size and age structure. — The length of smolts at first migration (Table 2.1 and 2, Fig. 2.4) in Nulahugyuk Creek (Table 2.3) falls well within the published range (eg. 180 – 240mm; Johnson 1980; Johnson 1989). Interestingly, the mean smolt length was very similar to the estimate of eight inches (203mm;Jenness 1922), which ethnographer Diamond Jenness made at the same location in 1916 of what he postured were "…young fry, two seasons old, making their way down to the sea." The age at first migration (3-5 years) is on the lower end but within the published range for the region (3-11 years; Johnson 1980; Johnson 1989; Swanson et al. 2010). The similarity between the current smolt age distribution and the age at first migration estimated from Sr profiles of adult otoliths confirms that this life-history trait is consistent between generations.

Adults were similar in mean and maximum length and weight to those in the nearby Coppermine River, Nu population (Fig. 1;Gillman and Kristofferson 1984), but larger than in many other populations in the Canadian Arctic (Roux et al. 2011). Nulahugyuk Creek arctic char also had similar maximum ages to those in Coppermine river, which are lower than many other high latitude populations (Roux et al. 2011). The large size and short life-span of these two populations mean that they have among the highest lifetime growth rates in the region (Table 3; Fig. 6; Gillman and Kristofferson 1984; Swanson et al. 2010). Such a rapid growth but relatively short life span (12-14 years) suggests that the Dolphin Union Strait – Coronation Gulf area (Fig. 2.1) might be one of relatively high productivity where arctic char may have fewer total reproductive events but greater fecundity than in surrounding areas where fish may have life spans twice as long but reach similar or smaller sizes. For example, the length and weight of adult arctic char in this study had substantial overlap with the length and weight at sexual maturity for the Nauyuk Lake population, however, this size was reached at a much earlier age in our study population (8-12 years vs. 10–18 years; Johnson 1980). The fact that natural mortalities were larger than other upstream migrating adults suggests that migration success is likely size limited, perhaps due to increase likelihood of stranding for larger individuals, which would also make them more susceptible to predation. Furthermore, the size limitation may also explain the relatively short life expectancy when compared to Coppermine River char (12 vs. 14 years).

The length-weight relationship of Nulahugyuk Creek arctic chars aligns well with other populations in the region (Johnson 1980). As was found in Nauyuk Lake, this relationship differed between life-history stages (Table 3; Johnson 1980). For juveniles, the observed reduction in the intercept compared to upstream adults likely owes to their feeding and growing

in an oligotrophic environment compared to the anadromous adults. In adults, the lower intercept in downstream migrants is almost certainly due to the high energy costs associated with spawning and overwintering in freshwater where anadromous adults do not typically feed (Dutil 1986; Johnson 1980). These differences in length-weight relations are also apparent in the lower condition factor that is found in char coming from freshwater compared to those coming from the ocean (Table 2.2). The decreased condition factor in seaward adults parallels that seen in Nauyuk Lake, where it was estimated that the cost of overwintering and spawning could be up to 46% of total body energy (Dutil 1986).

Migration pattern. — The seaward adult migration began shortly after the outflow of Hingittok Lake was free from ice obstruction. This timing is in line with that commonly observed in most other systems (Johnson 1980). The smolt migration was also similar to other populations in that it began shortly (nine days) after the adult downstream migration and was highly variable between years (Johnson 1980). As Nulahugyuk creek was likely still navigable for smolts when sampling ceased on July 16th 2014, we may have missed a significant portion of the 2014 smolt migration, however, that would mean that the timing of the run in 2014 was substantially later than in 2012.

To the best of our knowledge, the timing of the adult return migration is the earliest ever documented in the Canadian Arctic, beginning in late June and concluding before in the end of July. All other documented upstream migrations either start or continue well into August and usually into September (Table 4; Johnson 1980). The only known upstream migration that is similar in timing occurs entirely in freshwater, when arctic char migrate from Nauyuk Lake up Willow Creek, a small intermittent stream, to their spawning grounds at Willow Lake (Johnson

1980; Johnson 1989). Like Nulahugyuk Creek, water levels in Willow Creek are too low to permit passage into August (Gyselman 1984; Johnson 1980) necessitating an early migration.

The adult char migrating upstream in Nulahugyuk Creek had not previously migrated downstream in the same year. For returning adult char to arrive as they do, they would likely have to migrate directly from their overwintering grounds with little to no time spent feeding at sea, and indeed, all observed migrants had empty stomachs. There are many possible creeks and rivers such as Noahognik, Kogluktuaryuk and the Coppermine river that are within an accessible distance and could be utilized as overwintering habitat (Fig.1; Gillman and Kristofferson 1984; Jenness 1922). This overwintering theory is supported by the capture of char in Nulahugyuk Creek that were tagged near the mouth of the Coppermine River in a previous study. Furthermore, in a study conducted on the Nauyuk Lake arctic char, Nauyuk char were identified in nearly every freshwater system in the area (<250km) in which there was a significant fishery, within the same year the fish were tagged (Gyselman 1984).

Fidelity. — In semelparous salmonids, homing and straying are terms with relatively simple and rigid definitions. In reference to these fish's once in a life time return migration, homing is the act of returning to natal spawning grounds for the purpose of reproduction, while straying is entering and reproducing in a non-natal freshwater system (Quinn 1993). Under these definitions, benefits to homing include that individuals return to a location where their parents had reproductive success and that they may be uniquely adapted to (Quinn 1993). Straying, however, allows salmon to potentially colonize new systems that may have less competition, greater productivity and may not be as challenging to access. In iteroparous salmonids, the definitions of homing and straying are not as clear. Individuals make multiple migrations over the course of their lives and may utilize more than one freshwater system. Furthermore, it is

often difficult and costly to determine whether or not straying individuals have entered other freshwater systems to spawn or simply to overwinter. Here we use the terms in respect to the annual migration regardless of reproductive status. Under these definitions, reported ranges of annual stray after accounting for at sea mortality vary from 2.2 to 66% (Gyselman 1994; Jensen et al. 2015). The upper end of this range (66%) occurred in a single year for the Nauyuk Lake population, however, there was annual variation in the rate of stray of 33-66% that resulted in a mean straying rate of 53% (Gyselman 1994). Using a genetic population assignment method for populations in the Cumberland Sound region, NU, Moore et al. (2013) produced course estimates of straying rate between 15.8 and 46.5% and more refined estimates that ranged from 15.8 to 25.5%. In our study, there was no appreciable return of either smolts or adults within the same year of their downstream migration, suggesting that there was a near 100% annual straying rate for both life history stages. The near complete absence of fish between the ages of five and eight, and the size of 300 and 550mm suggests that once smolts enter the sea, they do not return until they have reached or are near reproductive maturity. Increased fidelity among reproductive individuals has been identified in the Cumberland Sound region, where individuals identified as strays were much more likely to be overwintering than reproducing (Moore et al. 2013), while in Nauyuk Lake, small, likely non-reproductive char, and char that had spawned in the previous fall, tended to have a lower fidelity than those that were in reproductive size classes but had not spawned in the previous year (Gyselman 1994). Furthermore, in our study, all but one of the eleven upstream migrants examined appeared to be in suitable condition for spawning in the fall, and all but one of the five seaward adults examined appeared to have spawned in the previous year. In addition to the on going tagging study taking place in the region, incidence of

Nulahugyuk char mixing with surrounding populations for the purpose of reproduction could be relatively easily resolved through genetic methods.

In a study of the migratory window of Dieset River Arctic char in Svlabard, Norway, Svenning and Gullestad (2002) found that in some years with high air temperature, flows could become to low to permit the return of juvenile char in late August. The authors presumed that all individuals stranded at sea likely died overwinter, however, our results suggest that these restrictive migratory conditions may lead to the use of non-natal freshwater systems for overwintering rather than the stochastic high mortality events suggested. Furthermore, there are other systems that support anadromous char populations on Svalbard that could provide suitable alternative overwintering habitat when the migratory window of Dieset River closes early.

The suggested pattern of fidelity of Nulahugyuk Creek arctic char would mean that they benefit from aspects of both straying and homing strategies. Their low fidelity and use of alternative overwintering habitat while immature or between reproductive events would limit the number of lifetime migrations that they would need to make through Nulahugyuk Creek, which has a narrow migratory window, and is thermally and physically challenging to navigate (Fig.1). Their return upon reproductive maturation would provide access to proven breeding grounds and would help maintain genetic isolation from surrounding populations, perhaps allowing for the development and persistence of local adaptations (Moore et al. 2013; Quinn 1993). The idea of utilizing "convenient" overwintering habitat rather than returning annually to natal waters has been discussed previously (Armstrong and Morrow 1980; Moore et al. 2013), and Johnson (1980) suggested that this may be an adaptation to the variable flows of streams that many arctic salmonid populations rely on for migrations; The Nulahugyuk Creek arctic char population provides one of the most pertinent examples in support of this hypothesis to date.

In our study, upstream migrating juveniles were assumed to be smolts intending to move out to sea that mistakenly re-entered the trap after their release downstream. This is supported by a number of observations; their captured frequency was highly correlated with the release of smolts moving downstream ($R^2 = 0.79$), there was no apparent difference in size between the groups as there would be if the upstream migrants had fed at sea, and most convincingly, smolts released on the downstream side of the trap were frequently observed swimming directly into the upstream trap following release, likely in an attempt to flee researchers working nearby. Despite this, we cannot say with certainty that some juveniles did not come from nearby freshwater outlets or that some downstream migrants briefly (hours-days) ventured out in to brackish water before returning as has been noted in other systems (Johnson 1980). This behavior would be interesting to quantify as it is indicative of juveniles assessing their seawater tolerance before migration, however, it does not affect any of our primary conclusions, as these individuals evidently did not spend any meaningful amount of time at sea. Upstream migrating adults that were previously captured in the downstream trap the same year were also assumed to have mistakenly re-entered the trap rather than be attempting a return migration as most recaptures occurred with one day of release. In 2014 only one of 471 tagged downstream adults was captured moving upstream more than a week after its initial release, and this individual had been recaptured in the interim indicating that it likely never went to sea.

Management Implications. — The Nulahugyuk Creek arctic char population provides examples of the challenges of managing a species with extensive life history diversity, particularly in an era of accelerated global climate change. Our results suggest that nearly all migrants in Nulahugyuk Creek utilize non-natal systems to overwinter and consequently they are likely mixing with other, more heavily exploited populations without management consideration (Roux

et al. 2011). While many arctic char fisheries are recognized as mixed stock, the extent of straying in our population exemplifies the need for the development of associated tools such as a thorough genetic stock assignment library to allow researchers and managers to better resolve stock composition. Furthermore, the basic nature of our findings suggests that there are likely still life-history aspects of anadromous arctic char that are not understood well enough to make well-informed management decisions. For example, if as we propose that some Nulahugyuk Creek arctic char overwinter in the Coppermine River and are among the first to enter the sea in spring, they may be disproportionately targeted by the early gillnet fishery that occurs immediately after break up at the River's mouth.

The Nulahugyuk Creek arctic char are also an example of a population that is likely vulnerable to the effects of global climate change. The creek already has a narrow migratory window caused by a rapid decline in discharge between early June and late July, and already experiences temperatures that are well beyond the optimal range and near lethal levels (Elliott and Elliott 2010; Quinn et al. 2011a). These conditions have likely already worsened as a result of climate change and will continue to decline as air temperatures rise causing a reduction in the amount of spring snowpack and an increase in water temperature within the creek (ACIA 2005; Wrona et al. 2006c).

While life history diversity certainly presents difficult management challenges, it can also protect species against exploitation and environmental change (Schindler et al. 2010). Nulahugyuk Creek arctic char possess unique life history attributes that may also make them an ideal source population if the aforementioned changes in snowpack and temperature make other surrounding systems uninhabitable for the current inhabitants that are not adapted to comparatively harsh migratory conditions and a short migratory window. In addition to the stabilizing effect of life

history diversity within a species, this diversity can also directly benefit other dependent species. For example, in sockeye salmon, variation in run timing serves to increase the feeding window of predators such as Grizzlies and Gulls, as these predators can have prolonged access to salmon by moving stream to stream based on the timing of a local run (Schindler et al. 2013). This phenomenon has not been studied for predation of arctic char, however, given the unique timing of the Nulahugyuk run and the prevalence of grizzly and gull predation on char that we observed, it is possible that this run significantly extends the period of availability of arctic char for these predators. Given the potential benefits of life history diversity, when management plans are being developed, the intent should be to not only maintain individual population size, but also to preserve their unique traits (Potter et al. 2003).

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Tables:

Table 2.1. Length, weight, and condition (K) of Nulahugyuk Creek arctic char at various life-history stages

	Length (mm)	Weight (g)	Condition
2012			
US Adult	659±41^{A}	3198±723^{A}	1.09±0.11^{A}
DS Adult	630±75^{B}	ID	ID
Smolt	186±25^{C}	51±22^{B}	0.77±0.06^{B}
2014			
US Mortality	789±51^{D}	5767±995^{C}	1.13±0.12^{A}
US Adult	729±54^{E}	4268±998^{D}	1.09±0.09^{A}
DS Adult	717±54^{F}	3139±752^{A}	0.84±0.09^{C}
Smolt	193±21^{C}	61±21^{B}	0.81±0.07^{D}

Data presented as mean ± standard deviation

Dissimilar letters indicate significant differences (α=0.05)

ID, Insufficient data; US, Upstream; DS, Downstream

Table 2.2 Otolith based age (years) estimated of Nulahugyuk Creek arctic char at various life-history stages

	N	Mean ± SD	Min.	Max.
Adults	37	10.5±0.9	8	12
AFM	18	4.0±0.8	3	5
Smolts	16	4.3±0.7	3	5

AFM: age at first migration

SD: standard deviation

Table 2.3. Nulahugyuk Creek arctic char length-weight and growth models

Component	Coefficient	SE	*t*	*p*
Length-weight model[1]				
$\log_{10}$ a	-5.251	0.016	-330.9	<0.001
b	3.068	0.006	507.6	<0.001
Life-history adjusted length-weight model[2]				
$\log_{10}$ a	-4.784	0.098	-48.6	<0.001
b	2.931	0.035	83.8	<0.001
US adult	Reference term			
DS (DS adult)	-0.098	0.005	-21.6	<0.001
Sm (Smolt)	-0.160	0.019	-8.4	<0.001
Growth model[3]				
L_o	183.0	10.58	17.30	<0.001
a	565.3	22.03	25.65	<0.001
b	1.539	0.141	10.93	<0.001
c	5.854	0.097	60.13	<0.001

[1] $\log_{10} W = b \cdot \log_{10} L + \log_{10} a$

[2] $\log_{10} W = b \cdot \log_{10} L + DS \cdot (1/0) + Sm \cdot (1/0) + \log_{10} a$

[3] $L = L_o + a \cdot \exp(-e^{(-(t-c)/b)})$

W = wet weight (g); L = Fork length (mm); t = age (years)

Table 2.4. Timing of the upstream migration of various anadromous arctic char populations in North America

Name	Period of upstream migration	Reference:
Nauyuk River	early August - mid-September	(Johnson 1989)
Firth River	late July - late September	(Glova and McCart 1974)
Fraser River	mid-July - late September	(Dempson and Green 1985)
Ikaluit River	mid - late August	(Read 2003)
Meladine River	August – mid-September	(McGowan 1992)
Rat River	August	(Gillman and Sparling 1985)
Diana River	mid-August - early September	(McGowan 1987)
Ekalluk River	late August - early September	(McGowan 1990)
Jayco River	late August - mid-September	(McGowan 1990)
Halovik River	mid-August - early September	(McGowan 1990)
Lauchlan River	late August - mid-September	(McGowan 1990)
Nulahugyuk Creek	late June - late July	Present study

Figures

Fig 2.1. Study area in northwest mainland Nunavut, Canada. Nulahugyuk Creek was the primary study site; Arctic char captured here feed in the Coronation gulf and Dolphin and Union Sound region and are thought to overwinter in navigable freshwater systems in the area.

Fig 2.2 Physical migratory environment of Nulahugyuk Creek, NU. Photos taken at a standardized location demonstrating the navigability of a representative riffle section mid- (A; early July) and post migration (B, early August). Creek discharge (C) and water temperature (D) values shown in white, grey, and black are from 2012, 2013, and 2014 respectively.

Fig 2.3 Daily number of arctic char migrating passed the fish trap by life history stage. The counts for each life history stage are shown separately for the 2012 (grey) and 2014 (black) field seasons.

Fig 2.4 Length frequency distributions of migrating arctic char captured during the 2012 and 2014 field seasons. The data is shown as percentages of either smolts (dark grey) or adults (light grey).

Fig 2.5 The relationship between length and weight over various life-history stages. Upstream migrating adults, downstream adults and smolts are shown in dark grey, white, and light grey respectively. The back transformed two-factor length-weight model is shown by the solid black line with the 95% prediction interval shown by the dashed grey lines. For model parameters see Table 3.

Fig 2.6 Back-calculated growth in Nulahugyuk Creek arctic char. Boxes represent the interquartile range around the median, lower and upper whiskers represent the 10^{th} and 90^{th}

percentile respectively and dots represent outliers. The Gompertz growth model is depicted by the solid grey curve with the 95% prediction interval shown by the dashed lines. For model parameters see Table 2.3.

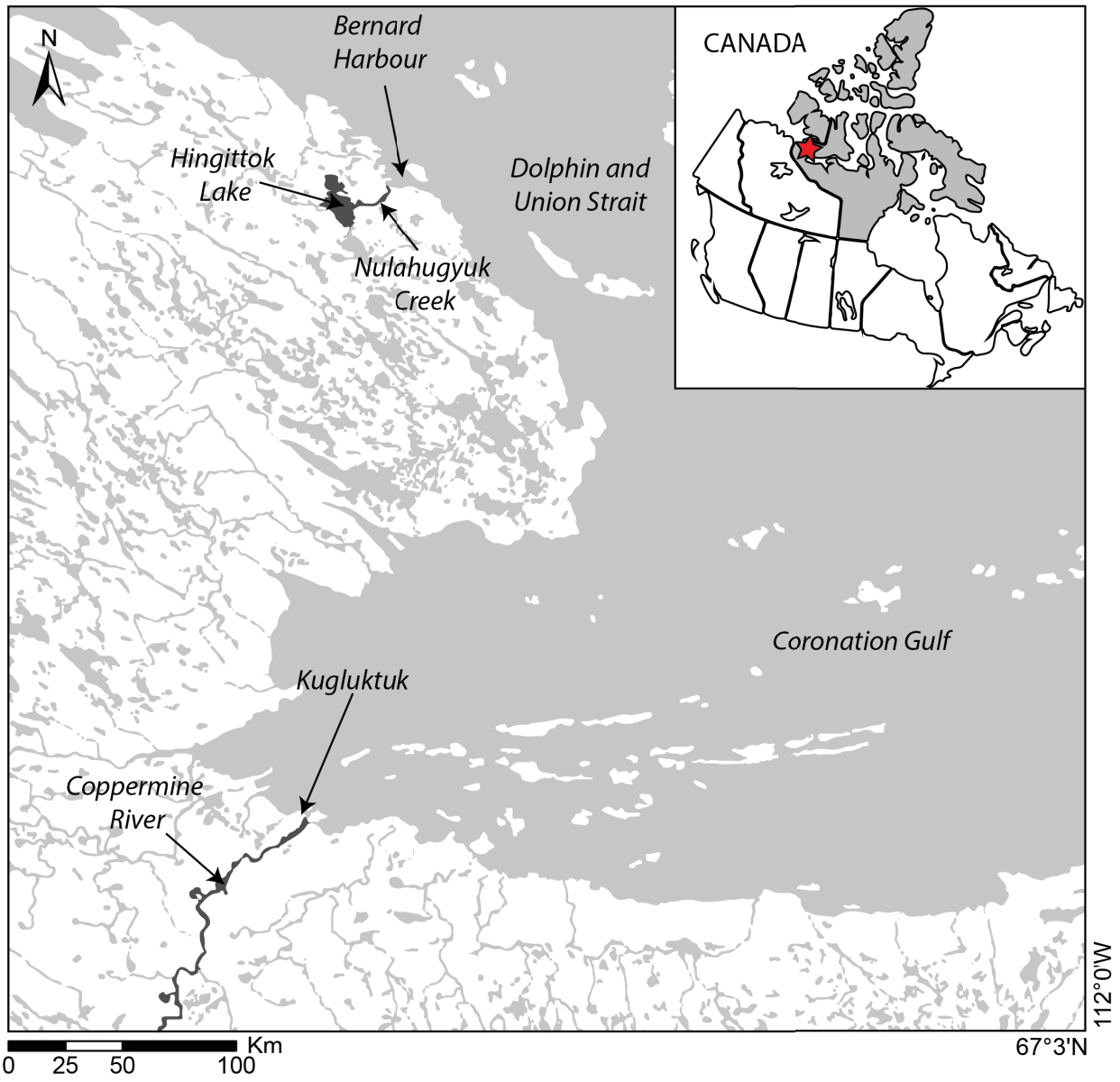
N
Bernard Harbour
Hingittok Lake
Dolphin and Union Strait
Nulahugyuk Creek
CANADA
Coronation Gulf
Kugluktuk
Coppermine River
0 25 50 100 Km
67°3'N
112°0'W

Figure 2.1

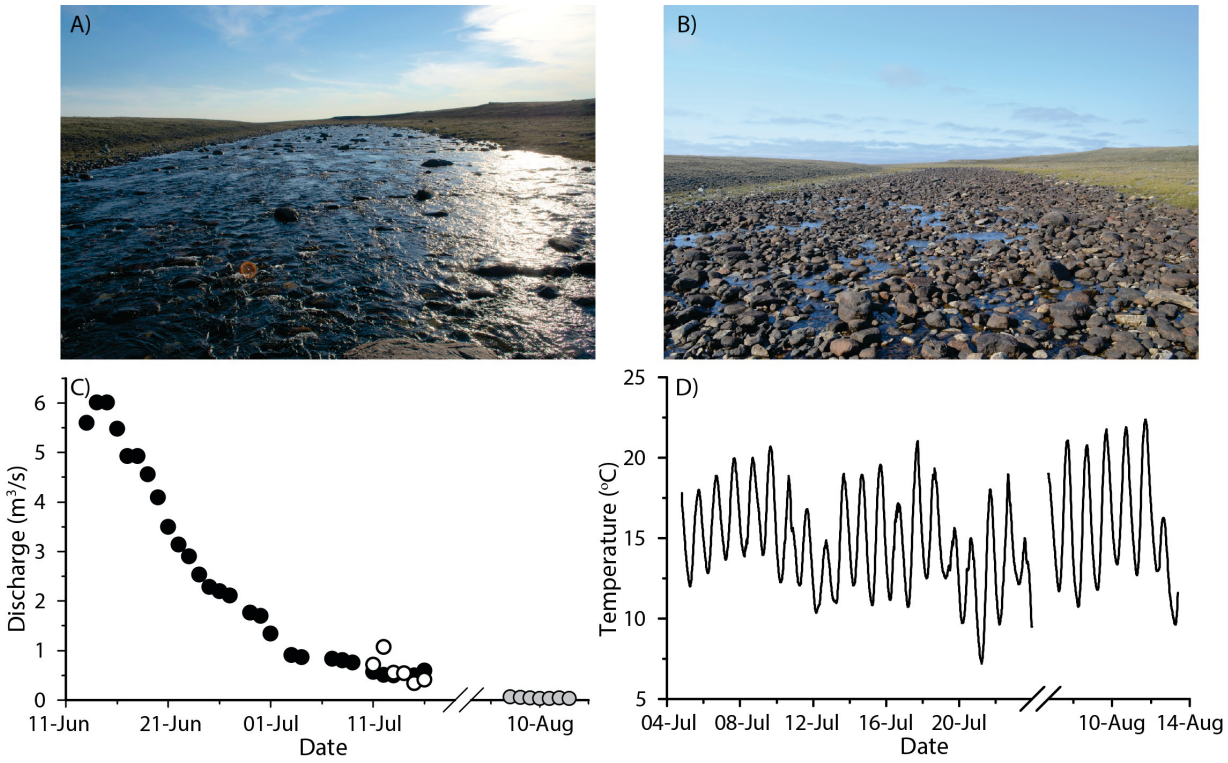
A)
B)
C)
Discharge (m³/s)
0
1
2
3
4
5
6
11-Jun
21-Jun
01-Jul
11-Jul
10-Aug
Date
D)
Temperature (°C)
5
10
15
20
25
04-Jul
08-Jul
12-Jul
16-Jul
20-Jul
10-Aug
14-Aug
Date

Figure 2.2

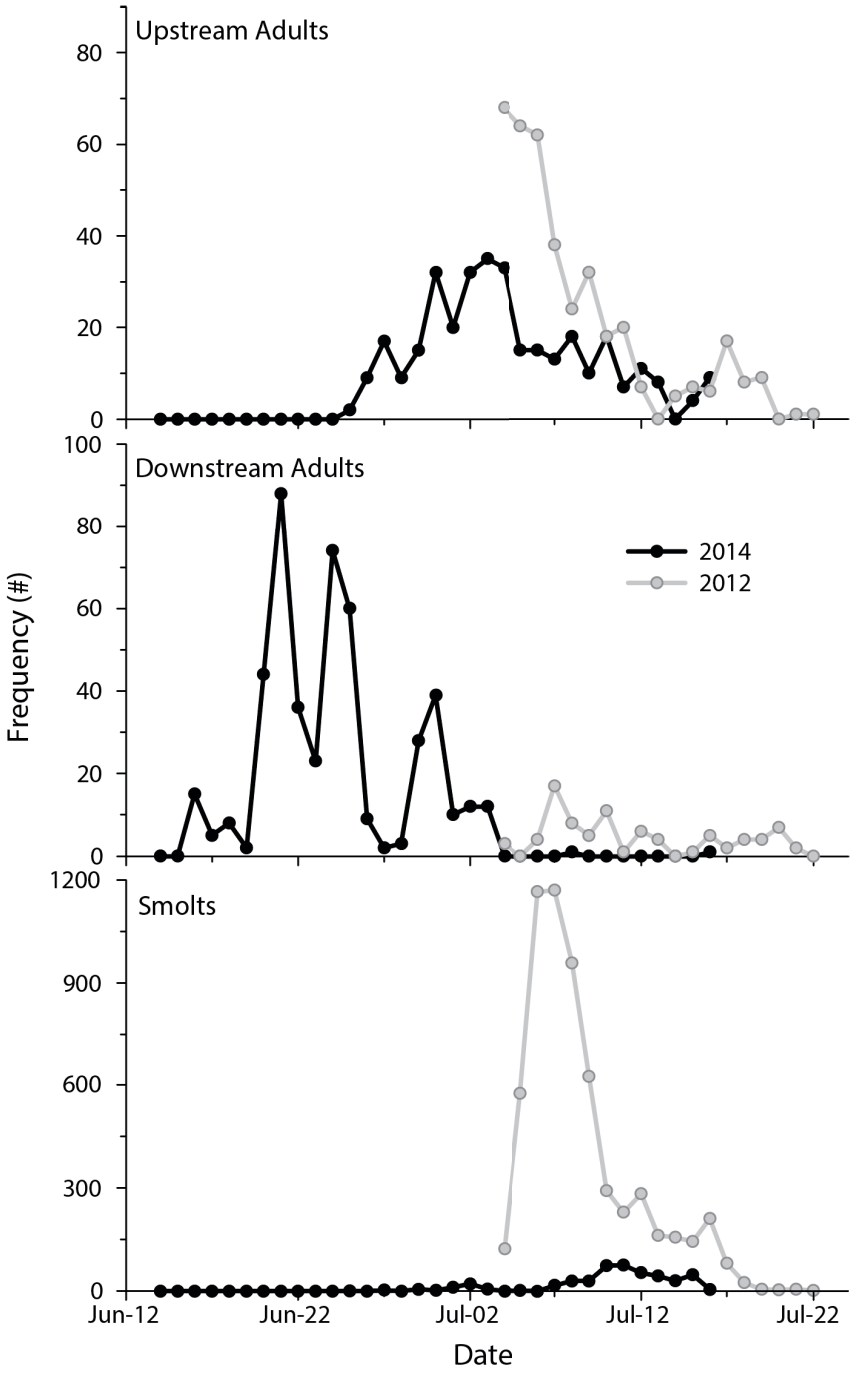
Upstream Adults
Downstream Adults
Smolts
2014
2012
Frequency (#)
Date
Jun-12
Jun-22
Jul-02
Jul-12
Jul-22

Figure 2.3

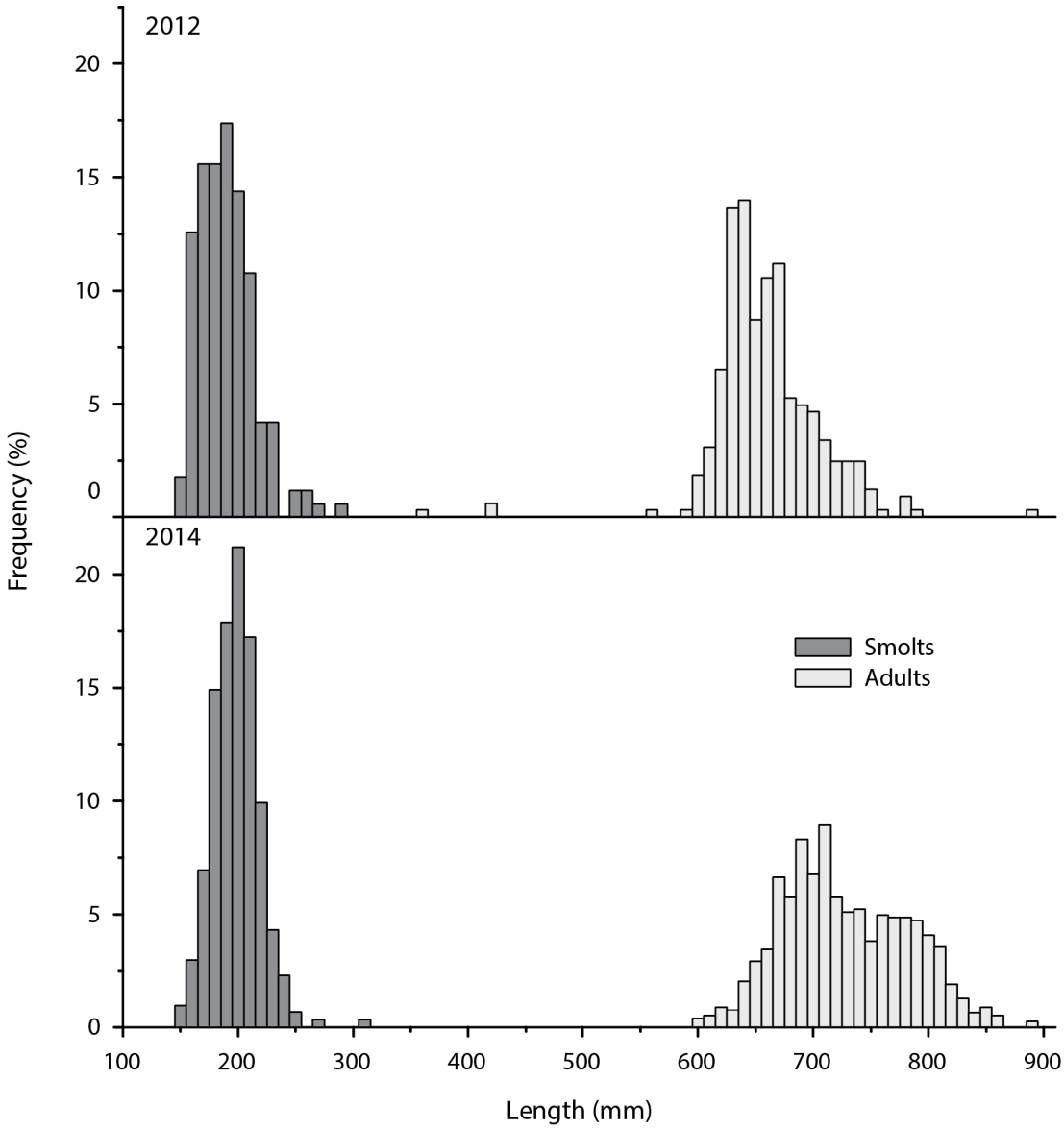
2012
2014
Frequency (%)
Length (mm)
Smolts
Adults
0
5
10
15
20
100
200
300
400
500
600
700
800
900

Figure 2.4

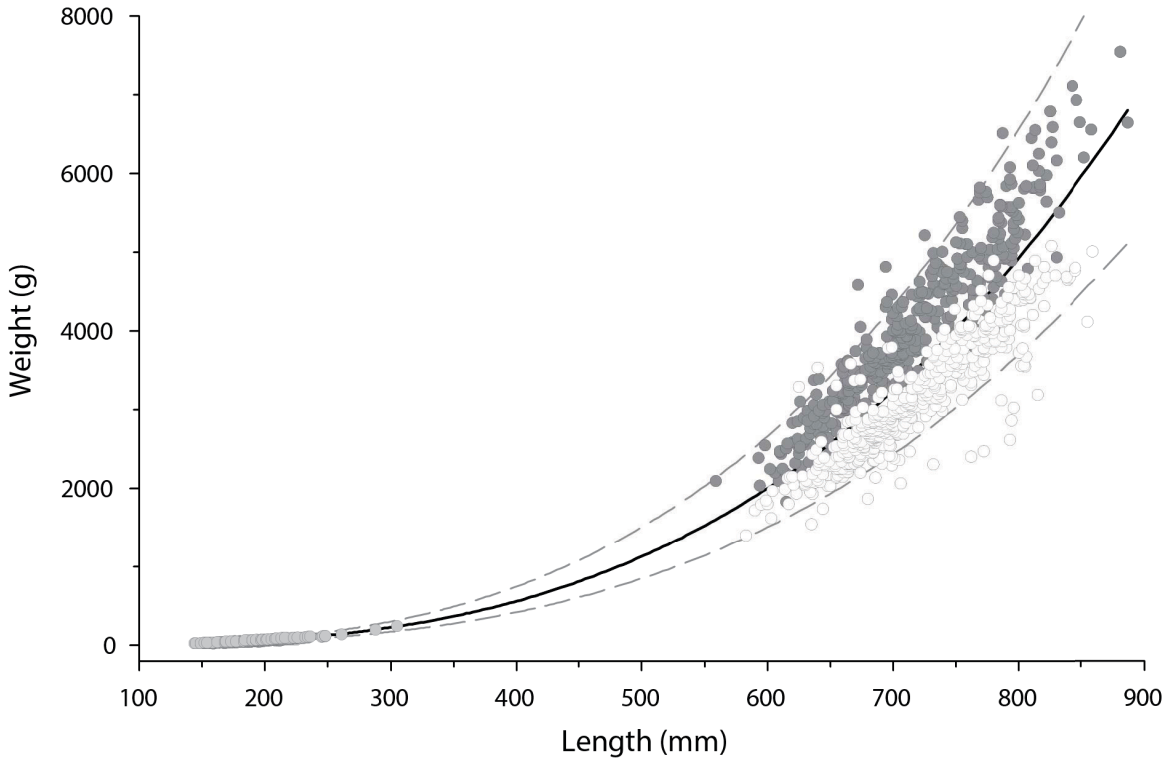
8000
6000
4000
2000
0
Weight (g)
100
200
300
400
500
600
700
800
900
Length (mm)

Figure 2.5

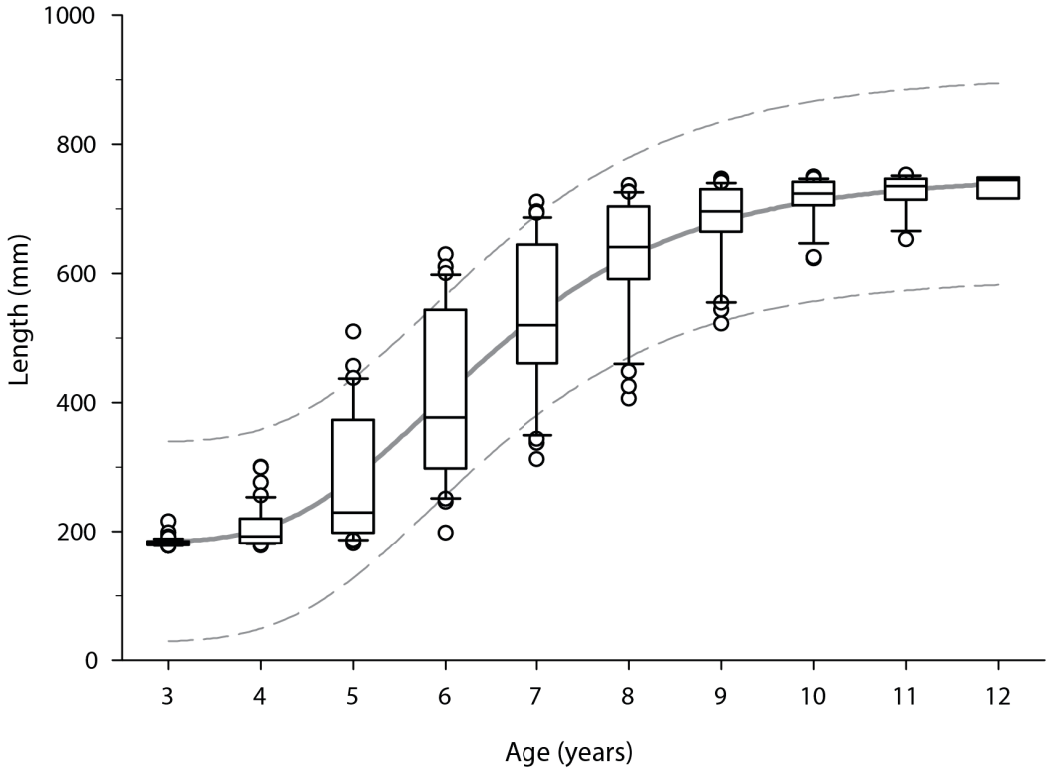

Figure 2.6

Chapter 3: Repeat swimming performance and physiology of arctic char (*Salvelinus alpinus)* and rainbow trout (*Oncorhynchus mykiss)* exposed to realistic diurnal temperature fluctuations.

Abstract

Arctic char are considered a typical cold-water stenotherm, however, we have identified a population that annually migrates through a warm (>21°C), physically challenging creek that undergoes large daily temperature fluctuations (>10°C). Contrary to expectation, in-field repeat exercise tests on juvenile char showed no substantial effect of temperature on initial swimming performance, however there appeared to be threshold for repeat swimming performance near 20°C above which recovery may be impaired. To further investigate this phenomenon we exposed juvenile arctic char and rainbow trout, a temperate reference salmonid, to simulated diurnal temperature scenarios in lab. As in the field, an initial exercise test did not reveal impaired performance at warmer temperatures, however, in a second test conducted after a brief recovery, swimming performance was significantly reduced in char but not in rainbow trout. This impaired repeat swimming performance after warming was associated with a reduced ability to increase routine aerobic metabolism at warm temperature. Diurnal warming also had more pronounced effects on blood composition and plasma glucose and lactate in arctic char than rainbow trout. These results suggest that the physiological performance of arctic char is more subject to impairment by thermal variation than temperate salmonids. This relative thermal limitation may constrain their migratory success under current conditions in some arctic streams and may put them at a competitive disadvantage for migratory habitat as more temperate species expand their ranges northward.

Introduction

The exercise physiology of salmonid species and populations is often adapted to function optimally under a relatively narrow range of frequently encountered temperatures (Eliason et al. 2011; Farrell 2009b). As global climate change progresses, deviations from historical freshwater thermal regimes result in an increase in exposure of migrating salmon to temperatures above these optimal ranges (Eliason et al. 2011; Farrell et al. 2008a), which has already been associated with instances of impaired migratory performance (Farrell et al. 2008a; Hinch et al. 2012; Martins et al. 2012; Richter and Kolmes 2005). Extensive research on the effects of temperature on the migratory physiology of Pacific salmon indicates that cardiorespiratory limitations at warm temperatures are an important contributor to this impaired performance and to determining thermal tolerance in general (Eliason et al. 2011; Farrell et al. 2008a; Farrell 2009b; Farrell et al. 2009).

Thermal physiology in salmonids has typically been studied at constant temperatures to which salmon have been previously acclimated (eg. Eliason et al. 2011; Farrell 2009b), at rates of temperature change that may not represent common natural variation (eg. 2°C/hr, Clark et al. 2012; Clark et al. 2008; Penney et al. 2014; Steinhausen et al. 2008), or at point-sampled temperatures in wild fish (Farrell et al. 2003; Rodnick et al. 2004). Environmental exposure more commonly occurs on diurnal to seasonal scales, or acutely in an irregular manner as fish transition between different thermal environments such as when moving between the ocean, rivers and lakes and when undertaking vertical migrations (Farrell 2009b; Friedland et al. 2001; Mathes et al. 2010; Rikardsen et al. 2007; Rodnick et al. 2004). The rate at which temperature change is experienced can have a pronounced effect on fishes tolerance to that variation (Dowd et al. 2015; Galbreath et al. 2004; Mora and Maya 2006). This effect may result in important

differences between the tolerance and response of salmonids to realistic and typical laboratory tested thermal regimes(Dowd et al. 2015).

In addition to the study of natural thermal regimes, the thermal physiology of more northerly distributed salmonids has also received relatively little attention (Penney et al. 2014), despite the fact that the effects of global climate change are most pronounced at higher latitudes (ACIA 2005; Wrona et al. 2006c). Among northern salmonids, the arctic char (*Salvelinus alpinus*) is of particular interest as it has the most northerly distribution of any freshwater fish and they possess extensive life history diversity that facilities its existence throughout the global arctic in a wide range of environments (Klemetsen 2010; Klemetsen et al. 2003; Roux et al. 2011). While this expansive, northern distribution and life history diversity may help the stability the species in the long term (Schindler et al. 2010) it also means that some populations likely utilize life-histories and habitats that make them particularly vulnerable to changing thermal regimes. For example, we identified one anadromous population in which individuals spend nearly all of their lives in cold (<11°C), thermally stable lacustrine and marine environments, but migrate between these environments via Nulahugyuk Creek (Fig.1), a shallow, physically challenging creek that undergoes large diurnal temperature fluctuations (>10°C per day) over broad a range of temperatures from ~7 to 21°C (Fig. 2).

In the present study we utilized field and laboratory methods to determine the effects of diurnal temperature fluctuations on arctic char migratory physiology. In the field, we assessed the repeat swimming performance of juvenile char, over the naturally occurring range of temperatures that char were observed migrating through. Based on field results, in the laboratory we exposed arctic char, to a simulated diurnal temperature fluctuation representative of the upper end of the range naturally encountered by migrating char in Nulahugyuk Creek and assessed

their behavior, repeat swimming performance, aerobic metabolism and several sub-organismal stress related endpoints. In a companion study (Chapter 4), we assessed biochemical and transcriptional endpoints and related them to the findings of the present study.

All laboratory endpoints were also assessed in rainbow trout (*Oncorhynchus mykiss*), which serves as a temperate reference species. Rainbow trout were selected because their thermal physiology has been well studied, they could be reared under nearly identical conditions to char, and like arctic char they are iteroparous and facultatively anadromous. In general, we expected that both species would exhibit physiological impairments at peak diel temperatures, but that arctic char would be much more sensitive to warming over the tested range and would therefore recover to a lesser extent during diel cooling.

Materials and Methods

Field sampling

Field sampling took place in the summers (June – August) of 2012–2014 near the mouth of Nulahugluk Creek, Nunavut (NU; 68.75°N 114.8°W; Fig 1). Fish were captured migrating between their freshwater and marine environments (Hingittok Lake and Bernard Harbour, NU, Fig 1) using a two-way fyke net 300m upstream from the mouth of the creek. Water temperature was recorded at the trap every 15 min using an automated temperature logger (HOBO Water Temperature Pro, Onset Computer Corporation, Bourne, Massachusetts). Detailed temperature and discharge data can be found in Chapter 2.

In 2012, creek side swimming performance tests were conducted on a subset of juvenile arctic char over the range of naturally occurring temperatures to assess the effect of natural temperature

variation on cardiorespiratory performance. These test were conducted as described below for the laboratory-based research.

Laboratory animals

Arctic char were obtained at six months post hatch from Elkview Farms (Red Deer, Alberta, Canada). The char were hatched in December 2012 at Elkview Farms but ova and sperm were obtained from the broodstock at the B&B Freshwater Fish Farm (Gunton, Manitoba). This broodstock originated from the Nauyuk Lake, NU population, which like our study population is in the central arctic and it also possesses some important life history similarities (Chapter 2). Rainbow trout were hatched at the University of Alberta (UofA; Edmonton, AB) aquatics facility, in December 2012. In May 2013 trout and char were transferred to the same housing rack at the UofA and separated into several 30L flow through tanks and held at similar density in 11°C dechloirinated municipal tap water. Fish were fed trout pellets twice daily (Nu-Way Trout Grower-Finisher, Hi-Pro Feeds, Okotoks, Alberta) and held under a simulated local light-dark cycle. Following all experiments fish were sacrificed, weighed to the nearest gram, and measured to the nearest mm. The ventricle was weighed to the nearest 0.001g and expressed as VM or relative ventricular mass (RVM) i.e. a percentage of body mass. Condition factor was calculated as $K = Mass/Length^3 * 10^{-5}$.

Diurnal temperature treatments.

Fish were exposed to one of three temperature treatments (Fig. 2) in a "bow-tie" swim-tunnel previously described by Tierney et. al (Tierney et al. 2011). Briefly, this swim tunnel has two large (25 cm^3), low flow chambers connected by a long, narrow (100 x 10 x10 cm), high flow section which allows fish to select between a range of water speeds and move between upstream

and downstream flow refugia. Flow in the center section was set at 35 cm s^{-1}, a speed commonly encountered by migrating char in Nulahugyuk Creek, NU, and ranged in the end sections from 35cm s^{-1} down to speeds low enough that fish could rest on the bottom without swimming. Control and diurnal warming treatments lasted between 16 and 24hrs with variation in duration occurring only in the 11°C component of the treatment (Fig. 2). All fluctuation treatments lasted for 24hrs as the entire duration was necessary to achieve the required temperatures at the selected rate of variation. Temperatures were achieved using Autoresp software (Loligo Systems, Tjele, Denmark) to control two solenoid valves that regulated the flow of cold or warm water into the swim tunnel.

Repeat swimming performance and gait transition

Immediately following the temperature treatment fish were transferred to a 90L swim-tunnel respirometer with a 70x20x20 cm test section (Loligo Systems, Tjele, Denmark) that was held at the final temperature of the respective treatment (Control and Diurnal fluctuation: 11°C ; Diurnal warming: 21°C). Fish were allowed to acclimate at a low water speed (0.5 body lengths s^{-1}; bl s^{-1}) for 30 min after which the speed was increased by 0.5bl s^{-1} every ten minutes until the fish fatigued and was therefore not able to remove itself from the rear gate within five seconds. Fish were then allowed to recover for 30 min at 0.5 bl s^{-1}. To assess the ability of fish to recover following the first exercise test, fish were given a second test except speed was increased every minute rather than every ten. The standard Brett equation (Brett 1964) was used to calculate the critical swimming speed for each test. A small electrical charge (6v) was applied to the rear gate to motivate fish to swim throughout the duration of both tests.

The gait transition speed (U_{GT}), an estimate of the speed at which the primary mode of swimming changes from steady to burst and coast, was determined from video using automated visual tracking software (Ethovision XT8, Noldus Information Technology Inc., Leesburg, Virginia) to generate ground speed and positional data. U_{GT} was defined as the speed at which the fish carried out three or more bursts per min and continued for the duration of the test (MacNutt et al. 2006). Rather than manually scoring bursts as was previously done, a burst was defined as a bout of upstream acceleration in 99th percentile of the data for the trial. This method produced U_{GT} estimates that were well within the published range (MacNutt et al. 2004; MacNutt et al. 2006).

Aerobic metabolism

Oxygen concentration was recorded every second during the swimming performance test from the start of the 30 min acclimation to the end of the 30 min recovery using an anode-cathode oxygen probe placed in the manufacturer specified port in the conventional swim tunnel. The probe was attached to the same DAQ unit and software as the swim tunnel. All swimming performance tests started at 100% O_2 saturation and the swim tunnel was sealed for the duration of the tests unless oxygen concentration dropped below 7.5mg/L, in which case the tunnel was briefly flushed with fresh water. Routine metabolic O_2 consumption rate ($MO_{2routine}$) was taken as the minimum [O_2] slope over five minutes that had an $R^2 > 0.90$ within the acclimation period. The metabolic rate for each step within the swim test was taken as the maximum [O_2] slope over one minute that had an $R^2 > 0.90$ in that step, which always occurred in the later half of the step as fish tend to take a few minutes to adjust to swimming at a new speed. Post-exercise oxygen consumption (POC) rates were calculated as the slope of [O_2] over each five minute increment of the 30 min recovery period following fatigue in the first swim test. In calibration trials, no

background oxygen consumption was detected. Changes in water velocity interfered with O_2 detection, but profiles stabilized within two minutes of the change, and because of the way MO_2 values were calculated the values from this period of each step were not utilized.

Blood analysis

Blood was drawn by caudal puncture using at 21-gauge needle and 5ml syringe that had been pre-rinsed with lithium-heparin (100 IU/ml; Sigma-Aldrich, Oakvillem Ontario). Three to five pre-hepranized microcapillary tubes (Thermo Fisher Scientific, Waltham, Massachusetts) were filled at the start of sampling and the rest of the blood was transferred into microcentrifuge tubes. Microcentrifuge tubes were spun for five minutes at 5000g after which plasma was transferred into new microcentrifuge tubes and stored until analysis at -20°C. Capillary tubes were spun for three minutes at 10,000g and hematocrit (Hct) was then measured as the height of the packed red blood cell column over that of the entire sample using digital calipers. The final Hct value was taken as the average Hct of at least three capillary tubes. After spinning, two drops of plasma were applied to test strips for lactate and glucose meters (Lactate: Lactate Plus, Nova Biomedical, Waltham, Massachusetts; Glucose: Accu-chek Nano, Roche, Basel, Switzerland). Glucose readings were verified to be accurate by comparison with plasma glucose values obtained from the nuclear magnetic resonance (NMR) based metabolomics analysis that was conducted on a subset of plasma samples (Chapter 4), however, lactate values obtained from the meter were adjusted to better represent NMR concentrations using the linear relationship with plasma lactate values obtained in the NMR analysis. In addition to the subset of individuals sampled in our companion study, plasma lactate and glucose concentrations were measured in all individuals in the present study as elevations in their concentrations are commonly used as secondary indicators of stress and have been associated with early mortality

in migrating salmon (Cooke et al. 2006). Plasma osmolality was determined though freeze-point analysis using a Model 3300 osmometer (Advanced Instruments, Norwood Massachusetts)**.** Plasma cortisol was measured by using a commercial enzyme-linked immunosorbent assay kit (Kit # 402710, Neogen Corp., Lexington, Kentucky, USA) and following the manufactures recommended protocol with two changes, ethyl acetate was used as an extraction solvent, and the solvent evaporation took place in a fume hood without a nitrogen stream or vacuum as in Canavello et al (Canavello et al. 2011).

Data analysis

All statistical analyses were conducted using R Studio (Team 2014). Figures were produced using ArcGIS (ESRI, Redlands, California), Sigmaplot 11.1 (Systat Software, San Jose, California) and Adobe Illustrator CS4 (Adobe systems, San Jose, California).

For laboratory animals, differences in all morphometric variables, swimming performance, recovery, $U_{GT,}$ Hct, plasma osmolality, cortisol, lct, and glu, were assessed using two-way analyses of variance with species, treatment and their interaction as predictor variables followed by Tukey's Honest significant difference test to allow for pairwise comparisons. For swimming performance, length was included as a covariate. For $MO_{2routine}$ and MO_{2max} VM, Cort, Hct, lct, and glu, body weight was included as a covariate. Covariates were removed from the analysis if they or their interactions were not statistically significant (α=0.05). Student's t-tests were used to analyze difference in FL, mass, and K between wild and captive char.

MO_2 data were analyzed using linear-mixed effects models (LMMs) generated using the lme4 package (Bates et al. 2013) and back fit using the lmertest package (Kuznetsova et al. 2014). Two separate MO_2 models were created for each species, one for the duration of the $U_{Initial}$ test

and another for the recovery period. Treatment and the log of body mass were included as fixed predictor variables in both MO_2 models. Time into the exercise test was included in the $U_{Initial}$ MO_2 model, while time post fatigue was included in the recovery MO_2 model. The individual fish ID was included as a random predictor variable to account for the fact that multiple MO_2 values recorded from the same individual are not independent. The marginal and conditional R^2 values were reported to describe the proportion of variation explained in each model by just the fixed effects (marginal) or by both random and fixed effects (conditional).

Field swimming performance and recovery data were analyzed using linear and quadratic regressions against temperature and the model with the lowest residual standard error was selected. Upon visual inspection of U_{Repeat} and recovery ratio vs. temperature relationships the two data points available for 21°C appeared to be outliers on an otherwise positive correlation, so a second analysis was conducted with the values excluded (Table 2, Fig. 3). In all regression models all possible interactions were assessed. If an interaction effect was not significant it was removed from the model as long as no higher order interactions were significant. To ensure assumptions of the various analyses were met normality of residuals was assessed using Shapiro-Wilks tests and visual analysis of q-q plots, and equality of variances using Levene's test when appropriate. If assumptions were violated Tukey's ladder of transformation was employed until a suitable transformation was identified. In all analyses interactions were treated as being multiplicative.

Results

Study animals.

In the laboratory study, rainbow trout were generally smaller than the arctic char (Fork length: -32%, $F_{2,39} = 21.9$,$p = 3.3*10^{-5}$; Mass: -13% , $F_{2,39} = 17.0$ $p = 1.8*10^{-4}$; Table 1) but had a similar condition factor (+4% , $F_{2,39} = 2.0$ $p = 0.17$; Table 1). VM co-varied with body mass but was still significantly lower in trout after accounting for mass (-25%, Species: $F_{1,38} = 88.7$, $p = 2.0*10^{-11}$, Mass: $F_{1,38} = 64.4$, $p = 7.3*10^{-10}$; Table 1). For comparison with previous studies, RVM was also analyzed and was not-surprisingly also lower in trout (-27%, $F_{1,38} = 20.5$, $p = 6.0*10^{-5}$; Table 1). There were no statistically significant morphometric differences between treatments ($F_{2,39} < 2.1$, $p > 0.13$). The wild juvenile arctic char used in swimming performance tests had a nearly identical average FL to captive char (-0.6%, $t_{52} = 0.2$, $p = 0.81$; Table 1) but had a significantly lower mass and condition factor. (Mass: -22%, $t_{47} = 3.7$, $p = 5.0*10^{-4}$; K: -27%, $t_{47} = 8.8$, $p = 1.4*10^{-11}$; Table 1).

Field swimming performance and recovery

In wild arctic char, $U_{Initial}$ had a weak quadratic relationship with temperature over the tested range (Table 2; Fig. 3). U_{Repeat} and consequently, recovery ratio, both exhibited linear positive relationships with temperature after the removal of data points sampled at 21°C (Table 3.2; Fig. 3.3). Only two individuals were tested at 21°C, however, their relatively low U_{Repeat} and recovery ratio may indicate a threshold for recovery near 21°C.

Laboratory swimming performance and recovery

Swimming performance and recovery ratio varied between diurnal temperature treatments in different manners for the two species. Specifically, U_{Initial} increased in trout following warming but did not change in char (Table 3.3; Fig. 3.4). U_{Repeat} also increased in trout following warming but decreased in char. Consequently recovery ratio did not change with warming in trout but

substantially deceased in char . Interestingly, for both species all but one metric of swimming performance ($U_{Repeat,}$ BL s^{-1}) and recovery following the complete diurnal fluctuation were numerically intermediate between the control and diurnal warming groups (Table 3.2, Fig,3.4). Even after accounting for treatment effects, comparison of least squares means showed that arctic char had lower initial swimming performance than rainbow trout ($U_{Initial}$: -19% $p<0.001$). U_{GT} was not different between species under control conditions or following diurnal fluctuation but numerically increased in trout and decreased in char following warming which resulted in a significant difference between species following warming (Table 3.2).

Aerobic metabolism during fatiguing exercise and recovery

Treatment effects on aerobic metabolism differed between trout and char (Table 3.3 and 3.4, Fig. 3.5). In trout, $MO_{2routine}$ and MO_{2max} both increased substantially following warming (Table 3.3) and were intermediate between control and warming following the complete fluctuation. In char, only minor numerical increases were found following warming and the fluctuation group was indistinguishable from control (Table 3.3). The LMMs revealed that in both species over all treatments metabolism increased with water velocity (time into test) (Table 3.4). Comparisons between LMMs showed that for arctic char during the $U_{initial}$ test, treatment did not significantly explain a portion of the variance in the data, however, recovery toward routine metabolism was lower in the warming group (Fig. 3.5) and so the warming treatment was a significant predictor variable in the recovery MO_2 model (Table 3.4). In trout, treatment variables explained unique portions of the variation in both exercise and recovery models and agreed with the trend that for the exercise test MO_2 was lowest in the control, intermediate in the fluctuation treatment and highest in the warming treatment. During recovery, the model suggests that the warming and control group recovered at a similar rate, while the fluctuation treatment group recovered faster.

Blood analysis

In trout, Hct did not differ between treatments but was lower following warming in char and numerically intermediate following the complete fluctuation (Table 3.3). There were no apparent changes in plasma osmolality between species or treatments (Table 3.3). Overall, plasma cortisol was lower in arctic char than in rainbow trout and numerically decreased following warming in char, however, no pairwise comparisons were significant (Table 3.3). Overall, plasma glucose and lactate concentrations increased following diurnal warming, although numerically to a greater extent in arctic char (Table 3.3). Both metabolites had intermediate concentrations following the complete fluctuation except for lactate in rainbow trout which was similar to control (Table 3.3).

Discussion

Arctic char are commonly considered to be a classical cold-water stenotherm and among the least thermally tolerant of all salmonids. However, we have recently identified one population that annually migrates through a shallow creek that experience drastic diurnal temperature fluctuations with maximal temperatures exceeding 21°C (Chapter 2; Fig. 3.1). Our results suggest that in terms of aerobic exercise performance, arctic char are likely more tolerant to thermal variation over environmentally relevant scales than previously thought, but as expected, they are more sensitive than related temperate species. Specifically, arctic char were able to maintain their initial swimming performance when brought from 11 to 21°C over 12 hours, however their ability to recover from this initial bout of fatiguing exercise was impaired while it was not impaired in rainbow trout. Arctic char also exhibited more pronounced effects of diurnal warming on blood composition and in plasma glucose and lactate levels, and char had an overall

lower capacity for aerobic exercise. Together, my results suggest that more temperate salmonids such as the rainbow trout likely already possess a competitive advantage for migratory habitat in the north over some northern salmonids under current summer conditions, which could allow for displacement as winter conditions at higher latitudes become more permissive of temperate species.

Initial swimming performance and aerobic metabolism

Swimming performance values were very similar between the field and laboratory for arctic char (Lab: 3.30 vs. 3.55 BLs^{-1}; Fig. 3 and 4), and were consistent with previous research for both arctic char and rainbow trout (e.g. Alsop and Wood 1997; Beamish 1980; Pettersson et al. 2010). Relative to their length, arctic char had a much lower swimming ability than rainbow trout, regardless of temperature (Table 2; Fig. 4). Trout improved swimming performance following warming from 11 to 21°C, which agrees with previous results following warming from 12-14 to 24°C over ~5hr in a desert population that experiences large diurnal fluctuations (Gamperl et al. 2002). This improvement was not surprising considering that when acclimated to similar conditions to those used in the present study, rainbow trout do not loose equilibrium until ~28°C (Carline and Machung 2001). A recent study on arctic char, however, found that under similar conditions, they exhibit a loss of equilibrium at ~23°C (Penney et al. 2014), so it is surprising that arctic char were able to maintain $U_{Initial}$ following warming from 11 to 21°C. Diurnal warming substantially increased $MO_{2Routine}$ and active MO_2 in trout, while only minor increases were observed in char (Table 2 and 3; Fig 5), which is consistent with differences in the effect of temperature on MO_2 between atlantic salmon *(Salmo salar*) and arctic char (Penney et al. 2014). Following the complete diurnal fluctuation, rainbow trout had intermediate $U_{Initial}$ and MO_2 suggesting that the physiological changes underlying their improved performance and elevated

MO_2 following warming were not fully reversed during within the diurnal cooling phase. This is consistent with a recent study in atlantic salmon that showed that smaller diurnal fluctuations could increase metabolic rate relative to being held at a constant temperature (Oligny-Hébert et al. 2015). All changes in routine and active MO_2 were proportional so there were no differences in aerobic scope between treatments. Based on an abundance of research salmonids, aerobic scope likely follows a bell shape thermal performance curve (Eliason et al. 2011; Farrell 2009b), so the lack of apparent difference in aerobic scope between treatments suggests that 11 to 21°C may be on opposite sides of the curve under the present acclimation temperature and rate of warming. Between species under control conditions $MO_{2Routine}$ was similar, however, MO_{2Max} was much higher in trout than in char and consequently so was aerobic scope; this difference persisted across treatments. The comparative low aerobic scope observed in char likely contributed to their relatively low swimming performance, and agrees with previous research on temperature induced measurements of aerobic scope that revealed that adult arctic char had a lower aerobic scope than Atlantic salmon (Penney et al. 2014). Beyond differences in thermal physiology our findings pertaining to swimming performance and MO_2 support the notion put forth by Beamish (Beamish 1980) that arctic char, and members of the genus *Salvelinus* in general, are not as proficient aerobic swimmers as their relatives in the genera of *Oncorhynchus* and *Salmo*.

Impaired repeat swimming performance following diurnal warming

Following diurnal warming in the laboratory experiments arctic char had drastically reduced repeat swimming performance, indicating an impaired ability to recover from fatiguing exercise and a relatively greater disruption of contributing biological processes (Jain et al. 1998; MacNutt et al. 2004). Interestingly in the field, an increase in recoverability was seen over the tested

range of temperatures up to 20°C, after which repeat swimming appeared to fall off precipitously, however, the number of individuals tested above 20°C was too small (N=2) to perform any meaningful threshold or breakpoint analysis. A reduction in the ability to recover from exercise in migrating char is concerning as salmonid migrations typically occur in bouts of strenuous exercise, followed by periods of lower exertion to recover, as opposed to single prolonged swimming efforts, and periods of prolonged exertion are associated with reduced migratory success (Hinch and Bratty 2000). This impaired recoverability may pose an even greater threat in shallow rivers such as Nulahugyuk Creek (Fig.1) where stranding and therefore aerobic fatigue are very common and thermal refugia are limited (Chapter 2). Arctic char we not able to increase $MO_{2Routine}$ to the same extent as rainbow trout when brought to 21°C and following a fatiguing exercise test at this temperate they did not recover to the same extent as either other treatment group, or as trout.

The decreased ability to increase MO_2 with temperature suggests that the performance of some component of the cardio respiratory system or cellular respiration was thermally limited relative more temperate species which agrees with previous findings in non-exercised char when compared to Atlantic salmon (Penney et al. 2014). It is also consistent with the notion in other salmonids that thermal tolerance in largely determined by the capacity to deliver and utilize oxygen (Eliason et al. 2011; Farrell et al. 2009). The fact that arctic char also had a longer recovery time also supports this notion, as impaired aerobic performance at warm temperature would necessitate a greater utilization of anaerobic metabolism to achieve the same $U_{initial}$ and would therefore incur greater oxygen debt. The prolonged recovery likely meant that the scope for aerobic performance at the start of the U_{repeat} was reduced and therefore limited repeat performance (Lee et al. 2003).

Blood physiology in relation to recoverability

The low Hct following warming in arctic char was likely the result of either an increase in fluid retention, or a failure to increase red cell volume. Increased fluid retention may have occurred as a compensatory mechanism to maintain or increase mean arterial blood pressure if cardiac output was limited at high temperatures as is common during exercise at warm temperatures in salmonids (Eliason et al. 2011; Farrell 2009a). However, it may have also been a result of increased mobilization of other metabolites (e.g. Lactate glucose, and amino acids; Chapter 4). If the change in Hct was associated with increased fluid retention, it would explain why the drastic increases in metabolite concentrations identified in a companion study (Chapter 4) did not result in increased plasma osmolality, and why plasma cortisol concentration appeared to decreased. Alternatively, as Hct increases with exercise (Jain and Farrell 2003), diurnal warming may have impaired the release of new red blood cells normally stimulated by exercise. Regardless of the cause, the reduction in Hct likely decreased the oxygen carrying capacity of the blood and may have contributed to the observed differences in recoverability.

Arctic char exhibited much more pronounced increases in plasma glucose and lactate than those observed in trout. This suggests that arctic char were struggling to meet aerobic cellular energy demands and were increasingly mobilizing glucose and utilizing anaerobic pathways to offset aerobic short falls (Jain and Farrell 2003; Tierney and Farrell 2004). Furthermore, these metabolites are common secondary indicators of stress and have been associated with early mortality in other salmonids it also likely means that in general the fish were in poorer physiological condition (Cooke et al. 2006).

Conclusions

For arctic char a relatively limited athletic ability paired with a lower ability to recover from exercise following naturual thermal variation will likey result in reduced migratory success, such as that found in pacific salmon (Farrell et al. 2008b; Martins et al. 2012) and make them particularily vulnerable to competition from Pacific and Atlantic salmon whose ranges appear to be expanding in the north (Dunmall et al. 2012; Jonsson and Jonsson 2009). Pink salmon may be of particualr concern as they are already being caught in the western arctic (Dunmall et al. 2012), are highly proficient swimmers, and are among the most tolerant salmonids to thermal variation (Clark et al. 2011; MacNutt et al. 2006).

It is important to note, that in both species throughout the study, results for fish that had experienced a complete diurnal fluctuation were intermediate to those that had experienced just diurnal warm and those under control conditions. These results suggest that within, the natural time scale of a day fish exposed to thermal variation do not return to the same physiological state they are in under static conditions. This finding provides support for the emerging concept that diurnal temperature variation and not just static temperatures, averages, or thermal maxima, should be considered when designing experiments intended to advance our understanding of how fish will response to natural scenarios (Dowd et al. 2015; Oligny-Hébert et al. 2015).

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Tables:

Table 3.1. Mophometrics of wild and lab reared juvenile arctic char and rainbow trout

	Arctic Char (lab)	Arctic char (wild)	Rainbow trout
N	20	35	25
Fork Length (mm)	201±4	199±4	174±4*
Mass (g)	98±6.2	73.0±3.6*	67.1±4.2*
Condition (K)	1.19±0.03	0.86±0.2*	1.24±0.02
Ventricle mass (g)	0.14±0.01	N/A	0.08±0.01*
RVM (%)	0.15±0.01	N/A	0.11±0*

* Indicates significant difference from lab reared arctic char (α 0.05)

Data are shown as mean ± standard error

RVM = Relative ventricular mass

Table 3.2 Regression models of the initial ($U_{Initial}$) and repeat (U_{Repeat}) swimming performance and recovery ratio (RR) of wild arctic char over the tested range of naturally occurring temperatures

	Coefficient	SE	*t*	*p*
$U_{Initial}$ model				
Constant	-66.7	77.2	-0.86	0.394
Temperature	-0.45	0.29	-1.53	0.136
Temperature^2	15.9	9.57	1.66	0.107
U_{Repeat} model				
Constant	5.48	24.0	0.23	0.821
Temperature	4.96	1.51	3.28	0.003
RR model				
Constant	0.57	0.29	1.99	0.059
Temperature	0.04	0.02	2.07	0.050

$U_{initial}$: $R^2 = 0.14$, $F_{2,31} = 2.53$, $p = 0.096$

U_{repeat}: $R^2 = 0.32$, $F_{1,23} = 10.77$, $p = 0.003$

RR: $R^2 = 0.16$, $F_{1,23} = 4.29$, $p = 0.050$

Table 3.3 Swimming performance, aerobic metabolism and blood analysis of rainbow trout and arctic char exposed to control, simulated diurnal fluctuation or diurnal warming treatments.

	Rainbow trout			Arctic char		
	Control	**Fluctuation**	**Warming**	**Control**	**Fluctuation**	**Warming**
Swimming Performance						
$U_{initial}$ (cm s^{-1})	71.4±5.1^{a}	82.0±3.5^{ab}	90.9±3.5^{b}	64.7±3.2^{ac}	64.4±5.1^{ac}	70.0±3.1^{ac}
U_{repeat} (cm s^{-1})	86.9±5.7^{a}	95.1±5.7^{a}	99.2±5.6^{a}	75.6±3.7^{a}	71.7±11.2^{ab}	39.6±11.0^{b}
Recovery (U_r-U_i)	15.5±3.5^{a}	13.0±3.4^{a}	9.5±4.3^{a}	11.0±4.5^{a}	7.2±7.0^{a}	-30.4±10.0^{b}
U_{GT} (% U_i)	92.2±3.8^{ab}	93.2±2.8^{ab}	95.2±0.5^{a}	92.1±0.9^{ab}	93.4±0.6^{ab}	87.8±1.6^{b}
Aerobic metabolism						
$MO_{2routine}$(mg kg^{-1} min^{-1})	4.36±0.43^{a}	6.21±0.88^{ab}	7.76±0.6^{b}	4.34±0.38^{a}	4.64±0.59^{a}	5.88±0.52^{ab}
MO_{2max} (mg kg^{-1} min^{-1})	10.73±1.06^{a}	13.42±1.53^{ab}	15.2±0.44^{b}	9.64±0.78^{a}	9.17±0.89^{a}	10.49±0.68^{a}
Scope (mg kg^{-1} min^{-1})	6.37±1.15	7.21±1.07	7.44±0.65	5.3±0.53	4.53±0.59	4.61±0.65
Factorial scope	2.61±0.32	2.33±0.24	2.04±0.16	2.24±0.14	2.03±0.16	1.84±0.14
Blood analysis						
Hematocrit (%)	41.3±1.8^{a}	40.5±1.6^{ab}	43±2.1^{a}	42±2.3^{a}	37.3±2.3^{ab}	33±1.6^{b}
Osmolality (mOSM)	343±7	334±7	367±13	326±2	360±19	346±9
Cortisol (ng mL^{-1})	99±8.4	105.2±16.4	95.2±13.6	92.8±14.9	71.2±10.9	52.3±7.4
Plasma Glucose (mM)	12.6±1.5^{a}	14.8±1.5^{a}	17.7±2.4^{ab}	9.9±0.7^{a}	11.7±2.1^{a}	21.5±0.9^{b}
Plasma Lactate (mM)	19.3±2.1^{ab}	18.6±1.8^{ab}	22.7±0.9^{b}	15.2±0.8^{a}	17.2±1.2^{ab}	20.5±0.5^{ab}

Dissimilar superscript letters of the same capitalization indicate significant differences within rows (α 0.05).

Data are shown as mean ± standard error

Table 3.4 Linear mixed-effects models of arctic char and rainbow trout aerobic metabolism during and following (recovery) a fatiguing exercise test under reference conditions (11°C) or following a diurnal warming or fluctuation treatment. Water velocity was increased by 0.5 BL s^{-1} every ten minutes until fatigue during the exercise test, and held constant at 0.5 BL s^{-1} during the recovery period.

Fixed effect	Coefficient	SE	DF	*t*	*p*
Arctic char					
Exercise MO2 model (m =0.21 c =0.83)					
Constant	30.81	3.27	23	9.44	0.000
Time (min)	0.33	0.03	104	11.52	0.000
Recovery MO2 model (m = 0.56 c = 0.86)					
Constant	44.46	2.92	22	15.23	0.000
Fluctuation	3.03	4.51	26	0.67	0.508
Warming	4.29	4.66	23	0.92	0.367
Time	-0.64	0.09	61	-7.46	0.000
Fluctuation*Time	-0.17	0.14	62	-1.17	0.248
Warming*Time	0.54	0.14	61	3.97	0.000
Rainbow trout					
Exercise MO2 model (m = 0.71 c = 0.79)					
Constant	-100.86	23.78	53	-4.24	0.000
Log(mass)	29.59	5.67	54	5.22	0.000
Time	-0.99	0.32	184	-3.12	0.002
Fluctuation	6.56	3.07	19	2.14	0.045
Warming	15.61	2.96	19	5.27	0.000
Log(mass)*Time	0.29	0.08	184	3.82	0.000
Recovery MO2 model (m = 0.66 c=0.87)					
Constant	-79.42	18.00	36	-4.41	0.000
Log(mass)	27.94	4.15	36	6.74	0.000
Fluctuation	3.85	3.55	35	1.09	0.285
Warming	14.62	3.55	35	4.11	0.000
Time	3.00	0.73	121	4.11	0.000
Log(mass)*Time	-0.85	0.17	121	-4.95	0.000
Fluctuation*Time	-0.31	0.12	113	-2.60	0.011
Warming*Time	0.03	0.12	114	0.25	0.800

Fish ID was included in all models as a random effect
m =marginal R^2
c= conditional R^2

Figures

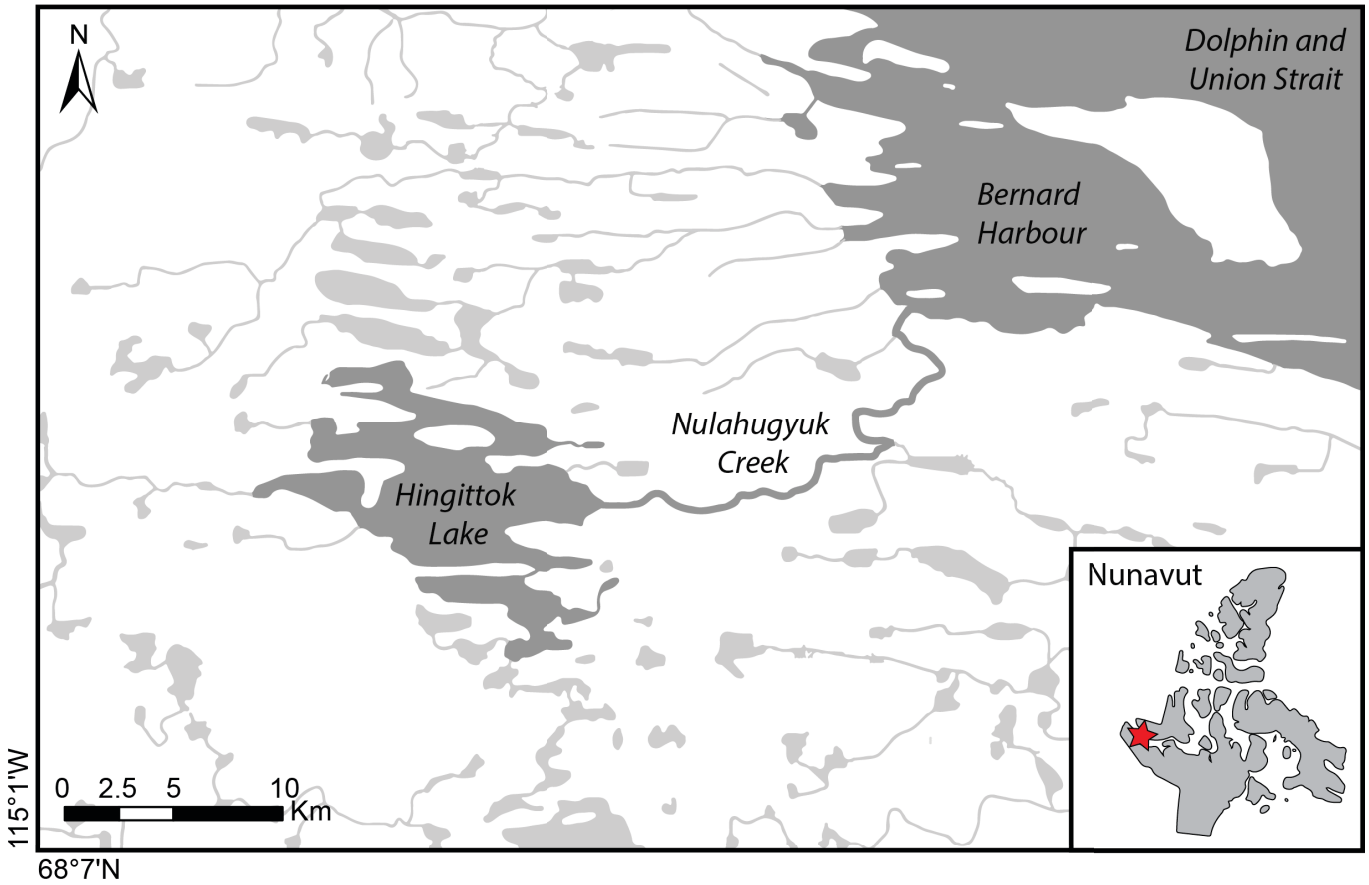
Fig. 3.1. Map of the study area in northwest Nunavut, on the Dolphin and Union Strait in the Arctic Ocean. The study population migrates annually between Hingittok Lake and Bernard Harbour via Nulahugyuk Creek.

Fig. 3.2. Natural diurnal temperature fluctuations recorded in Nulahugyuk Creek in 2012 (A) and the resulting laboratory study design (B). The simulation was designed to assess the effects of diurnal temperature variation similar to the blowup in (A) on repeat swimming performance and related sub-organismal physiology.

Fig. 3.3 Initial (top) and repeat (middle) swimming performance and recovery ratio (bottom) of wild juvenile arctic char over a range of naturally occurring temperatures. The red circle denotes individuals that were removed as outliers from their respective regression analyses based on visual inspection. Regression models are in Table 3.2. The individuals removed were the only two tested above 20°C and may indicate a threshold for recoverability.

Fig. 3.4 Initial and repeat swimming performance and recovery ratio of laboratory reared juvenile rainbow trout and arctic char following simulated diurnal warming or fluctuation. Dissimilar letters indicate significant differences between treatments within a species (α 0.05). Error bars represent standard error of the mean.

Fig. 3.5 Oxygen consumption (MO_2) during an initial exercise test (solid lines) and recovery (broken lines) by laboratory reared juvenile rainbow trout and arctic char exposed to diurnal temperature treatments. Error bars represent standard error of the mean. Summary statistics of routine and maximum MO_2 are in Table 3.3, and linear mixed effects models for describing the data are in Table 3.4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Dolphin and
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115°1'W
68°7'N

Figure 3.1

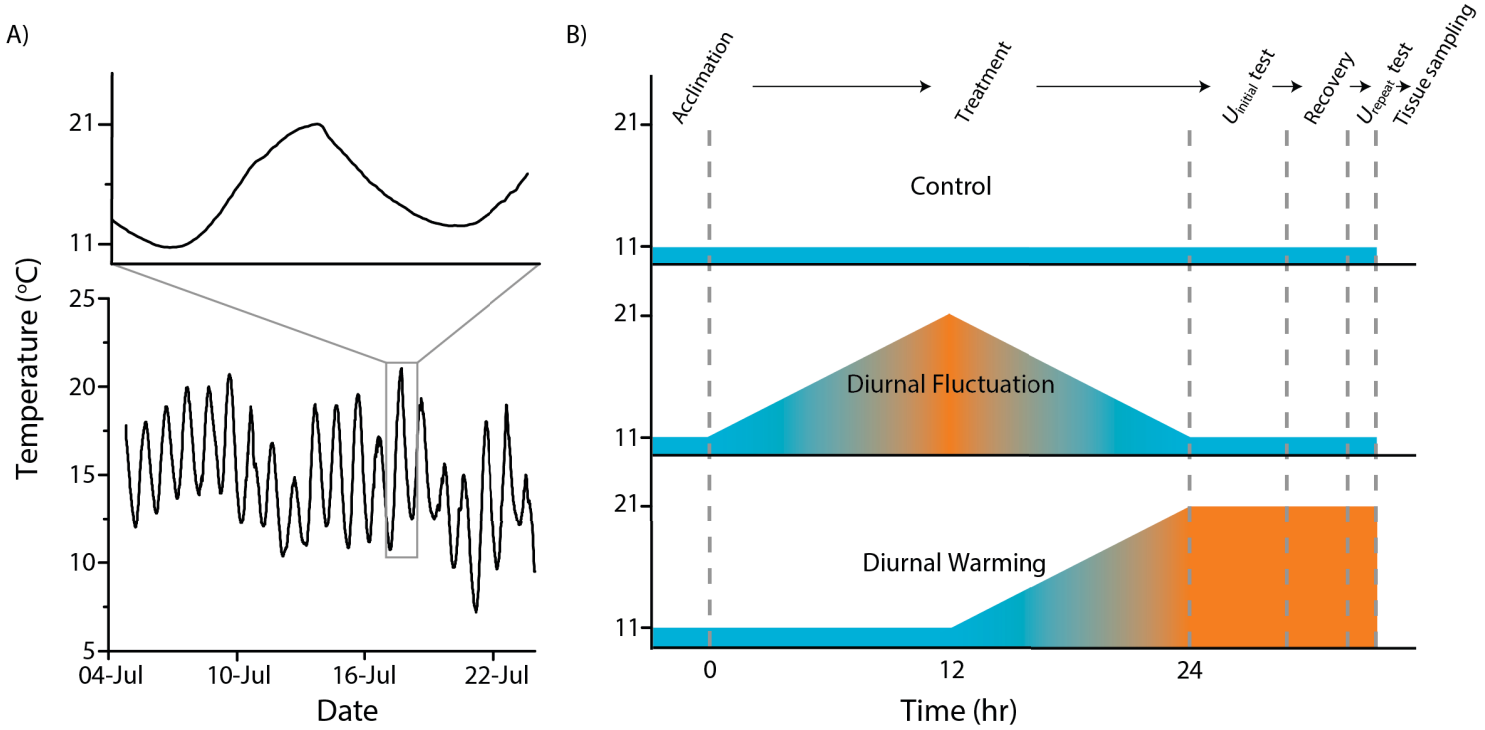
A)
B)
Temperature (°C)
21
11
25
20
15
10
5
04-Jul
10-Jul
16-Jul
22-Jul
Date
Acclimation
Treatment
$U_{initial}$ test
Recovery
U_{repeat} test
Tissue sampling
Control
Diurnal Fluctuation
Diurnal Warming
0
12
24
Time (hr)

Figure 3.2

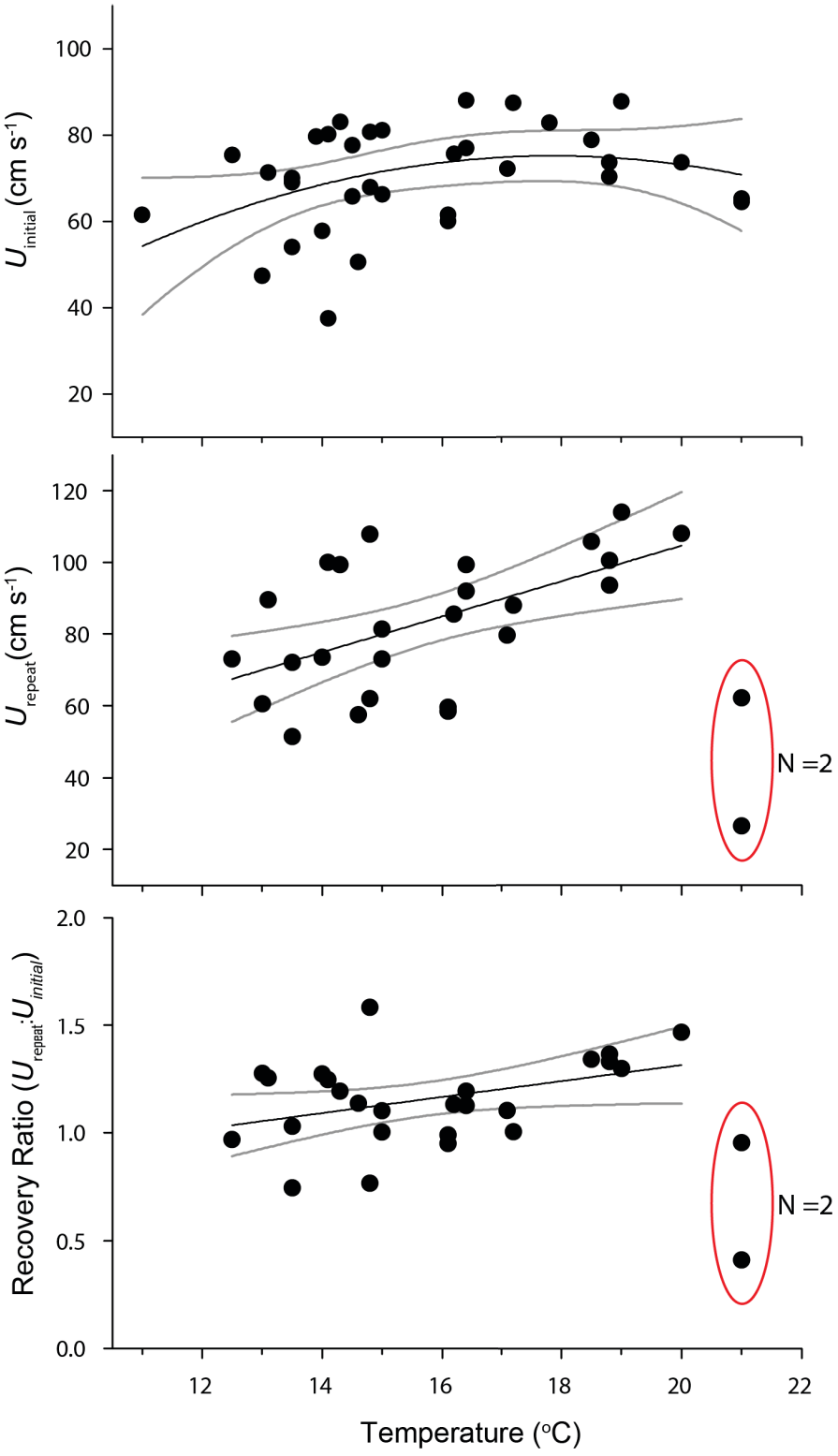
100
80
60
40
20
$U_{initial}$ (cm s^{-1})
120
100
80
60
40
20
U_{repeat} (cm s^{-1})
N =2
2.0
1.5
1.0
0.5
0.0
Recovery Ratio (U_{repeat}:$U_{initial}$)
N =2
12
14
16
18
20
22
Temperature (°C)

Figure 3.3

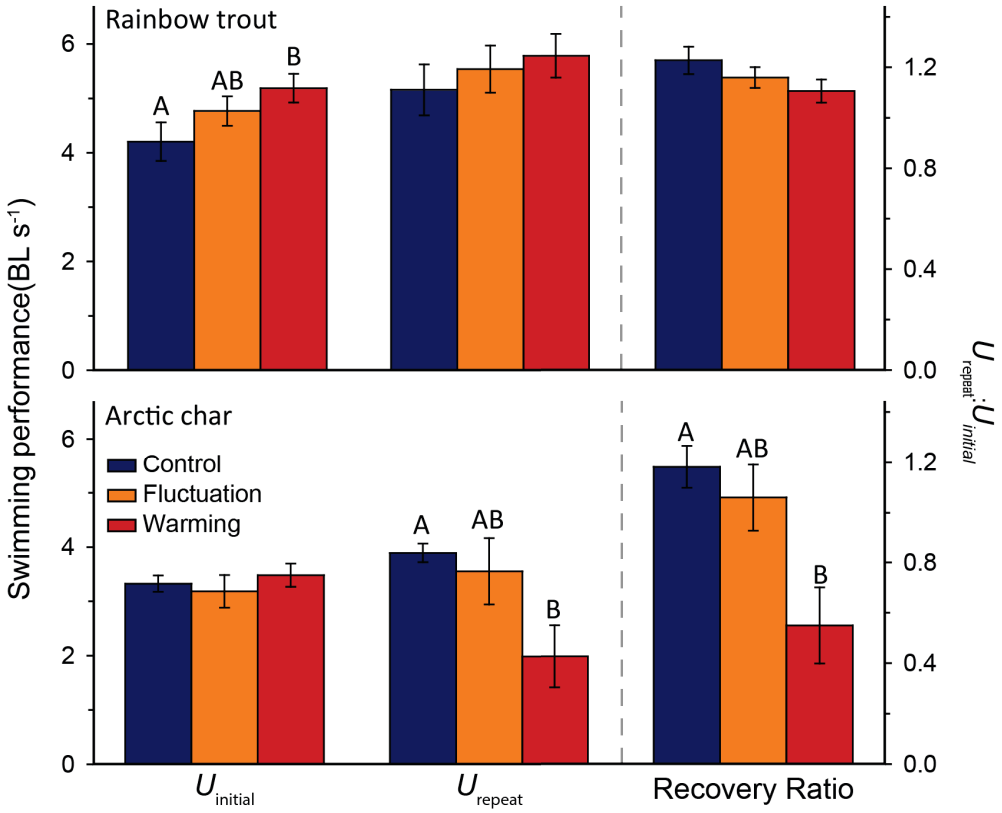
Rainbow trout
Arctic char
Control
Fluctuation
Warming
A
AB
B
A
AB
B
A
AB
B
Swimming performance(BL s-1)
0
2
4
6
0.0
0.4
0.8
1.2
Urepeat·Uinitial
Uinitial
Urepeat
Recovery Ratio

Figure 3.4

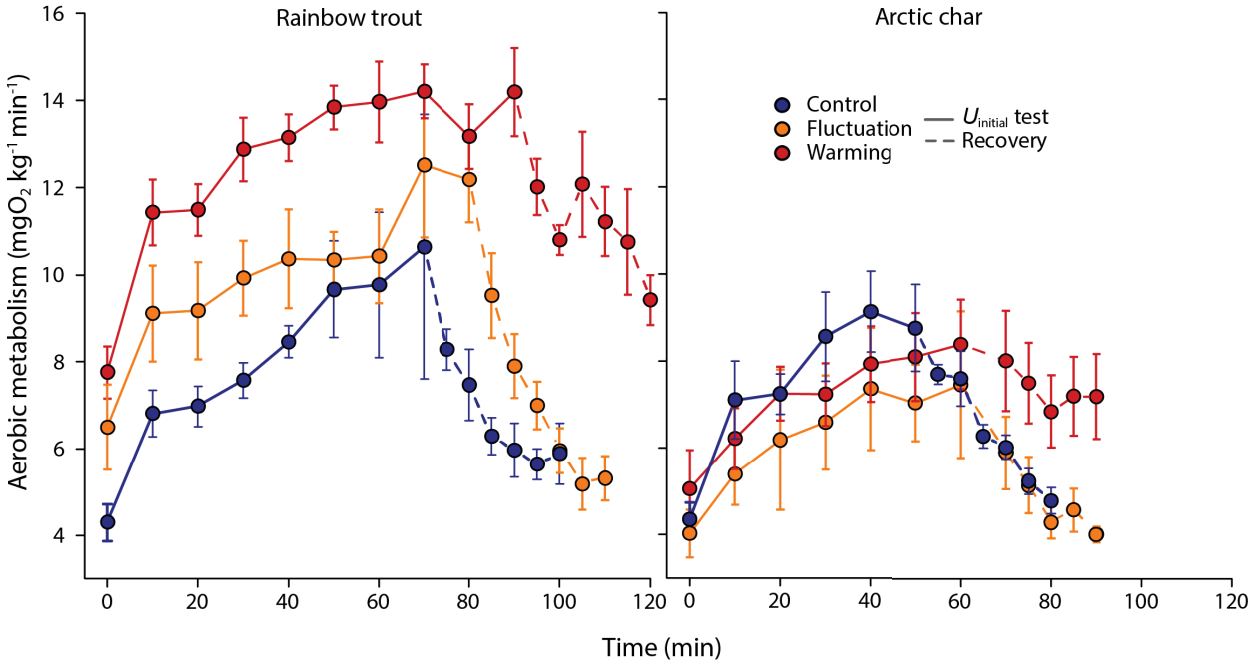
Rainbow trout
Arctic char
Control
Fluctuation
Warming
$U_{initial}$ test
Recovery
Aerobic metabolism (mgO_2 kg^{-1} min^{-1})
Time (min)
16
14
12
10
8
6
4
0
20
40
60
80
100
120

Figure 3.5

Chapter 4: Diurnal temperature fluctuations induce drastic biochemical and transcriptional changes in exercised arctic char (*Salvelinus alpinus)* and rainbow trout (*Oncorhynchus mykiss)*

Abstract

In a companion study (Chapter 3), I exposed arctic char and rainbow trout to simulated diurnal temperature fluctuations that are representative of the upper end of those currently experienced by migrating arctic char in Nulahugyuk Creek, Nu. Following this exposure fish were subjected to fatiguing exercise tests and measures of their recoverability, aerobic and anaerobic metabolism, and stress were assessed. Following diurnal warming, arctic char had an impaired ability to recover from fatiguing exercise and generally exhibited signs of insufficient energy production. In the present study I carried out metabolomic and qPCR analysis on plasma and gill samples from the same fish to identify biochemical and transcriptional changes associated with my previous results. Following diurnal warming both species exhibited substantial shifts in metabolite profiles and exhibited strong heat shock responses, however in both cases the magnitude of response was typically greater in char than trout. Among the key differences between species was a substantially higher accumulation of plasma succinate in char, indicating a greater loss in mitochondrial functionality, and an order of magnitude higher induction of gene expression for heat shock proteins (HSP) such as HSP90β, and HS70. A similar pattern of heat shock response was also identified in wild adult arctic char that naturally encountered warm water compared to those that were in cooler conditions, however the magnitude of response was substantially lower. Following a complete simulated diurnal fluctuation the heat shock response had largely recovered in trout but was still significantly elevated in arctic char. Together these

results indicate that current thermal conditions in the Canadian Arctic are sufficient to substantially disrupt critical biochemical process and cause severe thermal stress in arctic char. Furthermore, these findings suggest that anadromous salmonids that are adapted to historical arctic conditions may already be at a competitive disadvantage during their summer migration when compared to more temperate species whose ranges are expanding northward.

Introduction

As a result of global climate change, migrating salmonids encounter temperatures outside of their optimal range at increasing frequency (Farrell et al. 2009). In response to these temperatures, changes in gene expression and biochemistry occur that may be protective and compensatory, or as a result of the disruption of numerous biological processes (Iftikar et al. 2014; Jeffries et al. 2012b; Quinn et al. 2011b). For researchers, these changes can serve as biomarkers of thermal stress (Iftikar et al. 2014) and may provide insight into the mechanisms underlying the reductions in functionality that are observed at higher levels of organization (Cooke et al. 2006; Iftikar et al. 2014; Jeffries et al. 2012b).

Studies that involve the biochemical responses of salmonids to environmental stressors often focus on a few key metabolites and related enzymes. For example, the concentrations of plasma glucose and lactate as well as the activities or concentrations of related enzymes, citrate synthanse (CS) and lactate dehydrogenase (LDH) are often studied as indicators of the supply to, and functionality of aerobic and anaerobic fuel systems (Berli et al. 2014; Mathes et al. 2010). Furthermore, these are all targets that are relatively easily assayed using commercially available kits and analyzers (Cooke et al. 2006; Mathes et al. 2010; Stoot et al. 2014). The continued development of the field of metabolomics has increasingly allowed researchers to study many metabolites simultaneously in more complex studies to give a more complete picture of biochemical responses to various stressors. Recently, metabolite profiles were used to identify thermal sensitivity in wrasse species (Iftikar et al. 2014). These found the mitochondria of difference species had different thermal sensitivities that corresponded changes in their metabolite profiles. Metabolite profiles have also been proposed as a way to screen for thermal stress and general disruption of homeostasis in wild fish (Iftikar et al. 2014)

The study of transcriptional changes has also benefitted greatly by recent advances in transcriptomic technology that are now widely being applied in the field of environmental physiology (Evans et al. 2011; Jeffries et al. 2012b; Quinn et al. 2011a; Quinn et al. 2011b). Recently multiple studies have used transcriptomic techniques to identify changes in gene expression related to thermal tolerance and heat stress in salmonids. Among the genes identified changes in the expression of members of the heat shock family and of genes involved in cell cycling were common (Jeffries et al. 2012b; Quinn et al. 2011b). The target genes in the present study were selected based on these previous studies and fell into two broad categories based on their cellular function. Cyt-C2, Casp3, and Casp8 all code for proteins involved in the signaling of apoptosis, and Ubq, GRPP, Serpinh1, HSP90β-2, HSP90β, and HS70 are all broadly involved in the maintenance of protein functionality and cycling. Except for Ubq, the non-apoptosis related genes are all part of the heat shock family. Together, the proteins encoded by the target genes help maintain cellular homeostasis or terminate the cell if homeostasis is significantly disrupted following environmental perturbations such as thermal variation.

As with other areas of thermal physiology such as those addressed in the companion article (Chapter 3), the effects of temperature on heat shock proteins and apoptosis related gene expression, or on the metabolome have not been extensively studied under realistic thermal regimes, or in arctic char outside of the aquaculture setting (Quinn et al. 2011b). Specifically, no study has investigated either of these endpoints in response to large but realistic diurnal temperature fluctuations.

In the present study, our objectives were to (i) determine the relative transcriptional and biochemical sensitivity of arctic char, a model northern salmonid and rainbow trout, a model temperate salmonid, to realistic diurnal temperature regimes, (ii) assess the utility of previously

proposed biomarkers for thermal stress under these realistic thermal regimes, and (iii) begin to establish how the observed changes are related to each other, and to the metrics of stress, metabolism and whole-organism performance addressed in our companion study (Chapter 3). To achieve these objectives we conducted a nuclear magnetic resonance (NMR) based quantification of a large suite of plasma metabolites and used quantitative polymerase change reaction (qPCR) analysis to quantify changes in the expression of target genes following exposure to simulated or natural diurnal temperature fluctuations.

Materials and Methods

Field sampling

Field sampling took place in the summers (June – August) of 2012–2014 near the mouth of Nulahugluk creek, Nu (68.75°N 114.8°W). Fish were captured migrating between their freshwater and marine environments (Hingittok Lake and Bernard Harbour, Nu,) as in Chapter 3. Gill tissue samples were only taken from fish that were harvested by locals or from moribund individuals that were not likely to survive. Several filaments were clipped from the middle of the first arch and transferred into 1.5 or 2mL microcentrifuge tubes containing 1mL of RNAlater (Life Technologies, Grand Island, New York). Samples were stored in field at -20°C in a portable freezer, shipped by air to the University of Alberta (UofA, Edmonton, Alberta) on ice, and then stored -20°C until use. Fish sampled in field were classified as having experienced warm (>19°C) or cool (<15°C) water conditions within the previous 24 hours.

Laboratory Sampling

In the laboratory, all plasma and gill tissue samples were taken from individuals used in the companion study (Chapter 3) following their exposure to a temperature treatment and repeat

swimming performance tests. Briefly, ten to fifteen month old rainbow trout and arctic char were exposed to a complete diurnal temperature fluctuation (11 to 21 to 11°C), diurnal warming (11 to 21°C) or control conditions (11°C), following which they were put through an endurance and a sprint swimming performance test separated by a 30 min recovery period. Plasma samples were obtained as previously described (Chapter 3) but were further filtered through 200 kda Nanosep microcentrifuge filters (Pall Corporation, Port Washington, New York) 4°C for 30min at 10,000g prior to NMR analysis. Gill tissue was taken immediately following sacrifice. The entire first arch was excised and the filaments were trimmed away from the cartilage and placed in RNAlater overnight at 4°C before being transferred to -20°C until use.

Metabolomics

Three plasma samples from each treatment group and both species (N_{total} = 18) were supplied to Chenomx Inc. (Edmonton, Alberta) for analysis. NMR spectra were generated using Varian four-channel VNMRS 700 MHz NMR spectrometer (Varian, Palo Alto, California) with an auto tuning 1H/13C triple resonance biomolecular probe. Spectra were analyzed using Chenomx NMR suite 7.7 to generate metabolite concentrations within each sample.

Quantitative PCR

RNA was extracted using mechanical disruption and Trizol reagent (Life Technologies). For mechanical disruption, the tissue was ground with a micropestle homogenizer and vortexed with ~0.5mL of 2.3 mm zirconium dioxide beads (BioSpec, Bartlesville, Oklahoma). RNA was treated with DNAse (DNA-free, Life Technologies) and cDNA was made using the SuperScript First-Strand synthesis system (Life Technologies). qPCR was run using the SybrGreen method and primers (Table. 4.1) on a 7500 Fast Real-Time PCR System (Life Technologies). Raw ct

values were exported and used to generate relative quantitation (RQ; fold change) values using the **ΔΔ** ct method with correction for differences in primer efficiencies.

Data analysis

All data analysis was carried out using R Studio (Team 2014). Data presentation was done using R Studio, Sigmaplot 11.1 (Systat Software, San Jose, California) and Adobe Illustrator CS4 (Adobe systems, San Jose, California).

For broad scale analysis of laboratory qPCR and metabolomics data Principle components analyses (PCA) were conducted on each data set. 68% confidence limit ellipses were plotted for each treatment group to show separation of transcript and metabolite profiles between species and treatments. Metabolomics data was further analyzed using heat maps of fold change from the control group for both species and select pairwise comparisons were made with t-tests using the Benjamini-Hochberg procedure to control for false discover rate. qPCR data was analyzed using species and treatment as predictor variables in a two way analysis variance followed by Tukey's honest signi ficant difference post hoc test.

Results

Metabolite profiles following diurnal simulations

In rainbow trout and arctic char the principle components analysis of metabolite profiles showed a large separation in component space between fish that had experienced diurnal warming compared to those that had experienced a complete fluctuation or were held under control conditions (Fig. 4.1). There was a less pronounced separation between the control and fluctuation groups. Across all treatment groups there was a clear separation between species (Fig. 4.1).

At the individual metabolite level, in both species many amino acids including alanine, iso-leucine, leucine, phenylalanine, serine, taurine and tyrosine showed large increases in their concentration following exposure to diurnal warming but in most cases were at near control levels in the diurnal fluctuation group (Table 2.2; Fig. 4.2). Important energy metabolites including glucose and lactate were also elevated following diurnal warming but to a lesser extent in rainbow trout than in arctic char. In both species the concentration of succinate, an important intermediate of the citric acid cycle, was also higher in diurnal warming than control groups, and had returned to or below control levels following diurnal fluctuation (Table 2.2; Fig. 4.2). The increase in succinate was much larger for char than trout. Interestingly, plasma creatine was highly variable between individuals but in general was elevated following warming in rainbow trout but not arctic char.

The separation in plasma metabolite profiles between species (Fig. 4.1) was also apparent at the individual metabolite level. Alanine, asparagine, glutamate, glutamine, hydroxyproline, isoleucine, methionine, phenylalanine, serine, tyrosine and valine were all markedly higher in arctic char than rainbow trout in a manner that was independent of the diurnal temperature treatment (Table 2.2; Fig. 4.2).

qPCR following diurnal simulations

In rainbow trout and arctic char the principle components analysis of transcript profiles for arctic char showed the largest separation between control and the diurnal warming treatment, with the diurnal fluctuation treatment being intermediate (Fig. 4.3). In rainbow trout there was smaller separation between the warming and control group but in the same direction as seen in arctic

char (Fig. 4.3). Rainbow trout profiles in the diurnal fluctuation group had substantial overlap with the control group, which was not observed in arctic char (Fig. 4.3).

The separation observed between the control and the warming group in the PCA analysis was apparent at the individual target gene level. Specifically, members of the heat shock protein family including GRPP, Hsp90β, HSP70 were all highly induced following exposure to diurnal warming in both species. However, this induction occurred to a much greater extent in char than in trout, and char also showed significant induction of Serpinh1 and Hsp90β-2, which was not seen in trout (Fig. 4.4). Following the complete diurnal temperature fluctuation the expression of all thermally induced genes were near control levels for rainbow trout but, Serpinh1, Hsp90β and HS70 were all still substantially elevated in arctic char (Fig. 4.4). In arctic char Cyt-C2 was numerically increased following diurnal warming and fluctuation but due to high variation this difference was not statistically significant (Fig. 4.4). No significant differences in the expression of Casp3, Casp8 or Ubq were seen between any treatment groups for either species.

qPCR in wild adult arctic char

Compared to char at relatively cool temperatures, wild arctic char that had experienced water temperatures above 19°C in the past 24 hours induced expression of the same suite of heat shock genes as was seen following simulated warming in lab, excluding GRPP (Fig. 4.5). The induction of heat shock gene expression in wild adults however, was not nearly at the same magnitude as in lab. Interestingly, wild char at warm temperatures and juveniles in lab under simulated warming conditions exhibited a similar magnitude increase in the expression of Cyt-C2 (Fig. 4.5).

Discussion

Summary

Temperature is well established as one of the most important environmental factors that shapes the physiological performance of salmonids and ectotherms in general(Currie 2011; Farrell 2007), however metabolite and transcript profiles have not been thoroughly studied under natural thermal regimes, and are particularly unknown in northern fishes. In the present study I identify that arctic char and rainbow trout exhibit drastic metabolomic and transcriptional responses to diurnal temperature variation that is representative of current conditions in the Canadian arctic. While both species exhibited these responses, arctic char appeared to be more sensitive and less able to recover to a basal state following thermal variation.

Metabolite profiles following diurnal simulations

The overall change in metabolite profiles following diurnal warming indicates significant disruption of biochemical processes with warming. The specific metabolites involved provide further insight into which specific processes may be affected. The substantial increases observed in various plasma amino acids is indicative of an increase in proteolysis (Mommsen 2004). Increased protein degradation serves a number of purposes. Following exercise without thermal variation arctic char and salmonids in general increase proteolysis to supply amino acids for rebuilding damaged tissue and replenishing the general supply of cellular substrates (Barton et al. 1995; Mommsen 2004). Because all fish in the current study were exercised to a similar extent the most likely purpose is that these amino acids are being used to regenerate components involved in ATP production because of the heightened cost of being at warm temperatures. One particular amino acid that may serve this purpose is alanine, which drastically increased in

concentration after warming. In addition to lactate, alanine is used by hepatocytes in gluconeogenesis to replenish glucose (Suarez and Mommsen 1987). Migrating salmonids also use their protein reserves to fuel sexual maturation so the depletion of these reserves caused by swimming through warmer water would likely decrease reproductive output (Mommsen 2004).

The pattern of accumulation of succinate in plasma between treatment groups and species provides particular insight into potential mechanisms underlying the observed changes in aerobic metabolism (Chapter 3). Succinate is oxidized by complex II of the citric acid cycle (Succinate-dehydrogenase) and its accumulation is an indication of impaired mitochondrial functionality (Grieshaber et al. 1994). A number of fish species have now been shown to exhibit increased succinate levels during thermal stress (Iftikar and Hickey 2013; Iftikar et al. 2014; Pörtner and Knust 2007). However, the extent of accumulation observed in the present study, 5 and 10 fold increase in trout and char respectively (Table 4.2), appears to be unprecedented. The likely explanation for this is that fish in our study were subjected to fatiguing exercise tests that resulted in maximal aerobic output and thus any succinate accumulation as a result of mitochondrial dysfunction at warm temperatures would have been magnified. Although both species exhibited accumulation of plasma succinate following diurnal warming, it was much greater in char suggesting that that they experienced a relatively far greater loss of mitochondrial functionality. In addition to succinate accumulation in heat stressed fish, Iftikar et al (Iftikar et al. 2014) also observed a marked reduction in the respiratory control ratio (RCR), a metric of mitochondrial efficiency. Interestingly, a substantial (~50%) reduction in RCR was also found following acute thermal stress in arctic char when compared to atlantic salmon at similar temperatures (Penney et al. 2014), agreeing with differences in succinate levels in char and trout found in the present study. The increase in plasma lactate and glucose following diurnal warming

agrees with the my findings in chapter 3 and with previous research (Jain and Farrell 2003) that suggest there is increase glucose metabolism through anaerobic pathways following temperature stress. This increase in anaerobic metabolism is thought result from and inability to increase aerobic supply of ATP to match increased demand, potentially as a result of mitochondrial dysfunction as discussed above, or as a result of limited oxygen supply by the cardiorespiratory system (Eliason et al. 2011; Farrell et al. 2009).

While they are not necessarily related to the differences in sensitivity to thermal variation, overall differences between species in their metabolite profile and specific metabolite concentrations may reveal fundamental physiological differences. For example, there were eleven amino acids that were in higher concentration in all treatment groups for arctic char than rainbow trout. This suggests that following fatiguing exercise arctic char had an overall higher level of proteolysis than rainbow trout, and were likely using amino acids as a fuel source to a greater extent. A similar difference can be found between sockeye salmon that extensively utilize protein reserves to fuel upstream migration, and atlantic salmon that do not (Mommsen 2004). Hydroxyproline was among the amino acids that were generally at a higher level in char than in trout. Hydroxyproline is primarily found in collagen, and its high concentration in plasma indicates that structural proteins such as collagen were likely not protected from the elevated proteolysis exhibited by char (Mommsen 2004), and thus you would expect a greater structural turnover in char than in trout.

qPCR following diurnal simulations

The broad function of the selected target gene is to regulate apoptosis and protein homeostasis under environmental stress. As such, the overall difference between transcript profiles of fish

under control conditions and those that had experienced simulated diurnal warming suggests that this thermal variation was sufficient to substantially perturb cell homeostasis and induce a compensatory response. Given the larger separation in char than in trout, the disruption of homeostasis was likely more severe in char, suggesting that even over temperature ranges currently encountered in the Arctic, they are more thermally sensitive than temperate salmonids.

Indications of this heightened thermal sensitivity are also found in the expression of a number of individual gene targets. For example, inductions of HSP90β and HS70, classical members of the inducible heat shock response, were over an order of magnitude higher in char than in trout. Furthermore, in arctic char the heat shock response was still highly apparent after the completion of the diurnal fluctuation, while it had been reduced back to control levels in rainbow trout. This difference was reflected in the transcript profile PCA as well, in which there was significant overlap between the control and diurnal fluctuation groups for rainbow trout, but for arctic char the distribution was intermediate to the control and diurnal warming group. In the wild, these char would immediately begin another diurnal cycle before they had recovery from their last. This aligns well with indications in chapter 3 and assertions in a growing number of studies that thermal variation and thermal history matter for an organism's performance and underlying physiology as opposed to just the instantaneous, or average temperatures (Currie 2011; Dowd et al. 2015; Oligny-Hébert et al. 2015; Quinn et al. 2011b).

The magnitude of the inductions of heat shock protein gene expression in char and trout in the diurnal warming group are among the largest identified in any salmonid despite the fact that higher temperatures or longer exposures are often used to induce these responses (Anttila et al. 2014; Fowler et al. 2009; Jeffries et al. 2012b; Palmisano et al. 2000; Quinn et al. 2011a; Quinn et al. 2011b). While different HSPs are known to act over different time scales (Currie 2011) the

extent of induction may suggest that in some cases the heat shock response of salmonids may be maximally induced over naturally relevant time scales for thermal variation. This particular study was not designed to test such a hypothesis but based on the magnitude of observed induction it certainly warrants further investigation.

qPCR in wild adult arctic char

Migrating adult arctic char had similar patterns of heat shock response to those under simulated diurnal temperature regimes, albeit at a lesser magnitude. This suggests that under current migratory conditions in Nulahugyuk Creek, Nu, arctic char are experiencing significant thermal stress(Currie 2011). Interestingly both wild and lab-tested char at warm temperatures also had elevated expression of Cyt-C2. When released from the mitochondrion, Cyt-C2 in the cytoplasm plays a signaling role in the initiation of apoptosis (Loeffler and Kroemer 2000). In wild sockeye salmon, Cyt-C2 gene expression was not induced by heat stress alone but was significantly elevated in moribund individuals at warm or cool temperatures (Jeffries et al. 2012b). As such, it is likely that the elevated expression in the present study in an indication of elevated apoptotic signaling at warm temperatures. Alternatively, Cyt-C2 is a critical component of the electron transport chain and expression may have been stimulated by the inability to meet aerobic metabolic demands.

One important difference between the fish sampled for qPCR analysis was that wild arctic char were adults and captive char were juveniles. Juvenile rainbow trout are known to have a more pronounced heat shock response than adults, and this may underlie a greater relative thermal tolerance (Fowler et al. 2009), which has multiple implications for the present study if it holds true for arctic char. Although the natural thermal variation experienced by wild adults was less

consistent between individuals and potentially less harsh than that experienced by lab-tested juveniles, part of the difference in the magnitude of their heat shock responses was likely a result of age or size. Furthermore, our laboratory studies revealed concerning physiological effects of diurnal temperature variation on juveniles, so if there is indeed a functional reduction in the adult heat shock response, the physiological impairments observed in lab would likely be more pronounced in adults.

The heat shock response has been successfully used as a biomarker for thermal stress in other salmonid species (Anttila et al. 2014; Jeffries et al. 2012b; Lund et al. 2002). It has also been confirmed to be robust in captive arctic char under non-realistic thermal regimes intended to simulate aquaculture settings or acutely induce loss of equilibrium (Quinn et al. 2011a; Quinn et al. 2011b). The heat shock response however, has not been previously assessed in arctic char under environmentally relevant thermal conditions in the lab or in the wild. In the present study the gene targets suggested as markers of thermal stress in previous studies showed robust induction in response to simulated diurnal warming and fluctuations in juveniles, and to natural thermal variation in wild adults. These finding indicate that the heat shock response has utility as an indicator of thermal stress for arctic char under current environmental conditions.

Conclusion

Migrating sockeye salmon exposed to warm but environmentally relevant temperatures exhibit large inductions of gene expression associated with cytoprotection and also exhibit changes in biochemistry such as elevated plasma lactate and glucose levels that are indicative of increasing energy supply to meet demand (Jeffries et al. 2012a; Jeffries et al. 2012b). These molecular and biochemical changes are markers of thermal stress but are also indicative of a only partially

successful compensatory response as they coincide with a reduction of aerobic scope, cardiac output, and in some cases increased incidence of early mortality (Cooke et al. 2006; Farrell et al. 2008b; Jeffries et al. 2012b; Mathes et al. 2010). In parallel to this, in the present study, the observed metabolite changes following diurnal warming were likely, at least in part, a result of a response meant to supplement ATP production to meet increased demand at warm temperatures. The observed changes in transcript profiles should have helped cells maintain homeostasis and protein functionality. Given the losses in performance observed in chapter 3, these compensatory responses were at best, partially successful at mitigating thermal stress in arctic char. Rainbow trout under the same conditions were able to maintain or improve performance suggesting that their compensatory response was sufficient. Together these findings suggest that under the extremes of current migratory conditions observed in the Canadian Arctic, arctic char would likely be at a competitive disadvantage to rainbow trout and other temperate salmonids who's ranges are expanding northward as their own habitats become more thermally restrictive.

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Tables:

Table 4.1 List of primers sequences for target genes in the qPCR analysis

Target name	Abbr.	Primer sequence	Efficiency (%)		Source
			Arctic char	Rainbow trout	
Elongation factor 1-alpha (Endogenous control)	EF1a	F-CCCCTCCAGGACGTTTACAAA R-CACACGGCCCACAGGTACA	94.15	100.86	(Quinn et al. 2011b)
70 kDa Heat Shock Protein	HS70	F-AAG ATC AGC GAG GAG GAC AA R-TGC CTG ATC TCC ACA GCA	98.84	85.66	(Quinn et al. 2011b)
Heat Shock Protein 90β	HSP90β	F-GCG TTG CCC ACC ATT AAC R-AAT GGG TAA CCT GGT CAG TGT C	90.25	102.0	(Quinn et al. 2011b)
Heat Shock Protein 90β-2	HSP90β2	F- GCGTTGCCCACCATTAAC R- AATGGGTAACCTGGTCAGTGTC	98.66	101.34	(Quinn et al. 2011b)
Serpin H1 precursor	Serpinh1	F-CTG GGA GGC AAA AAC AAC TG R-TTC CAC CAT TCT TTT CAC CAG	92.28	87.78	(Quinn et al. 2011b)
78 kDa glucose-regulated protein precursor	GRPP	F-CGACGGAGAGGACTTTTCAG R-TTGGGGATACGGGTAGAGC	94.45	93.70	(Rise et al. 2004)
Ubiquitin carboxyl-terminal hydrolase 8	Ubq	F-GAA ATG TTT GCT GGC AAC G R-CCA TGG AAC AGA GCT ACG ATG	81.8	102.92	(Quinn et al. 2011b)
Caspase 3	Casp3	TTTGGGAGTAGATTGCAGGG TGCACATCCACGATTTGATT	131.24	93.71	(Sánchez et al. 2011)
Caspase 8	Casp8	CAGCATAGAGAAGCAAGGGG TGACTGAGGGGAGCTGAGTT	98.72	83.79	(Sánchez et al. 2011)
Cytochrome c-oxidase Subunit 2	Cyt-C2	F-CGAGCGTGCAGATCTTATAGC R-CTTCTCCGCTGAACAGTTGATG	101.46	83.65	(Jeffries et al. 2012b)

Table 4.2 Concentration of select metabolites that exhibited substantial differences between diurnal treatment groups or species

	Arctic char			Rainbow trout		
	Control	Fluctuation	Warming	Control	Fluctuation	Warming
Alanine	957.1±80.7	1001±100	1521±186	317.1±65.3	430.7±70.3	686.2±103.7
Asparagine	229.8±17	322.9±46.5	293.3±76.8	53.9±0	84.9±6.3	45.9±3.2
Creatine	490.7±351.9	176.2±19.2	508.2±158.4	233.1±80.8	49.2±17.6	1760±1132
Glutamate	186.7±31.8	218.4±26.4	217±43.4	109.4±16.2	95.2±20.1	179.4±3.7
Glutamine	447.1±72.4	484.6±12.3	417.2±47	166.6±18.6	226.1±52.7	239.9±31
Hydroxyproline	373.3±59.6	429.6±139.3	365.2±10	81.4±8.9	117.1±36.3	94.7±10.5
Isoleucine	125.1±8.6	141.4±12.3	230.1±29.5	74.7±9.3	93.1±15.1	152.6±35.2
Leucine	181.3±14.4	240.3±14.6	332.7±31.4	128.3±15.5	164±29.3	238.7±50.6
Methionine	88.7±8.1	120.4±18.9	151.4±3	50.6±7.1	67.2±9.5	82.6±12.6
Phenylalanine	119±3.7	130.4±19.9	230.1±17.1	71.9±13.8	89.6±3.2	109.7±10.2
Serine	223.1±58.8	312.7±42.3	416±43.3	108.5±14.7	210.2±38.7	188.3±6.1
Taurine	484.6±30.8	773.3±77	1664±495	490.7±158.8	314.3±67.5	952±139.1
Tyrosine	74.9±7.7	96.6±19.9	192.3±20.1	39.2±14	34.3±5.1	64.2±15.5
Valine	330.9±13.2	420.9±20.4	497.7±54.4	204.9±23.2	276.3±43.2	350±78.4
Succinate	19.1±12.5	15.1±3.9	221±73.6	15.4±4.9	14.5±4.3	88±14
Glucose	8.3±1.2	9.4±1.4	20.3±1.5	8.3±0.9	16.2±3.7	15.5±2.8
Lactate	12.5±1.0	16.7±1.5	23.7±1.5	15.3±3168	17.1±4.3	20.4±0.9

Values represent mean ± standard error, N= 3 per group

All concentrations are in µM except for Glucose and Lactate which are in mM

Figures:

Fig. 4.1 Principle components analysis of plasma metabolite profiles in arctic char and rainbow trout exposed to simulated diurnal warming and fluctuations. Ellipses represent 68% confidence limits for each treatment group.

Fig. 4.2 Heat map showing the fold change in concentration of specific metabolites in the plasma of arctic char and rainbow trout following diurnal temperature treatments

Fig. 4.3 Principle components analysis of transcript profiles in arctic char and rainbow trout exposed to simulated diurnal warming and fluctuations. Ellipses represent 68% confidence limits for each treatment group.

Fig. 4.4 Relative quantitation of target gene expression following diurnal temperature treatments in lab reared juvenile arctic char and rainbow trout. Dissimilar letters indicate significant differences between treatment groups for a given target (α=0.05) and error bars represent standard error of the mean.

Fig. 4.5 Relative quantitation of target gene expression following natural exposure to warm (>19°C) or cool (<15°C) water in migrating adult arctic char. Asterisks indicate significant differences between warm and cool groups (α=0.05, * <0.01, **<0.001) and error bars represent standard error.

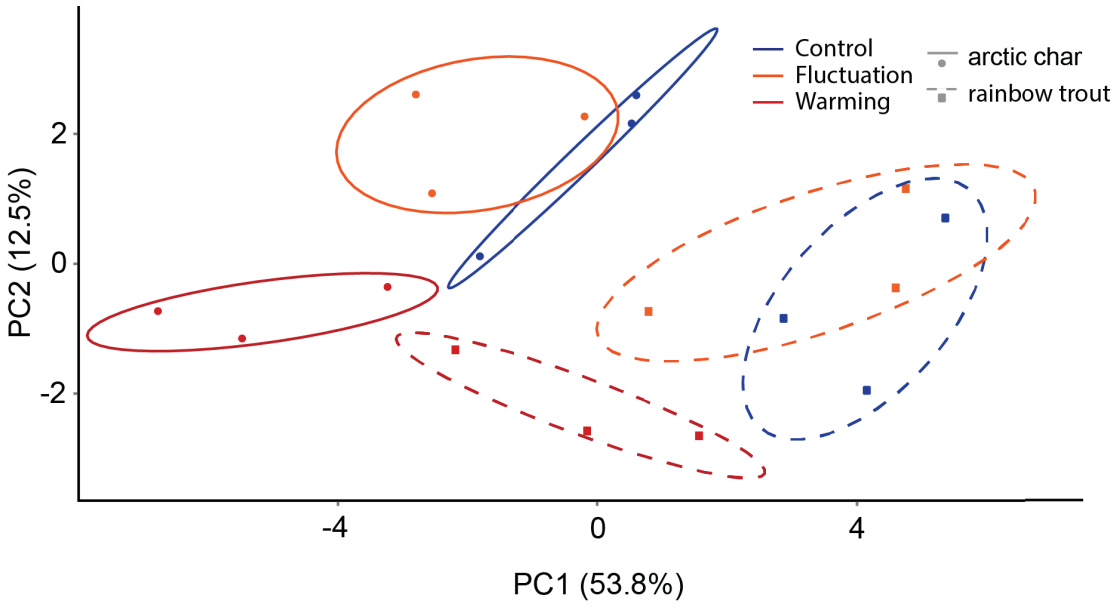
Control
Fluctuation
Warming
arctic char
rainbow trout
PC2 (12.5%)
2
0
-2
-4
0
4
PC1 (53.8%)

Figure 4.1

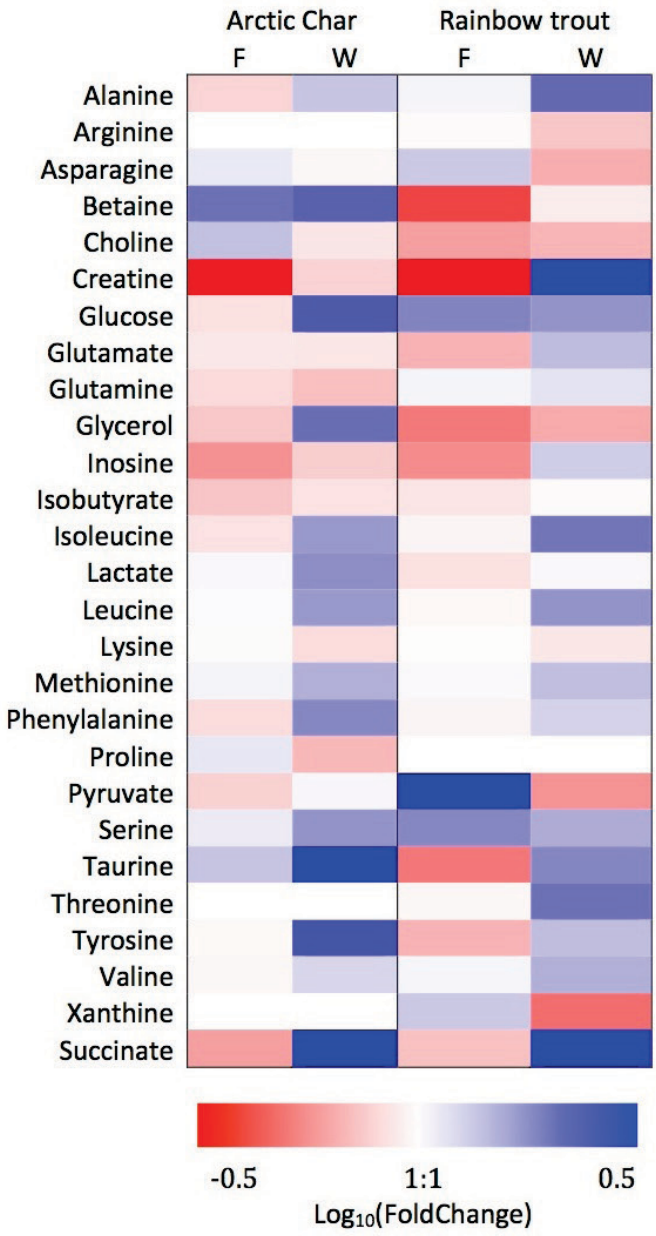
Arctic Char
Rainbow trout
F
W
F
W
Alanine
Arginine
Asparagine
Betaine
Choline
Creatine
Glucose
Glutamate
Glutamine
Glycerol
Inosine
Isobutyrate
Isoleucine
Lactate
Leucine
Lysine
Methionine
Phenylalanine
Proline
Pyruvate
Serine
Taurine
Threonine
Tyrosine
Valine
Xanthine
Succinate
-0.5
1:1
0.5
Log10(FoldChange)

Figure 4.2

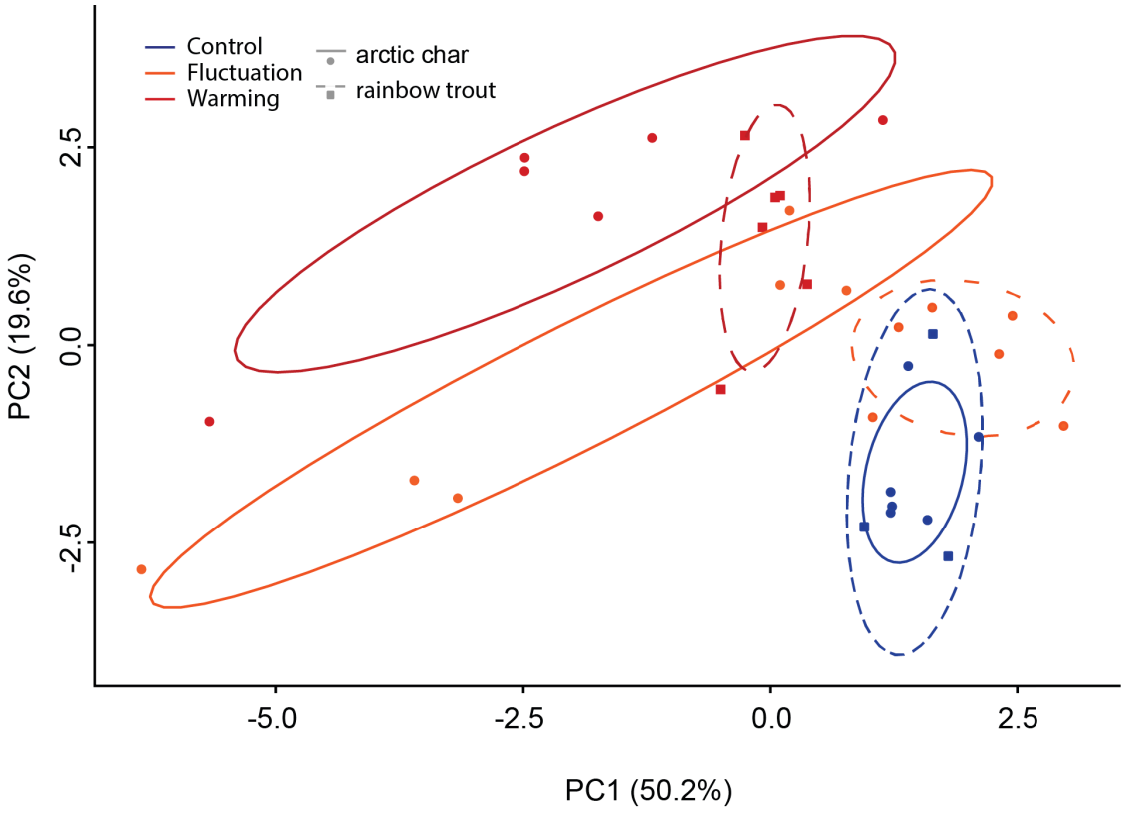
Control
Fluctuation
Warming
arctic char
rainbow trout
PC2 (19.6%)
2.5
0.0
-2.5
-5.0
-2.5
0.0
2.5
PC1 (50.2%)

Figure 4.3

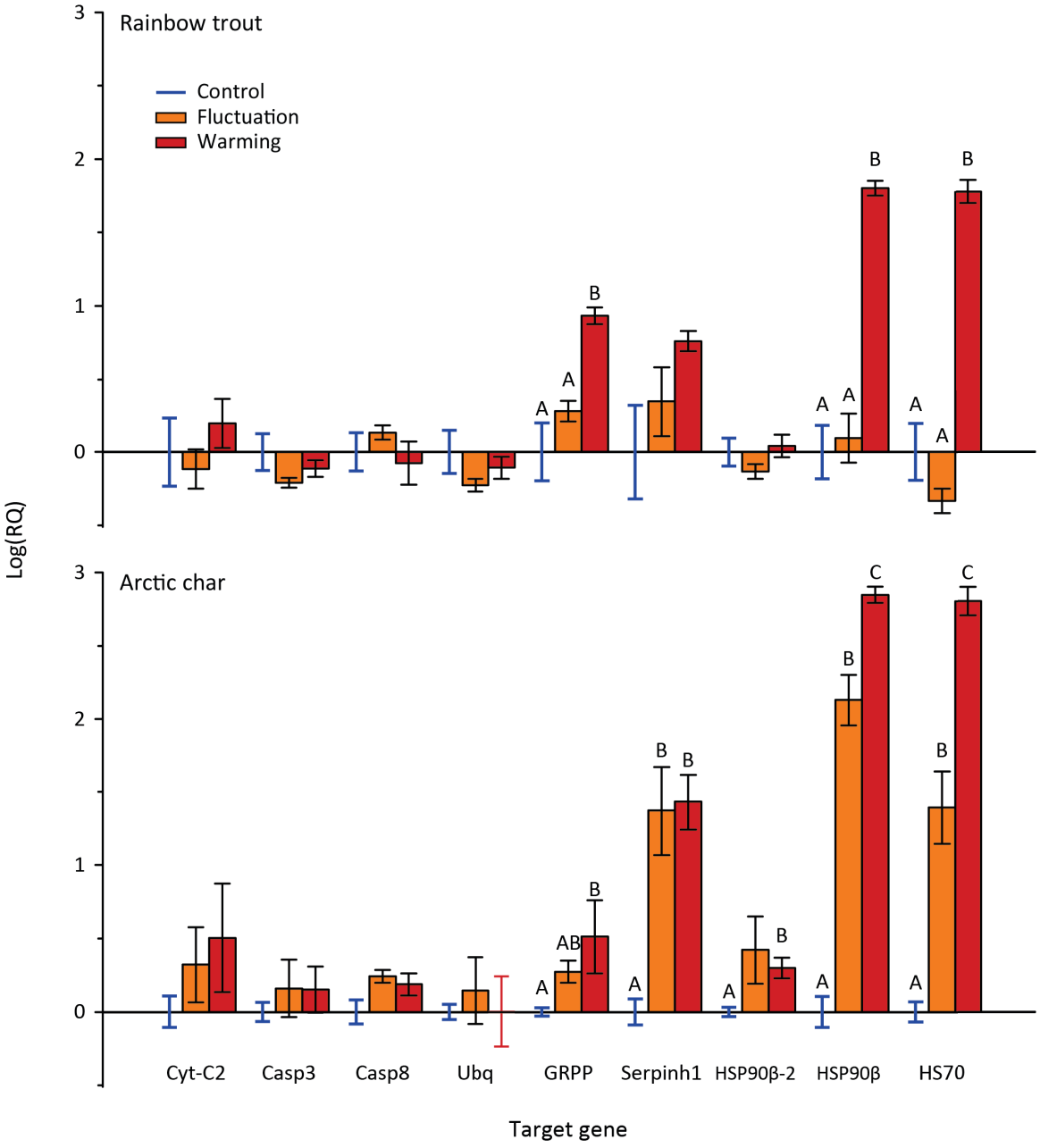
Rainbow trout
Control
Fluctuation
Warming
Arctic char
Log(RQ)
Target gene
Cyt-C2
Casp3
Casp8
Ubq
GRPP
Serpinh1
HSP90β-2
HSP90β
HS70

Figure 4.4

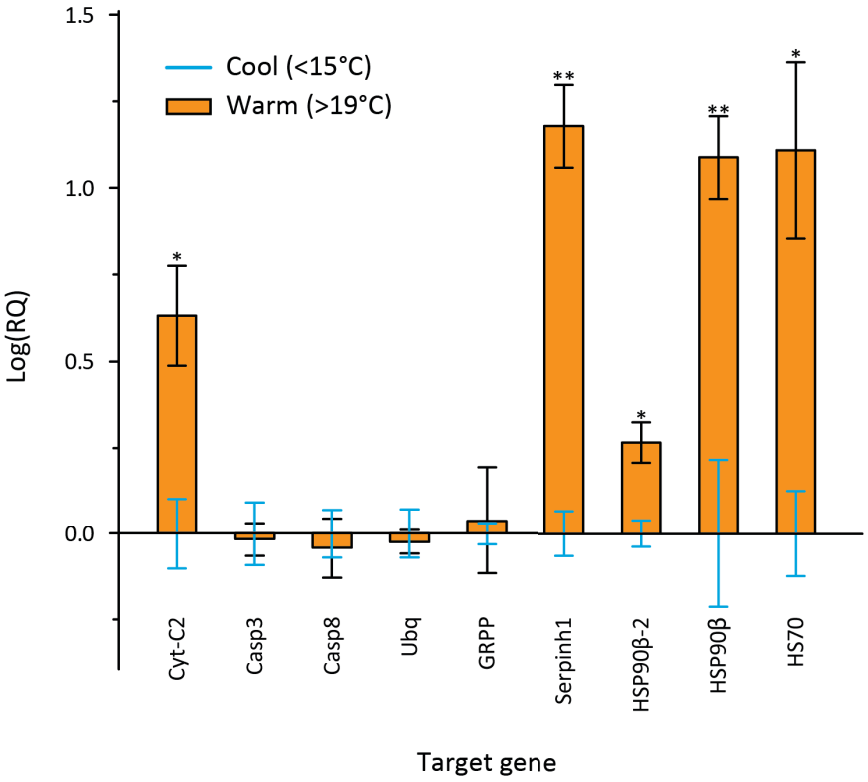
1.5
1.0
0.5
0.0
Log(RQ)
Cool (<15°C)
Warm (>19°C)
*
**
**
*
Cyt-C2
Casp3
Casp8
Ubq
GRPP
Serpinh1
HSP90β-2
HSP90β
HS70
Target gene

Figure 4.5

Chapter 5: General Conclusions

Summary of major findings

The unifying goal throughout my research was to identify characteristics of arctic char migratory life history and physiology that will shape their ability to cope with global climate change (GCC). I focused my research on the Nulahugyuk Creek arctic char migration, which was traditionally a vital Inuit fishery (Jenness 1922). Nulahugyuk Creek is generally shallow and experiences a rapid decline in discharge and large diurnal temperature fluctuations throughout the course of the migration. These conditions frequently lead to adult char becoming stranded in shallow water and juveniles and adults having to migrate through warm, variable water temperatures. The harsh nature of flow and thermal regimes in the creek allowed for the study of the life-history and physiology of arctic char under conditions represent the current extremes under which char migrate, but will encounter more frequently as GCC progresses.

Previous researchers have suggested that extensive life history diversity within char facilitates their existence in other extreme environments (Hammar 2014; Klemetsen 2010). In the present thesis I expanded our understanding of this diversity by identifying novel life history strategies that facilitate the persistence of arctic char in Nulahugyuk Creek. Specifically, these arctic char possess the lowest observed annual fidelity to their natal system (Gyselman 1994; Moore et al. 2013), and once smolts leave on their first migration they typically do not return for four to five years. Furthermore the upstream migration from the ocean to their spawning habitat is the earliest documented return run in northern Canada. These previously un-described life history strategies suggest that other populations of arctic char may possess the variation needed to adapt to more restrictive flows and temperatures in the future. Alternatively, if changes occur too

rapidly to permit local adaptation, populations already possessing these unique life history traits may be able to serve as source populations as other systems become uninhabitable to their current residents.

The alternative life history traits identified certainly lessen the exposure of Nulahugyuk Creek arctic char to even worse conditions, however the conditions they do face still present significant physiological challenges. I found that current diurnal temperature variation in Nulahugyuk Creek already appears to be sufficient enough to impair recovery from fatiguing exercise. This impaired recovery is likely a result of an inability to sufficiently increase cellular energy supply to meet increased demand at warm temperatures, despite the mobilization of significant energy reserved including glucose and numerous amino acids, and despite large increases in anaerobic metabolism. The general loss of performance in arctic char coincided with a potential reduction in mitochondrial functionality and a large compensatory transcriptional heat shock response response that was evidently insufficient. In the wild this impaired physiological performance and inability to recover from fatiguing exercise could result in longer recovery times at strenuous points in the migration such as in high flows or when fish become stranded. Furthermore, the exploitation of various energy reserves at warm temperatures (e.g. glycogen, protein) may deplete the energy available for growth or reproductive maturation (Mommsen 2004). Together these performance and energy losses could result in increased predator related mortality, reduced migratory success in general, and a reduction in growth or fecundity. In our comparative studies, relative to rainbow trout, arctic char exhibited a greater and prolonged disturbance to thermal variation representative of their current migratory conditions. This suggests that they may already be at a competitive disadvantage to more temperate salmonids whose ranges are expanding in the north. These findings are of particular concern, as the environmental conditions

studied will only become more common or worsen in the future (ACIA 2005; Reist et al. 2006b; Wrona et al. 2006b).

Future directions

In the present study I was able to identify population specific migratory life history traits that allow Nulahugyuk Creek arctic char to persist in an extreme environment by comparing their life histories to other previously studied populations. At the physiological level we only investigated the performance of one population of wild char and one strain of captive char and there were no other studies similar enough to begin addressing the extent to which thermal sensitivities of arctic char vary at the local environment or population level. There is well-established local adaptation in the migratory physiology of different populations of sockeye salmon that corresponds with the physical difficulty of their respective migrations and their thermal environments (Eliason et al. 2011). While the wild juvenile char in the present study were, able to maintain performance at least up to 20°C other populations that do not currently experience these conditions, or have as physically challenging migration may not have that ability and may therefore be susceptible to change. In the present study and those in more temperate species, cardiorespiratory or mitochondrial limitations appear to be responsible for performance losses at warm temperatures (Eliason et al. 2011; Farrell 2009b; Farrell et al. 2009; Schulte 2015). As such future studies in anadromous arctic char should investigate population specific differences in cardiac and mitochondrial physiology that may underlie differences in thermal sensitivity.

The current study was also not designed to assess the range of individual variation in thermal tolerance that exists within a given population. Quinn et. al (Quinn et al. 2011a) demonstrated that an aquaculture strain of arctic char possessed substantial individual variation in thermal

tolerance, potentially based on individuals ability to induce a heat shock response. As heritable variation within a trait is required for adaptation to occur, future studies in wild arctic char should assess the extent of existing variation in thermal tolerance as well as its heritability to determine potential for adaptation.

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