The effects of temperature acclimation and heating rate on the thermal tolerance of juvenile white sturgeon (*Acipenser transmontanus*)

by

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The following individuals certify that they have read, and recommend to the Faculty of Graduate and Postdoctoral Studies for acceptance, a thesis entitled:

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Abstract

Freshwater fish such as white sturgeon (*Acipenser transmontanus*) are particularly vulnerable to the effects of anthropogenic global warming; however, little is known about how acclimation to higher temperatures or rate of temperature increase affects their thermal tolerance. The Kenney dam on the Nechako River is home to the northern-most population of white sturgeon and is mandated to maintain water temperatures below 20°C for migrating sockeye salmon, but it remains unclear whether 20°C is an appropriate threshold for developing white sturgeon. To address this, 37-51 days post hatch (dph) and 66-80 dph juvenile white sturgeon were acclimated to one of four ecologically relevant temperatures (15°C, 18°C, 21°C, and 24°C) for two weeks, following which thermal tolerance (CT_{max}), size, condition factor, and survival were assessed. White sturgeon displayed highly plastic CT_{max} in response to acclimation, illustrated by a positive relationship between acclimation temperature and CT_{max} and large acclimation response ratios compared to other fish species. Acclimation to temperatures above 18°C was found to negatively affect condition factor, which suggests the presence of a sub-lethal threshold between 18°C and 21°C. Their highly plastic response to temperature was further demonstrated when the effect of heating rate (0.3°C/min, 0.03°C/min, 0.003°C/min) on thermal tolerance, somatic indices, and Hsp mRNA expression was assessed. White sturgeon CT_{max} was highest in the slowest heating rate, contrary to what has been observed in most other fish species. Hepatosomatic index decreased in all heating rates relative to control fish, indicative of the metabolic costs of thermal stress. Expression of *Hsp70* mRNA was increased in all heating rates relative to controls, whereas expression of *Hsp90a* and *Hsp90b* mRNA only increased in the two slower trials. Together these data indicate that while white sturgeon have a very plastic thermal response, acclimation to temperatures above 18°C may negatively affect overall health, indicated

by lower condition factor. As such, in the best interest of white sturgeon conservation, the operators of the Kenney dam may want to reconsider whether the 20°C threshold is appropriate.

Lay Summary

Freshwater fish such as the endangered white sturgeon are threatened by climate change induced warming. White sturgeon are culturally and economically important and are native to the west coast of North America. The Kenney dam on the Nechako River in British Columbia is currently mandated to maintain water temperature below 20°C; however, this threshold originated from research on sockeye salmon and may not apply to white sturgeon. To protect white sturgeon populations, this thesis investigates how juvenile white sturgeon will cope with warming temperatures, in hopes of better informing dam management decisions and species conservation. To do so, the effects of temperature and warming rate on traits such as thermal tolerance, survival, size, and other bodily indicators were assessed. Warmer temperatures were found to increase thermal tolerance, but negatively affect overall health. This suggests that river temperatures above 18°C may not be appropriate for white sturgeon conservation.

Preface

I conducted all of the research in Chapters 2 and 3 under the supervision of Dr. Colin Brauner (research questions, experimental design, and data analysis). William Bugg was a collaborator in Chapter 3. All experimental animals were treated according to the University of British Columbia Animal Protocol #A15-0266. I wrote all 4 chapters of this thesis and received editorial feedback from my committee members, Drs. Colin Brauner, Katie Marshall, and Patricia Schulte.

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List of Abbreviations

ARR Acclimation response ratio,

$$ARR = \frac{CT_{max}(T_{a2}) - CT_{max}(T_{a1})}{(T_{a2} - T_{a1})}$$

BSI Brain somatic index,

$$BSI = {Brain\ mass\ (g)}\Big/{Whole\ body\ mass\ (g)} \times 100\%$$

CF Condition factor,

$$CF = \frac{Whole\ body\ mass\ (g) \times 10^5}{Total\ length\ (mm)^3}$$

CSI Cardiosomatic index,

$$CSI = {Heart\ mass\ (g)}\Big/{Whole\ body\ mass\ (g)} \times 100\%$$

CSR Cellular stress response

CTM Critical thermal methods

CT_{max} Critical thermal maximum

CT_{min} Critical thermal minimum

dph Days post hatch

HSI Hepatosomatic index,

$$HSI = {Liver\ mass\ (g)}\Big/{Whole\ body\ mass\ (g)} \times 100\%$$

Hsp Heat shock protein

ILT Incipient lethal temperature

InSEAS Initiative for the Study of the Environment and its Aquatic Systems

IPCC	Intergovernmental Panel on Climate Change
LOE	Loss of equilibrium
mRNA	Messenger ribonucleic acid
NWSRI	Nechako White Sturgeon Recovery Initiative
OCLTT	Oxygen capacity limited thermal tolerance theory
T_a	Acclimation temperature
UBC	University of British Columbia
YSL	Yolk sac larvae

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Chapter 1: Introduction

Of the many freshwater fish species in North America affected by anthropogenic activity, white sturgeon (*Acipenser transmontanus*) has undergone severe population collapse (Hildebrand et al., 2016). As global temperatures continue to rise at rates predicted by the Intergovernmental Panel on Climate Change (IPCC), elevated water temperature will become increasingly problematic for developing white sturgeon (Chen et al., 2021).

The Nechako River in British Columbia, Canada, which is home to the northernmost population of endangered white sturgeon (Hildebrand et al., 2016), was dammed in the 1950s. Following the construction of the Kenney Dam, a water management program was developed, which led to the establishment of an upper water temperature threshold set at 20°C. This threshold temperature was established based on research conducted exclusively on migrating sockeye salmon (*Oncorhynchus nerka*; Macdonald et al., 2012). It remains unclear whether the 20°C threshold is an appropriate threshold for developing white sturgeon.

To better inform water management decisions and help prevent further population collapse, this thesis examines the effects of temperature acclimation and heating rate on juvenile white sturgeon as relatively little is known about the thermal biology of this species. The remainder of the introduction will broadly review the effects of temperature and thermal tolerance, acclimation, physiological indicators of temperature, and the life history of white sturgeon, which will lead to a discussion of the specific thesis objectives.

1.1 Temperature effects and thermal tolerance

Climate change is predicted to greatly impact global temperatures in the coming decades (Hassan et al., 2020; Hoegh-Guldberg et al., 2018). Northern regions of the globe are expected to be disproportionately affected by rising temperatures, having already experienced twice the global average of temperature increase of southern regions (Chen et al., 2021). In Canada, mean annual surface air temperatures have increased by 1.5°C from 1950 to 2010, with northernmost regions undergoing the largest increase (Vincent et al., 2012; Vincent et al., 2015).

Freshwater ecosystems are particularly susceptible to climate change because they are often overexploited environments that offer limited refugia and dispersal opportunities (Dudgeon et al., 2006; Hassan et al., 2020; Woodward, 2009). Accordingly, freshwater animals tend to have higher extinction rates than either terrestrial or marine species, particularly at higher latitudes. Freshwater ecosystems in northern latitudes are some of the most threatened in the world, and so require focused scientific study and heightened conservation efforts (Strayer and Dudgeon, 2010).

The impacts of anthropogenic global warming are especially significant for ectotherms such as fish, because their body temperature is directly influenced by the temperature of their environment. For ectotherms, temperature plays a critical role in determining population range, physiology, community dynamics, and survival (Boltaña et al., 2017; Crozier et al., 2008; Li et al., 2015; O'Gorman et al., 2016; Pankhurst and Munday, 2011; Schulte, 2015). All organisms have a defined thermal tolerance range in which they are able to function (Huey and Stevenson, 1979). Thermal tolerance extends from an organism's lower thermal limit to their upper thermal limit (Beitinger and Lutterschmidt, 2011). An organism's optimum temperature lies within this

range and is the temperature at which they function best (Schulte, 2015). When temperatures approach an animal's thermal limits, damage can be caused at all levels of biological organization (Abram et al., 2017; McArley et al., 2017; Schulte, 2015).

Thermal tolerance varies depending on developmental stage and consequently, an organism's most thermally sensitive life stage determines its vulnerability to global warming (Beitinger and Lutterschmidt, 2011; Dahlke et al., 2020). Some species display diurnal fluctuations in thermal tolerance, where thermal tolerance is highest during the day and lowest at night (Healy and Schulte, 2012). Thermal tolerance also fluctuates throughout the year; upper thermal tolerance is the highest in the summer when species are exposed to warmer weather and the lowest in the winter when ambient temperatures are lower (Schaefer et al., 1999; Sharma et al., 2015). In fish, thermal tolerance is generally the lowest during embryogenesis and spawning as these are the most oxygen-limited life stages (reviewed in Pörtner, 2001; Dahlke et al., 2020; Pörtner and Farrell, 2008). Early in development fish prioritize rapid growth – typical growth rates for larvae are 10-30% body mass per day. Consequently, they have less energy reserves to cope with abiotic stressors such as extreme temperatures (Rombough, 2011).

1.2 Measuring thermal tolerance

Multiple laboratory tests have been developed to assess thermal tolerance in ectotherms, including the incipient lethal temperature (ILT) and the critical thermal methodology (CTM; Cowles and Bogert, 1944; Fry et al., 1942). Both techniques are standardized, repeatable, and assess upper and lower thermal limits; however, CTM is preferentially used as it requires fewer

fish, it is fast to conduct, and its sub-lethal endpoint allows endangered animals to be assessed and then released back in to their environment (Beitinger and Lutterschmidt, 2011). Critical thermal maximum (CT_{max}) and critical thermal minimum (CT_{min}) tests are used to determine upper and lower thermal limits respectively. The endpoint for upper thermal tolerance occurs when the animal's locomotion becomes disorganized (Cowles and Bogert, 1944). In fish, this endpoint is characterized by a loss of equilibrium (LOE) where the fish is no longer able to right itself. To conduct CTMs, temperature is increased (or decreased) at a constant rate that is fast enough to prevent acclimation but slow enough that inner body temperature remains in equilibrium with the environment. Typical rates of temperature change in CTMs range between 0.1°C/min and 0.3°C/min, but there is a wide range cited in the literature (Beitinger and Lutterschmidt, 2011; Kingsolver and Umbanhowar, 2018; Lutterschmidt and Hutchison, 1997).

CT_{max} is commonly used to predict species' vulnerability to climate change (Sandblom et al., 2016; Sunday et al., 2012). Yet, while CTMs are easy to conduct in laboratories, their ecological relevance is less clear as the rates of temperature change are far greater than most temperature increases observed in nature (a rate of 0.3°C/min equates to 18°C/hour and 432°C/day;(Illing et al., 2020). Few studies in fish have directly compared the rate at which the temperature is increased (heating rate) during a CT_{max} trial to the final CT_{max} value. Those that have, typically revealed that faster heating rates resulted in higher CT_{max} values (Becker and Genoway, 1979; Illing et al., 2020; Mora and Maya, 2006). It is thought that slower heating rates result in lower CT_{max} values because the cumulative stress of prolonged heat exposure exhausts the animal (Rezende et al., 2014).

1.3 Acclimation

In light of long-term climate forecasts predicting drastic increases in temperature, research has begun to focus on how animals will adjust to survive these changes. Faced with increasing temperature, fish can either migrate, acclimate, or adapt (Fuller et al., 2010; Somero, 2010). Migration is often not feasible for many freshwater fish because of natural and anthropogenic ecosystem fragmentation, such as dams, waterfalls, and catchment divides (Rahel, 2007; Strayer and Dudgeon, 2010). Furthermore, climate change may be acting too rapidly for organisms to mitigate its affects through adaptation (Radchuk et al., 2019). Thus, the ability to successfully acclimate is critical for freshwater fish species.

Temperature acclimation is defined as the reversible physiological and biochemical changes that occur in response to thermal stress, and is crucial for enabling organisms to cope with natural and anthropogenic changes in their environment (Hazel and Prosser, 1974; Kingsolver and Huey, 1998). The timescale over which acclimation occurs on depends on a number of factors, including latitude, habitat, taxon, and body size (Morley et al., 2019; Rohr et al., 2018). Polar fish typically require 5 – 20 days to acclimate to temperature changes whereas tropical fish acclimate 2 – 4 times faster and are able to increase their thermal tolerance in 2 – 5 days (Bilyk and DeVries, 2011; Peck et al., 2014; Schmidt-Nielsen, 1990). Rohr and colleagues (2018) found that acclimation rate scales negatively with body size, with larger organisms (14g) taking longer to acclimate than smaller organisms (20mg). Most ectothermic vertebrates have some capacity for thermal acclimation. For instance, brook trout (*Salvelinus fontinalis*) acclimated to temperatures that span their thermal range (5°C – 25°C), were subsequently able to increase their thermal tolerance. However this was limited to acclimation temperatures of up to

20°C, beyond which no further increase in thermal tolerance was observed (Morrison et al., 2020).

1.3.1 Quantifying thermal acclimation

All organisms have a finite ability to acclimate, and upper thermal tolerance will eventually plateau even as the animal is acclimated to higher temperatures (Morrison et al., 2020). The difference between an organism's optimum temperature and the environmental temperature they experience is known as the thermal safety margin – as anthropogenic global warming continues, this margin will decrease (McArley et al., 2017; Sandblom et al., 2016). Another way to quantify an organism's capacity to acclimate is using the acclimation response ratio (ARR). ARR offers a metric of physiological plasticity that captures acclimation capacity. It is calculated as the ratio of the change in upper thermal tolerance (measured using CT_{max}), per degree change in acclimation temperature (T_a) (Claussen, 1977), as follows:

$$ARR = \frac{CT_{max}(T_{a2}) - CT_{max}(T_{a1})}{(T_{a2} - T_{a1})}$$

An ARR of 1 would indicate complete acclimation, where for example, a 5°C increase in acclimation temperature results in a 5°C increase in thermal tolerance. Conversely, an ARR of 0 indicates no acclimation (Claussen, 1977). ARR varies by taxa and by latitude (Morley et al., 2019; Rohr et al., 2018). Morley et al. (2019) found that the average ARR of tropical fish is significantly lower than mid- and high latitude species (0.26 versus 0.30 and 0.32). Juvenile lake sturgeon (*Acipenser fulvescens*) have higher ARRs (around 0.4) than most other species for similar latitudes, indicating a high degree of thermal plasticity (Bugg et al. 2020). This trait of a

high ARR may be general to all sturgeon as juvenile shortnose sturgeon (*Acipenser brevirostrum*), acclimated from 10°C to 15°C had an ARR of 0.78 (Zhang and Kieffer, 2014).

1.4 Physiological indicators of temperature effects on fish

Temperature can affect fish in a multitude of ways, depending on the duration of exposure. In fish, condition factor is a commonly used indicator of overall health (Bolger and Connolly, 1989). It is calculated using the relationship between fish mass and length, as follows:

$$Condition\ factor = \frac{Whole\ body\ mass\ (g) \times 10^5}{Total\ length\ (mm)^3}$$

While healthy condition factor varies by species, within a species, a higher condition factor is broadly indicative of better "condition". Declining condition factor is a common attribute amongst stressed fish, particularly in fish undergoing heat stress, and may be attributed to non-optimal temperatures for energy assimilation (Björnsson et al., 1989; Irons et al., 2007; Kappenman et al., 2009; Meffe, 1992; Yoon et al., 2019). Increased rearing temperature has been shown to lower condition in juvenile lake and shovelnose sturgeon (Kappenman et al., 2009; Yoon et al., 2019). Boucher et al. (2014) found the same trend in larval white sturgeon (*Acipenser transmontanus*).

Somatic indices of various organs are also used to quantify overall health of fish, including the: hepatosomatic index (HSI), cardiosomatic index (CSI), and brain somatic index (BSI). These indices are all calculated based on the relationship between the mass of the individual organ and the total mass of the fish.

$$Somatic\ index = \frac{Organ\ mass\ (g)}{Total\ mass\ (g)} \times 100\%$$

Like condition factor, HSI, CSI, and BSI are similarly affected by environmental stressors, but less is known about how they are affected by temperature especially on short time scales (e.g., hours to days; Eifert et al., 2015; Johansen et al., 2011; Medcalf et al., 2021). In addition to overall health, HSI can indicate changes to metabolism and lipid and glycogen reserves as a result of stress (Chellappa et al., 1995; Morrison et al., 2020). The HSI of two geographically distinct populations of juvenile lake sturgeon decreased as a result of a 30 day acclimation to higher rearing temperatures (Bugg et al., 2020). In species such as Arctic char (*Salvelinus alpinus*), heart rate increases in response to heat stress as animals increase their circulatory capacities to cope with increased tissue oxygen demand. This reaction may build heart muscle over time and increase CSI (Gilbert and Farrell, 2021; Gilbert et al., 2020). Finally, the brain uses a disproportionately high amount of energy relative to its weight because of the high metabolic demands associated with neural processing (Soengas and Aldegunde, 2002; Van Ginneken et al., 1996). During periods of heat stress, metabolic demand can overwhelm supply, which may result in the depletion of glucose reserves and decreased BSI (Schulte, 2015).

To mitigate the effects of heat stress, fish can acclimate and alter their thermal tolerance by regulating the expression of heat shock proteins (Hsps). Hsps are one facet of a set of cellular responses known collectively as the cellular stress response (CSR). The CSR is a highly conserved collection of cellular responses that maintain and restore protein homeostasis (Kültz, 2005). Hsps are similarly highly conserved and have been found in every organism studied to date (Fangue et al., 2006; Garbuz and Evgen'ev, 2017; Krebs and Feder, 1997). They are

grouped according to their molecular weights and there are three major families: *Hsp90s* (85-90kDa), *Hsp70* (69-73kDa), and low molecular weight Hsps (16-47kDa) (Garbuz and Evgen'ev, 2017). Unlike low-molecular-weight Hsps, some members of the *Hsp90* and *Hsp70* families are expressed constitutively (known as heat shock cognates) in cells when an organism is not stressed. When expressed constitutively, they aid with protein metabolism and act as molecular chaperones (Chen et al., 2018; Garbuz and Evgen'ev, 2017). Inducible Hsps are preferentially transcribed during periods of thermal stress and their presence, which can increase by multiple orders of magnitude in a matter of minutes, has been linked to increased thermotolerance (Tomanek and Somero, 2002). While there is not a 1:1 relationship between mRNA transcription and protein translation, increased transcription of Hsp mRNA has been found to lead to a similarly rapid increase in Hsp translation (Buckley et al., 2006; Mirault et al., 1977). In insects, studies have shown that inhibition of Hsp expression suppressed thermal tolerance, while additional expression increased it (Feder et al., 1996; Lu et al., 2016; Rinehart et al., 2007). Once upregulated, Hsps help mitigate cellular damage by binding and stabilizing proteins. Hsps then either catalyze protein refolding or, if the protein is damaged beyond repair, Hsps transfer the protein to a degradation pathway (Chen et al., 2018; Fangue et al., 2006). *Hsp70* upregulation appears to be the most conserved response to heat shock (Bugg et al., 2020; Werner et al., 2007; Yebra-Pimentel et al., 2020). In white sturgeon, acute heat shock increased *Hsp60* and *Hsp70* expression, the latter being the most upregulated and thus a more sensitive biomarker (Deng et al., 2009).

1.5 White sturgeon (*Acipenser transmontanus*)

White sturgeon (*Acipenser transmontanus*) are part of the genus *Acipenser*, which is comprised of 17 extant sturgeon species, which inhabit freshwater and estuarine water systems in Asia, Europe, and North America (Birstein and Bemis, 1997). All sturgeon are characterized by a number of traits: they are large, cartilaginous, benthic feeders that have long lifespans, mature slowly, migrate extensively, and spawn intermittently (Lebreton et al., 2004). Sturgeon are known as 'living fossils' because their morphology has remained largely unchanged relative to the earliest sturgeon fossil records from the Upper Crustaceous period (Gardiner, 1984). White sturgeon are the largest freshwater fish in North America, growing up to 6m long and weighing up to 800kg (Hildebrand et al., 2016). Their range extends along the West coast of North America, from Northern Mexico to Alaska, but they are typically confined to a few river basins, including: the Sacramento – San Joaquin, the Columbia, and the Fraser (Ruiz-Campos et al., 2011). After surviving for millennia, sturgeon populations have been devastated by human activity over the last 150 years, to the point where many species are in danger of extinction (Gessner et al., 2006; Hildebrand et al., 2016; Lebreton et al., 2004). In Canada, there are six distinct populations (Lower Fraser River, Mid Fraser River, Upper Fraser River, Upper Columbia River, Nechako River, and Kootenay River), four of which are considered endangered (Upper Fraser River, Upper Columbia River, Nechako River, and Kootenay River; Fisheries and Oceans Canada, 2014; Hildebrand et al., 2016).

Population decline has been caused by several anthropogenic activities including overexploitation through commercial fishing in the early 1900s. Despite a ban on commercial fishing, and the development of hatchery programs, white sturgeon populations continue to decline (Hildebrand et al., 2016). White sturgeon are particularly susceptible to anthropogenic

impacts due to their life history and requirement for different habitats throughout different life stages (Auer, 1996; Boreman, 2005; Jager et al., 2001; Parsley and Kappenman, 2000). White sturgeon have five defined life history stages – egg/embryo, yolk sac larvae (YSL), feeding larvae, juvenile, and adult (Deng et al., 2002; Hildebrand et al., 2016). The focus of this thesis on the early portion of the juvenile stage, which begins between 20 and 45 days post-hatch (dph) when white sturgeon metamorphose from their feeding larval stage, having developed a full complement of scutes and fins (Deng et al., 2002). Their early life stages are particularly challenging and are marked by multiple bottlenecks to survival and consequent high mortality rates (Hildebrand et al., 2016). Recovering from population collapse is especially difficult for white sturgeon as they take a long time to reach sexual maturity and reproduce infrequently (Boreman, 2005). Consequently, minimizing mortality during the first year of life is critical to white sturgeon population recovery (Gross et al., 2002). To reduce early life mortality, it is paramount that we gain a better understanding of the environmental factors that affect white sturgeon during this precarious period.

1.6 The Nechako River

The Nechako River, which continues to suffer from white sturgeon population collapse, has been impounded by the Kenney dam since the 1950s in order to supply electricity and smelt aluminum at Rio Tinto Alcan's nearby Kitimat plant (4Thought Solutions Inc., 2005). Saik'uz and Stellat'en First Nations, whose land on which the Kenney dam was built, have described environmental degradation and harm to salmon and white sturgeon populations since the dam was built (Bennett, 2021; Saik'Uz First Nation, 2019).

In addition to negatively altering habitats, dams may compound the effects of climate change and global warming (Mantua et al., 2010; Nilsson et al., 2005). Specifically, reduced flow rates in the summer can lead to increased water temperatures downstream. To mitigate this, many dams utilize spillways to increase water flow in order to keep water temperatures within an acceptable range (Pittock and Hartmann, 2011).

On the Nechako River, dam operators are able to effect water temperature downstream of the Kenney Dam by releasing surface water from the Nechako Reservoir via the Skins Lake Spillway. In the 1980s, a water management program was devised for the Nechako River, which established an upper temperature threshold for the river. This threshold was based upon research on migrating sockeye salmon (*Oncorhynchus nerka*) and was set at 20°C (Macdonald et al., 2012). However, due to the lack of research on the thermal tolerance of white sturgeon, it is unclear whether this 20°C threshold is acceptable. This thesis aims to fill this void. By understanding the thermal tolerance of the early life stages of white sturgeon from the Nechako River system and how temperatures around and above 20°C affect various somatic indices and thermal tolerance, this thesis addresses whether the 20°C threshold mandate is appropriate for white sturgeon.

1.7 Thesis objectives

The overall goal of this thesis was to investigate the effect of temperature acclimation and rate of temperature increase on the physiology of juvenile white sturgeon at various levels of biological organization, including mRNA expression of Hsps, size, somatic indices, thermal

tolerance, and survival. These experiments will provide valuable information on the temperature sensitivity and thermal tolerance of juvenile white sturgeon during their first summer of development, which will help inform conservation efforts and reservoir management decisions.

This thesis can be divided into two main objectives, which are as follows:

1.7.1 Determine the effect of temperature acclimation on survival, condition factor and thermal tolerance

To do this, juvenile white sturgeon were acclimated to one of four ecologically relevant temperatures (15°C, 18°C, 21°C, and 24°C) for two weeks. It was hypothesized that thermal tolerance, final condition factor, final size, capacity to acclimate, and survival would all be affected by acclimation temperature. Sturgeon acclimated to warmer temperatures were predicted to have increased thermal tolerance (measured by CT_{max}). Alternatively, if 20°C were above the thermal limit of juvenile white sturgeon, then capacity to acclimate (measured by ARR), final condition factor, final size, and survival would be lower in the higher acclimation treatments.

1.7.2 Determine the effect of heating rate during CT_{max} on thermal tolerance, somatic indices, and Hsp expression

To do this, juvenile white sturgeon underwent CT_{max} trials using one of three heating rates (0.3°C/min (a typical rate of heating during CTmax tests), 0.03°C/min, and 0.003°C/min). It was hypothesized that heating rate would affect thermal tolerance and Hsp expression. Sturgeon were predicted to have lower thermal tolerance and higher Hsp mRNA expression in

the slower heating rates. Furthermore, mRNA expression of HSP was quantified in high and low CT_{max} performers to determine if expression levels differed between these two groups. This thesis also sought to characterize the effect of heating rate on various somatic indices (condition factor, BSI, CSI, and HSI) as these metrics have not been measured after short-term thermal stress. If the duration (or amount) of stress was sufficient, then condition factor, BSI, and HSI would be expected to decrease, while CSI would be expected to increase with an increase in heating rate.

Chapter 2: The effects of acclimation temperature and age on thermal tolerance in juvenile white sturgeon

2.1 Introduction

Temperature has been coined the "abiotic master factor" as it affects physiology, behaviour, and ecology (Abram et al., 2017; Brett, 1971; Bugg et al., 2020; McArley et al., 2017). Ectotherms are especially sensitive to temperature changes as their internal body temperatures are dependent on environmental temperature (Hochachka and Somero, 2002; Schulte, 2015). In fish, early life stages are the most vulnerable to extreme temperatures (Dahlke et al., 2020; Pörtner and Farrell, 2008; Rombough, 1988). As temperatures increase worldwide due to climate change, more species will be exposed to temperatures that exceed their thermal range (Beitinger and Lutterschmidt, 2011; Dahlke et al., 2020). Increasing temperatures are predicted to decrease the abundance of freshwater habitats, which will disproportionately affect cold-water fish (Comte et al., 2013).

Temperature acclimation is the reversible physiological and biochemical changes in response to a thermal stressor and it allows organisms to shift their thermal tolerance range (Hazel and Prosser, 1974; Kingsolver and Huey, 1998; Lagerspetz, 2006). Acclimation to warmer temperatures can increase an organism's upper thermal limit and help organisms cope with the unprecedented environmental change brought about climate change (Bugg et al., 2020; McArley et al., 2017; Somero, 2010; Zhang and Kieffer, 2014).

Most studies used to inform the thermal tolerance range for juvenile white sturgeon have been conducted on southern populations – primarily from the Sacramento-San Joaquin Delta –

that experience warmer water temperatures than white sturgeon in Northern British Columbia (Cech et al., 1984; Deng et al., 2002; Deng et al., 2009; Wang et al., 1985). Growth of the juvenile white sturgeon (0.6g) native to the Sacramento-San Joaquin Delta is maximized at 20°C, which is the mean water temperature of their native nursery habitat (Cech et al., 1984; Lebreton et al., 2004). Above 20°C, there was increased mortality and growth was reduced, indicating that temperatures above 20°C may impact survival (Cech et al., 1984). Increased mortality above 20°C has also been observed in the embryonic and YSL phase of development in white sturgeon from the Sacramento-San Joaquin Delta and the Nechako River (Cech et al., 1984; Cheung, pers. comm; McAdam, pers. comm; Wang et al., 1985). While no studies have directly compared the thermal tolerance of white sturgeon from different river systems, independent studies have found that white sturgeon from more southern rivers have higher upper thermal limits than those from more northern rivers (Golder Associates Ltd., 2010; Wang et al., 1985). These findings demonstrate the need for population specific studies on thermal tolerance especially for conservation efforts.

The Nechako White Sturgeon Recovery Initiative (NWSRI) rears sturgeon at 15°C to maximize growth and survival as it mimics the natural environment (pers. comms. Steve McAdam). The aim of this thesis was to provide more insight on the effects of temperature on juvenile white sturgeon in the Nechako River. White sturgeon transition to their juvenile stage during the summer months when river temperatures are at their warmest (Hildebrand et al., 2016). The Kenney dam on the Nechako River in northwestern British Columbia is currently mandated to keep water temperatures below 20°C; however, this threshold was established for migrating sockeye salmon and did not take into account the white sturgeon (Macdonald et al., 2012). Despite this mandate, as a result of a record-breaking heat wave in British Columbia in

Summer 2021, water temperature on the Nechako River rose above 20°C for nearly four weeks throughout July and August, with maximum temperatures reaching 22.36°C. Average water temperature between June 1, 2021 and August 31, 2021 was 17.63 ± 2.86°C (Extracted from the Environment and Climate Change Canada Real-time Hydrometric Data website [https://wateroffice.ec.gc.ca/mainmenu/real_time_data_index_e.html] on September 20, 2021).

To better inform dam management strategies and determine whether the 20°C threshold is appropriate for early life stages of white sturgeon, Chapter 2 examined the effects of acclimation temperature on juvenile white sturgeon survival, final size, final condition factor, and thermal tolerance at two different ages. To address these questions, juvenile white sturgeon obtained from the Vanderhoof hatchery on the Nechako River were acclimated to one of four temperatures (15°C, 18°C, 21°C, and 24°C) for one of two, two-week trials. The acclimation temperatures were selected based on current and projected water temperatures on the Nechako River, with the mandated 20°C falling in the middle. The trials took place in July and August 2019, when rivers would be at their warmest. Following acclimation, CT_{max} and final condition factor were used as proxies for upper thermal tolerance and general health respectively. Previous research on the development of thermal tolerance by Cheung (2019) and Hines (pers. comms.) found that white sturgeon thermal tolerance plateaued by 40 and 54 dph respectively. Since white sturgeon in the current experiment underwent CT_{max} trials at 51 and 80 dph, it was predicted that thermal tolerance would have plateaued and not differ between age cohorts. It was hypothesized that CT_{max}, final condition factor, final size, and survival would all be significantly affected by acclimation temperature. Acclimation to warmer temperatures was predicted to increase thermal tolerance. If acclimation to temperatures in excess of 20°C was approaching (or above) the thermal limit of the fish, then it is predicted their capacity to acclimate (measured by

ARR), final condition factor, final size, and survival would be decreased in the 21°C and 24°C treatments.

2.2 Methods

2.2.1 White sturgeon broodstock and holding

In June 2019, 600 juvenile white sturgeon from four families (150 per family) were acquired through induced wild broodstock spawning at the White Sturgeon Recovery Facility in Vanderhoof, BC. The four families were created by crossing each female (4A0C59156C, 452A4D4A58, 6C00072619, 7F7D782004) with mixed milt from nine males (6C00072501, 6C00072551, 412466701E, 7F7B0B2B22, 0A1820245D, 0A1820507E, 7F7D7A574F, 4124705139, 4A0C480815). At 28 dph, the white sturgeon were transported by plane in plastic bags filled with oxygen and cooled by ice packs to the Department of Zoology at the University of British Columbia. Prior to the start of the experiment, the white sturgeon were held in a large flow-through holding tank (700L) in dechlorinated city water maintained at 15°C. Fish were held for one week to allow them to recover from transport stress before experiments were initiated. Fish were fed *ad libitum* three times daily with a hatchery mixture (EWOS #0 and #1, mysis crumble, and krill powder). The light:dark cycle was kept at 16h light:8h dark to mimic the natural environment at this time of year. Water quality was assessed daily by measuring ammonia, pH, and dissolved oxygen levels. Water quality was maintained within an acceptable range for the duration of the study (ammonia < 0.25ppm, 6.8 < pH < 7.2, dissolved oxygen > 90%). All experiments were approved by the University of British Columbia animal care committee in accordance with the Canadian Council for Animal Care, protocol number A19-0284-A005.

2.2.2 Temperature acclimation period

Two separate acclimation trials were conducted, one starting in July at 37 dph and the other in August at 66 dph. White sturgeon were haphazardly selected from the holding tank and assigned to one of four temperature acclimation treatments (15, 18, 21, or 24°C), each consisting of a 200L temperature controlled water bath with three replicate aquaria (20L) per temperature. Fish were transferred to their replicate aquaria with the water bath initially at 15°C. Then the temperature of the water bath was increased at a rate of 3°C/day until the respective target acclimation temperatures were reached at which point fish were allowed to acclimate to the respective temperature for two weeks. During this time, they were fed *ad libitum* three times daily with the same feed as above. Uneaten food and feces were siphoned from the bottom of the tank within one hour after each feeding.

All aquaria for each temperature treatment received inflowing water from a header tank that was maintained at the target temperature. Header tanks and water baths were heated using heater sticks (finnex TITANIUM 300+) controlled by temperature controllers (Fisher Scientific Traceable Digital Temperature Controller), and water pumps (VicTsing 400GPH) were used to ensure homogenous mixing. The system was designed so that there was no water mixing between replicate aquaria by ensuring that water flow was unidirectional. Each replicate aquarium was equipped with an air stone to ensure adequate aeration and water mixing. To minimize water fouling, a flow-through system was used, which allowed for around 20 turnovers per day. One aquarium per temperature treatment was equipped with a temperature logger (HOBO Tidbit MX2203) to allow for continuous temperature monitoring. Temperature in every aquarium was measured manually twice daily using a temperature probe (HANNA checktemp

1). Additionally, ammonia, pH, and dissolved oxygen were monitored, with measurements being taken once daily. Water quality was maintained within the same acceptable range as above for the duration of the study.

2.2.3 Thermal tolerance and critical thermal maximum testing

Thermal tolerance was assessed using a standard CT_{max} assay. Three CT_{max} systems were built using 5L aquariums. Each aquarium was equipped with an air stone, a water pump (VicTsing 80GPH submersible water pump) and a heater stick (finnex TITANIUM 300+). The water pump and heater stick were isolated from the rest of the aquarium using a mesh screen to ensure that fish were not able to directly contact with either. During the CT_{max} trial, temperature was continuously recorded using a temperature probe (HANNA checktemp 1).

Food was withheld 24 hours prior to the start of CT_{max}. The following day five fish were randomly selected and removed from each of the three replicates for a given temperature (n=15 per temperature treatment) to measure CT_{max}. The CT_{max} trials of all three replicates for each acclimation temperature were run concurrently. Prior to the trial, the fish were placed in the CT_{max} aquaria for one hour to allow them to become accustomed to their surroundings. For the CT_{max} trial, water temperature was increased at a constant rate of 0.3°C/minute, as described by Becker and Genoway (1979). CT_{max} was determined as the temperature at which a fish experienced LOE and was no longer able to right itself in response to two consecutive tail prods. At this point, water temperature and time from the start of the trial were recorded. After LOE, fish were removed and euthanized using 200mg/L MS-222 buffered with 400mg/L sodium bicarbonate.

2.2.4 Mass, total length, and condition factor

After fish were euthanized, total length (mm) and mass (g) were immediately measured. Fish were blotted dry using Kimwipes prior to being weighed (Sartorius B120S-0KR). Condition factor was then calculated, as follows:

$$Condition\ factor = \frac{Whole\ body\ mass\ (g) \times 10^5}{Total\ length\ (mm)^3}$$

2.2.5 Statistical Analysis

All statistical analyses were performed using R Studio (Version 1.1.423) and alpha was set at 0.05 throughout. Prior to analyzing the dataset, the tank effect on CT_{max} for each acclimation temperature was assessed using a one-way analysis of variance (ANOVA) with tank ID (A, B, or C) as the factor. There was no effect of tank on CT_{max} for any of the acclimation temperatures in either age cohort, so tank effect was excluded as a random effect.

The effect of acclimation temperature and age on survival was tested using a logistic regression. The effect of acclimation temperature and age on CT_{max} was tested using a two-way ANOVA, followed by a *post-hoc* Tukey HSD, with age cohort (37-51 dph or 66-80 dph), and acclimation temperature (15°C, 18°C, 21°C, and 24°C) as factors. The effect of acclimation temperature on final condition factor, mass, and length was assessed using a one-way ANOVA, followed by a *post-hoc* Tukey HSD, with acclimation temperature (15°C, 18°C, 21°C, and 24°C) as a factor. For final length and mass, the two age cohorts were analyzed independently because the fish were developing rapidly. Final condition factor of the two age cohorts was also analyzed independently because condition factors in the two 15°C acclimation treatments were

significantly different. The difference in final condition factor between the two groups was hypothesized to be due to the different feeding regimes employed at the Nechako White Sturgeon Recovery Initiative (NWSRI) and the aquatics facility at the aquatic facility (Initiative for the Study of the Environment and its Aquatic Systems [InSEAS]) at University of British Columbia (UBC). Assumptions of normality and homogeneity of variances were determined through visual inspection and all data met assumptions for these analyses.

2.3 Results

2.3.1 Effect of acclimation temperature and age on thermal tolerance and acclimation capacity

Thermal tolerance was significantly affected by acclimation temperature and age (two-way ANOVA, $F_{3,111} = 204.89$, $p < 0.0001$ and $F_{1,111} = 6.40$, $p < 0.05$; Table 2.1 and Figure 2.1). There was also a significant interaction between the two (two-way ANOVA, $F_{3,11} = 5.07$, $p < 0.005$). Thermal tolerance differed with age in the 18°C and 21°C acclimation treatments ($p < 0.01$ and $p < 0.01$ respectively), in which the 37-51 dph white sturgeon had a higher CT_{max}. Warmer acclimation temperature significantly increased CT_{max} in both age groups ($p < 0.001$), except between 21°C and 24°C treatments in the 37-51 dph white sturgeon. Average CT_{max} in the 15°C acclimation treatment was 30.28 ± 0.14°C for 37-51 dph fish and 30.20 ± 0.12°C for 66-80 dph fish. In the 18°C acclimation treatment, average CT_{max} was 32.08 ± 0.11°C for the 37-51 dph fish and 31.43 ± 0.18°C for the 66-80 dph fish. Average CT_{max} in the 21°C treatment was 33.11 ± 0.12°C for the 37-51 dph fish and 32.6 ± 0.15°C for the 66-80 dph fish. Finally, in the 24°C treatment average CT_{max} was 33.57 ± 0.2°C in the 37-31 dph fish and 33.87 ± 0.13°C.

ARR remained relatively consistent between acclimation temperatures in the 66-80 dph white sturgeon (at a value of about 0.4), but the ARR of the 37-51 dph white sturgeon decreased as acclimation temperature increased (Table 2.2), from a value of 0.6 at 15-18°C to about 0.15 at 21-24°C.

2.3.2 Effect of acclimation temperature on size, condition factor, and survival

Final length and mass did not differ between acclimation treatments at the end of the two-week acclimation ($p > 0.05$; Table 2.3). Survival was not affected by acclimation temperature or age ($p > 0.05$; Table 2.4). Final condition factor differed significantly between acclimation temperatures in both 37-51 dph and 66-80 dph white sturgeon ($F_{3,103} = 5.95$, $p < 0.001$ and $F_{3,101} = 11.11$, $p < 0.0001$, respectively; Table 2.3; Figure 2.2). Condition factor for 37-51 dph white sturgeon was significantly lower between 15°C and 21°C ($p < 0.01$); 15°C and 24°C ($p < 0.05$); and 18°C and 21°C ($p < 0.05$). For 66-80 dph, the white sturgeon acclimated to 21°C and 24°C had significantly lower condition factor than those acclimated to 15°C and 18°C ($p < 0.05$). Morphometrics of both age cohorts are reported in Table 2.5.

Table 2.1 The effect of acclimation temperature and age on the CT_{max} of juvenile white sturgeon, from a two-way ANOVA. Asterisk (*) denotes significance at alpha = 0.05.

Measurement	Variables	d.f.	F	p-value
CT_{max}	T_A	3	204.893	<0.001*
	Age	1	6.404	0.01278*
	T_A x Age	3	5.068	0.00251*

Table 2.2 Acclimation response ratio of 37-51 dph and 66-80 dph juvenile white sturgeon.

Change in T_a(°C)	37-51 dph	66-80 dph
	ARR	
15-18	0.60	0.4
18-21	0.33	0.4
21-24	0.17	0.43

Table 2.3 The effect of acclimation temperature on various traits of 37-51 dph and 66-80 dph juvenile white sturgeon, from a two-way ANOVA. Asterisk (*) denotes significance at alpha = 0.05.

	Measurement	d.f.	F	p-value
37-51 dph	Mass	3	0.997	0.397
	Length	3	2.132	0.101
	Condition factor	3	5.951	<0.001*
66-80 dph	Mass	3	0.166	0.919
	Length	3	0.315	0.815
	Condition factor	3	11.11	<0.001*

Table 2.4 Effect of acclimation temperature and age on survival of juvenile white sturgeon, from a logistic regression.

Measurement	Variables	d.f.	Deviance (explained)	p-value
Survival	T_a	3	3.559	0.313
	Age	1	0.042	0.837
	T_a x Age	3	6.678	0.083

Table 2.5 Morphometrics for 37-51 dph and 66-80 dph juvenile white sturgeon at the end of a two-week acclimation to one of four temperatures (15°C, 18°C, 21°C, and 24°C). Final average mass and length ± standard error (sample size indicated in brackets). Statistical analysis for morphometric data in Table 2.3.

T_a(°C)	37-51 dph	66-80 dph
	Mass (g)	
15	0.45 ± 0.048 (n = 23)	1.24 ± 0.088 (n = 28)
18	0.41 ± 0.022 (n = 28)	1.26 ± 0.096 (n = 28)
21	0.48 ± 0.023 (n = 29)	1.17 ± 0.107 (n = 26)
24	0.44 ± 0.039 (n = 27)	1.14 ± 0.104 (n = 24)
	Length (mm)	
15	44.3 ± 1.75 (n = 23)	65.38 ± 1.88 (n = 23)
18	43.72 ± 0.89 (n = 28)	66.47 ± 2.07 (n = 28)
21	47.85 ± 0.97 (n = 29)	67.32 ± 2.15 (n = 29)
24	45.56 ± 1.52 (n = 27)	66.86 ± 2.12 (n = 27)

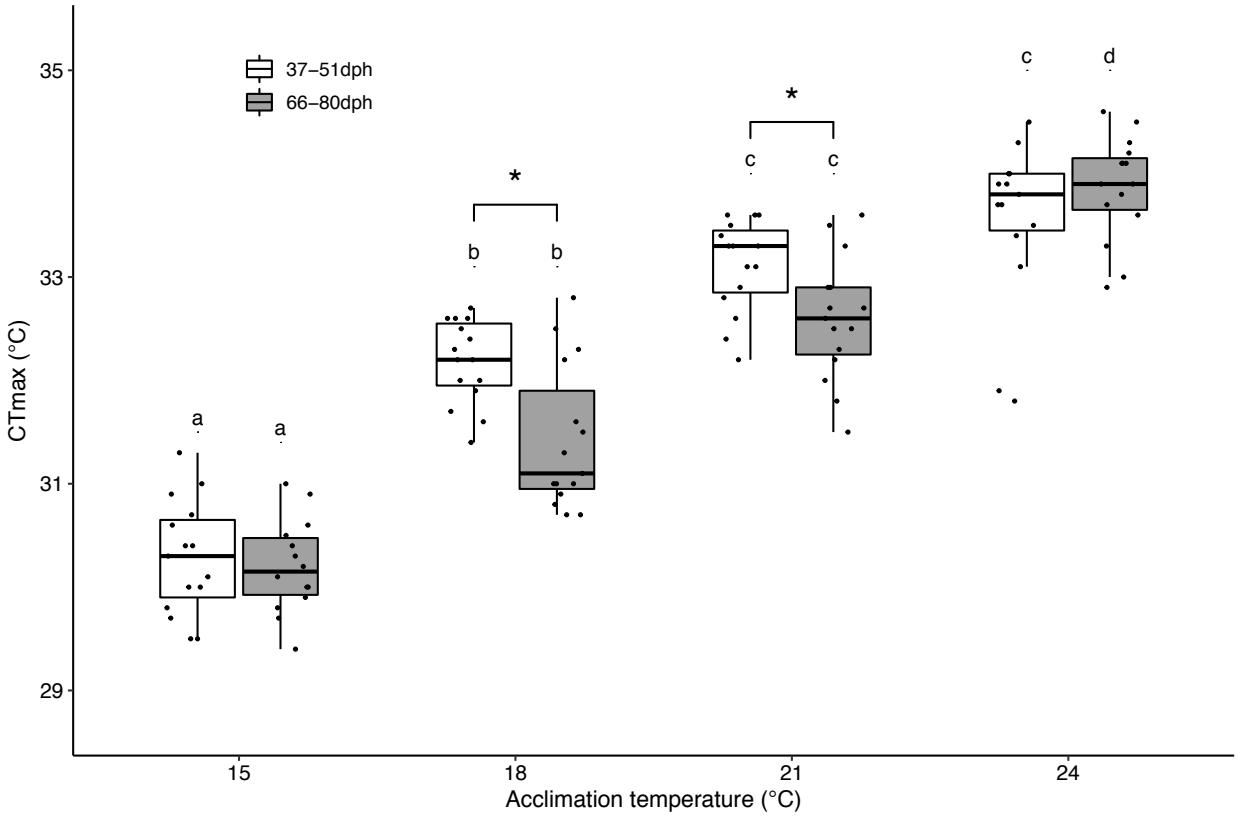

Figure 2.1 CT_{max} for juvenile white sturgeon following a two-week acclimation to one of four temperatures (15°C, 18°C, 21°C, and 24°C) and one of two age groups (37-51 dph and 66-80 dph). Black dots represent individual data points, while the boxplot represents group data. The horizontal line in the boxplot is the median, while the whiskers represent the maximum and minimum. Letters that differ indicate statistically significance a given age cohort. Asterisk (*) denotes a statistically significant difference between 37-51 dph and 66-80 dph sturgeon acclimated to the same temperature at alpha = 0.05. N= 15 per acclimation temperature per age cohort.

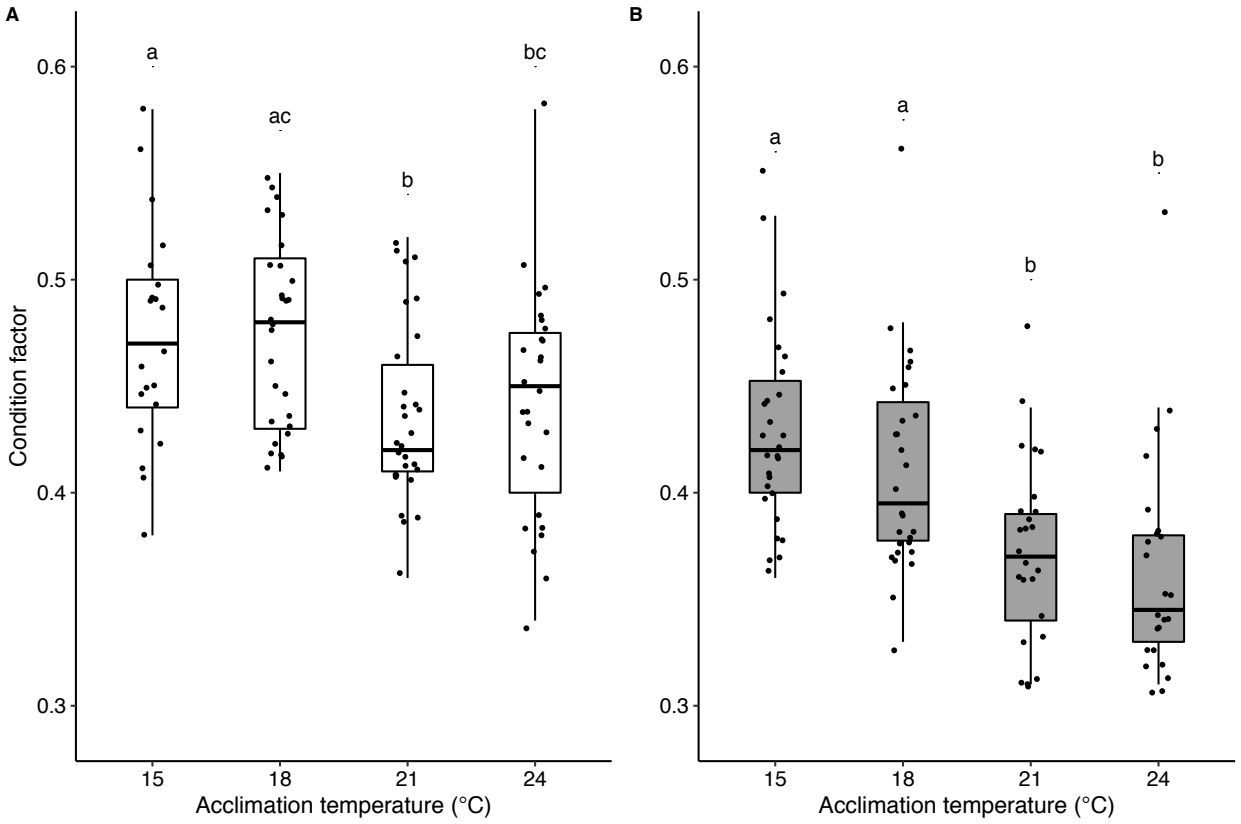

Figure 2.2 Final condition factor of 37-51 dph (**A**) and 66-80 dph (**B**) juvenile white sturgeon following a two-week acclimation to one of four temperatures (15°C, 18°C, 21°C, and 24°C). Black dots represent individual data points, while the boxplot represents group data. The horizontal line in the boxplot is the median, while the whiskers represent the maximum and minimum. Letters that differ indicate statistically significance a given age cohort at alpha = 0.05. For 37-51 dph sturgeon $N_{15°C} = 23$, $N_{18°C} = 28$, $N_{21°C} = 29$, and $N_{24°C} = 27$. For 66-80 dph sturgeon $N_{15°C} = 28$, $N_{18°C} = 28$, $N_{21°C} = 26$, and $N_{24°C} = 24$.

2.4 Discussion

The objective of this chapter was to assess the effects of acclimation temperature and age on thermal tolerance, survival, final size, final condition factor, and capacity to acclimate in juvenile white sturgeon to help inform conservation efforts on the Nechako River and determine whether the current 20°C upper water temperature mandate is appropriate. Broadly, the data indicated that while acclimation to warmer temperature conferred higher CT_{max}, overall condition decreased above 18°C indicating that the 20°C mandate may be too warm. There were effects of acclimation temperature and age on thermal tolerance, as well as an interaction effect between the two. Acclimation to warmer temperatures increased thermal tolerance for both age cohorts and interestingly the 37-51 dph sturgeon had significantly higher thermal tolerance (as indicated by CT_{max} values) than the 66-80 dph sturgeon following acclimation to 18°C and 21°C. Final mass, final length, and survival did not differ between temperature treatments. For both age cohorts, condition factor was lower in the 21°C and 24°C acclimation treatment compared to the 15°C treatment.

As predicted, and consistent with results of studies on multiple species of fish, the current findings indicate that acclimation to warmer temperatures confers greater thermal tolerance in juvenile white sturgeon (Becker and Genoway, 1979; Bugg et al., 2020; Fangue et al., 2006; Zhang and Kieffer, 2014). In 37-51 dph fish, CT_{max} increased progressively from about 30.3°C in fish acclimated to 15°C, to 33.6°C in fish acclimated to 24°C. In 66-80 dph sturgeon, CT_{max} increased progressively from 30.2°C in fish acclimated to 15°C to 33.9°C in fish acclimated to 24°C. The current study does not address the mechanisms through which acclimation occurs, therefore more research is required to understand the biological underpinnings of thermal tolerance. Bugg et al. (2020) found that acclimation affected juvenile lake sturgeon metabolic

rate, as well as the mRNA expression of transcripts of several genes related to heat shock (*Hsp70*, *90a*, and *90b*), hypoxia (*HIF-1α*), and the sodium-potassium pump (*Na+/K+ ATPase-α1*). As this is a closely related species, these potential thermal tolerance mechanisms warrant further research in white sturgeon and Hsp levels are measured in the experiments of chapter 3. The ability to acclimate and increase thermal tolerance will be paramount to species' survival as climate change not only drives an increase in mean water temperature, but also more extreme maximum temperatures.

While both age groups (37-51 dph and 66-80 dph) were able to increase their thermal tolerance in response to acclimation, the relationship between acclimation temperature and CT_{max} differed between the groups. The older sturgeon increased their thermal tolerance by the same amount between each acclimation temperature, which yielded a consistent ARR of about 0.4 in fish acclimated to higher temperatures. Other fish species from similar latitudes have a mean ARR of around 0.32 (Morley et al., 2019). In the younger sturgeon, the relationship between acclimation temperature and thermal tolerance was curvilinear and their ARR decreased progressively with temperature from 0.6 in fish acclimated from 15 to 18°C, to 0.33 in fish acclimated to 21°C and 0.17 in those acclimated to 24°C. Despite the younger sturgeon having significantly higher CT_{max} at 18°C and 21°C, their thermal tolerance was nearly identical to the older sturgeon at 24°C. These findings may indicate that within a specified temperature range, 37-51 dph white sturgeon experience a window of heightened thermal plasticity. The curvilinear relationship and decreasing ARR illustrates that the 37-51 dph white sturgeon were less thermally plastic at higher temperatures and may indicate that they have reached their capacity to acclimate to higher temperatures. Juvenile shortnose and lake sturgeon have similarly exhibited decreasing ARRs when acclimation temperatures were increased from 15°C to 20°C and 20°C to

24°C, respectively (Bugg et al., 2020; Zhang and Kieffer, 2014). The relatively constant ARR in the 66-80 dph could indicate that these animals still have the capacity to acclimate to even higher temperatures than 24°C. In the current study, both cohorts of juvenile white sturgeon exhibited relatively high thermal plasticity, which combined with studies on other sturgeon species, indicates that this characteristic may be a general trait of the sturgeon family (Bugg et al., 2020; Zhang and Kieffer, 2014).

Contrary to predictions, there was no effect of acclimation temperature on survival or final size. It could be that that the two-week acclimation period was too short to see any changes. In southern populations, increased mortality and inhibited growth has been reported in early juvenile white sturgeon acclimated to temperatures above 20°C for 30 days (Cech et al., 1984). However, consistent with predictions, condition factor decreased significantly with acclimation temperature within each age cohort studied here. Condition factor in the 15°C acclimated fish differed between the two age cohorts (0.49 in the 37-51 dph relative to 0.43 in the 66-80 dph); however, this was thought to be due to a change in feeding regime between the NWSRI and UBC, rather than a developmental trend. In a study where sturgeon were reared continuously at one facility from embryos to 80 dph, condition factor remained relatively constant at around 0.5 (Cheung, 2019). In both age cohorts in this study, the condition factor of white sturgeon acclimated to 21°C and 24°C was significantly lower than 15°C acclimated fish. In 37-51 dph sturgeon, condition factor decreased from 0.49 at 15°C to 0.44 for both 21°C and 24°C. In 66-80 dph sturgeon, condition factor decreased from 0.43 at 15°C to 0.37 and 0.36 for 21°C and 24°C, respectively. Decreased condition factor may be indicative of a reduction in overall health, which may result in lowered fitness. In Atlantic cod (*Gadus morhua*), decreased condition factor has been found to correlate with reduced recruitment (Rätz and Lloret, 2003). In lake sturgeon,

higher condition factor prior to a juvenile's first winter increases the likelihood of overwintering success (Yoon et al., 2019). These results highlight how early rearing environments are critical for long-term success and how a sub-lethal threshold in one environment may be fatal in other circumstances.

While juvenile sturgeon were able to acclimate to temperatures above 20°C, and acclimation to higher temperatures conferred higher thermal tolerance as indicated by CT_{max}, these temperatures may have negatively affected health in both the 37-51 dph and 66-80 dph white sturgeon as suggested by changes in condition factor. This may indicate a sub-lethal threshold between 18°C and 21°C that could affect an individual's ability to survive, particularly when exposed to multiple stressors. Follow-up studies should be completed to examine the effects of temperatures within the temperature range (18°C - 21°C) that condition factor was found to decrease over. In the absence of better resolution over this range, I recommend considering reducing the upper water temperature limit from 20°C to 18°C on the Nechako River. More research is also needed to elucidate the long-term effects of temperature in isolation, and in conjunction with other stressors likely to be experienced in the environment.

Chapter 3: The effect of heating rate on thermal tolerance in juvenile white sturgeon

3.1 Introduction

Under global warming, mean water temperatures and the frequency of extreme weather events are expected to increase and northern latitudes are predicted to be disproportionately affected by these changes (Hoegh-Guldberg et al., 2018; Vincent et al., 2015). As such, it is vital for conservation efforts to better understand of how organisms respond to temperature stress. Critical thermal tests (CTMs) were developed to assess the thermal limits of organisms (Cowles and Bogert, 1944). However, the ecological relevance of CT_{max} has been questioned because the rates of temperature increase used for the test far exceed those seen in nature – the conventional rate for CT_{max} is 0.3°C/min or 432°C/day (Beitinger and Lutterschmidt, 2011). The few studies that have used more ecologically relevant heating rates have found that faster heating rates result in higher CT_{max}, which suggests that current approximations of thermal tolerance based upon CT_{max} values may greatly overestimate thermal tolerance in nature (Becker and Genoway, 1979; Illing et al., 2020; Kovacevic et al., 2019; Mora and Maya, 2006). This discrepancy is especially relevant considering climate change, as laboratory measurements are used to help inform conservation efforts. When organisms undergo conventional CT_{max} tests, upper thermal tolerance is thought to be limited by either cardiac or neurological failure (Andreassen et al., 2020; Christen et al., 2018). At slower heating rates, upper thermal tolerance may be limited by a gradual accumulation of cellular damage that incurs from organisms spending prolonged time at temperatures where the rate of cellular damage exceeds the rates of repair (Rezende et al., 2014).

The effects of heat stress can be observed at all levels of biological organization (Schulte, 2015). At the whole body level, condition factor is commonly used as measure of overall health and has been found to decrease as a result of thermal stress (Boucher et al., 2014; Bugg et al., 2020; Morrison et al., 2020). At the organ level, changes in BSI, CSI, and HSI have been observed as results of stress (Bugg et al., 2020; Gilbert and Farrell, 2021; Soengas and Aldegunde, 2002). Alteration to heat shock protein (Hsp) transcription is an indicator of heat stress and is also a proposed mechanism to cope with heat stress (Somero, 2020). Hsps are ubiquitous in all species and upregulation at both the mRNA and protein levels can occur in minutes in response to thermal stress (Tomanek and Somero, 2000). mRNA expression of Hsps can be upregulated many fold and while there is not a 1:1 relationship with protein translation, upregulation does lead to a similar pattern of protein translation (Buckley et al., 2006; Mirault et al., 1977). Hsps may help cells cope with the effects of thermal stress by mitigating damage caused by malfunctioning proteins; binding to and stabilizing proteins at risk of degrading; refolding or breaking down damaged proteins (Chen et al., 2018; Garbuz and Evgen'ev, 2017; Tomanek and Somero, 2000). The magnitude of Hsp upregulation has been found to correlate with thermal tolerance (Krebs and Feder, 1997; Tomanek and Somero, 2000). In rainbow trout (*Oncorhynchus mykiss*) fibroblasts, inhibition of Hsp synthesis prevented the development of thermal tolerance (Mosser and Bols, 1988). In insects, transgenic inhibition of Hsp expression was found to decrease thermal tolerance while activation had the opposite effect (Feder and Krebs, 1998; Feder et al., 1996).

Many populations of white sturgeon, including the Nechako River population, are endangered and despite their perilous status, little is known about how they respond to heat stress (Hildebrand et al., 2016). Water temperature on the Nechako River, home to the northernmost

population of white sturgeon, fluctuates daily at a rate of 0.004°C/min in the summer (Extracted from the Environment and Climate Change Canada Real-time Hydrometric Data web site [https://wateroffice.ec.gc.ca/mainmenu/real_time_data_index_e.html] on September 20, 2021). No known studies have examined how ecologically relevant warming affects the upper thermal tolerance of juvenile white sturgeon. Given this, in Chapter 3 my objective was to determine the effect of heating rate during CT_{max} assays on thermal tolerance, somatic indices and Hsp expression.

To address this objective, the thermal tolerance of juvenile white sturgeon was examined using three different heating rates during CT_{max} trials. Of the three heating rates that were tested, one was the conventional rate used by most research groups (0.3°C/min), one mimicked natural temperature changes that could be experienced in this river system (0.003°C/min) and the other was an intermediate rate (0.03°C/min). The thermal tolerance of juvenile white sturgeon was predicted to decrease at slower heating rates as observed in other fish species (Kovacevic et al., 2019). Little is known about the effects of short-term thermal stress on various somatic indices. Thus, in addition to assessing thermal tolerance, this study sought to characterize the effects of heating rate on condition factor, HSI, BSI, and CSI. If the duration (or amount) of thermal stress was sufficient to affect these variables, then condition, HSI, and BSI would be expected to decrease while CSI would be expected to increase. Finally, gill samples were also taken from the bottom eight and the top eight performers during the CT_{max} trials to measure mRNA expression of various Hsps (*Hsp 47*, *70*, *90a*, and *90b*). It was hypothesized that heating rate would affect Hsp expression. Hsp expression was expected to be upregulated by the end of the CT_{max} trials relative to controls. Additionally, . mRNA expression of HSP was quantified in high and low CT_{max} performers to determine if expression levels differed between these two groups.

3.2 Methods

3.2.1 White sturgeon broodstock and holding

In August 2020, 300 juvenile white sturgeon (Family 4: one female 0A1820543F, crossed with three males 4527276447, 0A1820505C, 7F7B04052A) were acquired through induced wild broodstock spawning at the White Sturgeon Recovery Facility in Vanderhoof, BC. At 60 dph, the white sturgeon were transported from the White Sturgeon Recovery Facility in Vanderhoof, BC by air in plastic bags filled with aerated water and cooled by ice packs to the Department of Zoology at UBC Vancouver in August, 2020. Fish were randomly divided and held for two weeks in two large flow-through tanks (700L), filled with dechlorinated city water maintained at 15°C. Fish were fed bloodworms twice a day *ad libitum*. The light:dark cycle was set to 16h:8h to mimic the natural environment at that time. Water quality was maintained for the duration of holding. All experiments were approved by the University of British Columbia animal care committee in accordance with the Canadian Council for Animal Care, protocol number A19-0284-A005.

3.2.2 The effect of heating rate on thermal tolerance

White sturgeon were haphardly selected from the two 15°C holding tanks for experimental CT_{max} trials to assess thermal tolerance. In each of the CT_{max} trials, temperature was increased at a constant rate of either 0.3°C/min, 0.03°C/min, or 0.003°C/min until LOE, which was defined as inability to right after two consecutive tail prods. CT_{max} was recorded as the water temperature at LOE. Once fish attained LOE, they were removed from the aquarium and euthanized with 200mg/L MS-222 buffered with 400mg/L sodium bicarbonate.

Five trials (45 fish per trial) were run sequentially over the course of two weeks. Three CT_{max} heating rates were used where temperature was increased at 0.3°C/min (taking 1 hour total), 0.03°C/min (taking 6 hours total), and 0.003°C/min (taking 4.5 days total). Because of the possibility of diurnal effects on thermal tolerance, an additional two trials (second 0.3°C/min and third 0.3°C/min) were completed to ensure CT_{max} and Hsp expression were not affected by time of day (Healy and Schulte, 2012).

For all trials, fish were randomly selected from the two large flow-through holding tanks and evenly divided between three aquaria **(**15L) that were placed within a larger water table (200L). For the first three trials (first 0.3°C/min, 0.03°C/min, and 0.003°C/min), 45 fish were used. For the finals two trials (second 0.3°C/min and third 0.3°C/min) 30 fish were used. Fish were weighed (Mettler Toledo, New Classic SG) and total length was measured prior to the start of CT_{max}. The fish were given one hour to recover from handling stress before CT_{max} started. The water table was aerated using air stones and water was circulated using multiple water pumps (VicTsing 400GPH). For the 0.3°C/min trials, the water in the water table was heated directly by immersed heater sticks (finnex TITANIUM 300+) and the heating rate was monitored using a temperature probe (HANNA checktemp 1). For the 0.03°C/min and 0.003°C/min trials, a sump (200L) was used to double the total water volume of the system, to ensure water quality over the much longer CT_{max} trials and allow for slower heating rates. Temperature controllers (Fisher Scientific Traceable Digital Temperature Controller) were used in conjunction with heater sticks (finnes TITANIUM 300+) to slowly increase temperature at the respective rates. Temperature was monitored using both a temperature probe (HANNA checktemp 1) and temperature logger (HOBO Tidbit MX2203) in each aquarium. For the 0.003°C/min trial, an infrared security camera (geeni GNC-CW020) was used to monitor the fish 24 hours a day. Prior to the start of the

trials, food was withheld for 12 hours, however if the trial lasted more than 12 hours (ie the 0.003°C/min trial) then feeding was resumed *ad-libitum* two times a day within the CT_{max} trial.

3.2.3 Post-trial sampling and somatic indices

After fish were euthanized, fish were blotted dry with kimwipes and then total mass (Mettler Toledo, New Classic SG) and total length were measured. Gill samples were immediately removed from the top eight (the last eight fish to reach CT_{max}) and bottom eight performers (the first eight fish to reach CT_{max}) during each CT_{max} trial and placed into RNA*later* (Thermofisher Scientific, Waltham, USA) and stored at -80°C for quantification of mRNA expression. Brain, liver, and heart were dissected out and weighed (Sartorius CP124S) and the somatic index for each was calculated ([tissue mass / body mass] x 100). Condition factor was calculated using the formula listed above in Section 2.2.4.

3.2.4 mRNA expression

Samples in RNA*later* were left at room temperature for 24 hours and then placed in a -80°C freezer. Sample order was randomized for all RNA analysis. Total RNA was extracted from homogenized tissue using a Qiagen RNeasy mini kit according to manufacturer's protocols. A Nanodrop (Nanodrop 2000, Thermofisher Scientific, Waltham, USA) was used to determine total RNA quantity and quality. Total RNA was stored at -30°C until further analysis. To treat total RNA with DNase, 1uL of total RNA from each sample was diluted with ultrapure water to a final volume of 8uL and then mixed with 1uL of both DNase 1 reaction buffer and DNase in a 96-well plate. This solution was incubated in a thermal cycler (BIO-RAD T100 Thermal Cycler) at 25°C for 15 minutes. Next, to inactivate the DNase, 1uL of 25mM EDTA was added, and the

solution was incubated at 65°C for 10 minutes. Complementary DNA (cDNA) was synthesized from the extracted RNA using a Quantabio qScript cDNA Synthesis Kit (Quantabio, Beverly, USA). To synthesize cDNA, 10µL of master mix (5µL nuclease-free water, 4µL 5x reaction buffer, 1uL reverse transcriptase) was added to each well and the plate was incubated at 22°C for 5 minutes, 42°C for 30 minutes, 85°C for 5 minutes, and then held at 4°C. The cDNA was stored at -30°C until further analysis.

mRNA expression in the sample was measured using quantitative polymerase chain reaction (qPCR). Forward and reverse primers were designed for the reference genes *RPS18* and *RSP6*, and the target genes *hsp47*, *hsp70, hsp90a*, and *hsp90b* (Table **3.1**). *RSP6* and *RPS18* were selected as reference genes because they displayed stable expression across all treatments. All reactions contained 0.075uL of both the forward and reverse primers, 7.5µL of SYBR Green Mastermix (Thermofisher Scientific), 5.85µL of ultrapure H_2O, and 2µL of cDNA. For both reference genes, cDNA was undiluted 1:1 with nuclease-free water and for the target Hsp genes a 1 cDNA:10 nuclease-free water dilution was used. The qPCR was performed with a BIO-RAD CFX96 Real-Time System on a 96-well plate. To measure mRNA expression, the following protocol was used: denaturation at 95°C for 10 min, 40 cycles of denaturation at 95°C for 15s, annealing at 60°C for 30s, and extension at 72°C for 30s. The qPCR was followed by conducting a melt curve. Expression of the gene of interest was normalized to the reference genes (*RSP6* and *RPS18*) using the Vandesompele method and expressed as a relative change compared the controls (Vandesompele et al., 2002).

Table 3.1 List of forward and reverse primers and their efficiencies for white sturgeon *Rsp6, Rps18, Hsp47, Hsp70, Hsp90a*, and *Hsp90b*.

Gene	Forward	Reverse	Efficiency
RPS6	GGACAGGTTGAAGAGCTTGC	ATCATCAAGAAGGGCGAGAA	93%
RSP18	TCTCTCCAGATCCTCACGCA	AAGGACGGCAAATACAGCCA	86%
Hsp47	GACTCCAACGCCTTCAAGAG	TGTGATCATGGCTGAGAAGC	100%
Hsp70	GAGAGGCTCATTGGAGATGC	AAACAGTGTTGCTGGGGTTC	100%
Hsp90a	GCAGAGGTTCTCGAACTTGG	AGACCCTGGTGTCTGTGACC	93%
Hsp90b	GCAACTTGGTCCTTGCTCTC	AGCTCTCAGTCTGGGGATGA	89%

3.2.5 Statistical analysis

All statistical analyses were performed using R Studio (Version 1.1.423) and alpha was set at 0.05 throughout. Prior to analyzing the dataset, the effect of aquaria on CT_{max} for each heating rate was assessed using a one-way ANOVA with tank ID (A, B, or C) as the factor. There was no effect of tank on CT_{max} for any of the heating rates, so effect of aquaria was excluded as a random effect. The CT_{max}, Hsp expression, and somatic indices of the three 0.3°C/min trials were assessed using a one-way ANOVA. There was no difference between the three 0.3°C/min trials so trials were pooled.

Each measurement was analyzed using a one-way ANOVA, followed by a *post-hoc* Tukey HSD with heating rate as the factor (control, 0.3°C/min, 0.03°C/min, or 0.003°C/min). Each variable (except CT_{max}) was also analyzed using a two-way ANOVA, followed by a *post-hoc* Tukey HSD, with CT_{max} performance (high performer or low performer) and heating rate (0.3°C/min, 0.03°C/min, or 0.003°C/min) as factors. Assumptions of normality and homogeneity of variances were determined through visual inspection and Hsp data was log 2 transformed. All data met assumptions for these analyses.

3.3 Results

3.3.1 Effect of heating rate on thermal tolerance

There were no diurnal effects on CT_{max} when fish were heated at 0.3°C/min ($p > 0.05$). Thermal tolerance was significantly affected by heating rate ($F_{2,135} = 515.2$, $p < 0.0001$; Table 3.2; Figure 3.1), with slower heating rates yielding significantly higher CT_{max} results. Average CT_{max} increased by about 5°C between the fast and slowest heating rates. Average CT_{max} in the conventional heating rate was 29.2°C ± 0.24°C and the trial lasted about 1.5 hours. Average CT_{max} in the 0.03°C/min trial was 31.3°C ± 0.24°C and the trial lasted about 8 hours. Average CT_{max} in the 0.003°C/min trial was 34.2°C ± 0.15°C and the trial lasted about 4.5 days.

3.3.2 Effect of heating rate and performance on somatic indices

Average somatic indices are summarized in Table 3.2. There was no significant effect of heating rates on BSI or CSI ($p > 0.05$; Table 3.3). For the top and bottom performers, there was no significant effect of heating rate or performance – or an interaction effect between the two – for condition factor, BSI, CSI, or HSI ($p > 0.05$ for all). HSI differed significantly between heating rates and controls ($F_{3,121} = 4.48$, $p < 0.01$) – controls had significantly greater HSI than sturgeon that experienced heating rates of 0.03°C/min ($p < 0.05$) and 0.003°C/min ($p < 0.01$; Table 3.2; Table 3.4; Figure 3.2). Condition factor was significantly affected by heating rate ($F_{2,153} = 8.23$, $p < 0.0001$) (Figure 3.3). However, condition factor did not differ significantly between controls and sturgeon exposed to different heating rate ($p > 0.05$ for all).

3.3.3 Effect of heating rate and performance on Hsp expression

There were no diurnal effects on the expression of any Hsps measured when fish were heated at 0.3°C/min ($p > 0.05$).

3.3.3.1 *Hsp47*

Expression of *Hsp47* was significantly affected by heating rate ($F_{3,94} = 3.26$, $p < 0.05$; Figure 3.4). Sturgeon in the 0.003°C/min group had significantly more *Hsp47* expression than sturgeon in the 0.03°C/min ($p < 0.05$). When performance was included, *Hsp47* expression was affected by heating rate ($F_{2, 72} = 6.47$, $p < 0.01$; Figure 3.5). In the 0.3°C/min group, high performers had significantly lower *Hsp47* expression than low performers ($p < 0.05$). Within high performers, *Hsp47* expression was significantly greater in sturgeon heated at 0.003°C/min than those heated at 0.03°C/min ($p < 0.01$) and 0.3°C/min (0.05).

3.3.3.2 *Hsp70*

Expression of *Hsp70* was significantly affected by heating rate ($F_{3,94} = 298.9$, $p < 0.0001$; Figure 3.4). *Hsp70* expression of sturgeon in the 0.03°C/min and 0.003°C/min trials did not differ significantly ($p > 0.05$), but both had significantly greater *Hsp70* expression than controls and sturgeon in the 0.3°C/min trial (all $p < 0.0001$). Fish heated at 0.3°C/min had significantly greater expression than control fish ($p < 0.0001$). When performance was included, there was a significant effect of heating rate ($F_{2,72} = 285.81$, $p < 0.0001$) and performance ($F_{1,72} = 46.84$, $p < 0.0001$) as well as an interaction effect between the two ($F_{2,72} = 5.89$, $p < 0.005$; Figure 3.5). High performers had greater *Hsp70* expression than low performers in in the 0.3°C/min and 0.003°C/min trials ($p < 0.0001$ and $p < 0.01$, respectively). For both high and low performers,

sturgeon heated at 0.3°C/min had significantly lower *Hsp70* expression than those heated at 0.03°C/min ($p < 0.001$ for both) and 0.003°C/min ($p < 0.001$ for both).

3.3.3.3 *Hsp90a*

Hsp90a expression was significantly affected by heating rate ($F_{3, 94} = 31.8$, $p < 0.0001$; Figure 3.4). *Hsp90a* expression was significantly lower in controls and fish heated at 0.3°C/min than fish that were in the 0.03°C/min ($p < 0.01$ and $p < 0.0001$) and 0.003°C/min trials (both $p < 0.0001$). When performance was included, *Hsp90a* expression was significantly affected by heating rate ($F_{2,72} = 69.13$, $p < 0.0001$; Figure 3.5). *Hsp90a* expression in high performing sturgeon heated at 0.3°C/min was significantly lower than those heated at 0.03°C/min ($p < 0.001$) and 0.003°C/min ($p < 0.001$). *Hsp90a* expression of low performing sturgeon increased significantly between each heating rate, with the slowest heating rate having the highest expression.

3.3.3.4 *Hsp90b*

Hsp90b expression was significantly affected by heating rate ($F_{3, 94} = 55.83$, $p < 0.0001$) (Figure 3.4). Controls and sturgeon heated at 0.3°C/min did not have significantly different *Hsp90b* expression. *Hsp90b* expression increased significantly as heating rate slowed (all $p < 0.0001$). When performance was included, *Hsp90b* expression was significantly affected by heating rate ($F_{2,72} = 67.59$, $p < 0.0001$; Figure 3.5). *Hsp90b* expression in high performing sturgeon heated at 0.3°C/min was significantly lower than those heated at 0.03°C/min ($p < 0.001$) and 0.003°C/min ($p < 0.0001$). *Hsp90b* expression in high performers was significantly lower in the 0.03°C/min than the 0.003°C/min trial ($p < 0.05$). *Hsp90b* expression of low

performing sturgeon increased significantly between each heating rate, with the slowest heating rate having the highest expression (all $p < 0.001$).

Table 3.2 Somatic indices of juvenile white sturgeon in control conditions or one of three heating rates. Average condition factor, BSI, CSI, and HSI with standard error and sample size are provided. Treatments that differ from controls are indicated with an asterisk (*) at alpha = 0.05.

Heating rate	
	Condition factor
Control (n = 20)	0.4124 ± 0.010 (n = 20)
0.3 (n = 105)	0.4440 ± 0.006 (n = 105)
0.03 (n = 45)	0.4148 ± 0.008 (n = 45)
0.003 (n = 45)	0.3920 ± 0.008 (n = 45)
	BSI
Control	0.0076 ± 0.0004 (n = 20)
0.3	0.0071 ± 0.0003 (n = 45)
0.03	0.0076 ± 0.0003 (n = 45)
0.003	0.0074 ± 0.0003 (n = 45)
	CSI
Control	0.0034 ± 0.0003 (n = 20)
0.3	0.0031 ± 0.0001 (n = 45)
0.03	0.0033 ± 0.0002 (n = 45)
0.003	0.0029 ± 0.0002 (n = 45)
	HSI
Control	0.0199 ± 0.0013 (n = 20)
0.3	0.0160 ± 0.0007 (n = 45) *
0.03	0.0164 ± 0.0007 (n = 45) *
0.003	0.0152 ± 0.0007 (n = 45) *

Table 3.3 The effect of heating rate on CT_{max}, somatic indices, and Hsp expression in juvenile white sturgeon, from a one-way ANOVA. Asterisk (*) denotes significance at alpha = 0.05.

Measurement	**d.f.**	**F**	**p-value**
CT_{max}	3	631.9	<0.001*
Condition factor	3	9.406	<0.001*
BSI	3	0.726	0.538
CSI	3	1.011	0.39
HSI	3	4.547	0.00443*
Hsp47	3	3.256	0.0251*
Hsp70	3	298.9	<0.001*
Hsp90a	3	31.8	<0.001*
Hsp90b	3	55.83	<0.001*

Table 3.4 The effect of heating rate and performance on various somatic indices and Hsp expression in juvenile white sturgeon, from a two-way ANOVA. Asterisk (*) denotes significance at alpha = 0.05.

Measurement	Variable	d.f.	F	p-value
Condition factor	Heating rate	2	6.677	0.002*
	Performance	1	0.031	0.860
	Heating rate × performance	2	1.631	0.203
BSI	Heating rate	2	1.332	0.275
	Performance	1	1.418	0.241
	Heating rate × performance	2	0.285	0.753
CSI	Heating rate	2	1.032	0.365
	Performance	1	0.716	0.402
	Heating rate × performance	2	0.123	0.885
HSI	Heating rate	2	1.273	0.290
	Performance	1	0.001	0.979
	Heating rate × performance	2	0.635	0.535
Hsp47	Heating rate	2	6.474	0.003*
	Performance	1	2.484	0.119
	Heating rate × performance	2	2.703	0.074
Hsp70	Heating rate	2	285.806	<0.001*
	Performance	1	46.836	<0.001*
	Heating rate × performance	2	5.887	0.004*
Hsp90a	Heating rate	2	69.13	<0.001*
	Performance	1	0.266	0.608
	Heating rate × performance	2	1.62	0.205
Hsp90b	Heating rate	2	67.585	<0.001*
	Performance	1	1.029	0.314
	Heating rate × performance	2	0.213	0.809

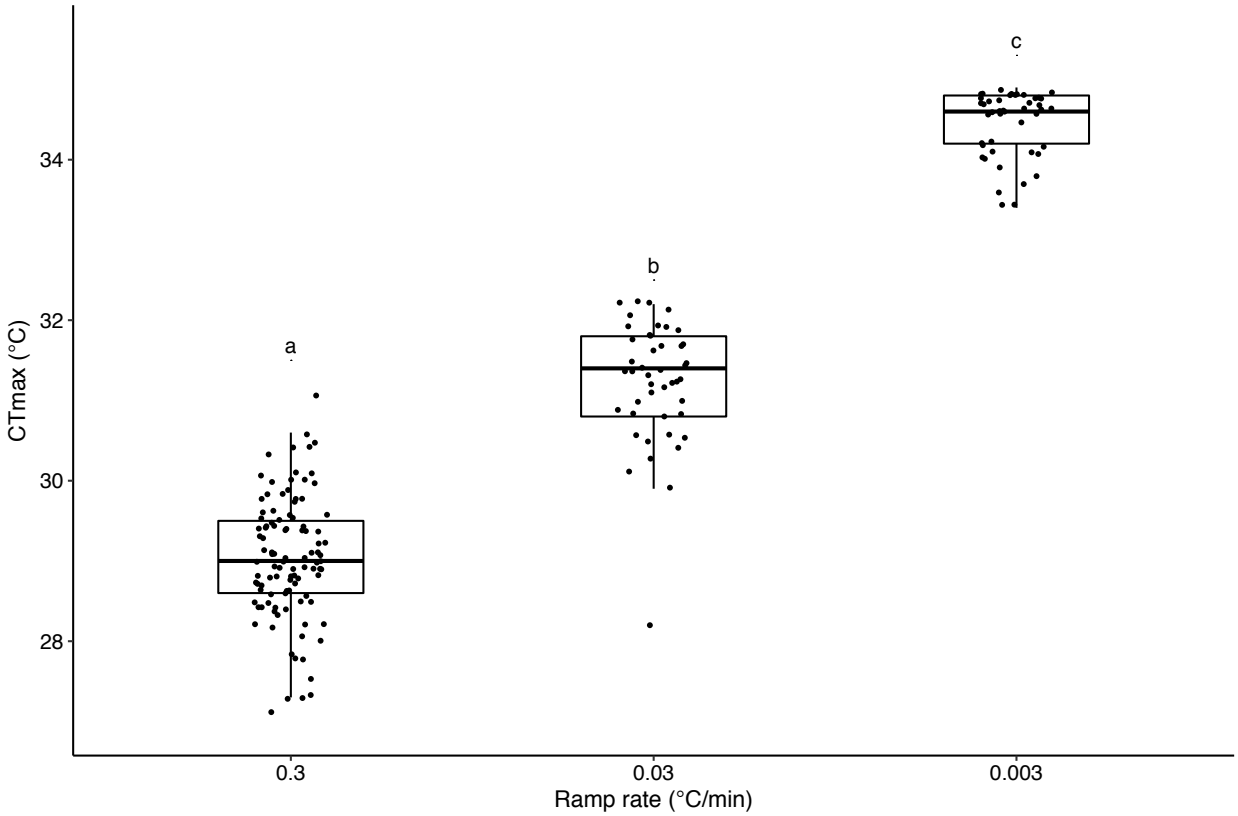

Figure 3.1 CT_{max} values of 15°C acclimated juvenile white sturgeon at three different heating rates (0.3°C/min, 0.03°C/min, and 0.003°C/min). Black dots represent individual data points and the boxplot represents group data. The horizontal line in the boxplot is the median, while the whiskers represent the maximum and minimum. Letters that differ indicate statistically significant at alpha = 0.05. N = 105 for 0.3°C/min and N = 45 for 0.03°C/min and 0.003°C/min.

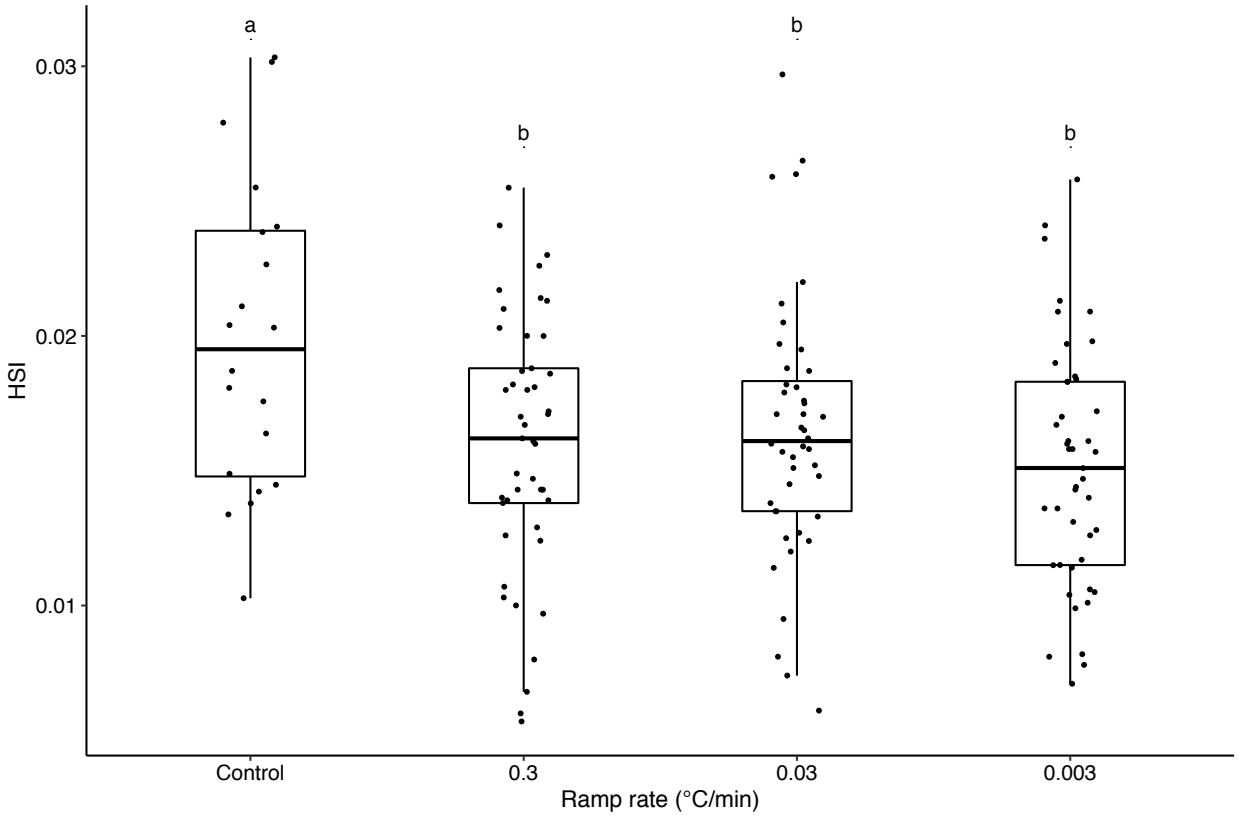

Figure 3.2 HSI of juvenile white sturgeon following CT_{max} trials at different heating rates (control, 0.3°C/min, 0.03°C/min, and 0.003°C/min). Black dots represent individual data points, while the boxplot represents group data. The horizontal line in the boxplot is the median, while the whiskers represent the maximum and minimum. Letters that differ indicate statistical significance at alpha = 0.05. N = 20 for control and N = 45 for 0.3°C/min, 0.03°C/min, and 0.003°C/min.

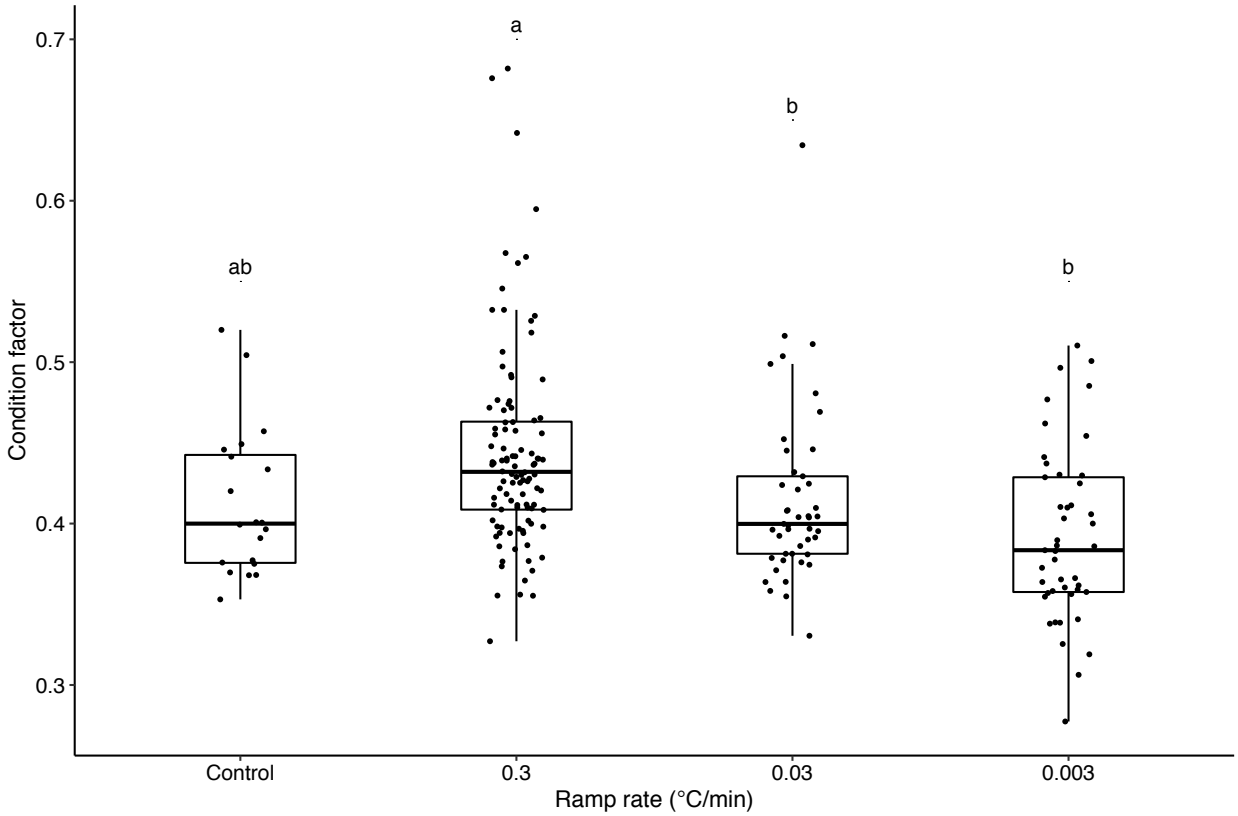

Figure 3.3 Condition factor of juvenile white sturgeon following CT_{max} trials at different heating rates (control, 0.3°C/min, 0.03°C/min, and 0.003°C/min). Black dots represent individual data points, while the boxplot represents group data. The horizontal line in the boxplot is the median, while the whiskers represent the maximum and minimum. Letters that differ indicate statistical significance at alpha = 0.05. N = 20 for control; N = 105 for 0.3°C/min; and N = 45 for 0.03°C/min, and 0.003°C/min.

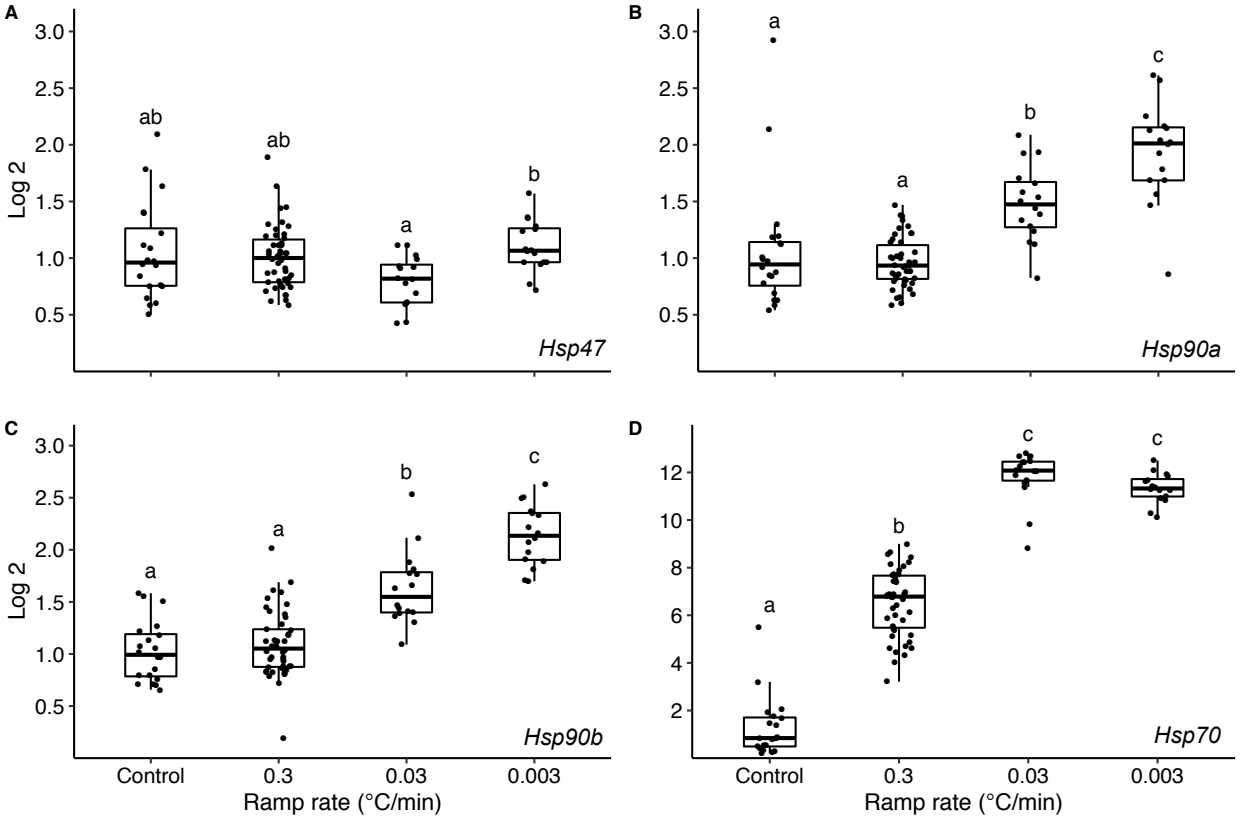

Figure 3.4 Relative expression of (**A**) *Hsp47,* (**B**) *Hsp90a*, (**C**) *Hsp90b,* and (**D**) *Hsp70* (log 2 scale) of juvenile white sturgeon following CT_{max} trials at different heating rates (control, 0.3°C/min, 0.03°C/min, and 0.003°C/min). Black dots represent individual data points, while the boxplot represents group data. The horizontal line in the boxplot is the median, while the whiskers represent the maximum and minimum. Letters that differ indicate statistical significance at alpha = 0.05. N = 20 for control; N = 48 for 0.3°C/min; and N = 16 for 0.03°C/min, and 0.003°C/min.

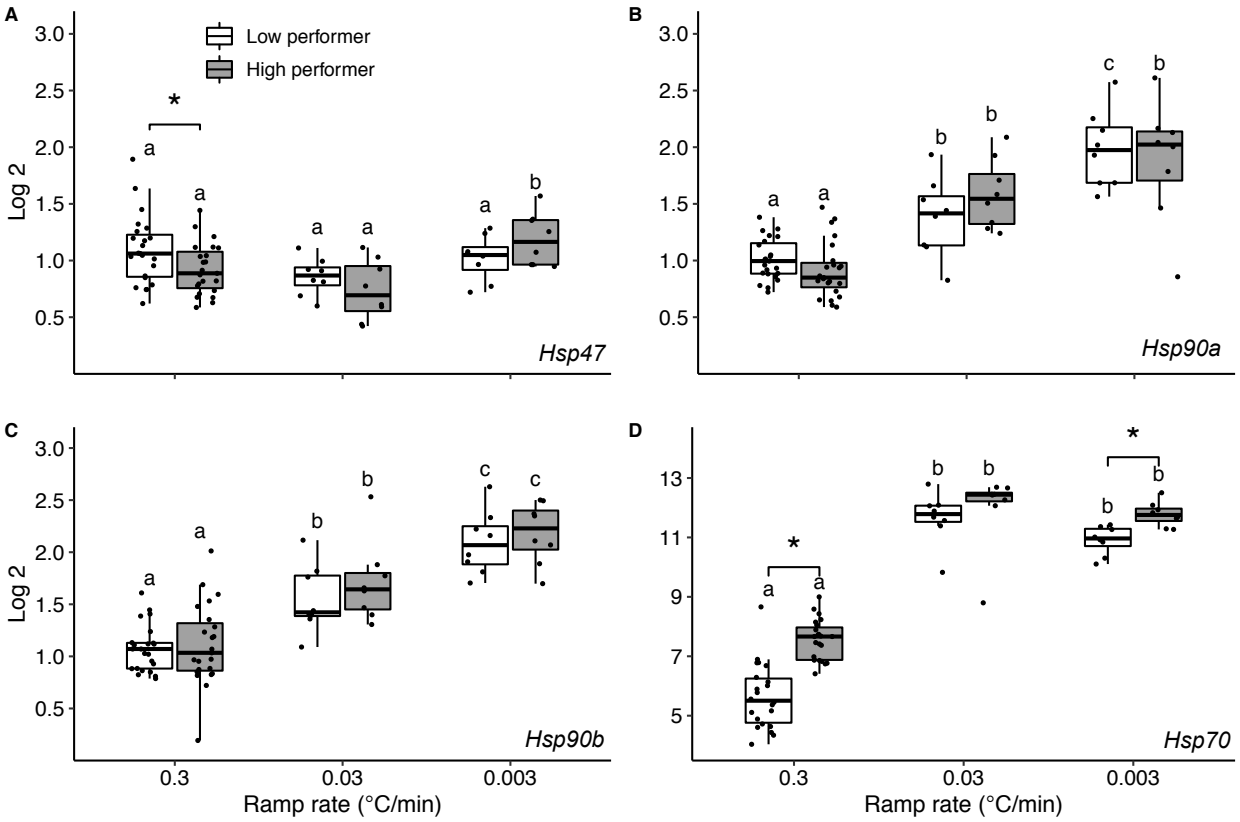

Figure 3.5 Relative expression of (**A**) *Hsp47,* (**B**) *Hsp90a*, (**C**) *Hsp90b,* and (**D**) *Hsp70* (log 2 scale) of low and high performing juvenile white sturgeon following CT_{max} trials at different heating rates (0.3°C/min, 0.03°C/min, and 0.003°C/min). Black dots represent individual data points, while the boxplot represents group data. The horizontal line in the boxplot is the median, while the whiskers represent the maximum and minimum. Letters that differ indicate statistically significance a given performance group. Asterisk (*) denotes a statistically significant difference between high and low performers with the same heating rate at alpha = 0.05. N = 24 in the 0.3°C/min trial and N = 8 in the 0.03°C/min, and 0.003°C/min trials per performance level (low and high).

3.4 Discussion

This chapter sought to determine how the heating rate used during a CT_{max} test affects thermal tolerance, Hsp expression, and somatic indices of juvenile white sturgeon. Notably, the relationship between heating rate and CT_{max} was the opposite of what was predicted, with CT_{max} increasing at slower rates of warming. HSI of controls was significantly greater than in any of the heating rates. Condition factor, BSI, and CSI were not significantly affected. *Hsp70*, *Hsp90a*, and *Hsp90b* expression was upregulated in accordance with the duration of heat stress. *Hsp47* expression was not affected by heating rate. There were no diurnal effects on CT_{max} or Hsp expression in the 0.3°C/min heating rate, unlike in the Atlantic killifish (*Fundulus heteroclitus)* where CT_{max} and *Hsp70-2* expression was greatest at midday (Healy and Schulte, 2012).

Unlike the majority of fish species studied to date, juvenile white sturgeon were able to rapidly adjust their thermal tolerance resulting in higher thermal tolerance in the slower heating rates (see review by Kovacevic et al., 2019). Given these data, the operators of the Kenney dam should consider the rate at which river temperatures are allowed to increase, as slower heating rates may allow white sturgeon time to increase their upper thermal tolerance. Zebrafish (*Danio rerio*) is the only fish species studied to date to show the same pattern as sturgeon in this study. Zebrafish fish display different trends depending on their initial acclimation temperature – when acclimated to supra-optimal temperatures (34°C), they had higher thermal tolerance when heated at 0.3°C/min relative to 0.025°C/min, whereas zebrafish acclimated to sub-optimal temperatures (22°C) were able to increase their thermal tolerance when heated at 0.025°C. In 22°C acclimated zebrafish, CT_{max} increased by 1.53°C, from 38.83°C in the 0.3°C/min trial to 40.36°C in the 0.025°C/min trial (Åsheim et al., 2020). In comparison, sturgeon were able to increase their thermal tolerance by 2.1°C when the heating rate was reduced from 0.3°C/min (CT_{max} of 29.2°C)

to 0.03°C/min (CT_{max} of 31.3°C). In the slowest heating rate (0.003°C/min), sturgeon were able to increase their thermal tolerance by 5°C to 34.2°C. In conventional CT_{max}, thermal tolerance is thought to be limited by a neurological and/or cardiac malfunction (Andreassen et al., 2020; Christen et al., 2018). Whereas in slower heating rates, fish are expected have lower thermal tolerance because it is thought to be limited by the accumulation of heat damage as organisms spend more time at temperatures where the rate of cellular damage exceeds the rate of repair (Rezende et al., 2014). Therefore, this data suggests that white sturgeon possess some physiological or biochemical mechanism(s) that allows for rapid acclimation. Åsheim and colleagues (2020) proposed Hsp production as a possible explanation for their findings in zebrafish, which may be involved in sturgeon as discussed below.

Juvenile white sturgeon displayed a highly plastic Hsp mRNA response. Modulation of Hsp mRNA expression is one proposed mechanism by which ectotherms are able to regulate their thermal tolerance in response to changing environments (Feder and Hofmann, 1999; Hochachka and Somero, 2002; Somero, 2020). It is important to note, however, that the relationship between mRNA transcription and downstream processes such as protein translation vary temporally and are not directly linked. The upregulation of transcription does not correlate 1:1 with upregulation of translation, but not surprisingly, there are reports of increased mRNA levels increasing protein levels of Hsps (Buckley et al., 2006).

Of the four Hsps examined, expression of *Hsp47* mRNA was the only one that remained relatively unchanged between controls and heating rate. The expression of *Hsp47* mRNA in response to heat shock is less conserved and more species-specific than the other Hsps studied (*Hsp70* and *Hsp90*; Mohanty et al., 2018). Previous studies have found variable expression patterns in response to acute heat shock – juvenile Atlantic sturgeon upregulated expression of

Hsp47, while juvenile brook trout did not alter expression levels (Mackey et al., 2021; Yebra-Pimentel et al., 2020).

Unlike *Hsp47* expression, which changed relatively little, expression of *Hsp90a*, *Hsp90b,* and *Hsp70* mRNA were all elevated in response to heat stress as hypothesized. The upregulation of these Hsps, which function as molecular chaperones, can help renature damaged proteins and prevent cellular apoptosis (Beere, 2004). Acute (*Hsp90a* and *Hsp70*) and constitutive (*Hsp90b*) expression depends on the severity and duration of the stressor (Chen et al., 2018; Somero, 2020). Therefore, it is interesting that both isoforms of *Hsp90* had very similar induction profiles. They were significantly upregulated in the two longer trials (0.03°C/min and 0.003°C/min) and neither was differentially expressed between high and low performers. Temperature and time are two confounding possible explanations as to why the *Hsp90* isoforms were not upregulated in the conventional CT_{max} (0.3°C/min). It could be that their activation is time dependent and the 0.3°C/min trial may not have lasted long enough to see upregulation. Alternatively, it could be that they are temperature dependent and the sturgeon in the conventional CT_{max} did not experience temperatures high enough to trigger *Hsp90* expression. A study that allowed for continuous sampling throughout CT_{max} trials at different heating rates would help tease apart these potential mechanisms.

The speed and extent to which juvenile white sturgeon can upregulate *Hsp70* expression, highlights the scope of their plasticity and may help explain why they show an opposite CT_{max} pattern to what was predicted. *Hsp70* expression plateaus between the two slower trials (0.03°C/min and 0.003°C/min), and there are many possible explanations for why this may be the case. Given the extent of *Hsp70* upregulation, it could be that cells are saturated and further upregulation would provide no additional advantage. *Hsp70* production requires a lot of energy

and thus further upregulation may be metabolically constrained (Han et al., 2011). While Hsps are preferentially synthesized during periods of stress and can be upregulated in a matter of minutes, it could be that on longer timescales, sturgeon are able to enlist some other physiological processes that can help them cope with heat stress (Tomanek and Somero, 2000).

Hsp70 was differentially expressed between high and low performers. High-performing sturgeon in both the 0.3°C/min and 0.003°C/min trials upregulated *Hsp70* expression significantly more than low performers. Additionally, while not statistically significant, the same trend is apparent in the 0.03°C/min trial. Importantly, both time and temperature are possible confounds in all three trials as high performers were exposed to higher temperatures and heat stress for longer durations than low performers. Thus, any difference between the groups may simply be due to the temperature and/or length of heat exposure. These findings merit further investigation in order to independently assess possible confounds. If time and temperature do not explain the variation between performers, then this finding may suggest that greater upregulation of *Hsp70* mRNA expression may protective and allow sturgeon to increase their thermal tolerance to a greater extent.

Condition factor, BSI, and CSI were not affected by heating rate. In studies that have found significant differences in these metrics, it has typically been after a longer exposure to stress (Gilbert and Farrell, 2021; Raspopov et al., 2017; Soengas and Aldegunde, 2002). Thus, it may be that the trials in the current study were too short in duration to cause any significant changes. HSI in all three trials was significantly lower than in the controls, but it did not differ between heating rates. Lower HSI may indicate the metabolic costs of coping with heat stress. Decreases in HSI have been observed in lake sturgeon following temperature acclimation, and in white sturgeon and three-spined sticklebacks, decreases in HSI have been linked to decreased

glycogen reserves (Bugg et al., 2020; Chellappa et al., 1995; Hung et al., 1990). Glycogen reserves are stored in the liver and they are a readily available source of energy that are utilized in bursts of activity (Goolish, 1991). Additionally, glycogen is bound to water (in humans 1 gram of glycogen binds 3 grams of water), so as glycogen reserves are diminished, water is released (King et al., 2018). It is possible that the observed decrease in HSI is in part due to water loss caused by glycogen catabolism, further reducing overall liver mass. Measuring changes in glycogen reserves as well as changes in dry mass are possible avenues for future research.

This study indicates that white sturgeon are able to rapidly adjust their thermal tolerance. A possible mechanism for this change is associated with Hsp expression as *Hsp70*, *Hsp90a*, and *Hsp90b* were all greatly upregulated in response to thermal stress. HSI decreased significantly, possibly because sturgeon are using glycogen reserve to cope with heat stress. To my knowledge, this is the first study to show a decrease in HSI in fish following an acute heat stressor (0.3°C/min). More research is needed to determine if other fish species experience this and whether glycogen depletion is behind the change. Future studies should be conducted to determine if rapid thermal acclimation is a trait common amongst all sturgeon species, or whether this is specific to white sturgeon.

Chapter 4: General Discussion and Conclusion

4.1 Thesis summary

The Kenney dam on the Nechako River is currently mandated to maintain water temperatures below 20°C, but it is unclear whether this is an appropriate upper temperature limit for white sturgeon. To provide conservation recommendations for the dam, I examined the effects of acclimation temperature, age, and heating rate on the upper thermal tolerance, somatic indices, and Hsp expression of juvenile white sturgeon. The results show that juvenile white sturgeon are able to acclimate to temperatures above 20°C over a two-week period, and that acclimation confers higher thermal tolerance. Their high ARRs relative to other species from similar latitudes suggest that white sturgeon have an unusually large capacity to acclimate to increased temperature. The plasticity of their thermal tolerance was further demonstrated when – contrary to predictions– their CT_{max} increased significantly at slower heating rates. A heating rate of 0.03°C/min resulted in 15°C-acclimated fish (trial lasted ~6.5hrs) having the same thermal tolerance as fish acclimated to 18°C for two weeks then heated at 0.3°C/min. An even slower heating rate (0.003°C/min; trial lasted ~4.5 days) allowed 15°C-acclimated fish to further increase their thermal tolerance to match the CT_{max} of the fish acclimated to 24°C for two weeks and then ramped at 0.3°C/min. In other words, the slower heating rates (Chapter 3) yield the same thermal advantage over a shorter time-course, as conventional heating rates following two-week acclimation (Chapter 2). To date, white sturgeon are one of the only fish species that show this trend (Åsheim et al., 2020; Kovacevic et al., 2019).

Despite their impressive ability to acclimate to higher temperatures, the condition factor of white sturgeon decreased following two weeks of acclimation to temperatures above 18°C (21°C and 24°C). These findings imply that although these fish are able to tolerate temperatures

above the 20°C upper temperature limit, there may be a sub-lethal threshold between 18°C and 21°C, above which their overall health may be negatively affected. In order to get better resolution within this temperature range (18°C - 21°C) more studies are required. In the absence of more information, I recommend considering reducing the upper temperature limit to 18°C to protect developing juvenile white sturgeon.

4.2 Limitations and future directions

4.2.1 Chapter 2

Mass and length were only measured at the end of the experiment. For the purposes of analysis, it was assumed that initial size was equal between fish at all acclimation temperatures; however, to draw conclusions about growth, future studies should take initial mass and length measurements prior to experimentation.

The current study did not observe increased mortality due to acclimation to temperatures up to 24°C, but it is possible that survival would decrease if sturgeon were exposed to high temperatures for longer durations, or if temperature were studied in conjunction with other stressors. Water temperature in the Nechako River exceeded 20°C for a total of four weeks in 2021. Given that climate change is predicted to continue to exacerbate temperature increases, more long-term acclimation studies are essential for understanding how these temperature changes will affect white sturgeon. Furthermore, white sturgeon have shown heightened vulnerability when temperature is one of multiple stressors; larval white sturgeon acclimated to warm temperatures (17.5°C) without adequate substrate experienced higher mortality rates relative to fish acclimated to the same temperature with proper substrate (Boucher et al., 2014). Temperature is one of six recognized factors that may be affecting the success of white sturgeon

in the Nechako River. Other stressors include water flow, predation, food availability, turbidity, and hypoxia. Designing factorial studies that more closely mimic the challenges of this natural environment will provide more ecologically relevant results.

4.2.2 Chapter 3

Given the decrease in HSI that was observed at all heating rates, future studies using white sturgeon should examine how energy reserves are affected by heat stress. Moreover, studies should be conducted to determine if lower HSI after acute heat stress is common across fish species, or whether it is unique to white sturgeon.

Because mRNA expression was only measured at the end of each trial, it remains unclear whether Hsp90a and Hsp90b upregulation was triggered by the duration of heat stress, or by the temperature of the water. Of the few studies that have looked at the time-course of Hsp expression during heat shock, none were conducted using sturgeon (Tomanek and Somero, 2000). Continuous sampling would help elucidate the trigger for Hsp90 upregulation in white sturgeon and would allow for the quantification of protein levels. This would allow researchers to better understand how the magnitude and timing of upregulation are connected between Hsp expression at the mRNA and protein levels.

4.3 Policy recommendation

The objective of this thesis was to gather information on juvenile white sturgeon thermal biology in order to better inform dam management decisions on the Nechako River in northern BC.

I sought to determine whether the current 20°C mandate is appropriate for developing juvenile white sturgeon. I found that while juvenile white sturgeon have a highly plastic thermal response, their condition factor decreased when reared at temperatures above 18°C for two weeks. These data suggest the existence of a sub-lethal threshold between 18°C and 21°C, beyond which the overall health of the animal may be affected. Moreover, in this thesis I examined the effects of temperature in isolation, yet this is rarely the only stressor white sturgeon will experience at a given time in nature. Studies have shown that when white sturgeon experience high temperatures in conjunction with other stressors, condition factor decreases more than when they are exposed only to temperature stress (Boucher et al., 2014). As a result of my findings, my policy recommendation is to consider lowering the current upper temperature limit in the Nechako River below the Kenney dam to 18°C.

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