

**THE EFFECTS OF EMBRYONIC INCUBATION TEMPERATURE ON SUBSEQUENT
DEVELOPMENT, GROWTH, AND THERMAL TOLERANCE THROUGH EARLY
ONTOGENY OF WHITE STURGEON**

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Abstract

An organism's phenotypic characteristics can be altered by environmental variations experienced during embryonic development. These changes can persist into adulthood. Increasing global temperatures are a current concern that may be particularly acute for species already threatened or endangered, such as the white sturgeon, *Acipenser transmontanus*. Given scant information on the effects of early embryonic temperatures on subsequent physiological parameters such as thermal tolerance, development, and growth in this species, the effects of global climate change on the future of white sturgeon populations is uncertain. To address this, white sturgeon embryos were incubated at 12, 15, and 18 °C until hatch, after which fish were reared at a common 15 °C for 80 days post-hatch. I conducted acute thermal tolerance tests through early ontogeny to determine how relative thermal tolerance (CT_{max}) changed and differed during this period while also sampling and evaluating various morphometric characteristics to determine the effects of embryonic temperatures on growth and development. Embryonic incubation at 12 °C with subsequent rearing at 15 °C increased larval development rate, growth rate, and the development of thermal tolerance; however, once developed CT_{max} averaged 29.9 for all embryonic rearing temperatures. For white sturgeon, embryonic temperatures appear to have discernable effects on growth and development up to 80 days post-hatch but not on thermal tolerance.

Lay Summary

Global temperatures are expected to continue to rise over the next century and this may have drastic effects on threatened and endangered populations of white sturgeon. Current research suggests that early environmental conditions experienced during embryonic development can have long term effects on the physical traits of an organism and impact survival. This thesis investigates the effect of early embryonic temperatures on traits like thermal tolerance, development, and growth in white sturgeon in hopes to better understand organismal responses to temperature and inform environmental managers on the best strategies to help conserve current sturgeon populations across their range. Using a variety of assessments, I found that embryonic temperatures had long term effects on the rate of development, rate of growth, and development of thermal tolerance in white sturgeon but no effects on thermal tolerance itself, once fish reached the early juvenile stage.

Preface

Chapter 2 of this thesis is co-authored by Katherine Cheung, Colin Brauner, and Steve McAdam. I conducted all of the research in chapter 2 (research questions, experimental design, experimentation, and data analysis) under the supervision of Dr. Colin J. Brauner. Susana Tran assisted, on occasion, with sample collection, and Monica McGrath assisted with the staging of yolk sac larvae. I wrote all 3 chapters of this thesis and received editorial feedback from my committee members, Drs. Colin J. Brauner, Steve O. McAdam, Jeffrey G. Richards, and Patricia M. Schulte.

All experimental animals were treated according to the University of British Columbia Animal Protocol #A15-0266.

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List of Abbreviations

AS	Aerobic scope
ATU	Accumulated thermal units
CTM	Critical thermal method
CT _{max}	Critical thermal maximum
dph	Days post hatch
HSP	Heat shock protein
ILT	Incipient lethal temperature
MMR	Maximum metabolic rate
Q ₁₀	Calculated factorial change in the rate of a biochemical reaction for every 10 °C increase in temperature
ROS	Reactive oxygen species
SMR	Standard metabolic rate
T _e	Embryonic incubation temperature
YSL	Yolk-sac larvae

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For my family,
I love you to pieces.

Chapter 1: Introduction

Environmental variation during embryonic development has been found to alter the phenotypic characteristics of an organism, which can persist into adulthood. Increasing global temperatures are a growing concern with little known information about the effects of early embryonic incubation temperatures (T_e) on subsequent thermal tolerance. This is of particular interest for threatened and endangered populations of white sturgeon as the temperatures in their river habitats are changing. This thesis utilizes a dynamic test method to assess thermal tolerance at multiple time points through early ontogeny while evaluating various morphometric characteristics to determine the effects of T_e on growth and development. The remainder of this introduction summarizes the challenges faced by white sturgeon in their natural environment and the effects of temperature on various levels of biological organization, from molecular to whole organism. It concludes with a summary of the current views of the mechanism(s) of thermal tolerance and the specific objectives and predictions of my thesis.

1.1 Plight of the white sturgeon

The Order Acipenseriformes represents an ancient lineage of fish that diverged 200 million years ago (Bemis et al., 1997; Bemis & Kynard, 1997). Extant Acipenseriformes (sturgeons and paddlefishes) inhabit rivers, estuaries, coastal, and inland seas in the northern hemisphere (Bemis et al., 1997; Bemis & Kynard, 1997). Many sturgeons and paddlefish are impacted by human activities such as overfishing, habitat degradation, pollution, and impoundment of riverine systems (Birstein et al., 1997) and are in danger of extinction.

White sturgeon (*Acipenser transmontanus*) are the largest freshwater fish in North America inhabiting three major river water systems, the Fraser, Columbia, and Sacramento (Scott and

Crossman, 1973). They have also been found along the west coast of North America from Alaska to northern Mexico (Hildebrand et al., 2016). They are long-lived species with delayed maturation. Females mature later than males, approximately 15-32 years and 12 years, respectively (Pacific States Marine Fisheries Commission, 1992). Upon reaching sexual maturity, spawning occurs approximately once every 5 years (Hildebrand et al., 2016; Scott & Crossman, 1973). White sturgeon have high fecundity and experience very high mortality rates early in development. The delayed maturation makes white sturgeon vulnerable to overharvest prior to spawning and the high mortality during early life stages makes them prone to anthropogenic effects that further increases mortality at this sensitive life-stage. These combined life history attributes make white sturgeon highly susceptible to human activity.

Within British Columbia, genetically distinct populations of white sturgeon reside in the Kootenay, Fraser, Columbia, and Nechako rivers (Drauch Schreier et al., 2012; Smith et al., 2002). Four of the six recognized populations in British Columbia are currently legally designated as endangered (Fisheries and Oceans Canada, 2014; Hildebrand et al., 2016). Heavy commercial exploitation in the early 1900s dramatically decreased the size of the lower Fraser population (Echols, 1995). Although commercial fishing has been banned, sturgeon numbers have not rebounded, likely due to habitat degradation, by-catch, and pollution (Hildebrand et al., 2016; Walters et al., 2005). This trend is consistent with data from other species of fish that have been overexploited (Hutchings, 2000, 2001). Populations in the Nechako, Kootenay, and upper Columbia rivers are affected by dams causing population fragmentation, decreasing genetic diversity (Drauch Schreier et al., 2012; Jager et al., 2001; Nelson & McAdam, 2012) and habitat alteration (Hildebrand et al., 2016, 2014; McAdam, 2015). Similar issues are faced by white

sturgeon populations that inhabit the Sacramento and mid- to lower- Columbia river systems in the United States (Hildebrand et al., 2016).

These anthropogenic effects may be the cause of various levels of recruitment failure in white sturgeon populations. While the lower Fraser, mid- and lower- Columbia, and Sacramento populations show episodic or consistent recruitment (Hildebrand et al., 2016), populations in the Nechako, Kootenay, and upper Columbia systems are experiencing chronic recruitment failure (Fisheries and Oceans Canada, 2014; Hildebrand et al., 2016). Evidence suggests that human activities are affecting the early life history of white sturgeon (Hildebrand et al., 2016), decreasing the number of sturgeon that eventually reach maturity. Because of this, white sturgeon are unable to replace the current aging population. Altered habitat caused by regulated river flow seems to be the likely cause of recruitment failure (McAdam, 2012, 2015; Paragamian et al., 2009).

The presence of dams along with flow regulation has changed hydrology patterns in the rivers inhabited by white sturgeon, and in turn has modified the river substratum which affects the early life stages of development in sturgeon. White sturgeon require gravel substrate when they are newly hatched. These yolk-sac larvae (YSL) use the interstitial spaces created by gravel substrate to hide and develop, emerging only after exhausting their endogenous yolk energy reserves. Altered flow regimes associated with dams can result in a decreased input of new gravel substrate if the source of the substrate is upstream of the dam, as well as infilling of downstream substrates when downstream river flow rates are decreased. In white sturgeon, larvae reared in the absence of gravel substrate show increased routine metabolic rates (Boucher et al., 2014), decreased yolk-sac utilization efficiencies (Boucher et al., 2014), and decreased length and wet weight over 46 days of development (Baker et al., 2014; Boucher et al., 2014;

Crossman & Hildebrand, 2014; McAdam, 2012). Additionally, larvae reared without gravel substrate show higher levels of cortisol (Bates et al., 2014), delayed gut development (Baker et al., 2014), and higher mortality rates in the first 35 days of development (Boucher et al., 2014). These characteristics indicate that energy is being diverted away from growth and development, resulting in larvae that are in poor condition at the onset of exogenous feeding, a life-stage characterized by high mortality. Thus, fish reared in inappropriate substrate may be more susceptible to predation and are likely to experience even higher mortalities in the wild (McAdam, 2012).

While the effect of altered water flows on the physical river habitat and subsequent impact on developing white sturgeon has received significant attention (Counihan & Chapman, 2018; Hildebrand et al., 2016, 2014), the effect of altered water temperature is much less studied, even though temperature regimes in sturgeon habitats are also subject to alteration by human activities. As average global temperatures continue to rise, so do river temperatures. In rivers where dams exist, downstream water is often warmer due to regulated water flow (Hamblin & McAdam, 2003). In the Nechako River, water flow is regulated by the Kenney dam to maintain downstream temperatures below 20 °C during summer months to reduce the impact on migrating salmon (Macdonald et al., 2012). In rivers that are not impounded, increased snowmelt during spring can lower overall river temperatures and hotter summers can result in higher than normal temperatures subjecting riverine fish like white sturgeon to increasingly variable temperatures. Tributaries and side channels could also experience higher summer temperatures as flow decreases and ambient temperature rises. Thus, there are many reasons that river temperature can vary which can impact the early life stages of riverine fish in general and sturgeon specifically.

Early in development, temperature has a direct effect on developmental rate. In salmonids, accumulated thermal units (ATUs, calculated by days post-fertilization x incubation temperature) can be used to predict time to hatch (Rombough, 1996); however, this has not previously been investigated in sturgeon and varies among ectotherms (Rombough, 2003). Exposure to higher temperatures causes white sturgeon embryos to hatch sooner but at a smaller size than those exposed to lower temperatures (Wang et al., 1985, 1987). When embryos are incubated at 20 °C, a smaller proportion hatch and those that do hatch display a greater percentage of physical abnormalities; at 24 °C no fertilized eggs are viable (Wang et al., 1985). These data indicate that the upper thermal limits of white sturgeon embryos lie between 20 °C and 24 °C.

In addition to negatively affecting embryonic development, higher temperatures also affect larval development. While larvae reared at higher temperature develop faster and have increased wet weight, they also have higher mortality and increased cortisol levels compared to their counterparts reared at a lower temperature (Bates et al., 2014; Boucher et al., 2014). The presence or absence of substrate has a large effect on survival at different temperatures. For example, Boucher et al., (2014) showed that larvae reared in gravel at 13.5 °C during the yolk-sac period had the highest survivorship in the first 45 days post-hatch (dph). Those reared at 17.5 °C without gravel showed the lowest survivorship. Larvae reared in bare tanks at 13.5 °C and those reared in gravel tanks at 17.5 °C showed no difference in survivorship and were intermediate to the two extremes. Thus, the effects of higher temperature on larval sturgeon development appear to be exacerbated by the lack of gravel substrate illustrating the complex interactions that exist between early environmental factors on larval development and overall survival (Boucher et al., 2014).

Most research on white sturgeon thermal biology has focused on embryonic and larval stages; less focus has been placed on the juvenile stage. Those that have focused on the juvenile stage have investigated the effects of rearing temperature on growth (Cech et al., 1984; Hung et al., 1993) or the effects of acclimation temperature on swimming performance and thermal tolerance (Hines et al., unpublished). In all these cases, studies were conducted on white sturgeon that were reared in absence of substrate (ie. bare tanks). Recent research has shown significant carry over effects of substrate rearing on metabolism that may affect later development and performance (Boucher et al., 2018).

White sturgeon spawn at different times of the year. Depending on the population, spawning can occur as early as February and as late as August (see summarized data in Hildebrand et al., 2016). Because the transition from larvae to juveniles typically occurs between 20 to 45 dph (Buddington & Christofferson, 1985; Deng et al., 2002), juvenile development occurs during the spring and summer when river temperatures may be on the rise. Average daily temperatures in these major water systems currently range from 16 – 22 °C (Fraser Basin Counsil, 2015; Patterson et al., 2007; USGS National Water Information System) with tributary and side channels likely exceeding this range. Understanding the long-term effects of a range of temperatures during early juvenile development and the interaction with substrates will be useful in understanding the impact of altered thermal regimes on sturgeon physiology and development and will have implications for conservation.

Reducing mortality in the first year of life for white sturgeon is essential to increasing recruitment and population numbers (Gross et al., 2002), aiding in sturgeon conservation. It is therefore important to improve our understanding of how different environmental factors affect white sturgeon physiology, tolerance to stressors, and general survival during the first year of

life. Given the high level of conservation concerns for all Acipenserids, this information can improve our understanding on the additional threat of thermal changes associated with climate change and river impoundment to inform conservation of this highly endangered group of fish.

1.2 Effects of Temperature

Temperature influences all levels of biological organization from molecular to whole organism. For poikilothermic ectotherms, changes in temperature can affect physiology, behaviour, and ecology. Because of the complex interactions between temperature and multiple levels of biological organization, this section will attempt to provide a brief summary of how organisms can maintain performance over a range of temperatures and what happens when these temperatures are exceeded using a bottom-up approach beginning with a discussion of the effects of temperature on biochemical reactions, protein and membrane structures, then integrating those to discuss effects on organelle and cellular function, to organs and organ systems, and finally to a whole organism's physiology, behaviour, and ecology.

Starting at the lowest level of biological organization, increasing temperature accelerates biochemical reactions by increasing molecular collisions and molecular kinetic energy above the activation energy. This increase, however, is not infinite. For enzyme-catalyzed reactions, temperatures beyond an optimal point alter enzyme conformation, decreasing binding capacities, and result in slower reaction rates (Somero, 1995). This occurs because an increase in temperature affects the weak bonds that maintain the secondary, tertiary, and quaternary structures of proteins (Fields, 2011; Somero, 1995). If temperatures are high enough, proteins can denature, resulting in complete loss of function. Additionally, denatured proteins can impact cell function by interacting with lipid membranes and aggregating and binding with normal

functioning proteins inactivating them (Bucciantini et al., 2002; Kampinga, 1993; Meredith, 2005). Changes to reaction rates given increasing temperatures can be quantified. For every 10 °C increase in temperature, a factorial change in the rate of a biochemical reaction, referred to as Q_{10} , can be calculated. Within an organism's physiological temperature range, Q_{10} s of enzyme-catalyzed reactions typically range from 1 to 3 (Schulte, 2011) where a Q_{10} of 2 means that an increase in 10 °C results in a doubling in the rate of reaction in question.

Besides influencing protein structure, temperature also influences the weak bonds associated with biological membranes affecting fluidity and their ability to create barriers between environments while allowing the passage of select solutes. The presence of biological membranes allows for the creation of different ion concentrations between the cell and its environment so membrane potentials can be generated. Membrane potentials are key for electrical conduction needed for neuronal communication and muscle contraction (Moyes & Ballantyne, 2011). Biological membranes are also needed for structural organization, chemical communication, and isolation of metabolic pathways within target organelles among other things (Hazel, 1995; Moyes & Ballantyne, 2011). A decrease in temperature can cause membranes to become too rigid, affecting diffusion capacity and the ability for embedded proteins to change conformation while an increase in temperature can cause membranes to become too fluid, affecting its ability to act as an effective barrier (Cossins et al., 1981; Hazel & Williams, 1990; Moyes & Ballantyne, 2011).

The effects of temperature on both membranes and proteins can influence higher levels of organization, like organelle and cellular function, which can in turn influence organs and organ systems within an entire organism. For mitochondria, changes in temperature can affect ATP production through changes in membrane fluidity and enzyme characteristics. Production of ATP

is dependent on the generation of a proton gradient across the inner mitochondrial membrane (Guderley, 2011). As electrons move through the electron transport system located on the inner mitochondrial membrane, protons are transported from the matrix to the intermembrane space, creating a proton gradient. This proton gradient is used by ATP synthase to phosphorylate ADP to ATP. In the end, the electrons are accepted by oxygen, reducing it to water, but in this process, reactive oxygen species (ROS) can also be produced which can damage cellular components. Increases in temperature have been found to increase ROS production, mitochondrial respiration, and phosphorylation rates, however, the overall efficiency of phosphorylation (based on oxygen consumption to ATP production) decreases (Iftikar & Hickey, 2013; Jarmuszkiewicz et al., 2015). This has been attributed to proton leak across the inner mitochondrial membrane. Increased membrane fluidity and activation of uncoupling proteins due to the presence of ROS increases proton movement down its electrochemical gradient without the production of ATP (Guderley, 2011; Hilton et al., 2010; Iftikar & Hickey, 2013; Jarmuszkiewicz et al., 2015; Kraffe et al., 2007). At high temperatures, decreased ATP production efficiencies could affect organ function and lead to organ failure (e.g. heart, Iftikar & Hickey, 2013).

Temperature also affects the transmission of electrical and chemical signals necessary for neuronal communication which are driven by brief and rapid changes in membrane potential. Changes in temperature affect the resting membrane potential, conduction velocity, and duration of action potentials (Vornanen, 2011). This change is due to ion channels embedded within the neuronal membrane. Changes in temperature affect the function of these embedded ion channels which in turn alter the conductance of ions across the membrane affecting membrane potential (Harper et al., 1990; Melani & Moran, 1998; Vornanen, 2011). Changes in resting membrane potential, conduction velocity, and duration of action potentials can affect the overall timing of

activity and levels of activity in an organism (Schleimer & Schreiber, 2016). At high temperatures, nervous system failure occurs through what is believed to be an imbalance in rates of ion flux which cause widespread generation of action potentials without recovery (Robertson & Money, 2012).

Changes that occur at the cellular and organ system level of organization, in turn, affect the whole organism. Changes in temperature can affect all aspects of performance, from metabolism to growth and development. Metabolism refers to the sum of all chemical reactions that occur within an organism. Metabolic rate, which is estimated by the rate of oxygen consumption, is a useful measure of metabolism by focusing on aerobic ATP production and, consequently, ATP consumption by an organism. Two measures of metabolism are routinely used in ectotherms: standard metabolic rate (SMR) and maximum metabolic rate (MMR). SMR refers to the minimum metabolism needed to keep an organism alive while MMR refers to the maximum aerobic metabolism that can be maintained for a sustained period (Beitinger & Fitzpatrick, 1979). The difference between MMR and SMR referred to as the aerobic scope (AS), can be a useful tool in assessing the aerobic capacity of an organism beyond SMR that can presumably be diverted to other processes (Claireaux & Lefrancois, 2007).

When temperature increases, SMR typically increases exponentially (Norin et al., 2014) as a result of the increased rate of the chemical reactions involved in metabolism and thus ATP consumed (Clarke & Fraser, 2004). Increases to SMR can have benefits and drawbacks depending on the organism's ecology and environmental conditions. Higher SMRs can allow organisms to increase their activity levels (e.g. for hunting), but in an environment where food is scarce, higher SMRs would be unsustainable (Sinclair et al., 2016). For MMR, an increase in temperature generally results in a continual increase of MMR until the upper end of their natural

range where a plateau or decrease can be seen producing an AS curve that is dome- or bell-shaped (Norin et al., 2014). This change in observed MMR and AS at an organism's upper thermal range is hypothesized to be a result of an inability for the circulatory and ventilatory system to match the increased oxygen demand of tissues known as the oxygen and capacity limited thermal tolerance (OCLTT, Fry & Hart, 1948; Iftikar & Hickey, 2013; Portner & Knust, 2007).

Growth, both somatic and reproductive, is accomplished when energy assimilated from food is greater than that required to maintain SMR (Beitinger & Fitzpatrick, 1979; Werner & Gilliam, 1984; Wootton, 2011a) and is also affected by changes in temperature. As temperature increases growth rate increases to an optimum point before decreasing (Wootton, 2011b). This observed decrease is likely due to an increase in SMR and a smaller AS. Additionally, the energetic costs associated with digestion and food assimilation does not decrease at higher temperatures (Clark et al., 2013; McLeod & Clark, 2016) further limiting energy that can be partitioned to growth. Under conditions where food is limited, the optimum temperature for growth shifts lower because SMR decreases at lower temperatures.

Development is also impacted by temperature where faster development during early life stages has been observed for individuals reared at higher temperatures (Pepin, 1991). Faster larval development times are typically associated with an increased growth rate. For some organisms, growth to a certain size is required before transition to a different life stage can occur (McLeod et al., 2015). A reduced time to achieve the next life stage can be beneficial as these transitions tend to be associated with changes in diet as well as habitat and can, therefore, decrease predation mortality (Anderson, 1988; Werner & Gilliam, 1984).

In addition to physiological changes, temperature can also alter organismal behaviour. Different species show thermal preferences where their performance can be optimized and mobile organisms actively seek out their preferred temperature (Crawshaw & Podrabsky, 2011). With increasing global temperatures, species distributions are altered as organisms move away from areas of higher temperature towards higher latitudes or altitudes where temperatures are cooler (Walther et al., 2002). Furthering our understanding of how organisms already at the peak of performance respond to increasing thermal stress is crucial.

1.3 Thermal Tolerance

From what has been outlined above, organisms have an optimum temperature at which they best operate and a thermal range at which they maintain normal function (Huey & Stevenson, 1979). Temperatures outside of this range can limit organismal performance which can impact survival. As temperatures increase due to climate change, organisms operating at the upper end of their preferred thermal range face increased thermal stress. In order for these organisms to survive, they must be able to tolerate the imposed stress.

Although the exact mechanism of thermal tolerance has yet to be elucidated, some organisms have been shown to exhibit phenotypic plasticity in thermal tolerance (Beitinger et al., 2000), where thermal range can be shifted higher or lower depending on the direction of the thermal exposure. An organism exposed to increased heat can undergo reversible physiological and biochemical changes, referred to as acclimation. These changes allow an organism to maintain performance even at temperatures outside of their normal range, but this requires time and can be energetically costly (Murren et al., 2015). Under these new physiological conditions, organisms

have been observed to have higher thermal tolerance when exposed to additional thermal challenges (Beitinger & Lutterschmidt, 2011; Peck et al., 2014).

In response to high temperatures, increased production of heat shock proteins (HSPs, Basu et al., 2002; Fangue, 2007), changes in protein concentration and isoform ratios (Cossins & Bowler, 1987), and modification of the lipid composition in biological membranes (homeoviscous adaptation, Cossins & Prosser, 1982; Hazel, 1995) have been observed in different organisms. The upregulation of HSPs seen in organisms exposed to heat stress is thought to help stabilize proteins or aid in the degradation and/or aggregation (through binding to HSPs) of proteins that have been destabilized by high temperature (Kampinga, 1993; Parsell & Lindquist, 1993). In doing so, this could prevent denatured proteins from interacting with membrane lipids and inactivating other proteins permitting maintained cellular function.

Altering protein concentration and isoform ratios can allow a cell to maintain similar levels of function even at increased temperatures (Fields, 2011; Somero, 1969). Certain organisms have protein isoforms with enzyme kinetics that are more suitable for higher temperatures due to substitutions in their amino acid sequence which alters the bonds holding their structure together (Cossins & Bowler, 1987; Somero, 1995). By increasing protein expression of these isoforms, a cell can maintain rates of chemical reaction even at higher temperatures because of the differential enzyme binding capacities.

Modification of membrane lipids by making them more rigid maintains fluidity at higher temperatures allowing membranes to continue to act as barriers in the cell (Cossins et al., 1981; Hazel & Williams, 1990). This could potentially help maintain the integrity of the inner mitochondrial membrane, preventing proton leak and sustaining ATP production efficiencies,

and help keep neuronal function by preventing increased conductance of ions across the membrane, allowing proper communication.

Though acclimation can help increase thermal tolerance, the extent to which an organism can acclimate is limited. Changes in thermal tolerance given changes in acclimation temperature are not perfectly compensated (Gunderson et al., 2017; Gunderson & Stillman, 2015). Additionally, the duration and magnitude of the thermal exposure must be taken into consideration in order to evaluate the outcome of an organism exposed to high temperatures (Bevelhimer & Bennett, 2000). Both chronic and acute thermal challenges, increase energetic costs associated with maintaining basal metabolism, leaving less additional energy to be partitioned towards other activities. Chronic exposures to sublethal temperature with little or no time for recovery can induce a stress response that suppresses immune function and inhibits growth compromising the health of the organism resulting in mortality (Eldridge et al., 2015) while acute exposure to extreme temperature could result in a collapse of physiological systems (e.g. circulatory or nervous system), as previously discussed, limiting oxygen delivery ultimately resulting in mortality (Portner & Knust, 2007; Somero, 2010).

In addition to acclimation temperature, diurnal and seasonal cycles, developmental stage, and overall thermal history can also influence thermal tolerance in some organisms (Beitinger & Lutterschmidt, 2011). Heat tolerance has been found to be higher during the midday than at night in common killifish (*Fundulus heteroclitus*; Healy & Schulte, 2012) and tropical catfish (*Clarias batrachus*; Varghese & Pati, 1997) and higher during the summer period than the winter in Atlantic stingray (*Dasyatis sabina*; Fangue & Bennett, 2003) and Indian hill trout (*Barilius bendelisis*; Sharma et al., 2015). These changes, triggered by thermocycling and photoperiod,

increase heat tolerance to mimic the changes seen in an organism's natural environment and allow them to better tolerate increased environmental temperatures at the appropriate time.

Thermal tolerance has also been observed to change as an organism progresses through different developmental stages (Bowler & Terblanche, 2008; Gilbert & Lattanzio, 2016; Klockmann et al., 2017; Komoroske et al., 2014; Miller et al., 2013). These differences in thermal tolerance at different life stages may be due to a complex interaction between behavioural compensation, energetic trade-offs, and thermal environments (Bowler & Terblanche, 2008). Behavioural compensation largely depends on mobility. Organisms with high mobility are capable of behaviourally regulating their body temperatures such that physiological changes which allow for increased thermal tolerance are not necessary (Bowler & Terblanche, 2008; Klockmann et al., 2017). Conversely, greater thermal tolerance is typically expected for stages where organisms are less mobile because they must endure the fluxes in environmental temperatures given little to no ability to behaviourally regulate their temperature. To increase thermal tolerance, energy must be expended (e.g. to increase HSP expression, alter membrane fluidity, or change enzyme concentrations) but this requires energy in excess of that required for resting metabolism. At life stages when development is rapid, limited AS means that organisms have little capacity to increase metabolic rate above routine metabolism (Portner & Farrell, 2008; Rombough, 2011), which may limit an organism's ability to increase their thermal tolerance at these life stages. Lastly, if thermal environments experienced during a life stage are stable, the ability to increase thermal tolerance may be selected against due to the associated metabolic costs.

From what has been discussed thus far, an organism's thermal tolerance is affected by thermal exposure over its lifetime. While acclimation refers to reversible changes in phenotype

that can occur throughout the life of an organism, changes in phenotype that occur early during embryonic development can be irreversible and long-lasting (Beaman et al., 2016; Wilson & Franklin, 2002). This developmental plasticity has been suggested by Beaman et al. (2016) to influence the acclimation capacity of an organism by setting the mean value for certain traits, and consequently the range at which that trait can be altered through acclimation. It has also been suggested that developmental plasticity could minimize energetic costs by preparing the organism for exposure to similar environments in the future, therefore decreasing the need for acclimation and the associated energetic costs. Recent research on flies (*Drosophila melanogaster*) demonstrated that higher temperatures experienced during embryonic development result in higher thermal tolerance in adulthood (Slotsbo et al., 2016). This interaction between developmental plasticity and the environment experienced by an organism will ultimately determine its performance under different thermal environments over its lifetime. To better understand how organisms will respond to climate change, it is important to understand how developmental plasticity and future environments interact.

Quantification of acute thermal tolerance typically uses one of two different methods, the static test method or the dynamic test method (Beitinger et al., 2000). The static test method, also known as the incipient lethal temperature (ILT) test, consists of exposing an organism to an abrupt change in temperature which is then maintained until mortality (Beitinger et al., 2000; Beitinger & Lutterschmidt, 2011; Lutterschmidt & Hutchison, 1997). Using this method, one can determine the time for 50% of the organisms to reach the incipient upper lethal temperature under a specified period of time (Beitinger & Lutterschmidt, 2011). In contrast, the dynamic method, also known as the critical thermal method (CTM), consists of exposing an organism to a constant rate of temperature change until a sub-lethal end response is observed (Beitinger et al.,

2000; Beiting & Lutterschmidt, 2011; Lutterschmidt & Hutchison, 1997). Using this method, one can determine the critical thermal maximum (CT_{max}) which is defined as “the thermal point at which locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to its death” (Cowles & Bogert, 1944). Although CT_{max} is only a relative measure of thermal tolerance, it is very repeatable and has a few advantages over the ILT test in that fewer organisms need to be employed, non-lethal endpoints are used and tests are relatively rapid, generally being completed within 2 h (Beiting et al., 2000; Beiting & Lutterschmidt, 2011). The CT_{max} value can also be used to compare thermal tolerance within and across species (Beiting & Lutterschmidt, 2011). These benefits of CT_{max} were the basis for my decision to use the CTM in this thesis.

1.4 Thesis Objectives

The general objective of this thesis was to investigate the role of developmental plasticity under varying T_e on subsequent development, growth, and thermal tolerance through different life stages in white sturgeon. To tease apart the effects of developmental plasticity from acclimation, embryos were incubated at three different temperatures (12, 15, and 18 °C) until hatch (to address developmental plasticity) and subsequently reared under a common temperature of 15 °C (to control for the effects of acclimation). This thesis incorporates CTM to assess thermal tolerance at multiple time points through early ontogeny and evaluates various morphometric characteristics to determine the effects of T_e on growth and development. This general objective can be broken up into the following:

Objective 1: Determine the effect of T_e on time to hatch, growth and development in white sturgeon through to yolk absorption

Because temperature has varying effects on organismal growth and development, I wanted to investigate the immediate effects of embryonic incubation on time to hatch, and initial growth and development through the YSL stage. During the YSL stage, energy for growth and development comes solely from endogenous stores so any differences observed through this stage is likely due to maternal effects (e.g. through differences in yolk provisioning and genetics, Burt et al., 2011; Rasanen & Kruuk, 2007) and differences in conditions experienced through embryonic development and following hatch. Because differences in maternal contributions have been found to significantly affect individuals, I predicted that familial differences would be observed in the parameters measured.

Wang et al. (1985, 1987) previously studied white sturgeon growth and development under constant rearing temperatures of 11, 14, 17, and 20 °C from fertilization to exogenous feeding. Under higher rearing temperatures, Wang et al. (1985, 1987) found that embryonic and YSL development was faster and that individuals at hatch were significantly smaller. Given these observations, I predicted that there would be differences in time to hatch and size at hatch but that this could be accounted for by expressing developmental time in ATUs similar to what has been observed in salmonids (Billard & Jensen, 1996).

Research by Martell et al. (2005) examined the effects of T_e on haddock (*Melanogrammus aeglefinus*) growth and development from hatch through to the juvenile stage in a similar setup to this study and found significant carry-over effects of T_e on growth and development from hatch to yolk absorption and from exogenous feeding on to the juvenile stage. Given these

observations, I predicted that growth and development of white sturgeon incubated at different T_e would also differ through the YSL stage.

Objective 2: Determine the effect of T_e on growth and thermal tolerance in white sturgeon from yolk sac absorption and the onset of exogenous feeding through to the early juvenile stage.

Because developmental plasticity can lead to lasting changes, I wanted to investigate whether temperatures experienced during embryonic development had lasting effects on growth from the onset of exogenous feeding through to the early juvenile stage. I also wanted to investigate whether thermal tolerance was affected by T_e , if this phenomenon was long-lasting, and how thermal tolerance changed through early development in white sturgeon. Again, I looked at whether family differences existed and persisted in these measured parameters through these life stages.

If growth associated with T_e persists through the feeding larval stage and into the juvenile stage similar to the study by Martell et al. (2005), I predicted that differences in growth in white sturgeon would still be observed at this time. And because thermal tolerance in adult flies has been shown to be affected by temperatures experienced during embryonic development (Slotsbo et al., 2016), I predicted that there would be significant effects of T_e on thermal tolerance from exogenous feeding through the juvenile stage. I also expected differences in thermal tolerance to be observed through development. Hines et al. (unpublished) found that thermal tolerance in white sturgeon increased over time and plateaued in the early juvenile stage from 54 dph through to 100 dph.

Chapter 2: The Effects of Embryonic Incubation Temperature on Subsequent Thermal Tolerance, Growth and Development of White Sturgeon

2.1 Introduction

Temperature influences biological organization from the molecular scale up to the level of the whole organism. These include changes to biochemical reaction rates (Schulte, 2011), protein and membrane structure (Cossins et al., 1981; Fields, 2011; Hazel & Williams, 1990; Moyes & Ballantyne, 2011; Somero, 1995), energy production and neural conductance (Guderley, 2011; Harper et al., 1990; Melani & Moran, 1998; Vornanen, 2011) and overall growth, development and metabolism (Clarke & Fraser, 2004; Norin et al., 2014; Portner & Knust, 2007). Particularly for poikilothermic ectotherms, changes in environmental temperature influence their physiology, behaviour, and ecology.

Poikilothermic ectotherms have an optimum environmental temperature for performance and a thermal range at which they maintain normal function (Huey & Stevenson, 1979). Ambient temperatures outside of this range can limit organismal performance which can ultimately impact survival. As global temperatures increase due to climate change, organisms operating at the upper end of their preferred thermal range may face increased thermal stress. Motile organisms inhabiting thermally heterogeneous environments are capable of behavioural thermoregulation and can actively seek out more favourable environments (Abram et al., 2017; Crawshaw & Podrabsky, 2011). Those that are incapable must tolerate the imposed stress, while those that cannot will likely perish.

Although the exact mechanism of thermal tolerance has yet to be elucidated, some organisms have been shown to exhibit phenotypic plasticity in thermal tolerance (Beitinger et al., 2000), by shifting their thermal range higher or lower depending on the direction of the thermal stress. An organism exposed to heat or cold stress can undergo reversible physiological and biochemical changes, referred to as acclimation. These can include, but are not limited to, upregulation of HSPs (Basu et al., 2002; Fangue, 2007), homeoviscous adaptation (Cossins et al., 1981; Hazel & Williams, 1990), and alteration of enzyme concentration and isoform ratios (Fields, 2011; Somero, 1995). These changes allow an organism to maintain performance even at temperatures outside of their normal range, but this requires time and can be energetically costly (Murren et al., 2015). Under these new physiological conditions, organisms have been observed to have higher thermal tolerance when exposed to additional thermal challenges (Beitinger & Lutterschmidt, 2011; Peck et al., 2014).

If exposure to high temperatures occurs early enough (e.g. during embryonic development), it can lead to long-lasting irreversible changes in phenotype (Beaman et al., 2016; Wilson & Franklin, 2002). These changes in phenotype, known as developmental plasticity, have been suggested by Beaman et al. (2016) to influence the acclimation capacity of an organism by setting the mean value for certain traits and consequently the range at which that trait can be altered through acclimation. In one study of fruit flies, exposure to high temperatures during embryonic development resulted in increased thermal tolerance in adulthood (Slotsbo et al., 2016).

Different life stages can also influence thermal tolerance in some organisms (Beitinger & Lutterschmidt, 2011; Bowler & Terblanche, 2008; Gilbert & Lattanzio, 2016; Klockmann et al., 2017; Komoroske et al., 2014; Miller et al., 2013). These differences may be due to a complex

interaction between behavioural compensation, energetic trade-offs, and the thermal environments experienced (Bowler & Terblanche, 2008).

In general, little is known regarding how developmental plasticity affects subsequent thermal tolerance through multiple life stages in an organism. With rising global temperatures, it is important to understand how these temperatures experienced during embryonic development shape thermal tolerance and how thermal tolerance changes through early life stages. This is of particular interest to species that are already of conservation concern, such as white sturgeon (*Acipenser transmontanus*), a long-lived and endangered fish endemic to North America (Bemis et al., 1997; Bemis & Kynard, 1997).

White sturgeon in Canada have been impacted by human activities like overfishing, habitat degradation, pollution, and impoundment of riverine systems (Birstein et al., 1997) and are in danger of extinction through much of their range. Habitat alteration caused by regulated river flow has had the largest effect on the early life history of white sturgeon decreasing the numbers that reach maturity resulting in chronic recruitment failure (McAdam, 2012, 2015; Paragamian et al., 2009). This altered habitat, when combined with increased environmental temperatures, has been shown to decrease survivorship within the first 45 dph (Boucher et al., 2014) illustrating the complex interactions that exist between early environmental factors on larval development and overall survival (Boucher et al., 2014).

While most of the thermal research on white sturgeon focuses on the embryonic and larval stages, less emphasis has been placed on the juvenile stage. Juvenile development for Canadian populations tends to occur during the summer months when river temperatures are on the rise. A better understanding of thermal tolerance in white sturgeon through early development can help

inform environmental managers on the best strategies to help conserve current sturgeon populations across their range and regulate river flow in impounded river systems.

The objective of this chapter was to investigate the role of developmental plasticity under varying T_e on development, growth, and subsequent thermal tolerance through different life stages in white sturgeon. This was broken down into two experiments with the first focusing on changes from hatch to the onset of exogenous feeding (0 – 13 dph; Series I) and the second focusing on changes from feeding through to the early juvenile stage (13 – 80 dph; Series II). In Series I, my objective was to determine the effect of T_e on time to hatch, growth and development from hatch through to yolk absorption while in Series II my objective was to determine the effect of T_e on growth and thermal tolerance in white sturgeon from the onset of exogenous feeding through the transition into early juveniles.

To investigate these objectives, fertilized eggs from the induced spawning of wild broodstock were transported to The University of British Columbia where they were incubated at 12, 15, and 18 °C until hatch. Once hatched, fish were reared out at a common 15 °C. Individuals from each temperature treatment were sampled at 0, 6, and 13 dph for growth measures and developmental staging to determine the effects of T_e on growth and development during the YSL stage (Series 1). Individuals were then tested for thermal tolerance using the CTM on 13, 16, 22, 30, 40, 50, 60, 70, and 80 dph, which bridged the time from the start of exogenous feeding through the transition into early juveniles, with morphometric measures taken to determine the effects of T_e on growth and thermal tolerance through these life stages (Series II).

2.2 Methods

2.2.1 *White sturgeon eggs and broodstock*

White sturgeon used in this study were obtained through induced spawning of wild broodstock conducted at the White Sturgeon Recovery Facility in Vanderhoof, British Columbia. Adult white sturgeon in reproductive condition were caught in the Nechako River in May 2017. Ovulation was induced on May 25, 2017, by administering luteinizing hormone releasing hormone analogue to females. Eggs from 3 females (Identification number 2522, 3622, and 5903) were collected and de-adheased (see Conte et al., 1988). These eggs were then independently fertilized with milt from the same 5 males (resulting in 5 crosses per female consisting of 1650 eggs per cross). Fertilized eggs from unique crosses were bagged separately and transported in a cooler to the Department of Zoology at the University of British Columbia within 24 h of fertilization. Once received, embryos were held at 15 °C for up to 3 h as embryos were distributed into 1 L McDonald jars.

Embryos from unique crosses (approximately 550 embryos from each) were grouped by maternal family and evenly distributed into 3 McDonald jars. In the end, each jar contained embryos from 1 female crossed with 5 males resulting in half-sibling families consisting of approximately 2750 eggs per jar. Half-sibling families are subsequently referred to by their maternal identification number as listed above. Families were used as replicates (1 McDonald jar per family) as temperatures were then adjusted at 1 °C h⁻¹ to the desired T_e of 12.4 ± 0.2 °C, 15.0 ± 0.2 °C, or 17.8 ± 0.4 °C until hatch (from this point forward referred to as 12, 15 and 18 °C). Following hatch, all fish were then held at 14.9 °C (herein referred to as 15 °C) for the remainder of the experiment. To simplify, individuals exposed to a T_e of 12 °C and post-hatch temperatures of 15 °C, T_e of 15 °C and post-hatch temperatures of 15 °C, and T_e of 18 °C and post-hatch

temperatures of 15 °C, will be henceforth be referred to as treatment 12/15, 15/15, and 18/15, respectively. Photoperiod was maintained at 12 h:12 h L:D throughout the experiment.

2.2.2 *Hatching jar setup and embryonic incubation*

McDonald jars (1 per family) at each T_e were set up in a recirculation system with overflow from each jar returned to a reservoir (a 100L Sterilite tote, 84 cm x 45 cm x 33 cm – LxWxH). A submersible pump (Pondmaster Mag Drive 5) within the reservoir drew water through a 15W or 18W UV sterilizer (Lifegard Aquatics QL-15 or Coralife 15601, respectively) before distributing it through a manifold to each McDonald jar within the system. Flow to each jar was adjusted to ensure adequate movement of embryos. A chiller (EcoPlus 1/4 HP) or submersible heater (Eheim Jager 150 W), hooked up to a temperature controller (Nema TR115SN), was used to hold and maintain the T_e of the reservoir which received a constant, slow inflow of fresh dechlorinated city of Vancouver tap water to allow a full reservoir exchange every 10 h.

On each of the two days of peak hatch, YSL from each McDonald jar at each T_e were placed into separate 37 L rearing tanks (68.5 cm x 38 cm x 18 cm – LxWxH) maintained at 15 °C. Hatched YSL from each jar were collected every few hours daily between 9 am and 5 pm. To keep track of the age of the fish through development, 5 pm was our designated end-of-day. Fish hatched after 5 pm would be considered to have hatched the following day. Density in each tank was maintained below 60 kg m^{-3} , which was determined to be an optimal rearing condition for white sturgeon (Hines, C., personal communication)

2.2.3 Husbandry and post-hatch rearing

A flow-through system was set up for the 18 rearing tanks described above (3 temperatures × 3 families × 2 days of hatch). Each rearing tank had 2 cm diameter holes (20 in total) cut along the length of both sides, 2.5 cm from the top. These holes were covered in screening to prevent fish escape but still allow removal of wastes. Incoming dechlorinated city water was warmed to 15 °C using multiple submersible heaters (Eheim Jager 250 W and Aqueon Pro 250 W) and supplied to a 750 L reservoir. A submersible pump (Pondmaster Mag Drive 12-B) distributed the water through a manifold to each rearing tank. During warmer weather when incoming city water surpassed 15 °C, heater sticks were removed and a chiller (EcoPlus 1/4 HP) was set up to cool reservoirs to the desired temperature. Outflow to each rearing tank was adjusted to maintain a complete turnover in 3 h or less.

Artificial substrate, a combination of 1" and 1.5" diameter sinking Bio-Spheres (Dynamic Aqua-Supply Ltd. Surrey, BC) was used to provide interstitial hiding spaces for the YSL similar to their natural environment (Boucher et al., 2014; McAdam, 2011). The substrate was removed when larvae were 13 dph and feeding was initiated. The timing of this coincides with yolk-sac absorption and onset of exogenous feeding (Conte et al., 1988; Deng et al., 2002). Larvae were hand fed to satiation 3 times daily on an EWOS diet (EWOS Canada, Surrey, B.C.) mixed with powdered krill and supplemented with *Artemia* (hatched daily). Excess food was siphoned out after 1 hour for the first two feedings but not for the last. The feed was left overnight and removed the following morning. Moribund individuals were removed each morning and totals noted.

2.2.4 Series I: Time to 50% hatch, wet weight, total length, and developmental staging of YSL

Daily estimates of hatch were noted during embryonic incubation to determine the time to 50% hatch. ATUs to 50% hatch were calculated by summing average temperature for each day of incubation until 50% of hatch occurred.

The effects of early T_e on growth and development of YSL were determined by sampling 10 fish from each family at 0, 6, and 13dph. Fish were euthanized in 200 mg L⁻¹ MS-222 buffered with 400 mg L⁻¹ sodium bicarbonate and stored in 10% neutral buffered formalin. Wet weight (g) and total length (mm) were measured 11 weeks after the sampling date. Samples were blotted dry on Kimwipes prior to weighing on an analytical balance (Sartorius CP124S, accuracy ± 0.1 mg) and total length was measured using digital calipers. Using the data obtained, condition factor was calculated as follows:

$$\text{Condition factor} = \frac{\text{wet weight (g)} \times 10^5}{\text{total length (mm)}^3}$$

Images of YSL under a stereomicroscope (Olympus SZX10) were taken by a digital camera (Canon EOS60) 9 months after sampling. Developmental stages were determined using the presence of external features as described by Dettlaf et al. (1993) and BC Hydro (2016): stage 36 – hatch; 37 – pectoral fin rudiment, opening of mouth; 38 – gill filament rudiments; 39 – digestive system rudiment divides into stomach and intestine; 40 – ventral fin rudiment; 41 – liver subdivided; 42 – complete liver division, pyloric appendage rudiment; 43 – ventral fin extends to preanal fin fold margin; 44 – complete yolk-sac absorption. The proportion of individuals sampled at each developmental stage was noted.

2.2.5 Series II: Wet weight, total length, and dry weight from the onset of exogenous feeding through the early juvenile stage

Wet weight (g), total length (mm), and dry weight (g) were measured for all fish that underwent CT_{max} testing. Wet weight was measured within 4 hours of the end of CT_{max} trials, while total length was measured after 11 weeks. These times used to standardize the potential effects of formalin storage on these measured parameters (Dang et al., 2000; Frimpong & Henebry, 2012; König & Borcherding, 2012; Treasurer, 1992; Yeh & Hodson, 1975). Dry weight was measured >5 months after fixation.

For wet weight, samples were blotted dry on Kimwipes prior to weighing on an analytical balance (Sartorius CP124S, accuracy ±0.1 mg). Total length was measured using digital calipers. For dry weight, samples were blotted dry before transferring onto an aluminum foil of known weight. Drying took place in an oven at 60 °C until the weight of the samples did not change by more than 0.2 mg over two consecutive days. Using the data obtained, condition factor and water content (%) were calculated.

Condition factor was calculated in the same manner as in Series I and water content was calculated as follows:

$$\text{Water content (\%)} = \frac{\text{wet weight (g)} - \text{dry weight (g)}}{\text{wet weight (g)}} \times 100$$

2.2.6 Series II: Thermal tolerance and critical thermal maximum protocols from the onset of exogenous feeding through the early juvenile stage

Critical thermal maximum (CT_{max}) was used as a proxy for thermal tolerance. This was assessed on larval and juvenile sturgeon at ages 13, 16, 22, 30, 40, 50, 60, 70, and 80 dph to

determine the effects of different T_e on thermal tolerance through the early stages of development. CT_{max} is defined as “the thermal point at which locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to its death” (Cowles & Bogert, 1944).

To determine CT_{max} , water temperature was increased at a constant rate of approximately 1.2 -1.3 °C every 10 min (7.2 - 7.8 °C h⁻¹). This is within the range recommended by Becker & Genoway (Becker & Genoway, 1979) for freshwater fish. Rates above 3 °C every 10 min (18 °C h⁻¹) cause a lag in core body temperature as the rate of heat transfer is slower than that of rising environmental temperature while rates below 1 °C every 10 min (6 °C h⁻¹) allow for thermal acclimation to occur during the CT_{max} test (Becker & Genoway, 1979); both result in inflated CT_{max} values and an overestimation of thermal tolerance with larger fish. The rate of 1 °C every 10 min (6 °C h⁻¹) has also been used in the majority of studies looking at thermal tolerance in other sturgeon species (summarized by Spear & Kieffer, 2016) making this rate useful for interspecies comparison.

While the majority of CT_{max} tests used loss of equilibrium (LOE) as an endpoint, LOE was difficult to observe in white sturgeon during preliminary trials. Sturgeon did not always lose equilibrium by exhibiting a “belly-up” response at CT_{max} . Some settled at the bottom of the tank and remained inactive. Nudging their caudal fins with a probe, at times, elicited a coordinated locomotory response indicating that CT_{max} had not been reached. This behavior made endpoint difficult to define. Some CT_{max} tests used opercular distention, associated with the onset of muscular spasm as an endpoint for fish, but at larval stages and in such small fish, this was determined inadequate. Based on preliminary trials, I used a lack of motor response following 3 consecutive tail prods as the endpoint.

Three independent CT_{max} systems were built, each consisting of 27 L of water placed into an insulated cooler (66 cm x 35.5 cm x 37 cm – LxWxH, Coleman 45.5 L) with 10 mesh baskets (12.5 cm x 10.5 cm x 12.5 cm – LxWxH) submerged approximately 8 cm from the surface of the water. The water temperature was adjusted to 15 °C and measured using a digital thermometer (Fisherbrand 4376EMD) calibrated against a laboratory standard mercury thermometer. Each CT_{max} system was heated with a 300 W (Finnex Titanium) heater stick and a circulation pump (Hydor Koralia Nano 240) and airstones were added to ensure adequate mixing and aeration of water.

Twenty-four hours prior to a CT_{max} test, 10 fish from a given family per treatment were netted and held without food in a 1.18 L plastic chamber within their respective rearing tanks. On the day of the CT_{max} test, fish from each family of a given treatment were randomly assigned to one of the 30 mesh baskets (10 fish per family x 3 families) and left to accustom to their surroundings for at least 1 h before testing commenced. CT_{max} trials started between 9 am and 11 am to minimize the potential effects of any diel rhythm on thermal tolerance (Healy & Schulte, 2012). Each CT_{max} system trial start time was staggered by 30 min and the order each system was used was randomized. The temperature was then increased at 1.2 -1.3 °C every 10 min until the endpoint for each fish was reached at which the temperature was noted as CT_{max}. Fish were immediately euthanized in 200 mg L⁻¹ tricaine methane sulfonate (MS-222) buffered with 400 mg L⁻¹ sodium bicarbonate and stored in 10% neutral buffered formalin for wet weight, total length, and dry weight measures (see below).

2.2.7 Statistical Analysis

Statistical analyses were performed using R software (version 3.5.0) through the RStudio platform (version 1.1.453). Data are expressed as mean \pm SEM (standard error). The effect of temperature treatment on differences of time to 50% hatch was analyzed using a one-way ANOVA using family as replicates ($N = 3$). Assumptions of normality and homogeneity of variances were determined through visual inspection. A post hoc Tukey pairwise comparison was run on the estimated marginal means generated by the linear model (R package *emmeans*) to determine which results were significantly different. Differences between total length and wet weight at hatch were determined using a one-way ANOVA on the mean trait values per family ($N = 3$). A post hoc Tukey pairwise comparison was run if significant differences were found.

The effect of treatment temperature, age, and the interaction of treatment temperature and age on each measured parameter was analyzed using a two-way ANOVA. An attempt to model individual data using a linear mixed-effects model was made with family as a random factor but this failed the assumption of homogeneity of variances for ANOVA, therefore, the mean values for each family were used rather than values from each individual tested ($N = 3$ at each time point). Due to differences in hatch numbers and survival, some families were not equally represented throughout the time course of the experiment resulting in an unbalanced sample size. To account for this in the statistics, marginal fitting (type II or type III sums of squares) was used (R package *car*). Assumptions were checked as mentioned above and natural log transformations were used for data that were not normally distributed.

Significant interactions were further investigated using a simple effects analysis where data were partitioned for each level within the factor, treatment or age, and post hoc Tukey

pairwise comparisons were run on the estimated marginal means generated by the linear model (R package *emmeans*) to determine which were significantly different. Where interactions were not significant, the interaction term was removed, and the two-way ANOVA rerun. Significant main effects of treatment and/or age were investigated further using post hoc Tukey pairwise comparisons similar to above.

To determine whether there were significant differences between families in any of the measured parameters, I utilized a linear mixed-effects model within each temperature with family as a fixed factor and age (dph) as a random factor ($N = 10$ individuals per family at each time point; R package *nlme*). Age was not a factor of interest in this particular question but was included as a random factor to account for the fact that each measured parameter would vary depending on the age of the individuals at which the measures were made. The effect of family was tested using ANOVA. Departures from assumptions of normality and homogeneity of variances were determined through visual inspection. Data that did not meet normality assumptions were natural log transformed.

2.3 Results

2.3.1 *Series I: The effect of embryonic incubation temperature on time to hatch, growth and development in white sturgeon from hatch through to yolk absorption (0 – 13 dph)*

The main objective of this series was to determine the effect of T_e on time to hatch, growth and development in white sturgeon from hatch through to yolk absorption.

2.3.1.1 Time to 50% hatch

Days to 50% hatch and ATUs to 50% hatch were both significantly affected by T_e (Table 2.1, one-way ANOVA, $F_{2,6} = 57, p < 0.0005$ and $F_{2,6} = 13.2, p < 0.01$, respectively). A Tukey post hoc comparison of days to 50% hatch indicated that embryos incubated at 12 °C took significantly longer to 50% hatch than those incubated at 15 and 18 °C ($p < 0.005$ and $p < 0.0005$, respectively), while those incubated at 15 °C took significantly longer to 50% hatch than those incubated 18 °C ($p < 0.05$). The effect of T_e on days to 50% hatch was as expected; however, contrary to my prediction, ATUs to 50% hatch for individuals incubated at 12 °C were significantly longer compared to those incubated 18 °C ($p < 0.01$) and those incubated at 15 °C not significantly different from the other two treatments. Familial differences in time to 50% hatch were only observed at 12 °C.

2.3.1.2 Growth through YSL stage (0 – 13 dph)

Familial differences attributable to maternal contributions were observed as predicted through the YSL stage but only for certain temperature treatments and parameters (Figure 2.1 – 2.3). Significant differences among families were found for wet weight in all three temperature treatments (one-way ANOVA for the fixed effect of family: 12 °C: $F_{2,85} = 39.6, p < 0.005$; 15 °C: $F_{2,75} = 3.7, p < 0.05$; 18 °C: $F_{2,89} = 11.1, p < 0.0001$) while total length and condition factor were only significantly different for those incubated at 12 °C (one-way ANOVA for the fixed effect of family, $F_{2,85} = 27.7, p < 0.0001$ and $F_{2,85} = 8.3, p < 0.01$, for length and condition factor respectively) and 18 °C (one-way ANOVA for the fixed effect of family: $F_{2,88} = 26.6, p < 0.0001$ and $F_{2,88} = 25.0, p < 0.0001$, for length and condition factor respectively).

When familial data were averaged and used as replicates, size at hatch for both wet weight and total length were not significantly different between temperature treatments as

predicted (Figure 2.4, one-way ANOVA with $N = 3$, $F_{2,6} = 0.064$, ns and $F_{2,6} = 0.53$, ns, respectively) but temperature did have significant effects on total length through the YSL stage (two-way ANOVA with $N = 3$ at each time point, treatment temperature*age: $F_{4,17} = 0.17$, ns; two-way ANOVA rerun without interaction, treatment temperature: $F_{2,21} = 4.7$, $p < 0.05$ and age: $F_{2,21} = 187.4$, $p < 0.0001$). A Tukey post hoc comparison of the estimated means indicated that YSL from treatment 12/15 was significantly longer than those from treatment 18/15 ($p < 0.05$) and means for those from treatment 15/15 not significantly different from either treatment. Treatment temperature also had significant effects on condition factor (two-way ANOVA on ln-transformed data with $N = 3$ at each time point, treatment temperature*age: $F_{4,17} = 0.16$, ns; two-way ANOVA rerun without interaction, treatment temperature: $F_{2,21} = 4.2$, $p < 0.05$ and age: $F_{2,21} = 111.6$, $p < 0.0001$). A Tukey post hoc comparison of the estimated means found that the condition factor of YSL from treatment 18/15 was significantly higher than treatment 12/15 ($p < 0.05$), while estimated means for treatment 15/15 was intermediate to the two. The effects of T_e on wet weight was not significant and no significant interactions were found between temperature treatment and age for any of the measured parameters. In general, wet weight and total length increased as fish aged, while condition factor decreased.

2.3.1.3 Development through YSL stage (0 – 13 dph)

Development through the YSL stage is affected by T_e , as predicted, with a significant interaction with age (Figure 2.5, two-way ANOVA using mean familial data with $N = 3$ at each time point, treatment temperature*age: $F_{4,17} = 11.3$, $p < 0.005$; treatment temperature: $F_{2,17} = 18.7$, $p < 0.0001$; age: $F_{2,17} = 2272.6$, $p < 0.0001$). Those incubated at 18 °C were more developed at hatch compared to those incubated at 12 and 15 °C (Tukey post hoc comparison with a simple effects analysis, $p < 0.0005$ and $p < 0.001$, respectively), with 82% exhibiting

rudimentary pectoral fin folds (Stage 37) compared to the 17% and 30% of individuals incubated at 12 and 15 °C, respectively (Figure 2.6). Most individuals at hatch when incubated at 12 and 15 °C did not have rudimentary pectoral fin folds (Stage 36). By 13 dph, the opposite was true with a greater proportion of individuals more developed in treatment 12/15 than treatment 18/15 (Tukey post hoc comparison with a simple effects analysis, $p < 0.05$) with 69% already completing yolk absorption (Stage 44) in the former compared to 33% in the latter. Individuals from treatment 15/15 were intermediate but not significantly different from either.

2.3.2 Series II: The effect of embryonic incubation temperature on growth and thermal tolerance in white sturgeon from the onset of exogenous feeding through transition into early juveniles (13 – 80 dph)

The main objective of this series was to investigate if any effects of T_e would persist through continued development at a constant rearing temperature of 15 °C, and if so, for how long. The measures addressed in this series are growth and thermal tolerance.

2.3.2.1 Growth from exogenous feeding through to the early juvenile stage (13 – 80 dph)

Familial differences were seen in some of the growth measures as predicted (Figure 2.7 – 2.9). Water content was significantly different between families for all treatment temperatures (one-way ANOVA utilizing linear mixed-effects models with $N = 10$ at each time point, 12 °C: $F_{2,241} = 19.1, p < 0.0001$; 15 °C: $F_{2,173} = 9.5, p < 0.0001$; 18 °C: $F_{2,231} = 7.9, p < 0.0001$). Significant familial differences in condition factor were found for treatment 12/15 and 18/15 (one-way ANOVA utilizing linear mixed-effects models with $N = 10$ at each time point, $F_{2,241} = 26.3, p < 0.0001$ and $F_{2,231} = 15.9, p < 0.0001$, respectively) and significant familial differences were also found in dry weight and total length for treatment 15/15 and 18/15, respectively (one-

way ANOVA on ln-transformed data utilizing linear mixed-effects models with N = 10 at each time point, $F_{2,173} = 3.9, p < 0.05$ and $F_{2,231} = 5.6, p < 0.005$, respectively). The early familial differences seen during the YSL stage only persisted for condition factor and total length for treatments 12/15 and 18/15, respectively. None of the differences found in the other measures were significantly different after the onset of exogenous feeding.

When familial data were averaged and used as replicates, T_e had significant effects on wet weight (two-way ANOVA on ln-transformed data with N = 3 at each time point, treatment temperature*age: $F_{16,42} = 0.94$, ns; two-way ANOVA rerun without interaction, treatment temperature: $F_{2,58} = 7.7, p < 0.005$ and age: $F_{8,58} = 596.2, p < 0.0001$), dry weight (two-way ANOVA on ln-transformed data with N = 3 at each time point, treatment temperature*age: $F_{16,42} = 0.82$, ns; two-way ANOVA rerun without interaction, treatment temperature: $F_{2,58} = 3.6, p < 0.05$ and age: $F_{8,58} = 416.3, p < 0.0001$), and total length (two-way ANOVA on ln-transformed data with N = 3 at each time point, treatment temperature*age: $F_{16,42} = 1.18, p > 0.05$; two-way ANOVA rerun without interaction, treatment temperature: $F_{2,58} = 12.4, p < 0.0001$ and age: $F_{8,58} = 443.5, p < 0.0001$) from exogenous feeding until the early juvenile stage, as predicted (Figure 2.10). Mean wet weight and total length for individuals from treatment 12/15 were estimated to be significantly larger than the other treatments (Tukey post hoc comparisons, $p < 0.05$ and $p < 0.005$, respectively). Estimated mean dry weights for treatment 12/15 were significantly larger than 18/15 (Tukey post hoc comparisons, $p < 0.05$), but treatment 15/15 was not significantly different from the other treatments. The only significant interaction was found between treatment temperature and age for condition factor (two-way ANOVA with N = 3 at each time point, treatment temperature*age: $F_{16,42} = 2.7, p < 0.01$; treatment temperature: $F_{2,42} = 14.1, p < 0.0001$; age: $F_{8,42} = 4.9, p < 0.0005$). For this parameter, significant differences between estimated

treatment means were only seen on 13 and 60 dph with condition factor being largest for 18/15 treatment and 15/15 treatment, respectively (Tukey post hoc comparisons, $p < 0.0005$ and $p < 0.05$, respectively).

In general, over the course of development from the onset of exogenous feeding to the early juvenile stage, wet weight stayed consistent from 13 – 30 dph before increasing while total length increased through this entire period. Dry weight was consistent between 13 and 16 dph and decreased to its lowest points between 22 and 30 dph before increasing (see Figure 2.10.1 for data redrawn on a smaller time scale, Tukey post hoc, $p < 0.05$) while water content peaked between 40 and 50 dph before decreasing and plateauing at 60 dph. Changes to condition factor over this period varied depending on temperature treatment but a decrease in condition factor was consistently observed before plateauing around 30 dph.

2.3.2.2 Thermal tolerance from exogenous feeding through to the early juvenile stage (13 – 80 dph)

As predicted, familial differences in CT_{max} were seen from exogenous feeding through to the early juvenile stage but only for treatment 12/15 (Figure 2.11, one-way ANOVA utilizing linear mixed-effects models with $N = 10$ at each time point, $F_{2,239} = 5.3$, $p < 0.01$).

When familial data were averaged and used as replicates, treatment temperature and age had significant effects on CT_{max} with an additional significant interaction between treatment temperature and age on CT_{max} (Figure 2.12, two-way ANOVA with $N = 3$ at each time point, treatment temperature*age: $F_{16,42} = 2.7$, $p < 0.01$; treatment temperature: $F_{2,42} = 9.1$, $p < 0.001$; age: $F_{8,42} = 461.9$, $p < 0.0001$). At 13, 22, and 30 dph, estimated mean CT_{max} for treatment 12/15 was the highest (Tukey post hoc comparisons using a simple effects analysis, $p < 0.05$). Changes in CT_{max} over this period, regardless of treatment temperature, were consistently lower at 13 and

16 dph before increasing and plateauing at 30 or 40 dph at approximately 29.9 °C matching my prediction that thermal tolerance changes through development. This increase in thermal tolerance, however, appears to occur earliest in the 12/15 treatment. Given that estimated mean CT_{max} was not different between temperature treatments at the plateau, T_e does not seem to have long-term carryover effects on thermal tolerance, counter to my previous prediction.

2.4 Discussion

The objective of this thesis was to investigate the role of developmental plasticity under varying T_e on development, subsequent growth, and thermal tolerance through different life stages in endangered white sturgeon. In general, the data indicated that treatment temperature had effects on development and growth as initial embryonic temperature treatments resulted in differences in time to hatch, progression through developmental during the YSL stage, total length, wet weight, and dry weight. While treatment temperature was found to affect the development of thermal tolerance via CT_{max} through to the early juvenile stage, little evidence was found to show that early developmental temperature affected thermal tolerance in the later juvenile stage.

2.4.1 *The effects of embryonic incubation temperature on development*

Embryonic incubation temperature affected both embryonic and larval development as indicated by differences in time to hatch and differences in progression through developmental stages. Embryonic development was faster under treatment 18/15 as observed by a shorter time to 50% hatch (7 days post-fertilization; 117 ATUs) compared to individuals under treatment 15/15 (9 days post-fertilization; 130 ATUs) and treatment 12/15 (12 days post-fertilization; 146

ATUs). This trend is consistent with previous findings across different species of marine and freshwater fish where embryonic development, on average, triples with every 10 °C increase in temperature ($Q_{10} \approx 3.0$), consequently decreasing the incubation period (Rombough, 1996).

In white sturgeon, Wang et al. (1985) found that incubation period ranged from 5, 7 – 8, and 10 – 13 days when embryos were incubated at 17, 14, and 11 °C, respectively. Similar observations were made by Jay (2014) where 50% hatch occurred at 6, 7, 8, and 10 days post-fertilization when white sturgeon embryos were incubated at 17.0, 15.5, 14.0, and 12.5 °C, respectively. In the present study, the days to hatch were higher than that reported in Wang et al. (1985) and Jay (2014) under similar T_e but these differences may be due, in part, to differences in handling times prior to reaching incubation temperatures. Embryos were held at 10 °C for about 16 h and then 15 °C for about 4 h during transport and sorting prior to being assigned to a treatment. It took 1 day before embryos reached their respective treatment temperatures.

Although ATUs are expected to standardize development across a range of temperatures (Billard & Jensen, 1996), ATUs to 50% hatch were significantly different given T_e in this study. In salmonids such as chinook (*Oncorhynchus tshawytscha*), ATUs to 50% hatch are expected to be roughly 527 – 525 ATUs for T_e ranging from 7.5 – 12.5 °C, respectively, while chum (*Oncorhynchus keta*) range from 542 – 533 ATUs for T_e ranging from 7.5 – 12.5 °C (Billard & Jensen, 1996). Thus, although they differ between species, they are relatively constant within species. No other studies have reported ATUs to hatch in white sturgeon except for Boucher et al. (2014); embryos incubated at 15 °C hatched at 105 ATUs. From the data provided in Wang et al. (1985) and Jay (2014), ATUs to hatch were determined by multiplying T_e with the incubation duration. Embryos incubated at 17, 14, and 11 °C hatched between 79 – 93, 96 – 109, and 105 – 143 ATUs, respectively (Wang et al., 1985) and embryos incubated at 17, 15.5, 14, and 12.5 °C,

hatched at 102, 109, 112, and 125 ATUs, respectively (Jay, 2014). Differences in ATUs to hatch under varying T_e are apparent in Wang et al. (1985) and Jay (2014) as well, adding to the body of evidence that there is a deceleration in development rate at lower temperatures (Hildebrand et al., 2016), a relationship clearly different than salmonids. In haddock, Martell et al. (2005) found that the length of each embryonic developmental stage was generally longer when embryos were incubated at lower temperatures compared to those reared under higher temperatures. In Columbia white sturgeon, Parsley (2010) found that time of neural tube formation took significantly longer under a cold T_e regime.

When comparing ATUs to hatch under similar T_e , embryos in this study hatched at higher ATUs across all T_e than Wang et al. (1985) and Jay (2014). These observations could be attributed to differences in parentage. Studies in salmonids have shown that parentage influences time to hatch (Beacham, 1988; Burt et al., 2012). Alternatively, interpopulation differences could explain these observations. Populations of white sturgeon are genetically distinct (Drauch Schreier et al., 2012; Smith et al., 2002) and each population is locally adapted to the environmental conditions of their riverine habitat. Progeny for the current study came from the Nechako population (northernmost) while those for Wang et al. (1985) and Jay (2014) came from the Sacramento (southernmost) and Upper Columbia (geographically intermediate) population, respectively. Populational differences in embryonic development rate under differing T_e have been observed in killifish and the common frog, *Rana temporaria* (DiMichele & Westerman, 1997; Laugen et al., 2003). In killifish, embryonic development exhibited countergradient variation where individuals from northern populations developed faster at all T_e compared to their southern counterparts (DiMichele & Westerman, 1997). Faster embryonic development in northern populations has been argued to compensate for slower development that

typically occurs under colder environmental temperatures so that in situ, the development between the populations would be similar even at different temperatures (DiMichele & Westerman, 1997). This countergradient variation was not found in common frog but differences in rates of embryonic development were seen given progeny from different population reared under different T_e (Laugen et al., 2003).

At hatch, individuals from treatment 18/15 were significantly more developed with a larger proportion of individuals with rudimentary pectoral fin folds (Stage 37) compared to treatment 12/15 and 15/15. Similar differences in stage at hatch have been observed in rough-skinned newts (*Taricha granulosa*; Smith et al., 2015) where individuals incubated at higher temperatures hatch more developed. Higher temperatures could signify impending increases in environmental temperature which result in a more rapid depletion of energy reserves as yolk-sac utilization efficiencies decrease (Boucher et al., 2014). Yolk-sac larvae which are more developed at hatch would have a selective advantage in that they could transition to exogenous feeding sooner to contend with depleting resources as well as develop a higher thermal tolerance sooner to deal with impending temperature increases. At lower T_e , faster embryonic development might not be necessary as environmental temperatures are not close to the upper limits of embryonic development. This difference in development in white sturgeon, however, did not persist post-hatch; at 6 dph, all individuals from all treatments were observed to be at the same developmental stage and at 13 dph, a greater proportion of individuals from treatment 12/15 had completed yolk absorption (Stage 44) and on average, were significantly more developed compared to those from treatment 18/15. Compensatory development appears to occur in individuals exposed to treatment 12/15 compared to treatment 15/15 and 18/15. This observation has not been reported in any other studies to date. In rough-skinned newts, individuals exposed

to higher T_e continued to develop faster compared to their counterparts (Smith et al., 2015) and in haddock, continued acceleration in gut development was observed in individuals exposed to higher T_e and exogenous feeding initiated sooner (Martell et al., 2005, 2006). In white sturgeon, it appears that lower T_e may convey some benefit that is not seen in other organisms.

2.4.2 The effects of embryonic incubation temperature on growth

At hatch, no significant differences in wet weight or total length were observed in white sturgeon which counters previous observations by Wang et al. (1985). Wang et al. (1985) found that larval white sturgeon from the Sacramento population were significantly smaller in wet weight and total length at hatch when incubated at 17 °C compared to 11 °C. However, another study of Columbia white sturgeon showed no difference in total length at hatch for embryos incubated at 17.0, 15.5, 14.0, and 12.5 °C (BC Hydro, 2016), consistent with the results in this study. One potential explanation for these differences could lie in the fact that Wang's (1985) study used embryos from only 1 cross between 1 male and 1 female, whereas the BC Hydro (2016) study used embryos from 2 genetically distinct families as replicates. Parental genetics could have played a role in the size at hatch under different T_e . The use of only one family may not be indicative of the average size at hatch from multiple genetic crosses.

Given common post-hatch rearing conditions between all T_e treatments, substantial differences were observed in total length from the YSL stage through to the early juvenile stage. Individuals from treatment 12/15 were estimated to be longer than those from treatment 15/15 and 18/15 throughout early development. While wet weight was not affected by T_e during the YSL stage, T_e had carry-over effects from post yolk absorption through to the early juvenile stage. Estimated mean wet weights post yolk absorption were greater for treatment 12/15 than

those from treatment 15/15 and 18/15. Overall, individuals from treatment 12/15 were estimated to be larger than all others. These results oppose similar thermal studies in haddock (Martell et al., 2005) and rough-skinned newt (Smith et al., 2015). However, if we take into consideration the differences observed in development between individuals incubated at different temperatures, the general trends are consistent between studies where development and size are positively correlated. Individuals that develop faster also tend to be larger. This correlation has also been observed in Atlantic sturgeon (*Acipenser oxyrinchus*) and shortnose sturgeon (*Acipenser brevirostrum*) where length at the onset of exogenous feeding was found to be independent of rearing temperature (Hardy & Litvak, 2004). Because YSL from the 12/15 treatment developed faster, they would be larger than same-aged individuals from other treatments. Exogenous feeding in these larvae would also initiate sooner resulting in earlier gains in wet weight, dry weight, and total length for treatment 12/15 through the early juvenile stage.

Condition factor, which is a ratio of wet weight to total length, was higher during the YSL stage compared to the feeding and early juvenile stages. This was due to the presence of large yolk reserves which increased wet weight without associated changes to total length. During the YSL stage, condition factor for individuals exposed to treatment 18/15 was greater than those exposed to treatment 12/15. This could be explained by the fact that YSL from treatment 12/15 were longer due to compensatory development and therefore condition factor would be smaller. Condition factor through exogenous feeding decreased as yolk reserves were depleted and condition factor remained relatively consistent through the early juvenile stage.

Dry weight at the start of exogenous feeding decreased from 16 dph, with the lowest dry weight observed at 30 dph. Due to low sampling resolution, we could not determine the exact inflection point when the minimum dry weight occurred and whether differences existed between

treatments. If development is faster for individuals in treatment 12/15, I would expect the inflection point to occur sooner than in treatment 15/15 and 18/15. Decreases in dry weight typically occur at the onset of exogenous feeding when lipid reserves are depleted as metabolism shifts from lipids to body protein although the timing of when these shifts occur and for how long they last vary depending on species (Kamler, 2008). This decrease in dry weight was also observed in Columbia white sturgeon but only to 22 dph following which it increased (McAdam, S. O., unpublished). Water content associated with wet weight tended to increase at the onset of exogenous feeding. At this time rapid growth and development occurred with increased cell division which typically increases water content and wet weight, but dry weight did not increase as body protein was catabolized during this period and not anabolized until after 30 dph.

2.4.3 The effects of embryonic incubation temperature on thermal tolerance

Critical thermal maximum, a proxy for thermal tolerance, was shown to be affected by a combination of treatment temperature and age. At 13, 22, and 30 dph, individuals from treatment 12/15 had the highest estimated CT_{max} , while estimated mean CT_{max} for individuals from treatment 18/15 was the lowest at 13 and 22 dph but not different from 12/15 at 30 dph. At all other times, CT_{max} was not different between treatments.

Changes in thermal tolerance through different life stages were also observed with an estimated mean CT_{max} of 24.1 °C at the start of exogenous feeding around 13 and 16 dph, increasing through 22 and 30 dph, and then plateauing from 30 dph onward at a mean value of 29.9 °C. An increase in thermal tolerance through early development has also been observed in lower Fraser white sturgeon. Hines et al. (unpublished) found that thermal tolerance increased from the start of their study, at 40 dph, then plateaued from 54 dph until the end of their study at

100 dph with mean thermal tolerance averaging 31.8 °C. The differences observed in CT_{max} values once plateaued could be driven by population differences. The lower Fraser population tends to experience warmer conditions during the summer months compared to the Nechako population, therefore, I'd expect CT_{max} to reflect this. Similar populational differences in CT_{max} have been observed in common killifish and diamondback watersnakes, *Nerodia rhombifer* (Fangue et al., 2006; Winne & Keck, 2005).

The increase in CT_{max} through early ontogeny may be driven by the transition through different life stages (Bowler & Terblanche, 2008; Gilbert & Lattanzio, 2016; Klockmann et al., 2017; Komoroske et al., 2014; Miller et al., 2013). Metamorphosis in white sturgeon, described as the transition between the feeding larval stage and the juvenile stage (Hildebrand et al., 2016), is poorly characterized and can occur anywhere between 20 and 45 dph (Buddington & Christofferson, 1985; Deng et al., 2002). Given the increased rate of development observed in treatment 12/15, the transition from feeding larvae to juveniles might have occurred sooner explaining the left shift in the transitional period of CT_{max}. When comparing these current results to those of Hines et al. (unpublished), the plateau in thermal tolerance occurred sooner at 30 dph as opposed to 54 dph. This could be explained by the fact that the YSL in the study by Hines et al. were not reared in substrate. Rearing YSL without substrate has been demonstrated to increase routine metabolic rate and consequently decrease aerobic scope (Boucher et al., 2018), as well as slow gut development (Baker et al., 2014) which could mean that less energy was available for growth and development. A slower rate of development would delay the transition from feeding larvae to the juvenile stage and delay the acquisition of thermal tolerance.

In the current study, early environmental temperatures experienced by white sturgeon have been shown to have long-lasting effects on development, growth, and development of

thermal tolerance with individuals hatching from 12 °C showing faster growth and development. However, no observed differences in CT_{max} at the early juvenile stage was found thus providing little evidence to support the idea that T_e has long term effects on thermal tolerance in white sturgeon. One possible explanation for these observations is that developmental thermal plasticity is not present in white sturgeon. Given the long lifespan of white sturgeon, establishing the basis for thermal tolerance during embryonic development may negatively impact them especially if early temperatures experienced are not indicative of future temperatures sturgeon may experience. Flies, by comparison, are more likely to experience similar thermal conditions during embryonic development and adulthood due to their shorter life cycles which may explain why Slotsbo et al. (2016) found significant effects of T_e on thermal tolerance in adulthood. An alternative explanation could be that the effects of developmental plasticity are short-lived under a common post-hatch environment. The advantages incurred when individuals are reared at higher T_e might not be beneficial post-hatch and could be energetically costly to maintain. Thus, post-hatch environmental temperatures and phenotypic plasticity may override any differences that would have been observable at hatch. Testing thermal tolerance immediately after hatch and through to 13 dph may provide more insight into the existence of developmental thermal plasticity and the duration of its effects.

This study has shown that variation in rearing temperature can produce different long-term responses within individuals of the same family. Although the temperatures used in this study are not fully representative of the thermal regimes that white sturgeon encounter in the wild nor are these a complete representation of potential responses, it is interesting to note that out of the temperatures tested, incubation of embryos at 12 °C seems to confer the most benefits within the measured responses. Simulating conditions similar to treatment 12/15 in a captive

setting for Nchako population white sturgeon could decrease the time required to produce larger individuals with possibly fewer mortalities than rearing at a constant 18 °C. This might be desired in the case of hatcheries hoping to release more progeny back into the wild given that larger individuals tend to be more adept at surviving.

Table 2.1 Time to 50% hatch for white sturgeon embryos incubated at 12, 15, and 18 °C.
 Significant differences in mean times to hatch are indicated by lowercase letters.

ATUs to 50% hatch				
T _e (°C)	Female 2522	Female 3622	Female 5903	Mean ± SEM
12	134	146	158	146 ± 6.9 ^a
15	130	130	130	130 ± 0 ^b
18	117	117	117	117 ± 0 ^c

Days to 50% hatch				
T _e (°C)	Female 2522	Female 3622	Female 5903	Mean ± SEM
12	11	12	13	12 ± 0.6 ^a
15	9	9	9	9 ± 0 ^b
18	7	7	7	7 ± 0 ^c

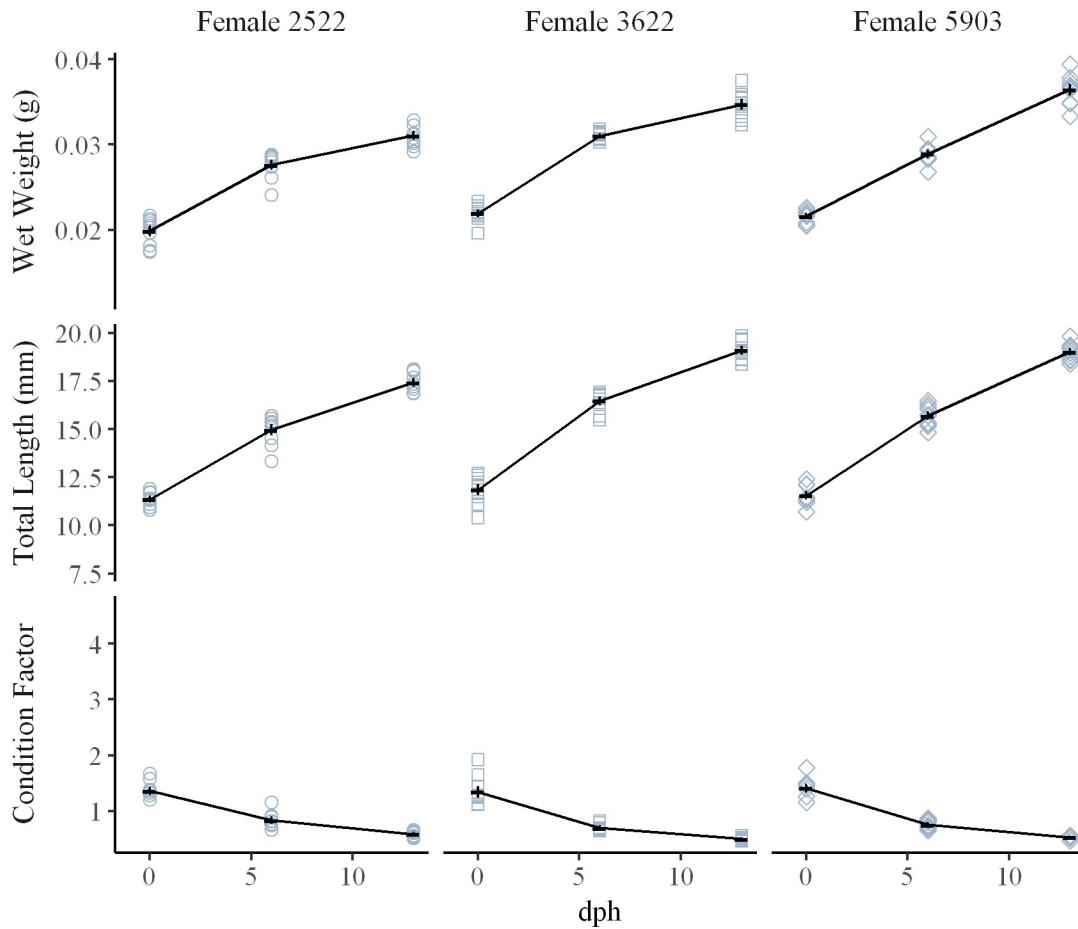


Figure 2.1 Changes through YSL development in wet weight, total length, and condition factor of three white sturgeon families incubated at 12 °C until hatch (Treatment 12/15). N = 10 fish per family at each time point. Open symbols represent individual data while familial means are indicated by the horizontal bar and error bars represent standard error. Significant familial differences were found for wet weight, total length and condition factor (one-way ANOVA utilizing a linear mixed-effects model, $F_{2,85} = 39.6$, $p < 0.005$).

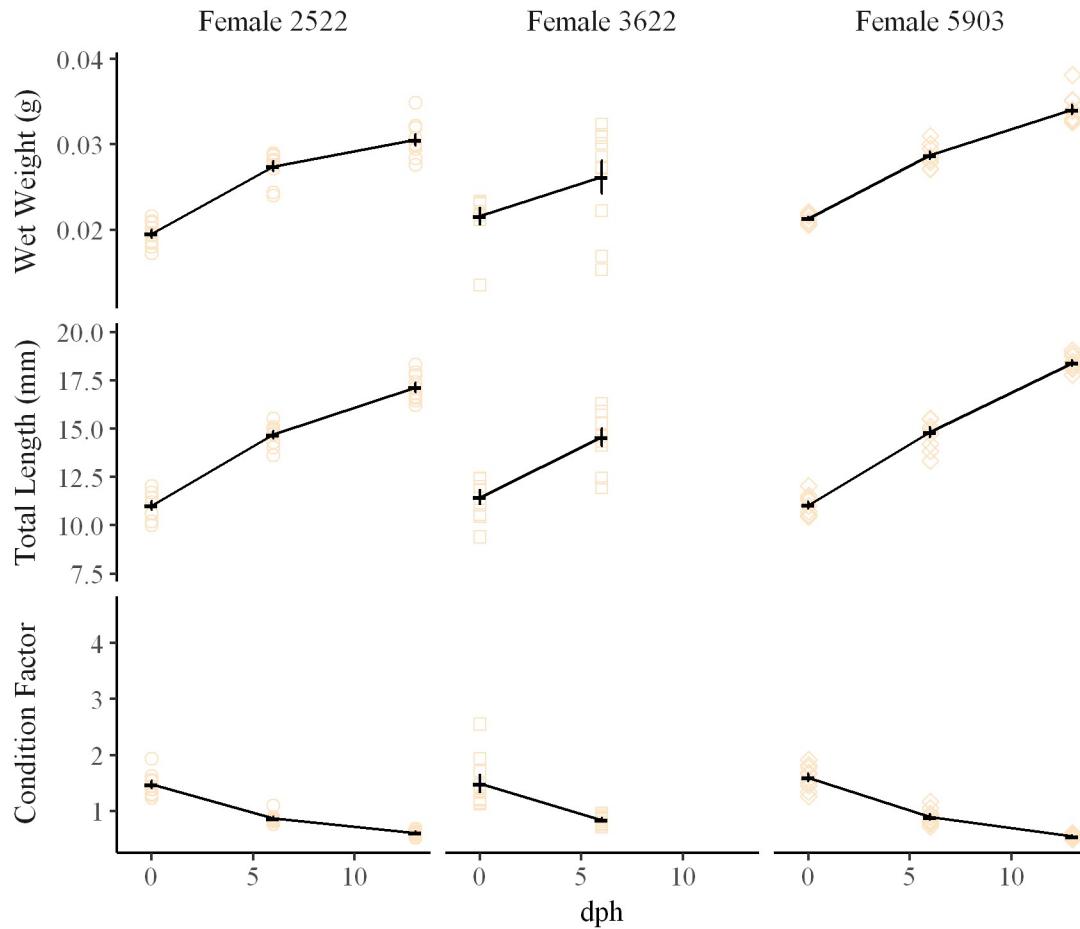


Figure 2.2 Changes through YSL development in wet weight, total length, and condition factor of three white sturgeon families incubated at 15 °C until hatch (Treatment 15/15). N = 10 fish per family at each time point. Open symbols represent individual data while familial means are indicated by the horizontal bar and error bars represent standard error. Due to low hatch success, female 3622 was not represented past 6 dph. Significant familial differences were found only for wet weight (one-way ANOVA utilizing a linear mixed-effects model, $F_{2,75} = 3.7, p < 0.05$).

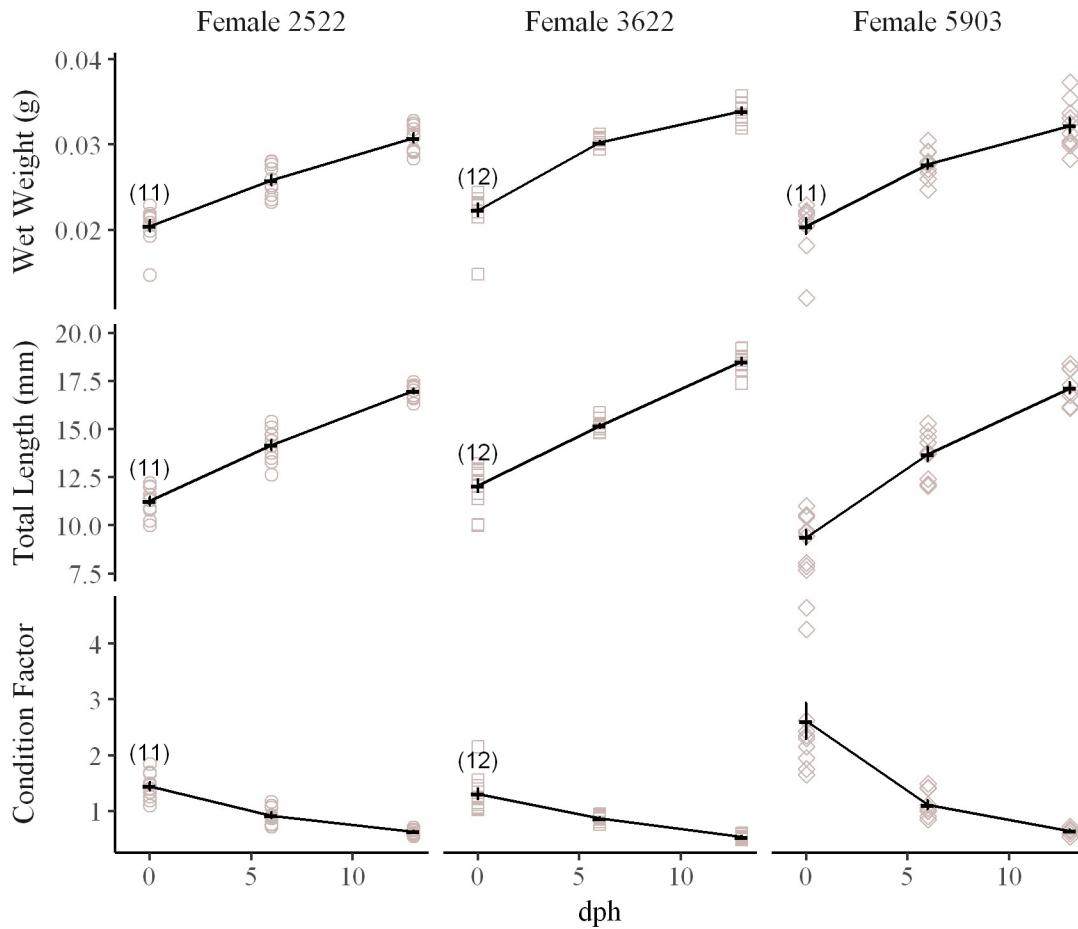


Figure 2.3 Changes through YSL development in wet weight, total length, and condition factor of three white sturgeon families incubated at 18 °C until hatch (Treatment 18/15). N = 10 fish per family at each time point unless otherwise noted in the figure. Open symbols represent individual data while familial means are indicated by the horizontal bar and error bars represent standard error. Significant familial differences were found for wet weight, total length and condition factor (one-way ANOVA utilizing a linear mixed-effects model, $F_{2,89} = 11.1, p < 0.0001$).

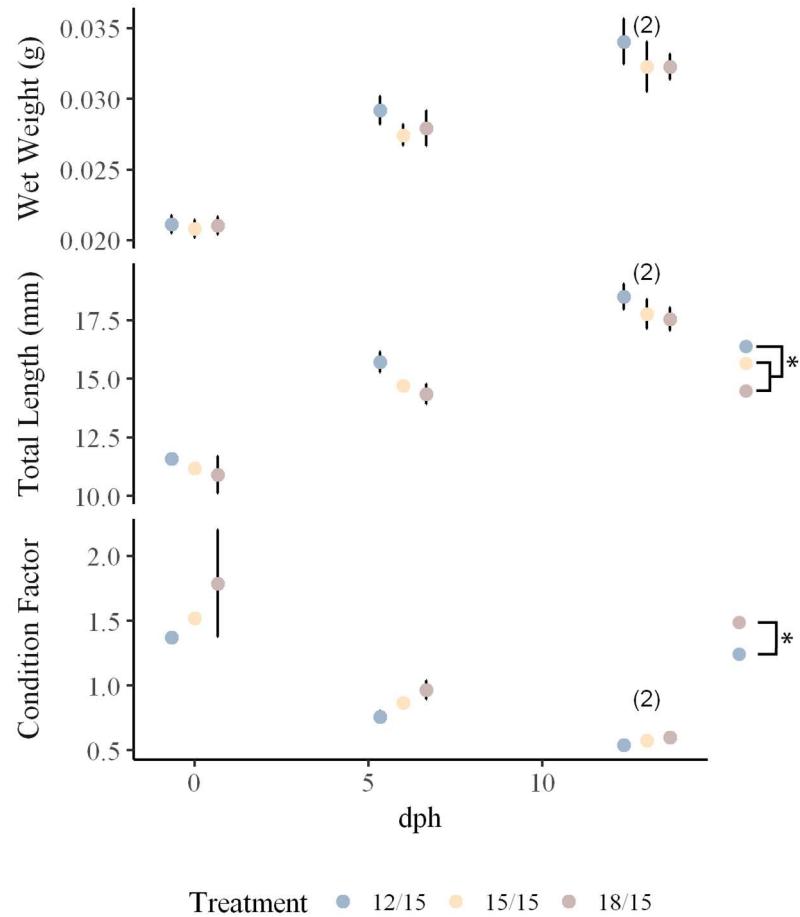


Figure 2.4 Overall means using averaged familial data of wet weight, total length, and condition factor of white sturgeon YSL incubated until hatch at 12 °C (Treatment 12/15), 15 °C (Treatment 15/15), and 18 °C (Treatment 18/15). N = 3 families at each time point in all cases unless indicated in the figure. Overall means are indicated by the circles and error bars represent standard error. No significant differences were found in wet weight and total length at hatch (one-way ANOVA, $F_{2,6} = 0.064$, ns and $F_{2,6} = 0.53$, ns, respectively) but significant effects of treatment temperature on total length and condition factor was found (two-way ANOVA without interaction, $p < 0.05$). Statistically significant differences between treatments are indicated by an * (Tukey post hoc, $p < 0.05$).

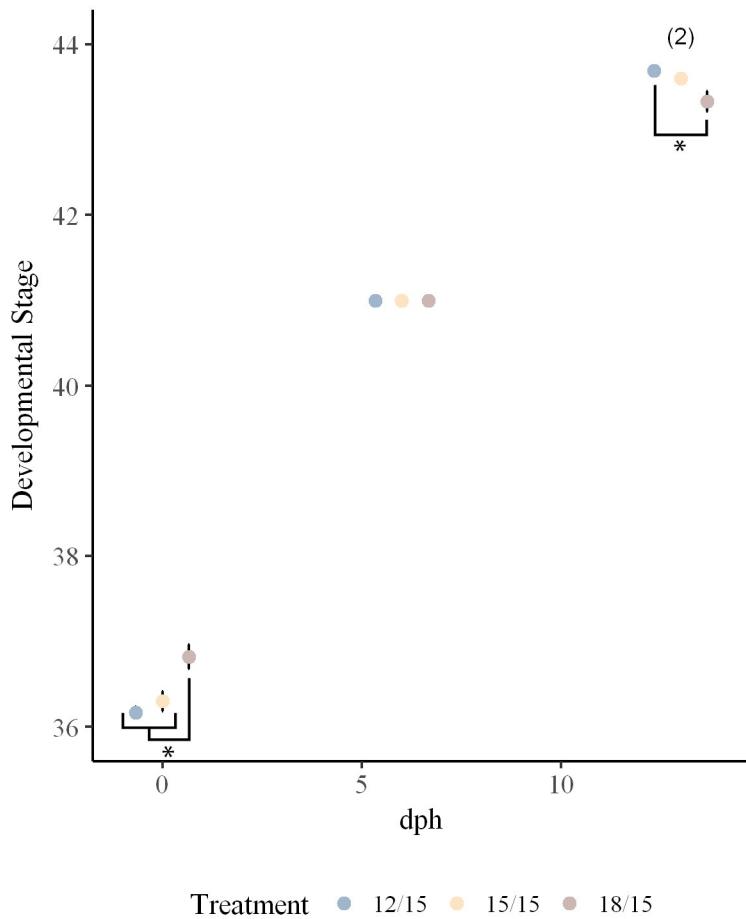


Figure 2.5 Overall mean developmental stage using averaged familial data of white sturgeon YSL incubated until hatch at 12 °C (Treatment 12/15), 15 °C (Treatment 15/15), and 18 °C (Treatment 18/15). N = 3 families at each time point in all cases except N = 2 for the 15/15 treatment at 13 dph. Means are indicated by the circles and error bars represent standard error. A significant interaction was found between treatment temperature and age on developmental stage (two-way ANOVA, $F_{4,17} = 11.3$, $p < 0.005$). Statistically significant differences between treatments are indicated by an * (Tukey post hoc using a simple effects analysis, $p < 0.05$).

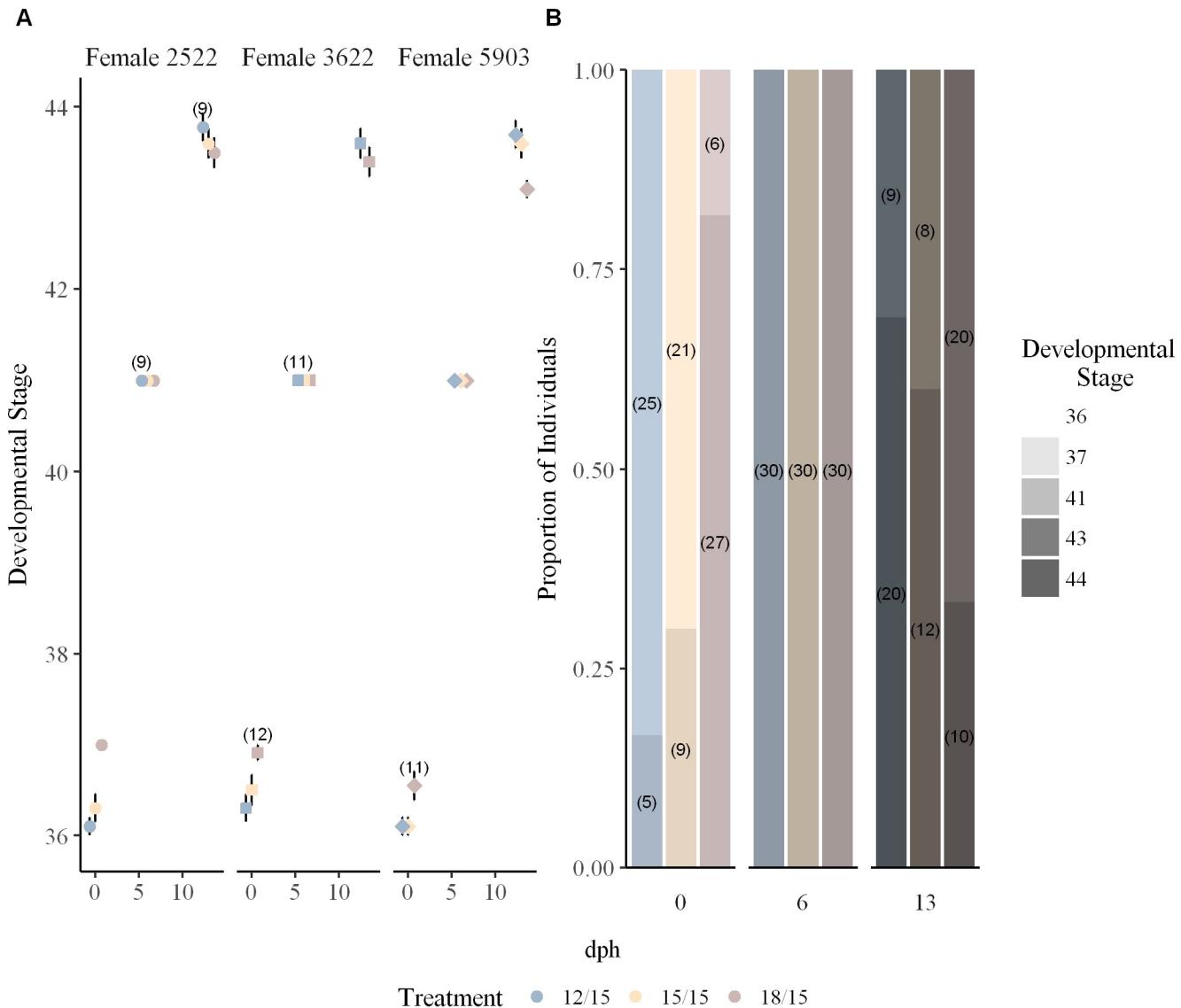


Figure 2.6 A) Mean developmental stages for YSL from three white sturgeon families incubated until hatch at 12 °C (Treatment 12/15), 15 °C (Treatment 15/15), and 18 °C (Treatment 18/15). N = 10 fish per family unless otherwise noted in the figure. Female 3633 from treatment 15/15 was not represented at 13 dph due to low hatch success. Means are indicated by the filled shapes and error bars represent standard error. B) Total proportion of sampled white sturgeon YSL at each developmental stage at 0, 6, and 13 dph for Treatment 12/15, Treatment 15/15, and Treatment 18/15. Number of individuals at each developmental stage are noted in brackets in each column.

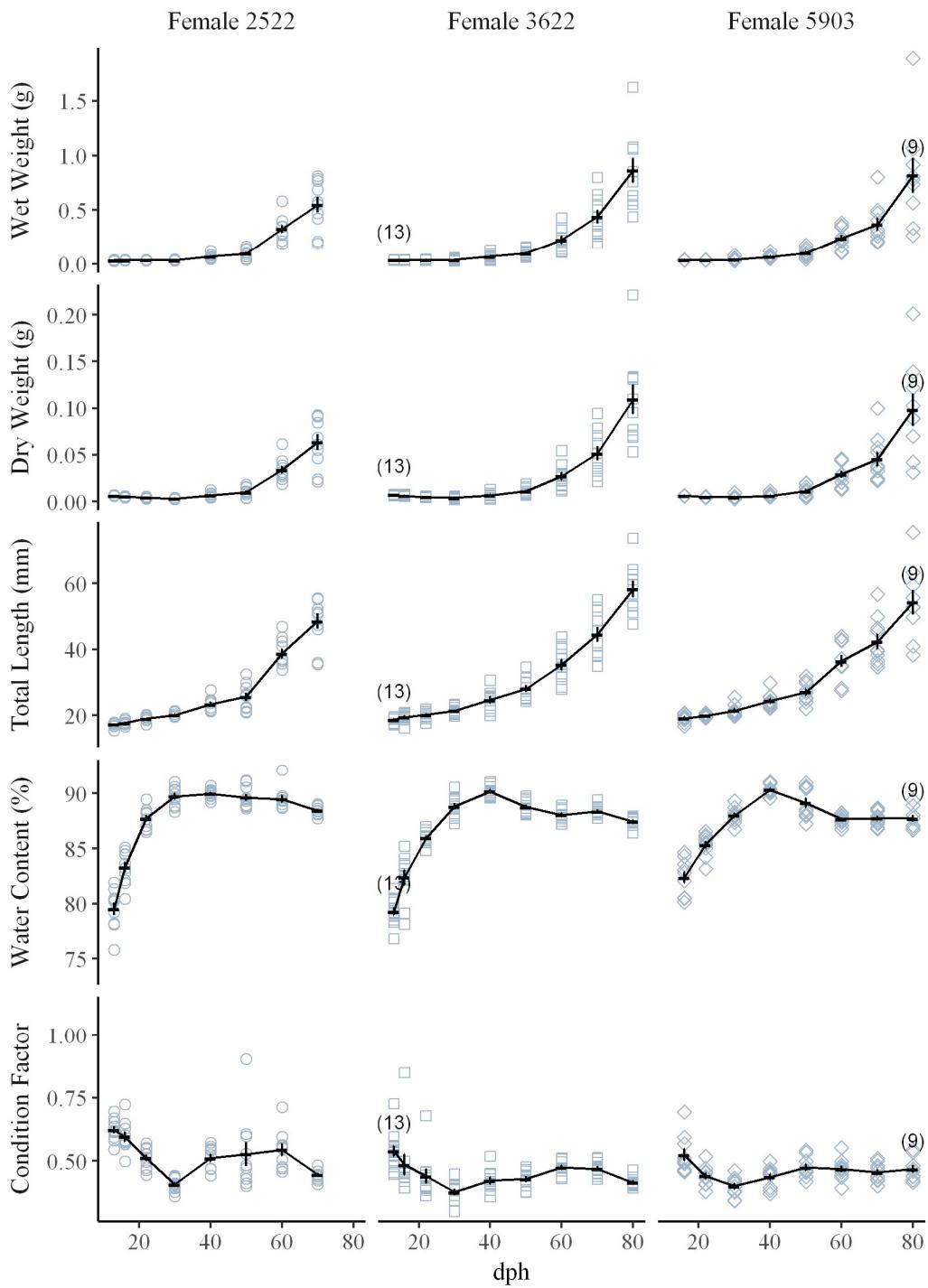


Figure 2.7 Changes in wet weight, dry weight, total length, water content, and condition factor of three white sturgeon families incubated at 12 °C until hatch (Treatment 12/15) from the onset of exogenous feeding to 80 dph. N = 10 fish per family at each time point unless otherwise noted in the figure. Open symbols represent individual data while familial means are indicated by the horizontal bar and error bars represent standard error. Significant familial differences were found for water content and condition factor (one-way ANOVA utilizing a linear mixed-effects model, $F_{2,241} = 19.1, p < 0.0001$ and $F_{2,241} = 26.3, p < 0.0001$, respectively).

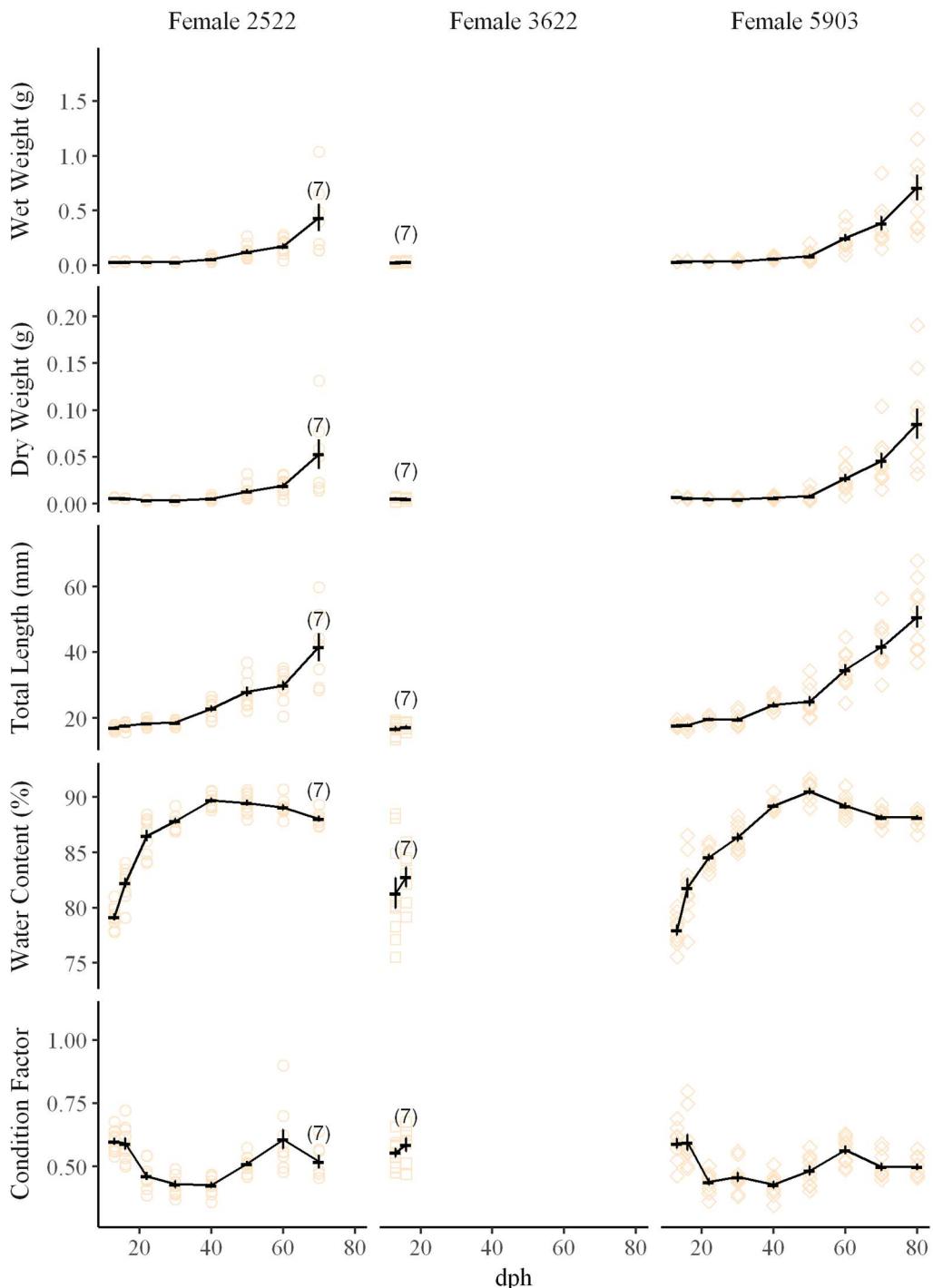


Figure 2.8 Changes in wet weight, dry weight, total length, water content, and condition factor of three white sturgeon families incubated at 15 °C until hatch (Treatment 15/15) from the onset of exogenous feeding to 80 dph. N = 10 fish per family at each time point unless otherwise noted in the figure. Open symbols represent individual data while familial means are indicated by the horizontal bar and error bars represent standard error. Due to low hatch success, female 3622 was not represented past 16 dph. Significant familial differences were found for dry weight and water content (one-way ANOVA utilizing a linear mixed-effects model, $F_{2,241} = , p < 0.05$ and $F_{2,173} = 9.5, p < 0.0001$, respectively).

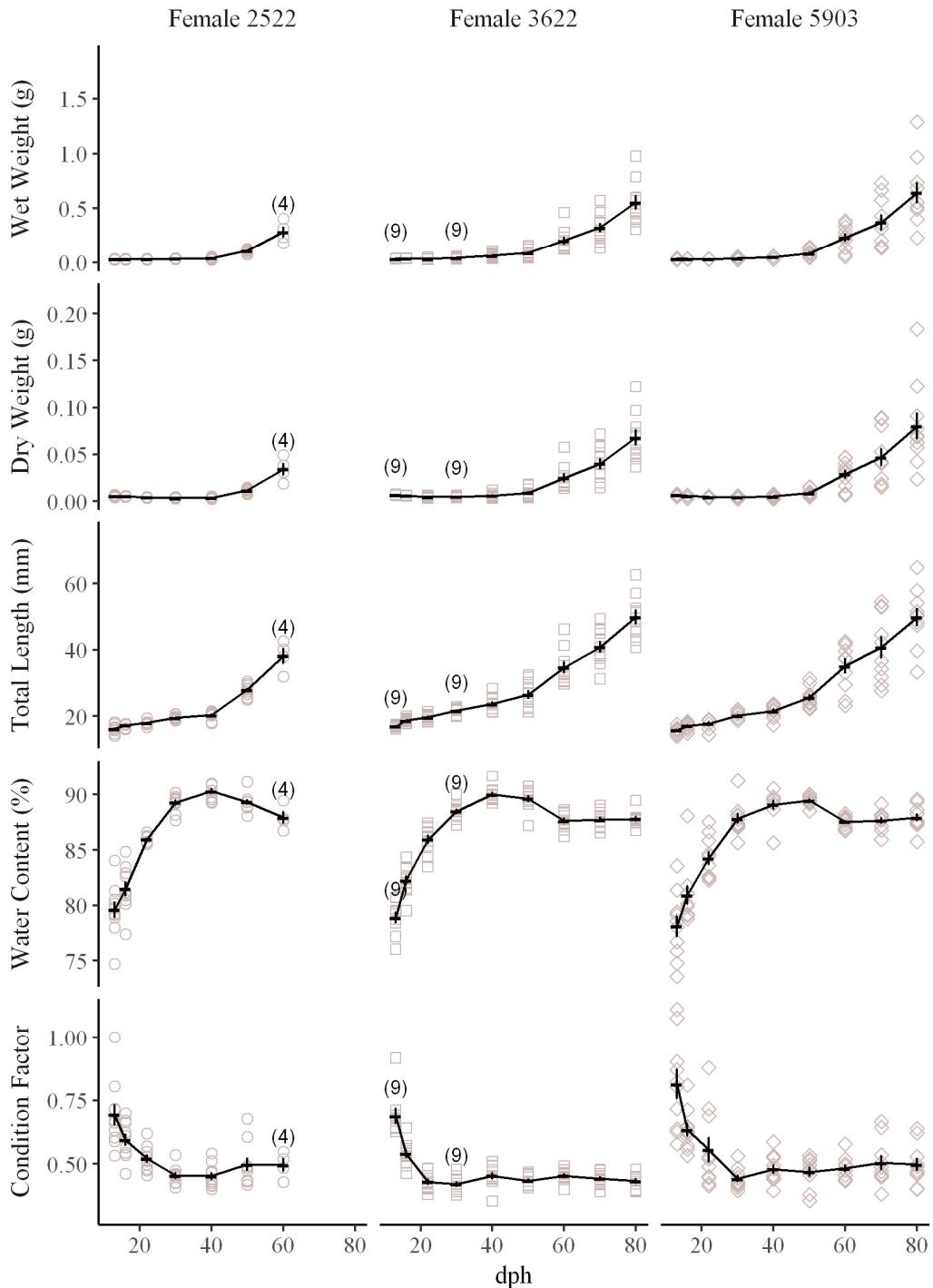


Figure 2.9 Changes in wet weight, dry weight, total length, water content, and condition factor of three white sturgeon families incubated at 18 °C until hatch (Treatment 18/15) from the onset of exogenous feeding to 80 dph. N = 10 fish per family at each time point unless otherwise noted in the figure. Open symbols represent individual data while familial means are indicated by the horizontal bar and error bars represent standard error. Significant familial differences were found for total length, water content, and condition factor (one-way ANOVA utilizing a linear mixed-effects model, $F_{2,231} = 5.6, p < 0.005$, $F_{2,231} = 7.9, p < 0.0001$, and $F_{2,231} = 15.9, p < 0.0001$, respectively).

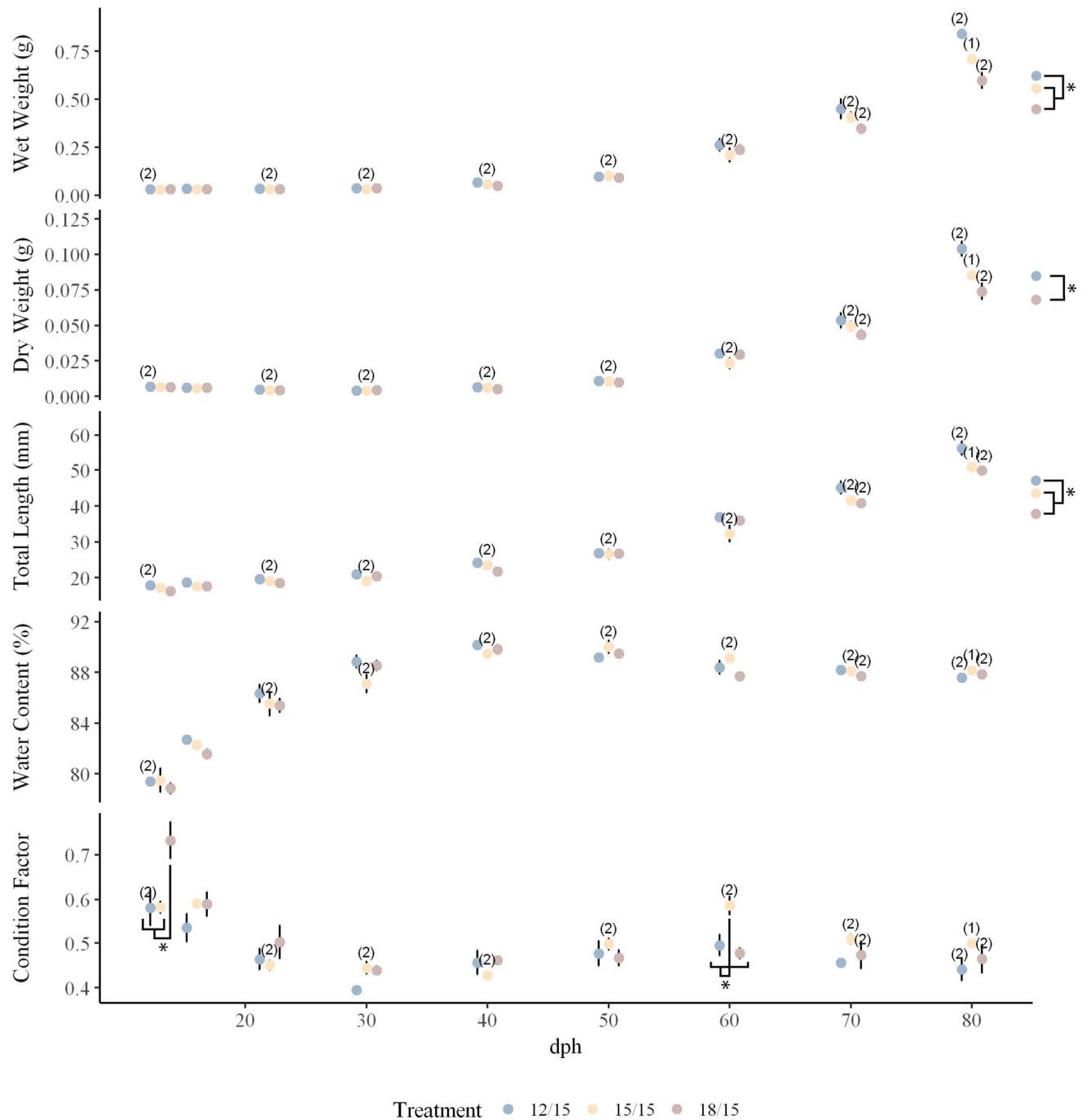


Figure 2.10 Overall means of wet weight, dry weight, total length, water content, and condition factor using averaged familial data of white sturgeon incubated at 12 °C (Treatment 12/15), 15 °C (Treatment 15/15), and 18 °C until hatch (Treatment 18/15) and then transferred to 15 °C through to 80 dph. N = 3 families at each time point unless otherwise noted in the figure. Overall means are indicated by the circle and error bars represent standard error. A significant interaction was found between treatment temperature and age for condition factor (two-way ANOVA, $F_{16,42} = 2.7, p < 0.01$). Treatment was found to have significant effects on all but water content (two-way ANOVA with interaction removed, $p < 0.05$). Statistically significant differences between treatments are indicated by an * (Tukey post hoc, $p < 0.05$).

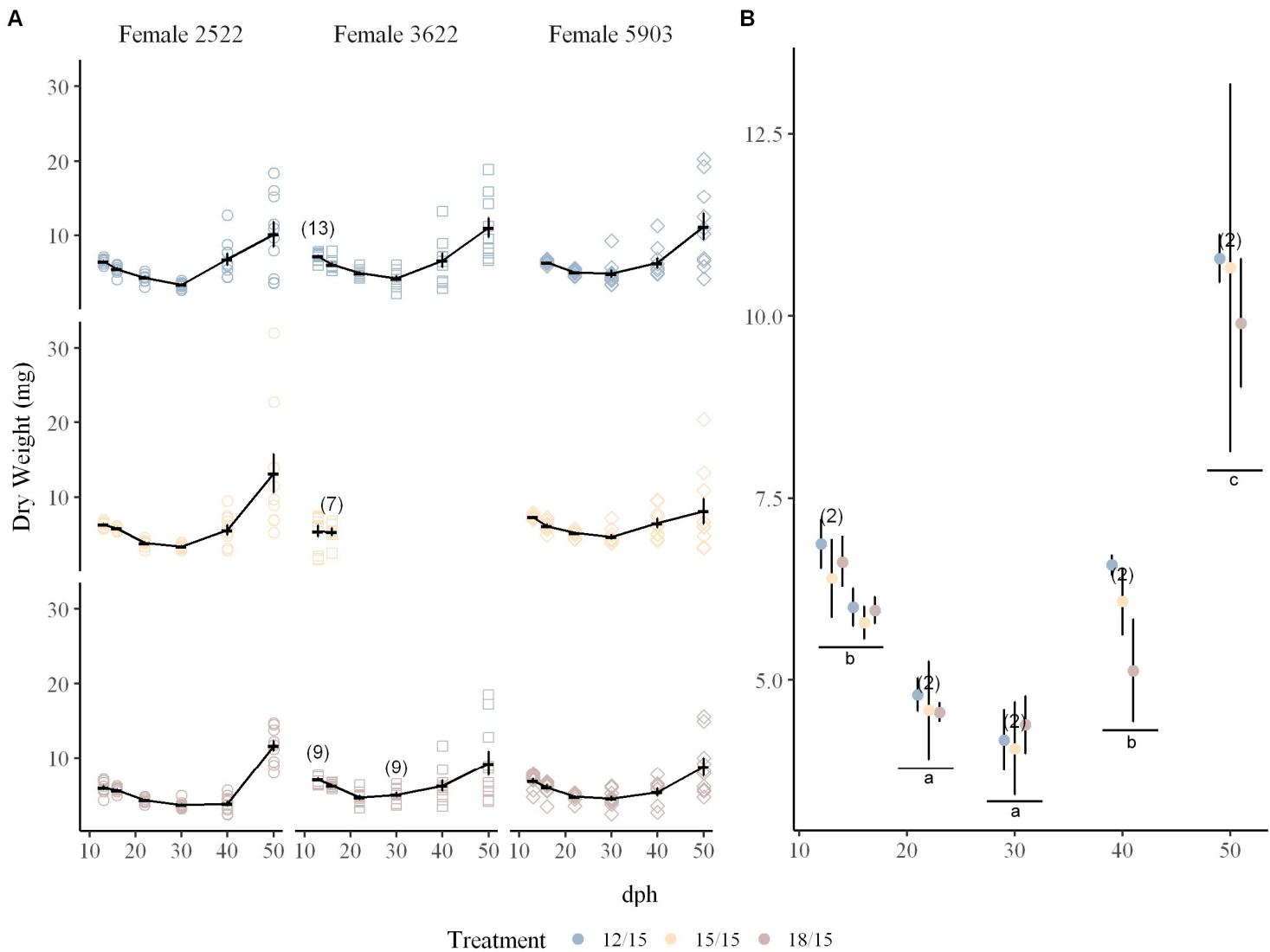


Figure 2.10.1. A redrawn figure concentrating on changes in dry weight (in mg) from exogenous feeding to 50 dph. A) Changes in dry weight for from onset of exogenous feeding to 50 dph for three white sturgeon families incubated at 12 °C until hatch (Treatment 12/15), 15 °C until hatch (Treatment 15/15), and 18 °C until hatch (Treatment 18/15). N = 10 fish per family at each time point unless otherwise noted in the figure. Means are indicated by the horizontal bar and error bars represent standard error. B) Overall means for dry weight using averaged familial data of white sturgeon incubated at 12 °C until hatch (Treatment 12/15), 15 °C until hatch (Treatment 15/15), 18 °C until hatch (Treatment 18/15) from the onset of exogenous feeding to 50 dph. N = 3 families at each time point unless otherwise noted in the figure. Overall means are indicated by the circle and error bars represent standard error. Statistically significant differences in dry weight at each age are denoted by letters (Tukey post hoc, $p < 0.05$). A significant decrease in dry weight was observed after 16 dph which did not increase again until after 30 dph.

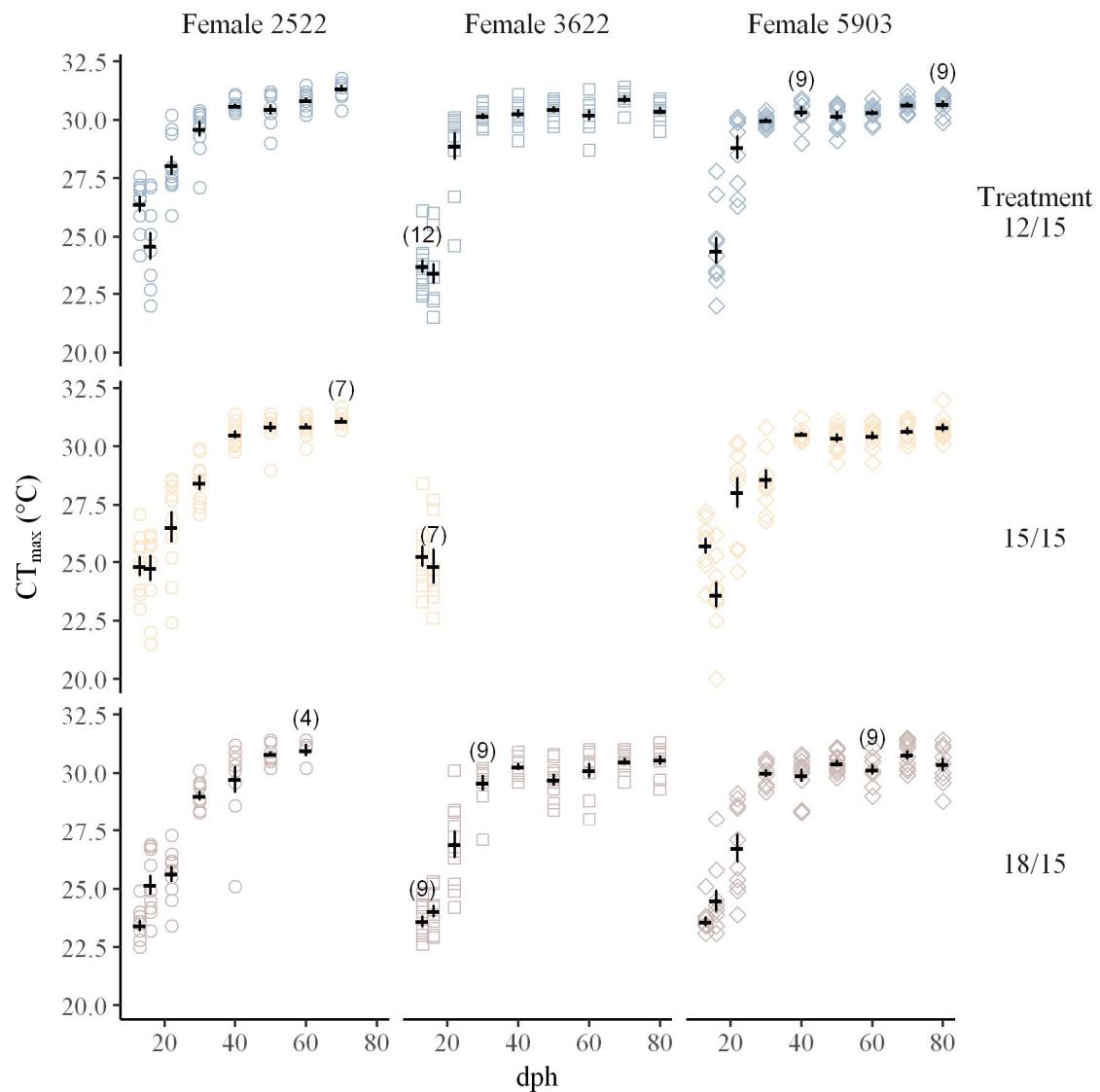


Figure 2.11 Changes in CT_{max} from the onset of exogenous feeding to 80 dph in three white sturgeon families incubated at 12 °C (Treatment 12/15), 15 °C (Treatment 15/15), and 18 °C (Treatment 18/15) until hatch and then reared at a constant temperature of 15 °C. N = 10 fish per family at each time point unless otherwise noted in the figure. Open symbols represent individual data while familial means are indicated by the horizontal bar and error bars represent standard error. Familial differences in CT_{max} were seen from exogenous feeding through to the early juvenile stage but only for treatment 12/15 (one-way ANOVA utilizing a linear mixed-effects model, $F_{2,239} = 5.3$, $p < 0.01$).

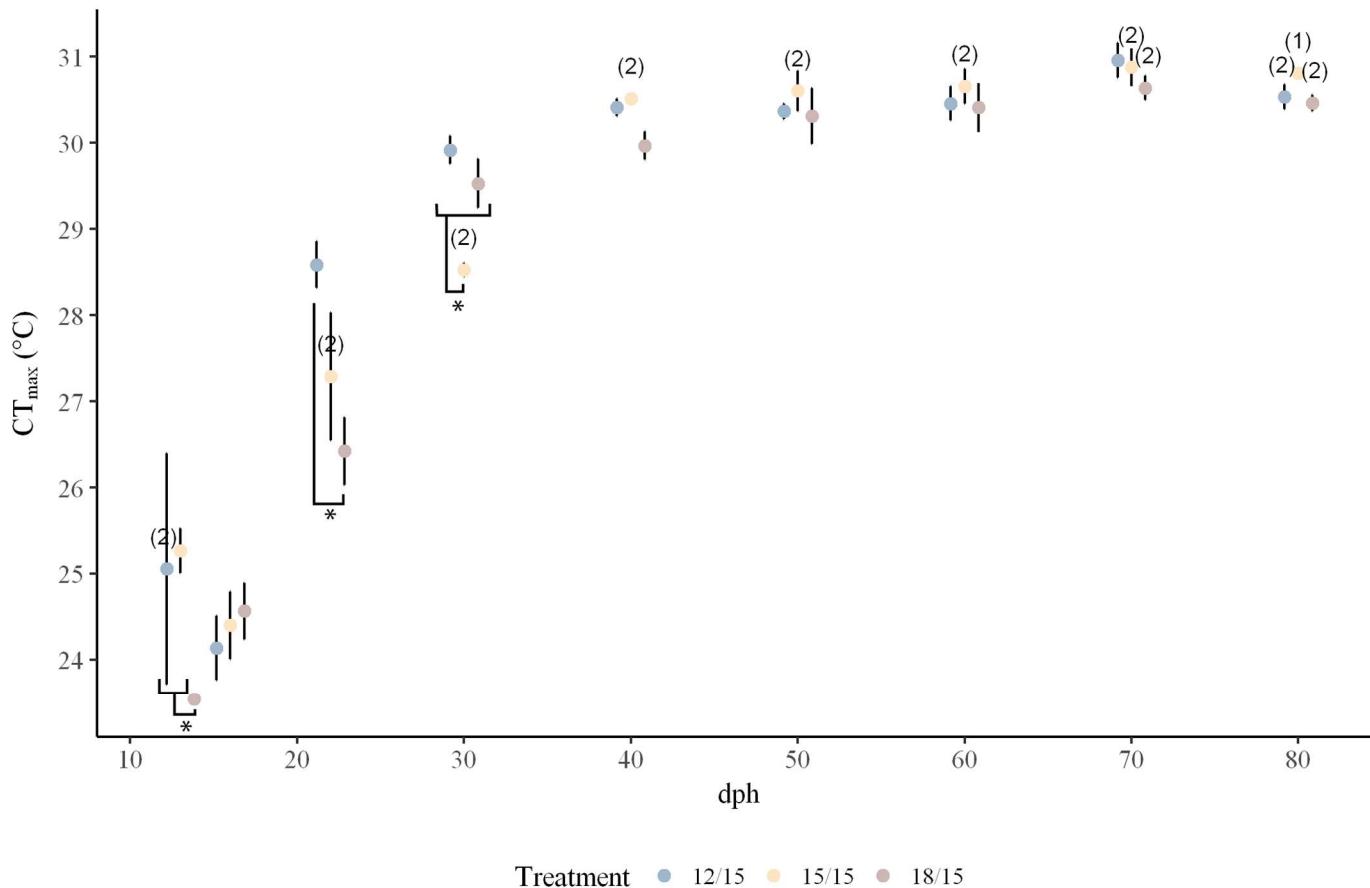


Figure 2.12 Overall means of CT_{max} using averaged familial data from the onset of exogenous feeding to 80 dph in white sturgeon incubated at 12 °C (Treatment 12/15), 15 °C (Treatment 15/15), and 18 °C until hatch (Treatment 18/15) and then reared at a constant temperature of 15 °C. N = 3 families at each time point unless otherwise noted in the figure. Overall means are indicated by the circle and error bars represent standard error. A significant interaction was found between treatment temperature and age (two-way ANOVA, $F_{16,42} = 2.7, p < 0.01$). Statistically significant differences between treatments are indicated by an * (Tukey post hoc using a simple effects analysis, $p < 0.05$).

Chapter 3: General Discussion and Conclusion

3.1 Thesis Summary

The overall objective of this thesis was to investigate the role of developmental plasticity under varying T_e on development, growth, and thermal tolerance through different life stages in white sturgeon. The ultimate goal is to better understand the effects of changing riverine temperatures on the early ontogeny of white sturgeon, to learn both about the basic life history of this threatened species and to use this information for white sturgeon conservation. In general, T_e was found to influence developmental rate through early ontogeny in white sturgeon which in turn, affected growth and development of thermal tolerance. Sturgeon exposed to treatment 18/15 showed rapid embryonic development but poor long-term responses in post-hatch development, subsequent growth, and delayed development of thermal tolerance. In contrast, sturgeon exposed to treatment 12/15 showed slow embryonic development but improved long-term responses in post-hatch development, subsequent growth, and earlier development of thermal tolerance.

From the results gathered, embryos incubated at 18 °C hatched in less time and at fewer ATUs than those incubated at 12 and 15 °C. At hatch, most individuals incubated at 18 °C were more developed, with rudimentary pectoral fin folds present, compared to those incubated at 12 and 15 °C. When reared from post-hatch at a common temperature, larvae exposed to treatment 12/15 showed compensatory development while the development of those exposed to 18/15 slowed over the following 13 days. At 13 dph, most of the individuals from treatment 12/15 had completed yolk absorption whereas most individuals from treatment 18/15 had not. Most individuals from treatment 18/15 at 13 dph were also less developed than those from treatment

15/15. With regards to growth, T_e had no effect on wet weight and total length of individuals at hatch but through the YSL stage, those exposed to treatment 12/15 were longer than those from 18/15 and 15/15. This was also consistently observed through the feeding and early juvenile stages. Wet weight and dry weight through the feeding and early juvenile stages for individuals from treatment 12/15 were also greater than those from treatment 18/15 and 15/15. Thermal tolerance, as approximated by CT_{max} , was shown to increase through early development and plateaued earlier for individuals exposed to treatment 12/15 compared to those from treatment 15/15 and 18/15. Critical thermal maxima, once plateaued, did not differ between treatments. Taken together, these findings indicate that for these 3 families generated from wild brood stock obtained from the Nechako, early rearing temperature has a large effect on subsequent growth and development, and that among the temperatures investigated here, incubation at 12 °C yielded the best performance. It should be noted that incubation of Nechako population embryos at 21 °C would not be recommended as it resulted in 100% mortality in preliminary trials. This upper thermal limit may only be applicable to this population as successful hatching has been observed at 21 °C in lower Fraser population white sturgeon (Baker, D., personal communication).

3.2 Research Limitations and Future Directions

While the goal of this thesis was to investigate the role of T_e on different measures of performance through early development in white sturgeon, the design of this experiment could not allow me to tease apart the effects of T_e from the effects of treatment temperature which consists of an interaction between pre- and post-hatch temperatures.

Changes in environmental temperatures during early ontogeny can dramatically affect development and metabolism. Across fish species, routine metabolism and development have

been found to roughly double with a 10 °C increase in environmental temperature during the early larval stage ($Q_{10} \approx 2.0$; Rombough, 1996). Individuals from treatment 12/15 experienced warmer post-hatch environmental cues. This increase in temperature would increase metabolism and development during the YSL stage which could explain the compensatory development observed in treatment 12/15. Conversely, individuals from treatment 18/15 experienced a relatively cooler post-hatch environmental temperature. This cooling would decrease metabolism and slow development during the YSL stage which could explain why individuals from treatment 18/15 were less developed compared to all other treatments at 13 dph. Looking at the mechanisms driving developmental rate given different pre- and post-hatch treatment temperatures are an area worthy of further investigation.

These differences in rates of development during the YSL stage associated with the interaction between pre- and post-hatch temperatures could also explain the variation in growth and development of thermal tolerance across treatments. At the onset of exogenous feeding when larval fish start actively searching for food, larvae of the same species have been found to reach similar lengths regardless of rearing temperature (Atlantic and shortnose sturgeon; Hardy & Litvak, 2004). Larval length invariably affects swimming performance which in turn influences feeding ability and predator avoidance (Martell et al., 2005). Because development and size appear to be correlated, YSL from the 12/15 treatment would be longer compared to same-aged individuals from the other treatments due to their increased rate of development associated with the compensatory growth described above. These longer individuals were observed in treatment 12/15 between 0 and 13 dph. Because exogenous feeding initiated sooner in individuals from treatment 12/15, those individuals were able to assimilate food earlier and grow more rapidly in relation to individuals of the same age from a different treatment, which was observed from 13

dph onward. Similarly, if thermal tolerance increases as development proceeds, as in other temperate species of fish (Rombough, 1996), then CT_{max} would also be expected to increase sooner in the 12/15 treatment associated with compensatory growth, as seen in the results of this experiment. Interestingly, higher temperature exposures during embryonic development in Nechako white sturgeon did not seem to increase heat tolerance later in development as seen in cases of flies (Slotsbo et al., 2016). It could be that developmental plasticity exists but the cost of maintaining thermal tolerance was too high and trade-offs were made given that post-hatch environmental cues did not match those experienced during embryonic development or it could be that developmental thermal plasticity does not exist in white sturgeon.

To better understand the role of developmental plasticity in subsequent development, growth and thermal tolerance in white sturgeon and to tease apart the effects of T_e from the effects of pre- and post-hatch environments, a full factorial study should be completed to allow for better insight into the observations of this study. Individuals from each T_e should be reared at 12, 15, and 18 °C post-hatch to parse out the effects of changing post-hatch temperatures from constant post-hatch temperatures and T_e . Sampling more frequently during the YSL stage will also help give better indications of when developmental changes are occurring and how individuals from each treatment respond to pre- and post-hatch temperatures. Following others who have studied sturgeon YSL development, a sampling interval of 12 h should be sufficient time to observe all the stages of development up to yolk absorption (Dettlaff et al., 1993; Jay, 2014; Wang et al., 1985). Lastly, earlier assessments of CT_{max} including points at hatch, 1, 2 and 4 days after may offer more insight as to whether developmental thermal plasticity exists and how long-lasting its effect is under varying post-hatch temperatures.

3.3 Ecological Implications and White Sturgeon Conservation

In the wild, increasing post-hatch temperatures would indicate impending seasonal warming of the riverine habitat, typically seen by spring spawning sturgeon. Faster development may allow sturgeon to take advantage of the increased food availability during warmer weather and increase their growth during this time. The increase in temperature could also signal upregulation of factors that confer thermal tolerance in anticipation of further temperature increases. While a reduction in post-hatch temperature would not be a normal occurrence in the wild, it could occur in impounded sections of the Columbia River. Spawning has been observed in the warmer Pend d'Oreille river and hatched larvae have been found to drift into the cooler mainstem Columbia river (McAdam, S.O., personal communication). This decrease in temperature would decrease metabolism and slow development which could be beneficial for a short period of time in that yolk reserves could be prolonged if food was not readily accessible in colder environments. However, slowed development over a long period may compromise individuals in the wild if they cannot find enough food before winter.

Although compensatory development along with increased growth and an earlier development of thermal tolerance was observed in Nechako white sturgeon exposed to a higher post-hatch temperature (treatment 12/15), it is uncertain how higher T_e , like 15 and 18 °C, and further increases in post-hatch temperatures of 18 and 21 °C, respectively, would affect these parameters. Additionally, it is unclear how larval development, growth, and development of thermal tolerance would change if temperatures increased more rapidly post-hatch (for example, a T_e of 12 to a post-hatch temperature of 18 °C compared to treatment 12/15). If riverine temperatures continue to rise with climate change and river impoundment, one would expect compensatory development to cease at some threshold temperature where routine metabolic rates

are too high to allow for substantial increases in growth and thus delay development of thermal tolerance. If this were to occur, increases in riverine temperatures would negatively impact juvenile sturgeon and further increase mortalities within the first year. Further investigation of the effects of global climate change and river impoundment on larval development, growth, and development of thermal tolerance in white sturgeon is needed.

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