Early Environmental Effects on Growth Rate, Muscle Development, and Swimming Performance in larval and age-0 Lake Sturgeon, *Acipenser fulvescens*

by

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Abstract

In fishes, differences in egg incubation, water temperature, and substrate can have a significant impact on phenotypic development. This is particularly relevant during the first year of life when growth rate peaks and influences an individual's life history trajectory. In the present study, Lake Sturgeon, *Acipenser fulvescens*, were reared in different environments for approximately one year. In the first year, sturgeon were reared in 3 temperature regimes (with or without substrate) that mimicked hatchery conditions or river temperature profiles. In the second year, embryos were incubated in MacDonald jars or adhered to substrate and newly hatched larvae were reared in different thermal environments (16, 18 and 20°C) for two weeks during the first month post-hatch. All treatments were then transferred to a common garden set-up where tanks were fed by river water at ambient temperature and natural light cycles. Fish were deprived of food when water temperature reached 1.5°C, then food was re-introduced when water temperature exceeded 2°C, approximately 4.5 months later. Yolksac volumes, body length and mass were assessed during both years. In the first year, there were differences in yolk sac absorption between substrate treatments in the first two weeks post-hatch, and differences in larval length between temperature treatments following the overwintering. In the second year, red muscle fibre area and myonuclear counts were assessed and volitional downward swimming and forced escape responses were tested. Growth rate in the first three months of life was highest in the 20°C group of fish but converged in all treatments during winter. Growth rate in fish raised in warmer waters during early life once again exceeded cooler water treatments post-winter, suggesting growth phenotype was fixed in early life, showing little phenotypic plasticity, and was temperature dependent. Thus, increased growing temperature during early life history results in development of a fast-growing phenotype post the first winter. Overall, there was an absence of significant differences between

results for muscle fibre area and myonuclear counts between incubation and temperature treatments and the presence of significant differences between time points indicating a strong evolutionary pressure to maintain plasticity in muscle structure and swimming performance.

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Chapter 1. General Introduction

In all organisms, interactions between the environment and genotype are flexible; the outcome for these interactions is expressed in terms of the capacity for phenotypic plasticity (Aubin-Horth and Renn 2009; Nylin and Gotthard 1998; Valladares et al. 2006). Early environmental experience, regardless of genetic makeup, has a significant impact on development in all organisms both in regard to proximate consequences of phenotypic development and the ultimate consequences of survival and therefore overall fitness (an individual's ability to compete and survive to reproduce successfully) of an organism. Environmental conditions shape phenotype and therefore reaction norms for subsequent environmental conditions later in life (Krubitzer and Kahn 2003; Nesan and Vijayan 2012; Monoghan 2008). Costs of poor early environmental conditions can be mitigated by phenotypic plasticity in adult life-history decisions when encountering improved environmental conditions (Auer 2010). The success of individuals depends on their ability to respond to environmental variability without compromising their fitness (Pörtner and Farrell 2008). Variability in environmental conditions exerts selection pressure that influences the evolution of phenotypic plasticity (Seebacher et al. 2012). Differences in rearing environments for fish can result in differences in behaviour (Maynard et al. 1996), growth rate (Gessner et al. 2009), survival post-stocking (Wiley et al. 1993; Maynard et al. 1996), muscle development (Johnston 2006) among many other factors. Development of these traits under different conditions can result in distinct phenotypes later in life for traits that are fixed early in life, or similar phenotypes for traits that are plastic depending on current conditions.

In the wild, one environmental condition that Lake Sturgeon, *Acipenser fulvescens,* are known to select following hatch is substrate. Despite being poor swimmers, yolksac larvae will drift to source suitable habitat for the avoidance of predators (Peterson et al. 2007) while using

their large yolksacs for energy until the onset of exogenous feeding. Lake Sturgeon yolksac larvae tend to prefer 20-50 mm gravel as substrate for this life stage (Hastings et al. 2012), which must have adequate interstitial space to be effective in decreasing predation (McAdam 2011). Substantial plasticity in the distances travelled prior to settling on a suitable habitat has been demonstrated in Lake Sturgeon yolksac larvae (Hastings et al. 2012). This plasticity appears to be retained in adults and used to maintain fitness as witnessed by the distribution and migration patterns of adults (Forsythe et al. 2012; Shaw et al. 2013).

In laboratory/aquaculture environments, the presence of substrate in rearing tanks influences sturgeon phenotypic development. Substrate presence has been shown to affect growth rate and survival in several sturgeon species (Boucher et al. 2014; Gessner et al. 2009). Substrate preference has also been shown to influence swimming behaviours of sturgeon larvae but may be species specific (Nguyen and Crocker 2006) demonstrating clear interactions between environment and developing phenotype.

Of all environmental parameters, temperature in the aquatic environment could be considered the most pervasive particularly in regard to growth during early life in White Sturgeon, *A. transmontanus,* (Boucher et al. 2014). Water temperatures have been shown to influence growth rates and mortality in Lake Sturgeon (Wang et al. 1987). Similarly, temperature of larval rearing environments can also affect muscle fibre development in several species of fish (Galloway et al. 1998; Galloway et al. 1999; Johnston et al. 1998; Macqueen et al. 2008).

Lake Sturgeon are part of the family Acipenseridae, with 27 species of sturgeon and paddlefishes across the northern hemisphere being classified as endangered (Nelson et al. 2013). The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) considers Lake Sturgeon as “Endangered” (species facing imminent extirpation from the area) in two of the three

designable units where populations are known to exist in Canada and classifies them as "Special Concern" in the remaining unit (COSEWIC 2017). The life history traits of sturgeon typically include long lifespan, slow growth, late sexual maturity, and irregular spawning behaviour (Scott and Crossman 1973).

Lake Sturgeon are most vulnerable to mortality during the first year of life in particular the period between fertilization to approximately 250 mm fork length when developmental abnormalities can be a major source of mortality. The increased risk of mortality to predation during early life can also have profound consequences on population recruitment (Forsythe et al. 2013) thus the early rearing environment is critical in understanding mitigative strategies to be used to aid in population recovery efforts (Auer and Baker 2002).

Wild populations of Lake Sturgeon have declined in the past centuries due to overfishing, pollution, and degradation of natural habitats in combination with the unique life history traits of the species (as described above) (Billard and Lecointre 2000; Bronzi and Rosenthal 2014; Peterson et al. 2007). Habitat degradation is due to a variety of factors including but not limited to hydro-electric development and mitigative strategies have included restrictions on fishing, habitat restoration and juvenile stock enhancement programs (Billard and Lecointre 2000) which are particularly important for regions where wild populations are near extirpation (Auer 2004). Restoration stocking of Lake Sturgeon using individuals raised in a hatchery environment during the first year of life has been used across the natural range of the species including New York (Jackson et al. 2002), western Lake Superior (Schram et al. 1999) and the Nelson River, Manitoba. However, our understanding of the effects of hatcheries on phenotypic development in Lake Sturgeon is limited. Previous research has shown that there was no significant difference in survival due to rearing environment or size at the time of release (Crossman et al. 2009). However,

it is important for the ultimate success of the stock enhancement programs that hatchery-reared individuals released into wild populations possess phenotypes similar to wild individuals.

The research proposed will inform hatchery practices currently employed in the rearing of Lake Sturgeon for conservation purposes to better understand interactions between the environment and developmental phenotype during the most critical and vulnerable stage of life for Lake Sturgeon, the first year. The research will aid in development of best practices for rearing of Lake Sturgeon destined for stock enhancement programs by providing a better understanding of the environmental impacts of rearing conditions on phenotypic development. Ultimately leading to the goal of improved population growth and sustainable populations. This research will test the prediction that a more complex environment during early life stages will result in a phenotype more reflective of the wild-type. Specifically, chapter 2 describes the effect of substrate, temperature, and incubation treatments on growth rate during the first year of life in two cohorts of Lake Sturgeon. Chapter 3 describes the effect of embryo incubation, temperature and age on muscle development during the first year of life in Lake Sturgeon. And chapter 4 describes the effect of embryo incubation, temperature and age on voluntary and forced swimming performance during the first year of life in Lake Sturgeon. I hypothesize that different rearing environments will influence the growth rate, muscle development and swimming performance of Lake Sturgeon throughout the first year of life.

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Chapter 2. Growth Rate

Introduction

In teleosts, it has been shown that in general, individuals with higher growth rates in early development have a survival advantage over slower growing individuals through enhanced resistance to food deprivation and better tolerance of environmental extremes which leads to greater fitness (Sogard 1997). In most fishes, growth rate peaks during early development (Kurita et al. 2004; Tracey and Lyle 2005) and is highly heritable, as shown in Coho Salmon, *Oncorhynchus kisutch,* (Tymchuk et al. 2006) but also depends on environmental factors such as temperature and incubation time (Crossman et al. 2014). Individual size is particularly relevant in northern species where survival during the first winter of life is critical for subsequent fitness (Cargnelli and Gross 1997). The survival and fitness of individuals in northern populations depends largely on stored energy reserves accumulated prior to the winter when food intake may not be sufficient to maintain body mass (Keast 1968). In northern populations, high growth rates early in life are associated with larger size, better energetic condition after the overwintering period, and therefore higher fitness levels (Cargnelli and Gross 1997).

Growth rate in fishes is a key performance metric that has a profound effect on an individual's life history trajectory, influencing physiological measures such as swimming performance which dictates the ability of fish to forage, escape predation or disperse (Stobutzki and Bellwood 1997), or homeostatic regulation of water and ion balance particularly in anadromous fish species (McCairns and Bernatches 2009). To increase growth rate and gain the accompanying advantages, trade-offs exist, such as altered swimming performance in transgenic Coho Salmon (Farrell et al. 1997), or immunity in Coho Salmon (Jhingan et al. 2003). A trade-off found in hatchery raised trout populations showed a behavioural change through an increased willingness to take riskier foraging behaviour in order to maximize growth rate, but this was not

observed in wild populations due to the increased risk of predation with such foraging behaviour (Biro et al. 2004).

Variability in growth rates among individuals in a population is important for maximizing age-specific survival and reproductive success (Roff 1984). In arctic char, growth rates among populations reared in isolation were more variable than those reared or fed in groups (Jobling and Reinsies 1986). This variation can have other environmental influences, for example Boucher et al. (2014) found that water temperature and presence of substrate affected growth rate and survival of White Sturgeon, *Acipenser transmontanus*, yolksac larvae. This variation in growth rate within populations can increase, decrease or remain stable over time (Pfister and Stevens 2002).

Water temperature is considered the most important environmental factor influencing fish development. Rearing temperature has been shown to affect growth rate, survival and body morphology in Coho Salmon, where higher temperatures led to smaller body sizes (Löhmus et al. 2010). The influence of temperature can occur during the incubation phase with higher temperatures decreasing the incubation time before hatch in Atlantic Salmon, *Salmo salar*, (Nathanailides et al. 1995). It can also occur later, in White Sturgeon, the highest rate of yolksac depletion occurred at the highest temperature (20 ˚C) (Wang et al. 1987). And White Sturgeon larvae at 46 days post hatch (dph), reared in 17.5 ℃ water were larger than those reared in 13.5 ℃ water (Boucher et al. 2014).

During the winter, temperatures in north temperate lakes and rivers often drops to near freezing and many systems are ice-covered for extensive periods in the year. In addition to the low overwinter temperatures, food becomes scarce during this period. The decrease in food availability means that fish cannot feed to satiation and this can negatively impact their growth rates as seen in yearling Rainbow Trout, *Salmo gairderi*, that received limited food and had lower growth rates

than yearlings fed to satiation (Weatherley et al 1979). Similarly, Channel Catfish, *Ictarulus punctatus,* that fed to satiation grew more than the fish that were partially fed or were starved for 3 months, and the starved fish decreased in mass over the length of the starvation period (Kim and Lovell, 1995).

Another environmental condition that can affect growth rates in sturgeon is the presence or absence and type of substrate. Substantial adaptive capacity to early rearing environments has been shown in Atlantic Sturgeon, *A. oxyrinchus,* where the presence of substrate influences growth rate (Gessner et al. 2009). In White Sturgeon larvae, those reared with small gravel (12-19 mm) substrate showed the highest survival rates (McAdam 2011). The onset of exogenous feeding in Atlantic Sturgeon happened one day later in larvae raised with gravel substrate compared to those raised in environments without any gravel substrate (Gessner et al. 2009). After the onset of exogenous feeding, those individuals raised over gravel had a growth rate, in wet mass, that was significantly higher than in the sand or control group due to compensatory growth (Gessner et al. 2009).

It is evident therefore that fish incubated and/or reared in environments with colder temperatures, less food availability and an absence of preferred substrate display a reduced growth rate. This chapter describes the effect of substrate and temperature on growth rate during the first year of life in two cohorts of Lake Sturgeon. In 2016, Lake Sturgeon, *A. fulvescens,* were reared from embryo with and without gravel substrate and in different temperature profiles. In 2017, Lake Sturgeon were reared from embryo in different temperature profiles. I hypothesize that different rearing environments will influence the growth rate of Lake Sturgeon. I predict that Lake Sturgeon reared in colder water with a lack of substrate would have lower growth rates than those reared in warmer water with substrate. Further, I predict that fish exposed to a prolonged drop in temperature

and deprivation of food (simulating an overwintering event) would exhibit a cessation in growth. When water temperatures were increased, and food was returned after winter I predict that fish that displayed a higher growth rate prior to winter, as a result of increased temperature during early rearing, would continue to exhibit that phenotype following winter.

Methods

2016-2017 cohort

Gamete collection

In May 2016 candidate female and male spawning adult Lake Sturgeon were captured by gill net (6 hours soak time) and placed in holding tanks on shore at Landing river in Northern Manitoba (55 deg 19' 30.91" N, 96 deg 54' 23.81" W). To induce final oocyte maturation and spawning behaviour, male and female fish were administered with an intra muscular injection of 10 µg/kg dose of Gonadotropin Releasing Hormone (GnRH) followed by a second dose of 20 µg/kg 12-20 hours later. This technique has been used routinely in the coordinated collection of eggs and milt in spawning adult Lake Sturgeon (Zubair et al. 2012). Once collected, fertilized or unfertilized eggs (see below) were transported from Landing River to the Manitoba Hydro Grand Rapids Fish Hatchery (GRFH) in Grand Rapids, Manitoba (Figure 2.1).

Figure 2.1. Map of Manitoba showing locations of spawning sites and Lake Sturgeon rearing. The dark green star indicates the Landing River spawning site. The light green star indicates the Grand Rapids Fish Hatchery rearing site in Grand Rapids, MB. Map modified from: https://www.thinglink.com/scene/581897367964352514

Fertilization and embryogenesis

Fertilized eggs had one of two destinations. For hatchery destined eggs, for every ml of eggs an equivalent amount of river water was added and immediately followed with approximately 20µl of sperm per ml of eggs evenly split between at least two males. The gametes were gently swirled for approximately 1.5 minutes to enable fertilization of the eggs. Water was then drained and a solution of river water plus Fullers earth (~20g.L^{-1}) was added to the fertilized eggs. The eggs were gently rolled by hand in this mixture to inhibit adhesion of the eggs in preparation for

incubation in MacDonald hatching jars at GRFH. On arrival at the hatchery embryos were disinfected by soaking in Ovadine for 10 min at a concentration of 100 ppm then rinsed with fresh water before they were added to the MacDonald jars where they were gently rolled for the duration of embryogenesis prior to hatch.

The eggs designated for the experimental setup at GRFH were transported unfertilized to the hatchery. Upon arrival at the hatchery these eggs were fertilized using the proportions described above and added to the experimental tanks in a multistressor unit (MSU) (Aquabiotech, Coaticook, QC, Canada). The MSU had three rows of six 9 L tanks, each row was evenly split with three tanks containing sinking bioballs to act as substrate and three tanks without substrate. The flow rate was kept even between tanks at the reccommended rate for the tank size. Eggs were allowed to adhere to the substrate, or base of the tanks in the MSU. The substrate was provided for developing larvae to use as refuge at hatch. The following temperature treatments were used for each row of the MSU:

1) Hatchery treatment – temperature profiles mimicked the temperature profile of the GenPop fish (see below)
2) Stocking treatment – temperature profiles mimicked the temperature of the GenPop fish until mid-October when switched to mimic ambient river temperature as closely as possible
3) Ambient treatment – the temperature followed the natural River temperature as closely as possible

Dissolved oxygen in all tanks of the MSU was maintained at 100% saturation and light levels were maintained by the MSU based on the GPS coordinates at Grand Rapids, hatchery throughout the year.

Hatch and on-growing of fish

Upon hatching, larvae in the hatchery were reared by staff in standard hatchery troughs supplied by well water held at 14-16 °C throughout the entire year. Light cycles were approximately 14 hours light: 10 hours dark until early September then 10 hours light: 14 hours dark for the remainder of the year. Fish reared by hatchery staff will henceforth be described as the general population (GenPop) treatment. To maintain optimal stocking density for growth throughout the year GenPop treatment fish were periodically removed for stocking purposes or sacrificed by hatchery staff. For the fish in the MSU that had substrate (sinking bioballs) in the tank during embryogenesis the bioballs were removed at the onset of exogenous feeding and were replaced with sand to reflect the typical substrate preference of Lake Sturgeon at this life stage.

All fish in the GenPop and experimental treatments were fed a diet of brine shrimp, *Artemia salina,* ad libitum 3 times a day at the onset of exogenous feeding for approximately 3 weeks. At which point a mix of brine shrimp and blood worm, *Glycera*, was fed to all fish with the proportion of bloodworm increasing as the fish grew when it became 100% of their diet after approximately 3 weeks. In early December 2016, the temperature of the MSU for the stocking and ambient treatments was reduced to reflect overwintering conditions (3.5˚C). It is recognized that actual river temperature during winter was less than this, however 3.5°C was the lower limit that the MSU could reliably maintain. Fish in the stocking and ambient treatments were deprived of food for 41 days as part of an overwintering event simulation. During the overwintering period, the daylight intensity was reduced from 100% to 10% for the stocking and ambient treatments to simulate a reduction in light in the river due to ice cover. Figure 2.2 illustrates the temperature profiles and accumulated thermal units for all treatments in the 2016-2017 cohort.

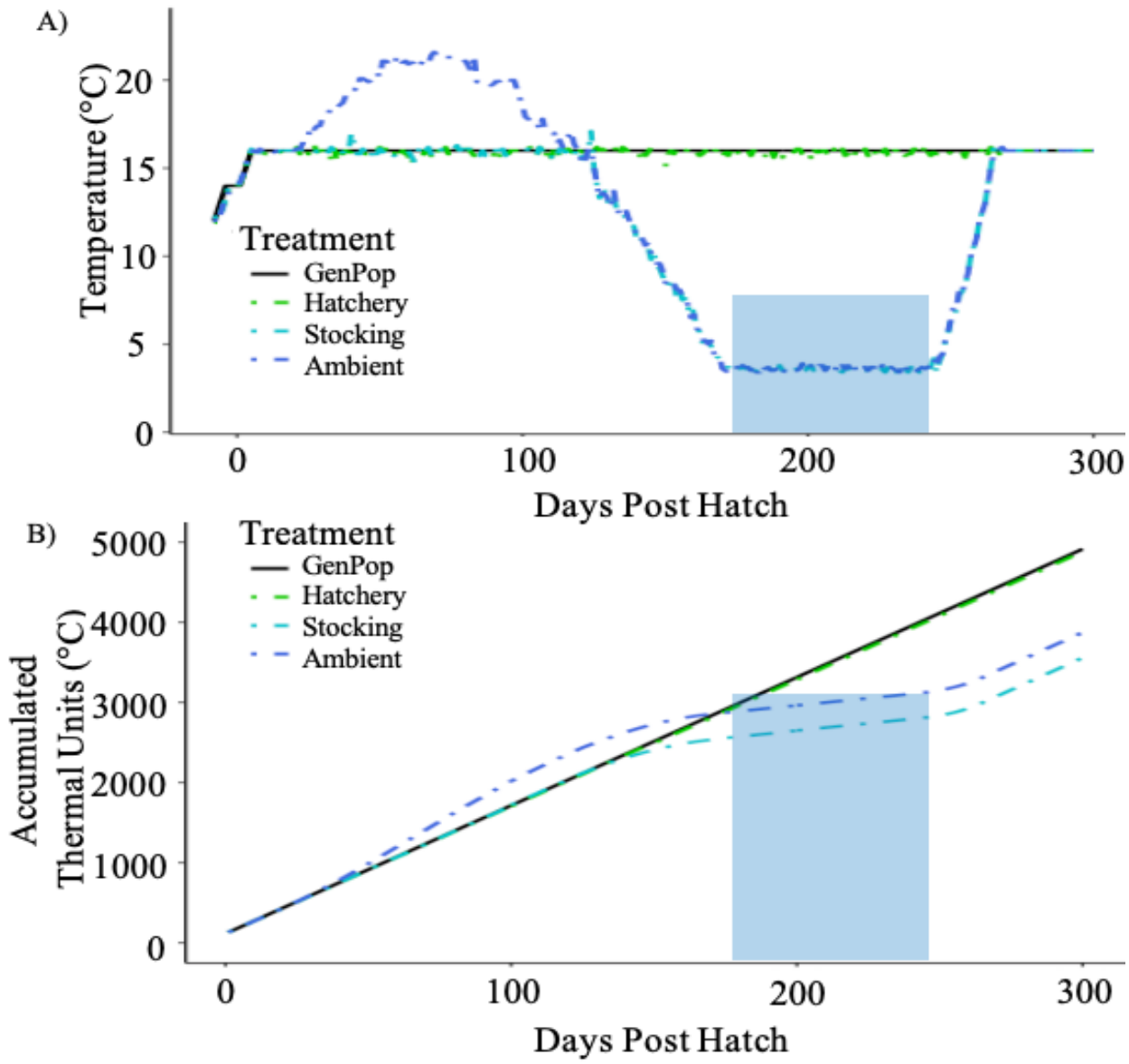


Figure 2.2. Temperature profiles of rearing tanks in the multistressor unit (dot-dashed lines) and general population (solid line) for Lake Sturgeon during the first experiment (2016-2017). The blue box marks the overwintering/food deprivation period of 41 days. GenPop data is shown as a solid black line. Hatchery data is shown as a dot-dashed green line. Stocking data is shown as a dot-dashed turquoise line. Ambient data is shown as a dot-dashed blue line. A) Temperature (˚C) each day from fertilization until end of experiment across all treatments. B) Accumulated thermal units (˚C) each day from fertilization until end of experiment across all treatments.

Sampling

Ten fish from each treatment were sampled every day from hatch until 16 dph, then every month for the remaining 10 months of the study to track growth. Fish were removed from their

respective treatment tanks and placed in a tank containing a solution of MS-222 (~100mg.L^{-1}) buffered with equal volumes of sodium bicarbonate. When equilibrium was lost the fish was removed and data were recorded as described below. The fish was then returned to their respective holding tank or sacrificed by further immersion in the MS-222 when being removed to keep densities equal.

Growth rate was assessed by recording fork length (0.1 mm), total length (0.1 mm), wet mass (0.01 g) and yolksac length, height and width at the yolksac stage, and gape size at the entrance of the mouth between jaws (vertical and horizontal via calipers) (0.1mm) were recorded once the yolksacs were absorbed. Yolksac volume (YSV, mm^3) was calculated using the equation: $YSV = (\pi/6) \cdot L \cdot H^2$ (Blaxter and Hempel 1966), where L is the yolksac length in mm, and H is the yolksac height in mm. Wet mass measurements began at 10 dph due to equipment availability constraints.

Statistical analysis

The growth metrics of fish in the GenPop and Hatchery treatments were compared at each time point to examine differences that could have arisen during embryogenesis and growth in different tank environments when embryos were incubated in a MacDonald tumbling jar and grown in hatchery trays or allowed to adhere to substrate as embryos and grown in MSU tanks. To compare the two treatments at each time point, data were analyzed using a T test with n=8-10, results with significance of $p<0.05$ are reported. Where data were not normally distributed, data were rank transformed to meet the assumptions of a normal distribution.

The growth metrics of fish in the MSU tanks with and without substrate and different temperature treatments were compared at each time point. For measurements at the yolksac stage, before temperatures became different in the 3 temperature treatments (from hatch until 15 dph), the data were pooled to compare Substrate and No Substrate at each time point using a T test with

n=8-10, results with significance of $p<0.05$ are reported. Where data were not normally distributed, data were rank transformed to meet the assumptions of a normal distribution. For measurements after the yolksac stage, when temperatures became different in the 3 temperature treatments (after 16 dph), two-way type II or III ANOVAs (Substrate x Temperature) were used depending on whether interaction effects were present or not, with n=4-10 and Tukey's HSD post hoc tests were used to determine which groups differed, results with significance of $p<0.05$ are reported.

2017-2018 cohort

Gamete collection, fertilization

Eggs and Sperm were collected from adult spawning fish caught at Landing River on the Nelson River in the same way as described above. Further, eggs were fertilized in one of two ways as described above; riverside and rolled in Fullers earth or at GRFH and added to the treatment tanks in the MSU. All treatments tanks in the MSU contained bioballs as substrate for embryos to adhere to and as refuge for yolksac larvae at hatch. Water supplying the MSU, dissolved oxygen and light levels to tanks in the MSU were also as described for the 2016-2017 cohort.

Embryogenesis

Conditions for GenPop fish in the 2017-2018 cohort followed those described for the 2016-2017 cohort. However, temperature treatment and time for fish in the MSU was different in the 2017-2018 cohort. All three temperature treatments began embryogenesis in the MSU at 12°C and this was increased by 0.5°C.day^{-1} to 14°C until larvae began to hatch.

Hatch and on-growing of fish

At hatch yolksac larvae were exposed to one of the following three temperature profiles (one for each row of the MSU):

1) Hatchery treatment: temperature was raised by 0.5°C.day^{-1} to a maximum of 16°C to match the standard raring temperature

2) Hatchery+2°C treatment: temperature was raised by 0.5°C.day^{-1} to a maximum of 18°C

3) Hatchery+4°C treatment: temperature was raised by 0.5°C.day^{-1} to a maximum of 20°C

At 24 days post hatch the water temperature in the above three treatments was reduced by 0.5°C.day^{-1} until it reached 15.5°C to reflect the surface water temperature of the Saskatchewan river at that time. Larvae from all treatments were then transferred to the following common garden experimental setup.

Rearing troughs supplied by surface water from the Saskatchewan river were equally divided into a total of eight sections. Environmental conditions (ambient river temperature, light levels and dissolved oxygen) were identical across each of the sections. Equivalent numbers of fish from each of the MSU treatments and from the hatchery were transferred to two of the eight sections in a haphazard order to create a total of four duplicate treatments in the common garden setup: Hatchery Transfer, Hatchery, Hatchery+2°C and Hatchery+4°C. A fifth and final treatment involved the GenPop treatment similar to that described for the 2016-2017 cohort, where sampling of the fish was exclusively from those remaining in the hatchery. Light levels for GenPop fish were controlled as described above. Light levels for experimental fish in the common garden experiment were ambient until mid-December 2017 when opaque corrugated plastic lids were placed on the tanks to simulate a reduction in light due to ice cover in the natural environment. Experimental and GenPop fish were stocked at similar densities and were fed in an identical fashion. However, in mid-October 2017 as the environmental temperature decreased feeding frequency for the experimental fish decreased to once every second, then once every fourth day until a cessation of feeding in mid-December 2017, at which point the water temperature was approximately 1.5°C. Food was withheld from the experimental fish for the next 4.5 months until the temperature in the tanks reached 2°C for 3 consecutive days. At this point food was provided

once every fourth day and feeding frequency increased to daily at three weeks after the first reintroduction of food. Experimental fish were allowed to grow and the experiment was terminated on June 30, 2018 when the water temperature had reached 18˚C. GenPop fish remaining in the hatchery were fed ad libitum twice daily on bloodworm throughout the year and were on-grown at an average temperature of 16˚C until release in April of 2018. The final sampling for the fish raised exclusively in the hatchery occurred on April 26, 2018. Figure 2.3 illustrates the temperature profiles and accumulated thermal units for all treatments in the 2017-2018 cohort. Figure 2.4 illustrates the treatments within the common garden setup. All described procedures and experiments were approved by the University of Manitoba's animal care committee (protocol#: F015-007) pursuant to the guidelines established by the Canadian Council for Animal Care.

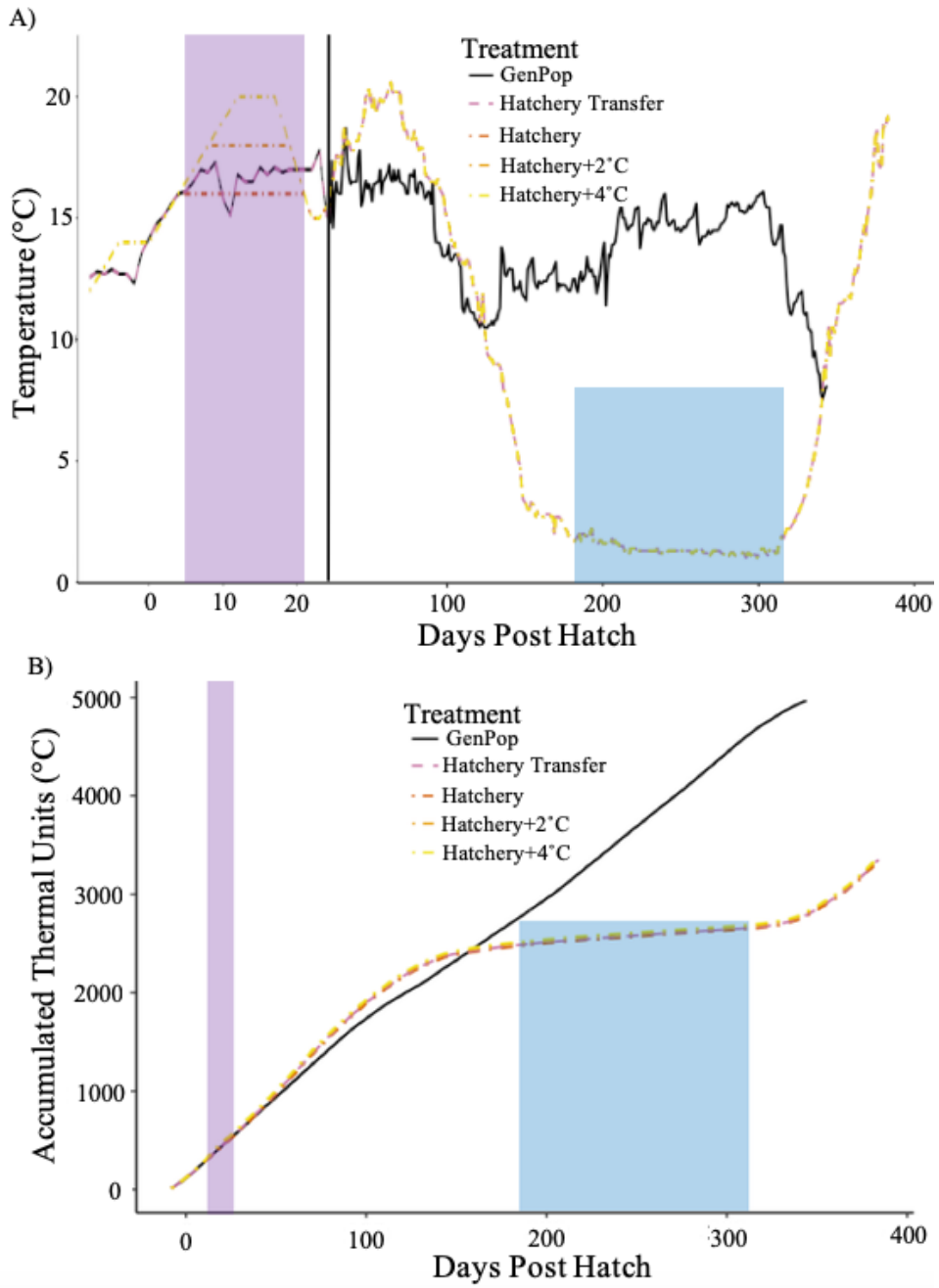


Figure 2.3. Temperature profiles of rearing environments in the experimental troughs and general population for Lake Sturgeon during the second experiment (2017-2018). The vertical black line indicates the transfer of fish to the experimental troughs and change in scale of x-axis. The purple box marks the period of different temperatures in the MSU. The blue box marks the overwintering/food deprivation period of 4.5 months. GenPop data is shown as a solid black line. Hatchery Transfer data is shown as a dashed purple line. Hatchery data is shown as a dot-dashed red line. Hatchery+2˚C data is shown as a dot-dashed orange line. Hatchery+4˚C data is shown as a dot-dashed yellow line. A) Temperature (˚C) each day from fertilization until end of experiment across all treatments. B) Accumulated thermal units (˚C) each day from fertilization until end of experiment across all treatments.

Figure 2.4. Incubation and rearing environments in the experimental troughs and general population for Lake Sturgeon during the second experiment (2017-2018). GenPop data is shown as black text. Hatchery Transfer data is shown as purple text. Hatchery data is shown as red text. Hatchery+2˚C data is shown as orange text. Hatchery+4˚C data is shown as yellow text.

Sampling

Ten fish from each treatment were sampled every day from hatch until 15 dph, sampling occurred again at 24 dph, when fish were transferred to the common garden experimental setup and then every month for the remaining 13 months of the study to track growth. Fish were removed from their respective treatment tanks and placed in a tank containing a solution of MS-222 (~100mg.L^{-1}) buffered with equal volumes of sodium bicarbonate. When equilibrium was lost the fish was removed and data were recorded as described above. The fish was then returned to their respective holding tank or sacrificed by further immersion in the MS-222, for examination of skeletal muscle development (see chapter 3). Any swimming performance test occurred prior to MS-222 exposure (see chapter 4).

Statistical analysis

The growth metrics of fish in the GenPop and Hatchery Transfer treatments were compared at each time point to examine differences that could have arisen between fish that remained in the

hatchery and those that were transferred to the common garden setup. However, the GenPop fish experienced a disease outbreak around 3 mph as well as increased density compared to the experimental troughs which could have slowed their growth at that time. To compare the two treatments at each time point, data were analyzed using a T test with n=8-10, results with significance of $p<0.05$ are reported. Where data were not normally distributed, data were rank transformed to meet the assumptions of a normal distribution.

The growth metrics of fish in the Hatchery Transfer and Hatchery treatments were compared at each time point to examine differences that could have arisen during embryogenesis when embryos were incubated in a MacDonald tumbling jar or allowed to adhere to substrate. To compare the two treatments at each time point, data were analyzed using a T test with n=8-10, results with significance of $p<0.05$ are reported. Where data were not normally distributed, data were rank transformed to meet the assumptions of a normal distribution.

The growth metrics of fish in the Hatchery, Hatchery+2°C, and Hatchery+4°C treatments were compared at each time point to examine differences that could have arisen from early incubation temperature differences before the transfer to the common garden setup. To compare the three treatments for each time point and to compare the time points for each treatment, data were analyzed using a one-way ANOVA with n=8-10 and Tukey's LSD post hoc tests were used to determine which groups differed, results with significance of $p<0.05$ are reported. Where data were not normally distributed it was rank transformed to meet the assumptions of a normal distribution. Where data could not be transformed to meet the assumptions of a normal distribution, data were analyzed using a non-parametric Kruskal Wallis test followed by a Dunn post-hoc analysis to determine significance.

Results

2016-2017 cohort

There were no significant differences between gape size for any treatments at any time point.

Yolksac larvae

Significant differences in yolksac volume were present between GenPop and Hatchery (No Substrate) (incubation) treatments at 3, 5, 6, 7, 9, 10, 11, 12, 13, 14 and 15 dph with GenPop fish having larger yolksacs and absorbing them slower (see figure 2.5a). Significant differences in yolksac volume were present between Substrate and No Substrate treatments at 4, 8, 9, 10, 12, 13 and 14 dph with Substrate fish having larger yolksacs and absorbing them slower (see figure 2.5b).

During the yolksac stage, significant differences in total length were present between GenPop and Hatchery (No Substrate) treatments at 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15 dph with GenPop fish having shorter total lengths (see figure 2.6a). Significant differences in total length were present between substrate treatments at 4, 5, 6 and 10 dph with Substrate fish having longer total lengths from 4-6 dph, then becoming shorter at 10 dph (see figure 2.6b).

Significant differences in wet mass were present between GenPop and Hatchery (No Substrate) treatments at 10, 11, 12, 13, 14 and 15 dph with GenPop fish having lighter wet mass values (see figure 2.7a). Significant differences in wet mass were present between substrate treatments at 10, 11, 12, 13 and 14 dph with No Substrate fish having greater body mass (see figure 2.7b).

Pre-winter

During the summer and fall grow-out stage, significant differences in total length were present between GenPop and Hatchery (No Substrate) treatments at 19 dph, 1, 3, 4, 5 and 6 mph with GenPop fish having shorter total lengths before 2 mph, becoming similar in lengths at that

stage and subsequently switching to becoming longer than Hatchery fish (see figure 2.8a).

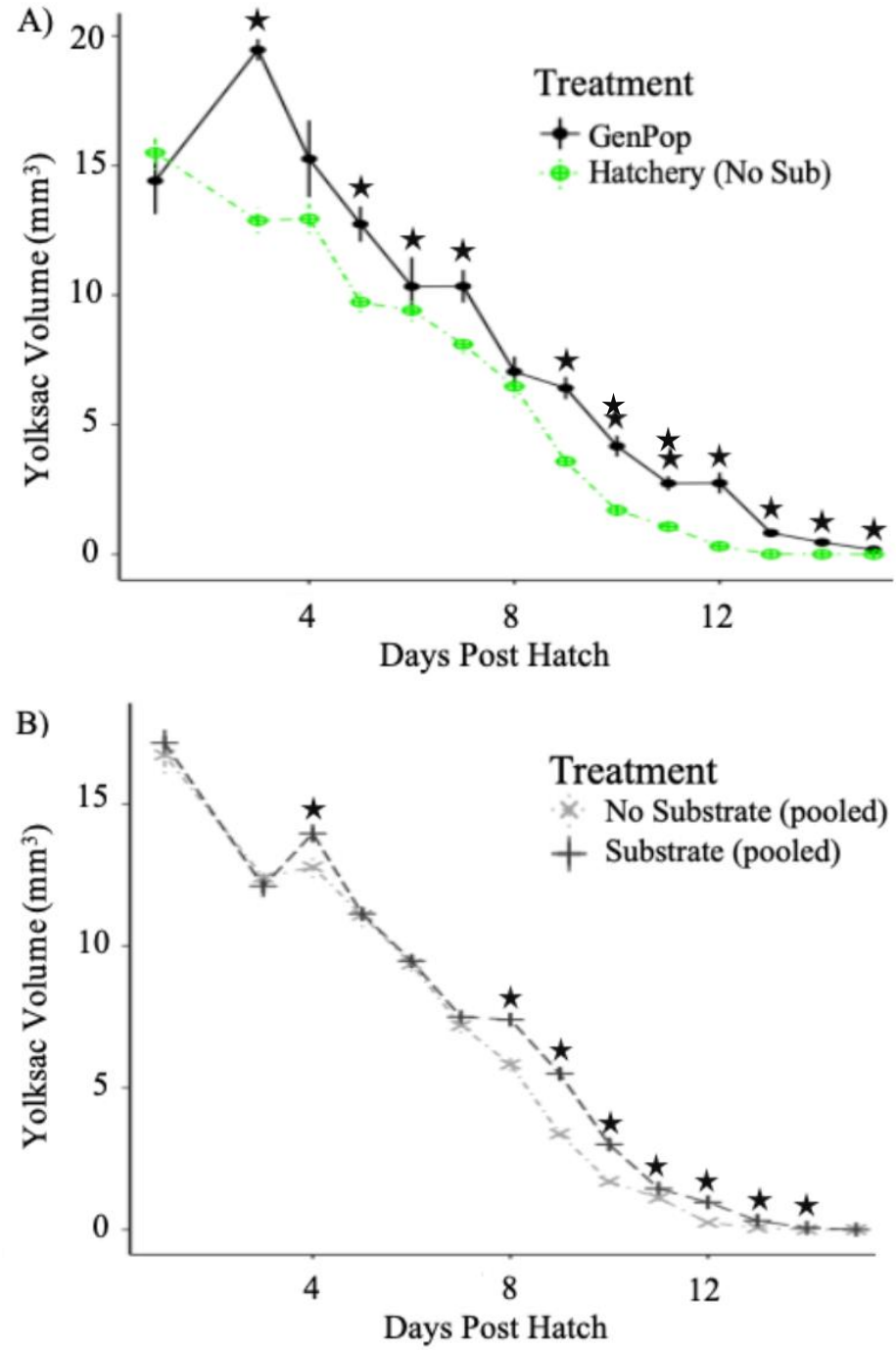


Figure 2.5. Mean larval yolksac volume (mm^3) with standard error from hatch until 15 dph for Lake Sturgeon during the first experiment (2016-2017). GenPop data is shown as a solid black line. Hatchery data is shown as a green line. No substrate data is shown as a lighter dot-dashed line. Substrate data is shown as a darker dashed line. A) Incubation comparison between GenPop and Hatchery without substrate. B) Temperature comparison between pooled No Substrate and Substrate treatments of the Stocking data. Differences within time points but between treatments are marked by black stars. n=8-10.

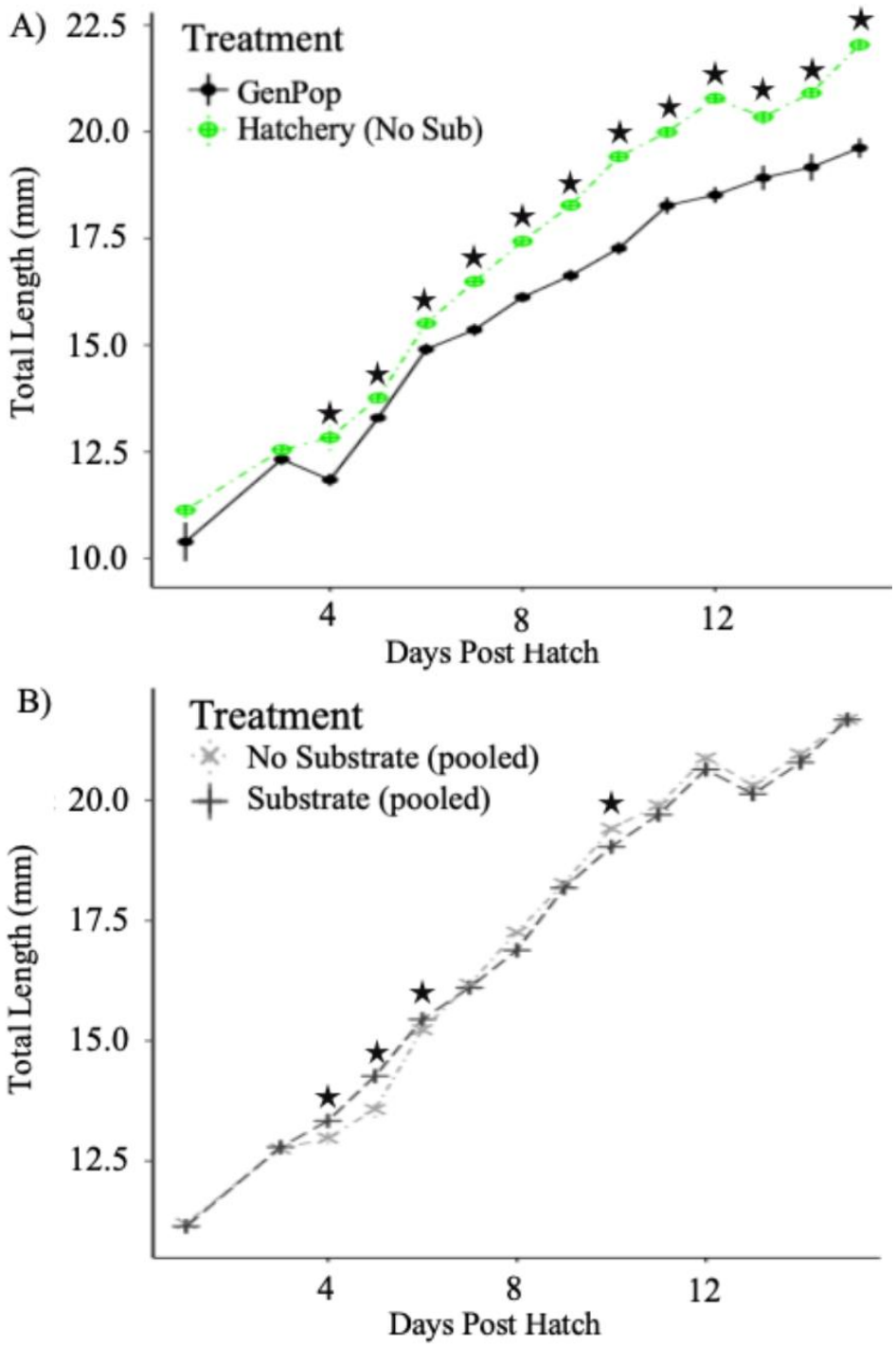


Figure 2.6. Mean total length (mm) with standard error from hatch until 15 dph for Lake Sturgeon during the first experiment (2016-2017). GenPop data is shown as a solid black line. Hatchery data is shown as a green line. No substrate data is shown as a lighter dot-dashed line. Substrate data is shown as a darker dashed line. A) Incubation comparison between GenPop and Hatchery without substrate. B) Temperature comparison between pooled No Substrate and Substrate treatments of the Stocking data. Differences within time points but between treatments are marked by black stars. n=8-10.

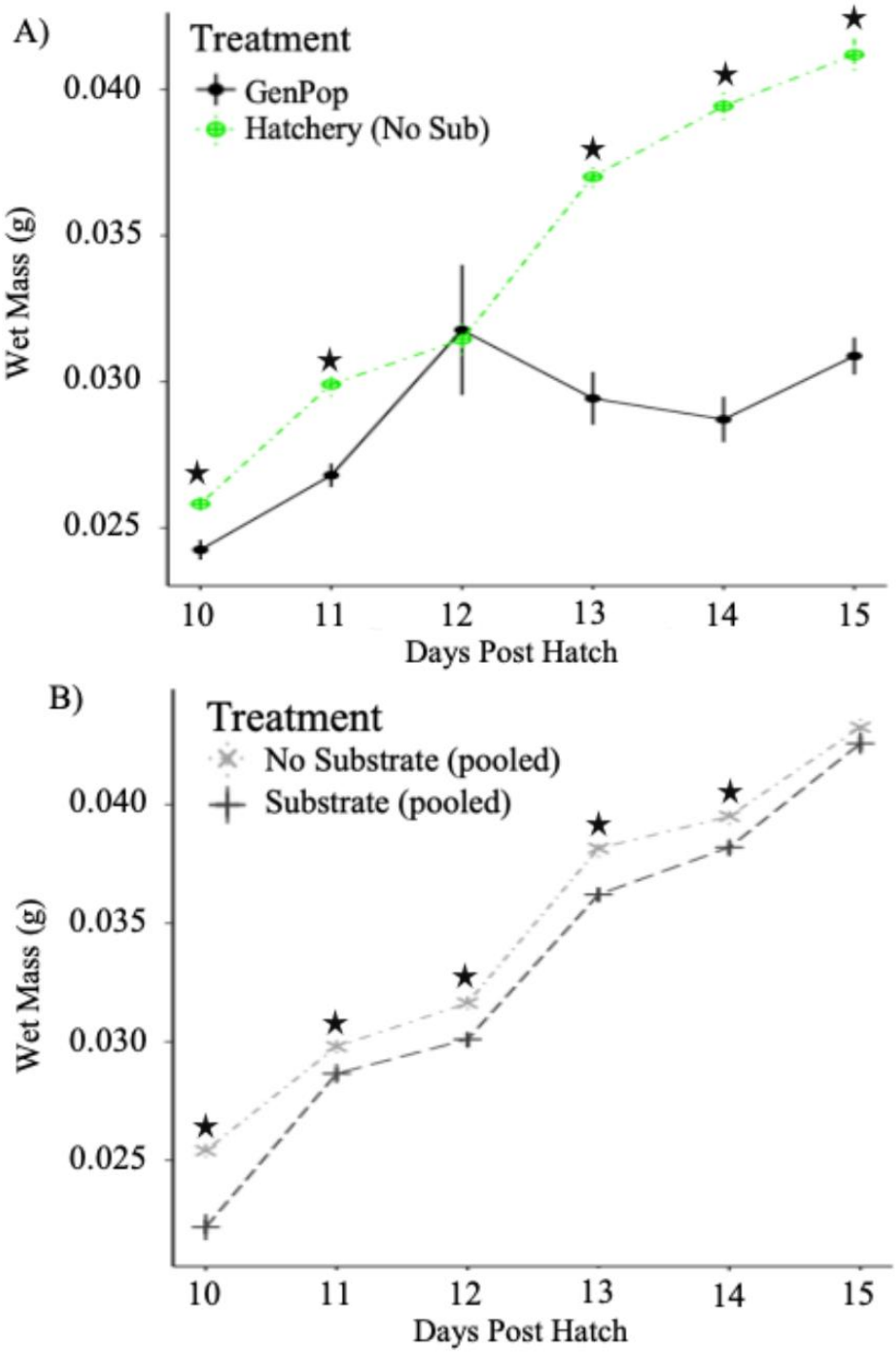


Figure 2.7. Mean wet mass (g) with standard error from hatch until 15 dph for Lake Sturgeon during the first experiment (2016-2017). GenPop data is shown as a solid black line. Hatchery data is shown as a green line. No substrate data is shown as a lighter dot-dashed line. Substrate data is shown as a darker dashed line. A) Incubation comparison between GenPop and Hatchery without substrate. B) Temperature comparison between pooled No Substrate and Substrate treatments of the Stocking data. Differences within time points but between treatments are marked by black stars. n=8-10.

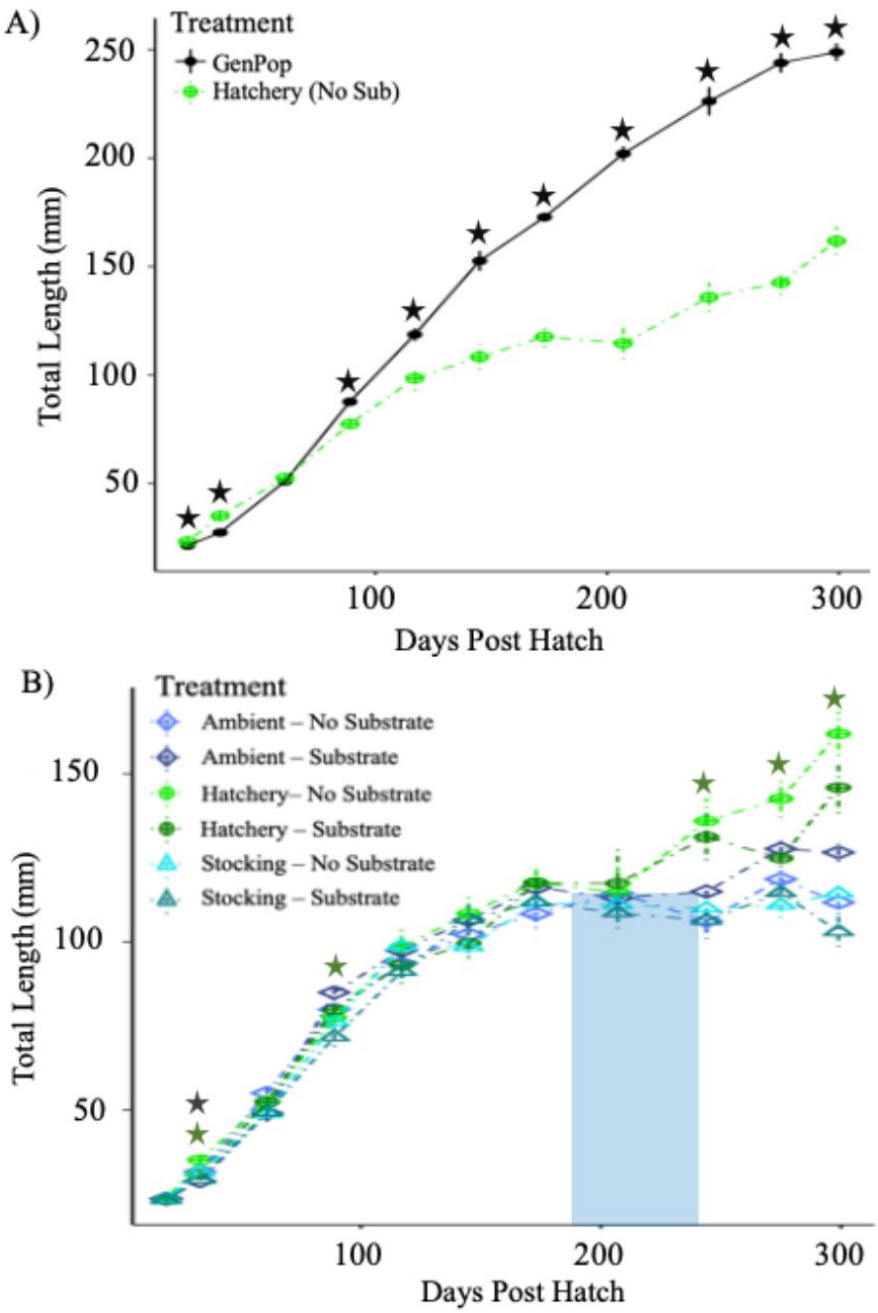


Figure 2.8. Mean total length (mm) with standard error from 19 dph until 300 dph for Lake Sturgeon during the first experiment (2016-2017). GenPop data is shown as a solid black line. Hatchery data is shown as a green line. Stocking data is shown as a turquoise line. Ambient data is shown as a blue line. No substrate data is shown as a lighter dashed line. Substrate data is shown as a darker dashed line. The blue box marks the overwintering starvation period for Stocking and Ambient treatments. A) Incubation comparison between GenPop and Hatchery without substrate. Differences within time points but between treatments are marked by black stars. B) Treatment comparisons between Ambient, Hatchery and Stocking treatments and No Substrate and Substrate. Differences within time points but between temperature treatments are marked by green stars. Differences within time points but between substrate treatments are marked by a grey star. n=8-10.

Significant differences in total length were present between substrate treatments at 1 mph with Substrate fish having shorter total lengths (see figure 2.8b). Significant differences in total length were present between temperature treatments at 1 and 3 mph with Hatchery fish having longer total lengths than Ambient and Stocking fish at 1 mph and Ambient fish having longer total lengths than Stocking fish at 3 mph (see figure 2.8b).

Significant differences in wet mass were present between GenPop and Hatchery (No Substrate) treatments at 19 dph, 1, 3, 4, 5 and 6 mph with GenPop fish having lighter wet mass before 2 mph, becoming similar in body mass at that stage and subsequently switching to becoming greater in mass than Hatchery fish (see figure 2.9a). Significant differences in wet mass were present between substrate treatments at 19 dph and 1 mph with Substrate fish having lighter wet mass (see figure 2.9b). Significant differences in wet mass were present between temperature treatments at 1 mph with Hatchery fish having greater wet mass values than Ambient and Stocking fish (see figure 2.9b).

Winter

During the overwintering period (7 mph), significant differences in total length were present between GenPop and Hatchery (No Substrate) treatments with GenPop fish having longer total lengths (see figure 2.8a). There were no significant differences in total length between substrate treatments. There were no significant differences in total length between substrate or temperature treatments.

Significant differences in wet mass were present between GenPop and Hatchery (No Substrate) treatments with GenPop fish having greater wet mass values (see figure 2.9a). There were no significant differences in total length between substrate or temperature treatments.

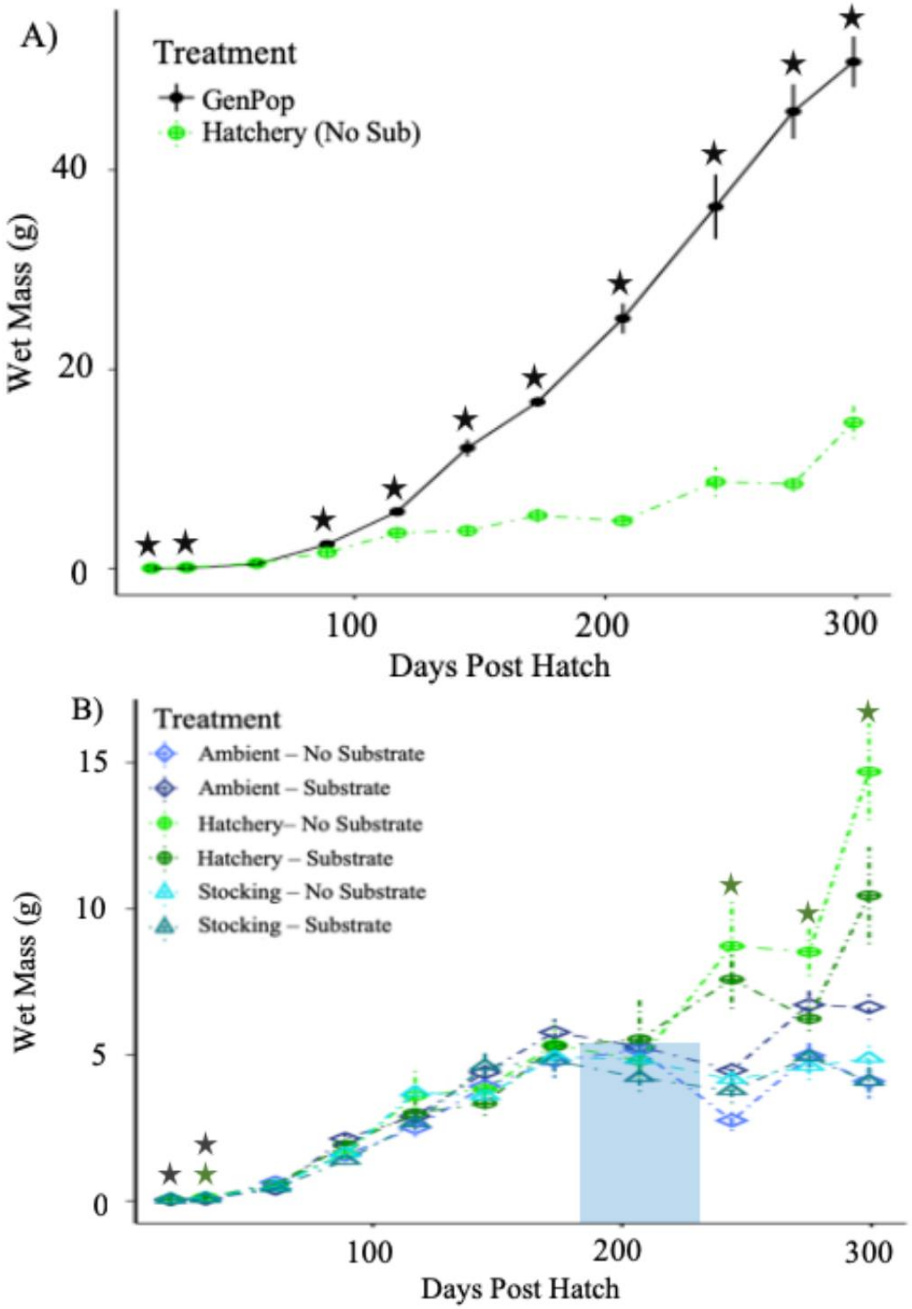


Figure 2.9. Wet mass (g) with standard error from 19 dph until 300 dph for Lake Sturgeon during the first experiment (2016-2017). GenPop data is shown as a solid black line. Hatchery data is shown as a green line. Stocking data is shown as a turquoise line. Ambient data is shown as a blue line. No substrate data is shown as a lighter dashed line. Substrate data is shown as a darker dashed line. The blue box marks the overwintering starvation period for Stocking and Ambient treatments. A) Incubation comparison between GenPop and Hatchery without substrate. Differences within time points but between treatments are marked by black stars. B) Treatment comparisons between Ambient, Hatchery and Stocking treatments and No Substrate and Substrate. Differences within time points but between temperature treatments are marked by green stars. Differences within time points but between substrate treatments are marked by a grey star. n=8-10.

Post-winter

After the overwintering period, significant differences in total length were present between GenPop and Hatchery (No Substrate) treatments at 8, 9 and 10 mph with GenPop fish having longer total lengths (see figure 2.8a). There were no significant differences in total length between substrate treatments. Significant differences in total length were present between temperature treatments at 8, 9 and 10 mph with Hatchery fish having longer total lengths than Ambient and Stocking fish at 8, 9 and 10 mph, and Ambient fish having longer total lengths than Stocking fish at 9 mph (see figure 2.8b). There were interactions between substrate and temperature at 9 and 10 mph.

Significant differences in wet mass were present between GenPop and Hatchery (No Substrate) treatments at 8, 9 and 10 mph with GenPop fish having greater wet mass values (see figure 2.9a). There were no significant differences in wet mass between substrate treatments. Significant differences in wet mass were present between temperature treatments 8, 9 and 10 mph with Hatchery fish having greater wet mass values than Ambient and Stocking fish (see figure 2.9b). There were interactions between substrate and temperature at 8, 9 and 10 mph.

2017-2018 cohort

Yolksac larvae

Significant differences in yolksac volume were present between Hatchery Transfer and Hatchery (incubation) treatments at 8, 9, 10, 11, 12 and 14 dph with Hatchery Transfer fish having larger yolksacs and absorbing them the slowest (see figure 2.10a). Significant differences in yolksac volume were present (after the temperatures began to diverge at 7 dph) between Hatchery, Hatchery+2°C, and Hatchery+4°C treatments at 10, 11, 12, 13, 14 and 16 dph with Hatchery fish having larger yolksacs and absorbing them the slowest (see figure 2.10b).

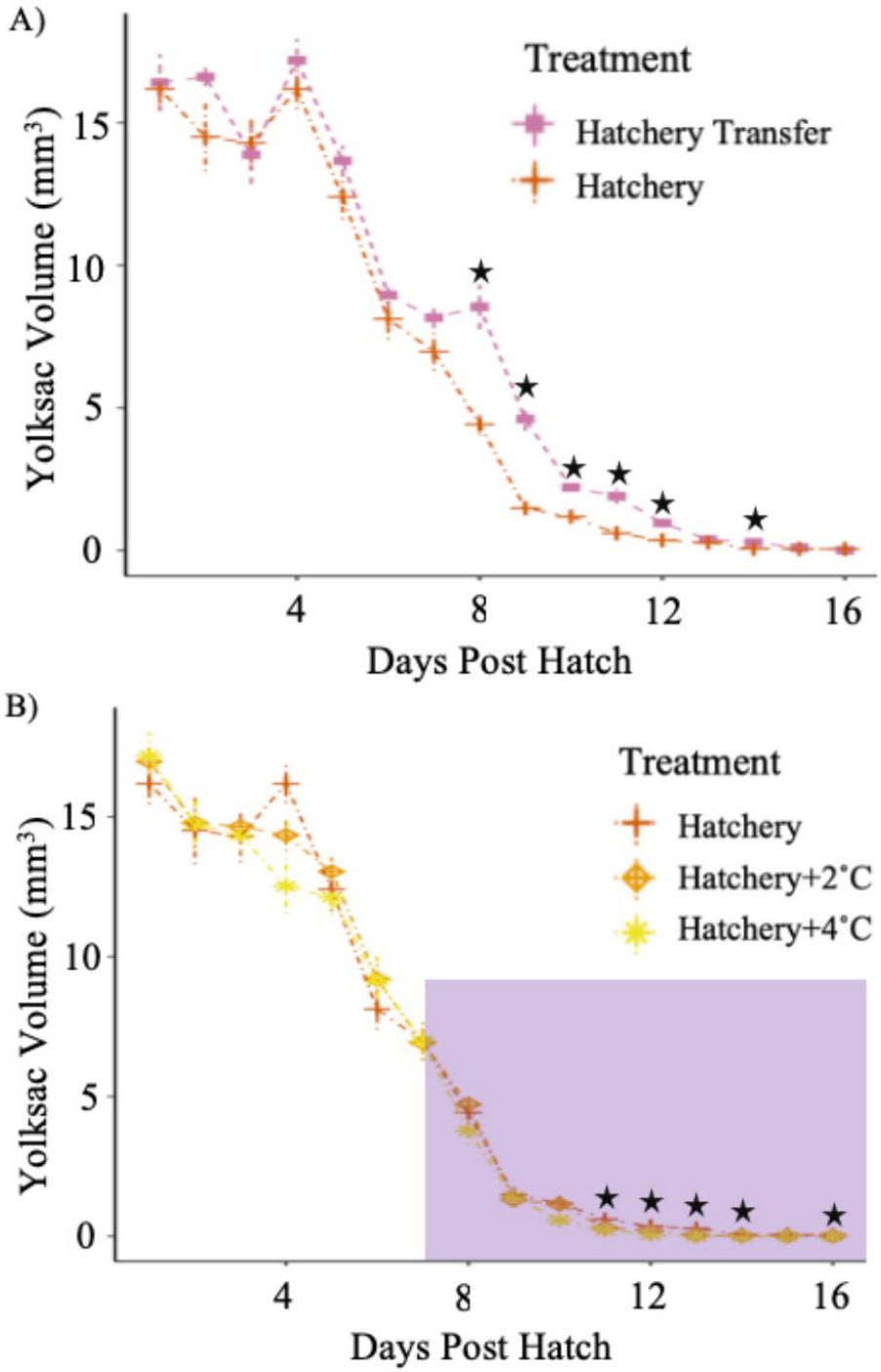


Figure 2.10. Mean larval yolksac volume (mm^3) with 95% confidence intervals over 15 days post hatch for Lake Sturgeon during the second experiment (2017-2018). The purple box marks the period of different temperatures in the MSU. Hatchery Transfer data is shown as a dashed purple line. Hatchery data is shown as a dot-dashed red line. Hatchery+2˚C data is shown as a dot-dashed orange line. Hatchery+4˚C data is shown as a dot-dashed yellow line. A) Incubation comparison between Hatchery Transfer and Hatchery. B) Temperature comparison between Hatchery, Hatchery+2˚C, and Hatchery+4˚C. n=8-10. Significant differences between treatments at each time point are marked by black stars.

Pre-transfer

Prior to transfer to the common garden setup, significant differences in total length were present between Hatchery Transfer and Hatchery treatments at 1, 3, 4, 5 and 8 dph with Hatchery fish having longer total lengths (see figure 2.11a). Significant differences in total length were also present between Hatchery, Hatchery+2°C, and Hatchery+4°C treatments at 8, 9, 10, 11, 12, 13, 14, 15, 16, 18 and 23 dph with Hatchery fish having shortest total lengths and Hatchery+2˚C and/or Hatchery+4˚C fish having longest total lengths (see figure 2.11b).

Significant differences in wet mass were present between Hatchery Transfer and Hatchery treatments at 2, 3, 4, 5, 8, 9, 10, 12 and 18 dph with Hatchery fish having lighter wet mass values at 2,3 and 18 dph, but illustrated greater wet mass values in between those time points (see figure 2.12a). Significant differences in wet mass were present between Hatchery, Hatchery+2°C, and Hatchery+4°C treatments at 13, 14, 15, 16, 18 and 23 dph with Hatchery fish having lighter wet mass values and Hatchery+2˚C and/or Hatchery+4˚C fish having greatest wet mass values (see figure 2.12b).

Pre-winter

After transfer to the common garden setup, during the summer and fall, significant differences in total length were present between GenPop and Hatchery Transfer treatments at 2, 4, 5 mph with Hatchery Transfer fish having longer total lengths (see figure 2.13a). There were no significant differences in total length between Hatchery Transfer and Hatchery treatments during summer and fall. Significant differences in total length were present between Hatchery, Hatchery+2°C, and Hatchery+4°C treatments at 5 and 6 mph with Hatchery fish having shortest total lengths and Hatchery+2˚C and/or Hatchery+4˚C fish having longest total lengths (see figure 2.13c).

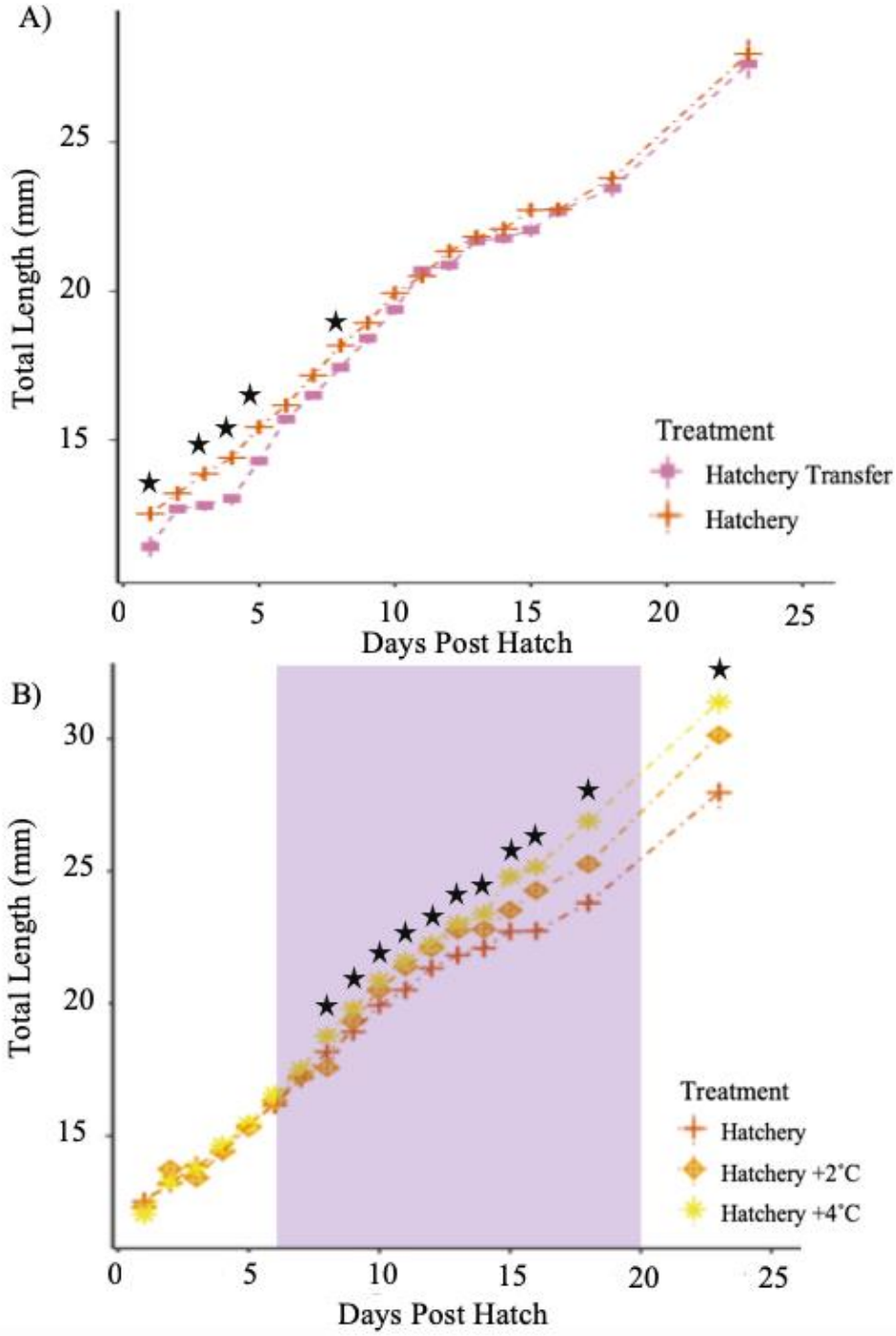


Figure 2.11. Mean total length (mm) with standard error from hatch until 25 dph for Lake Sturgeon during the second experiment (2017-2018). The purple box marks the period of different temperatures in the MSU. Hatchery Transfer data is shown as a dashed purple line and squares. Hatchery data is shown as a dot-dashed red line and crosses. Hatchery+2˚C data is shown as a dot-dashed orange line and diamonds. Hatchery+4˚C data is shown as a dot-dashed yellow line and stars. A) Incubation comparison between Hatchery Transfer and Hatchery. B) Temperature comparison between Hatchery, Hatchery+2˚C, and Hatchery+4˚C. n=10. Significant differences between treatments at each time point are marked by black stars.

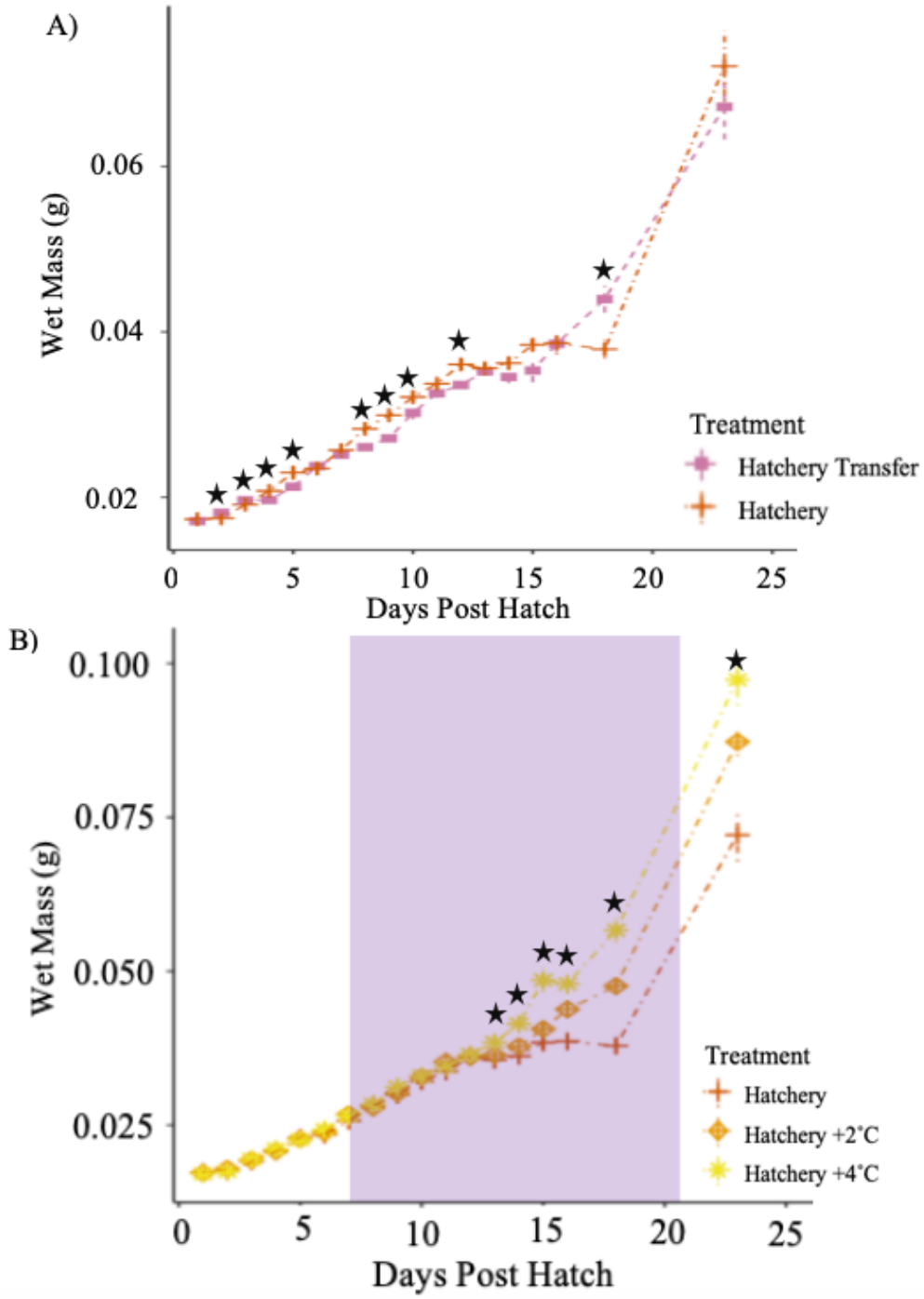
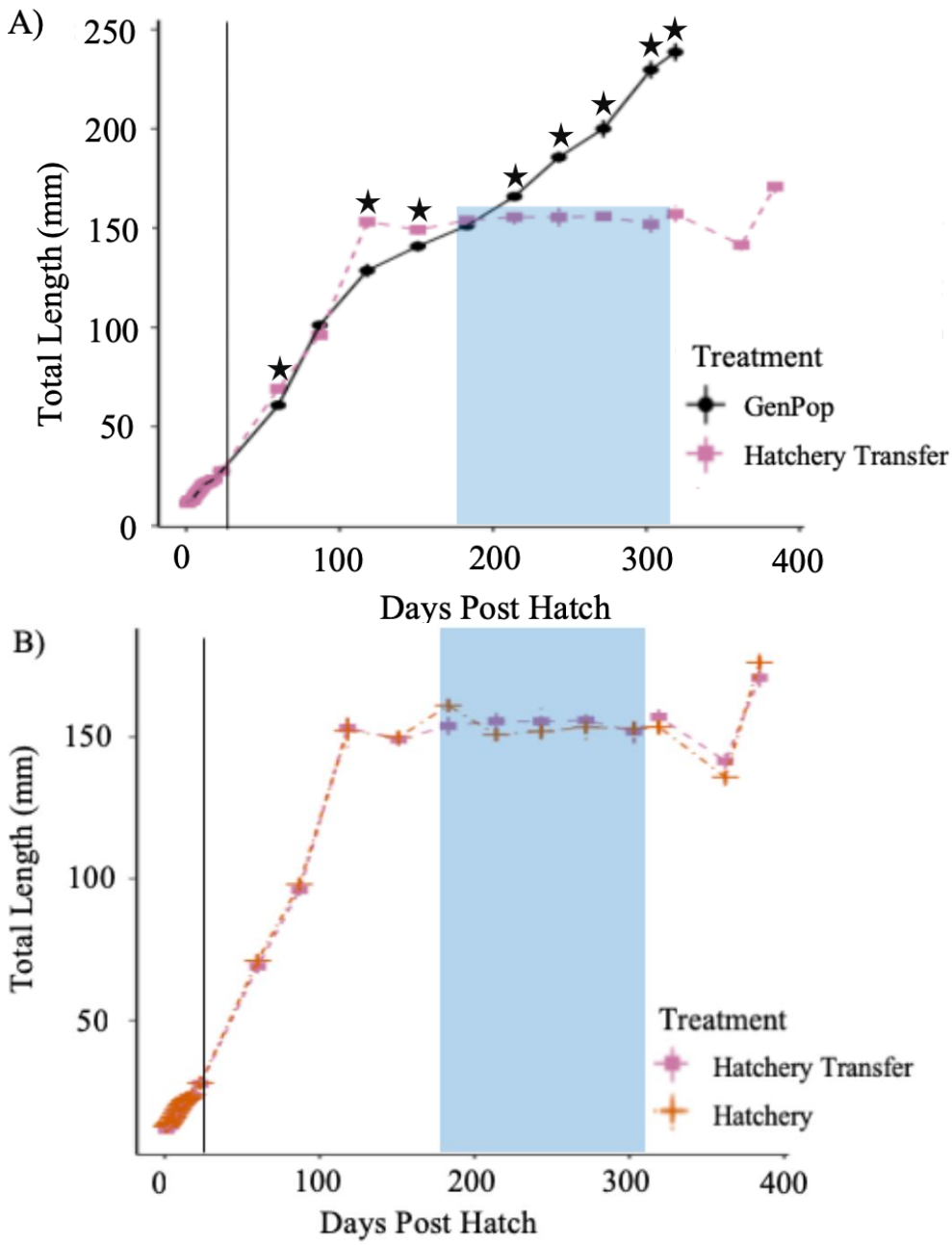


Figure 2.12. Mean wet mass (g) with standard error from hatch until 25 dph for Lake Sturgeon during the second experiment (2017-2018). The purple box marks the period of different temperatures in the MSU. Hatchery Transfer data is shown as a dashed purple line and squares. Hatchery data is shown as a dot-dashed red line and crosses. Hatchery+2˚C data is shown as a dot-dashed orange line and diamonds. Hatchery+4˚C data is shown as a dot-dashed yellow line and stars. A) Incubation comparison between Hatchery Transfer and Hatchery. B) Temperature comparison between Hatchery, Hatchery+2˚C, and Hatchery+4˚C. n=10. Significant differences between treatments at each time points are marked by black stars.


A)
Total Length (mm)
250
200
150
100
50
0
0
100
200
300
400
Days Post Hatch
Treatment
GenPop
Hatchery Transfer
B)
Total Length (mm)
150
100
50
0
100
200
300
400
Days Post Hatch
Treatment
Hatchery Transfer
Hatchery

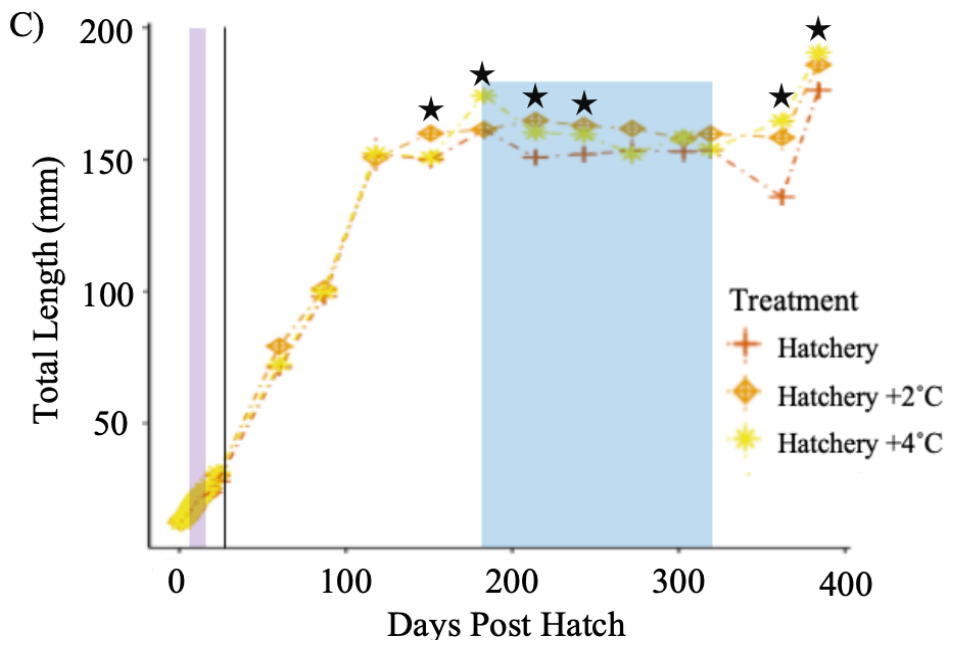
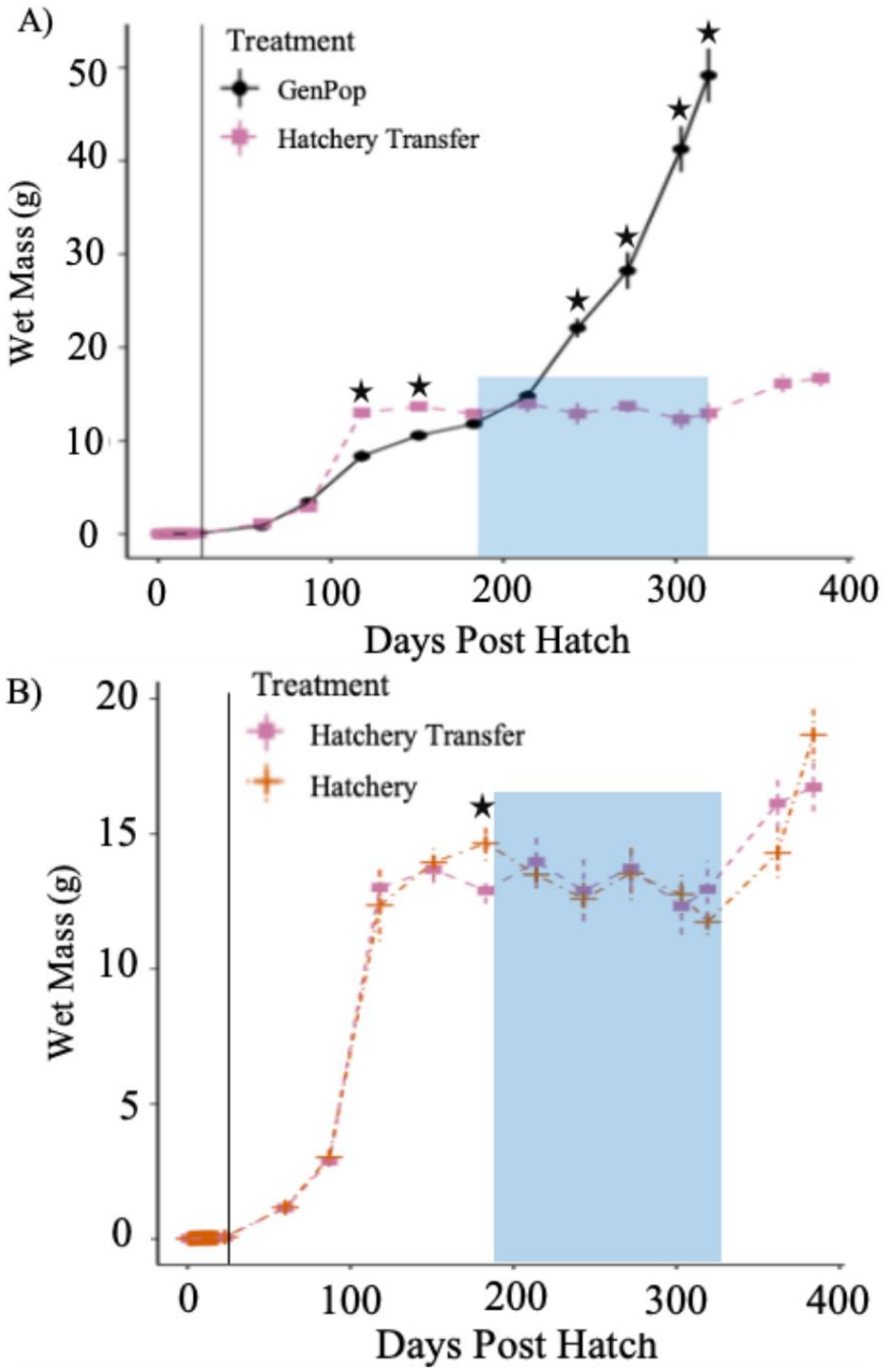


Figure 2.13. Mean total length (mm) with standard error from hatch until 400 dph for Lake Sturgeon during the second experiment (2017-2018). The vertical black line indicates the transfer of fish to the experimental troughs. The purple box marks the period of different temperatures in the MSU. The blue box marks the overwintering/food deprivation period of 4.5 months. GenPop data is shown as a solid black line and circles. Hatchery Transfer data is shown as a dashed purple line and squares. Hatchery data is shown as a dot-dashed red line and crosses. Hatchery+2˚C data is shown as a dot-dashed orange line and diamonds. Hatchery+4˚C data is shown as a dot-dashed yellow line and stars. A) Treatment comparison between Hatchery Transfer and Hatchery. B) Incubation comparison between Hatchery Transfer and Hatchery. C) Temperature comparison between Hatchery, Hatchery+2˚C, and Hatchery+4˚C. n=8-10. Significant differences between treatments at each time point are marked by black stars.

Significant differences in wet mass were present between GenPop and Hatchery Transfer treatments at 4 and 5 mph with Hatchery Transfer fish having greater wet mass values (see figure 2.14a). Significant differences in wet mass were present between Hatchery Transfer and Hatchery treatments at 6 mph with Hatchery fish having greater wet mass values (see figure 2.14b). Significant differences in wet mass were present between Hatchery, Hatchery+2°C, and Hatchery+4°C treatments at 6 mph with Hatchery+4˚C fish having greatest wet mass values (see figure 2.14c).


A)
Treatment
GenPop
Hatchery Transfer
Wet Mass (g)
0
10
20
30
40
50
0
100
200
300
400
Days Post Hatch
B)
Treatment
Hatchery Transfer
Hatchery
Wet Mass (g)
0
5
10
15
20
0
100
200
300
400
Days Post Hatch

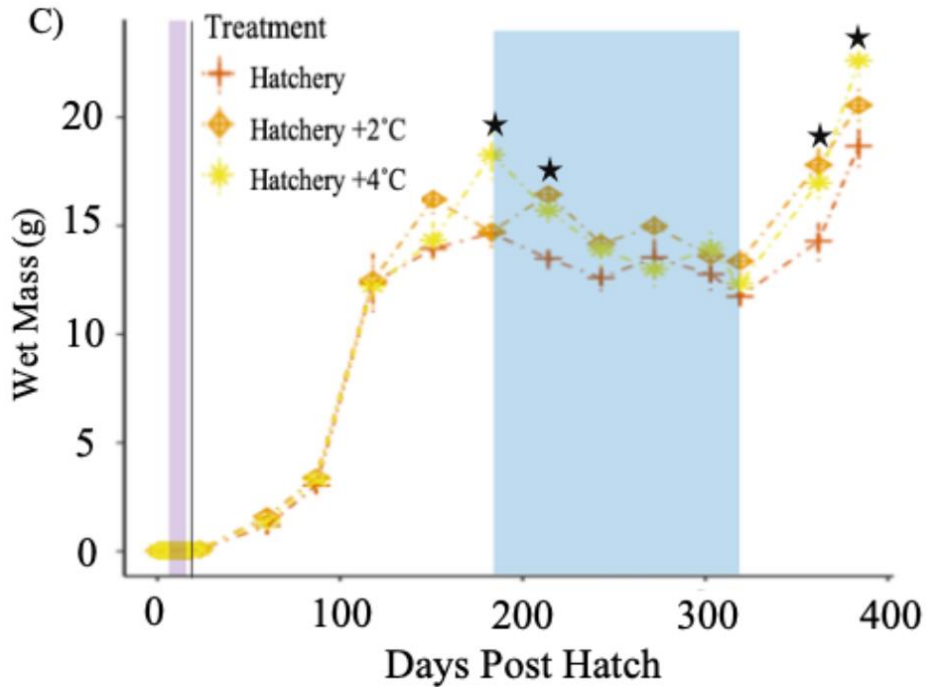


Figure 2.14. Mean wet mass (g) with standard error from hatch until 400 dph for Lake Sturgeon during the second experiment (2017-2018). The vertical black line indicates the transfer of fish to the experimental troughs. The purple box marks the period of different temperatures in the MSU. The blue box marks the overwintering/food deprivation period of 4.5 months. GenPop data is shown as a solid black line and circles. Hatchery Transfer data is shown as a dashed purple line and squares. Hatchery data is shown as a dot-dashed red line and crosses. Hatchery+2˚C data is shown as a dot-dashed orange line and diamonds. Hatchery+4˚C data is shown as a dot-dashed yellow line and stars. A) Treatment comparison between Hatchery Transfer and Hatchery. B) Incubation comparison between Hatchery Transfer and Hatchery. C) Temperature comparison between Hatchery, Hatchery+2˚C, and Hatchery+4˚C. n=8-10. Significant differences between treatments at each time points are marked by black stars.

Winter

After transfer to the common garden setup, significant differences in total length were present between GenPop and Hatchery Transfer treatments at 7, 8, 9, 10 and 11 mph with Hatchery Transfer fish having shorter total lengths (see figure 2.13a). There were no significant differences in total length between Hatchery Transfer and Hatchery treatments during winter. Significant differences in total length were present between Hatchery, Hatchery+2°C, and Hatchery+4°C treatments at 7 and 8 mph with Hatchery fish having shortest total lengths and Hatchery+2˚C and/or Hatchery+4˚C fish having longest total lengths (see figure 2.13c).

Significant differences in wet mass were present between GenPop and Hatchery Transfer treatments at 8, 9, 10 and 11 mph with Hatchery Transfer fish having lighter wet mass values (see figure 2.14a). There were no significant differences in wet mass between Hatchery Transfer and Hatchery treatments during winter. Significant differences in wet mass were present between Hatchery, Hatchery+2°C, and Hatchery+4°C treatments at 7 mph with Hatchery fish having lighter wet mass values and Hatchery+2˚C and Hatchery+4˚C (see figure 2.14c).

Post-winter

After recovery from overwintering, there were no significant differences in total length between Hatchery Transfer and Hatchery treatments during winter. Significant differences in total length were present between Hatchery, Hatchery+2°C, and Hatchery+4°C treatments at 12 and 13 mph with Hatchery fish having shortest total lengths and Hatchery+2˚C and/or Hatchery+4˚C fish having longest total lengths (see figure 2.13c).

There were no significant differences in wet mass between Hatchery Transfer and Hatchery treatments during winter. Significant differences in wet mass were present between Hatchery, Hatchery+2°C, and Hatchery+4°C treatments at 12 and 13 mph with Hatchery fish having lighter wet mass values and Hatchery+2˚C and/or Hatchery+4˚C fish having greatest wet mass values (see figure 2.14c).

Discussion

2016-2017 cohort

Yolksac larvae

Yolksac volumes of larvae that hatched from eggs incubated in tumbling jars (GenPop) were significantly larger than those that adhered to surfaces (Hatchery No Substrate) for the majority of the yolksac stage and the yolksacs of GenPop fish were fully absorbed a day after those of Hatchery fish. This means that the GenPop fish absorbed their yolksacs more slowly, likely

related to incubation method. There were also significant differences in yolksac volumes of larvae that were reared in different substrate treatments. Substrate treatment fish had significantly larger yolksac volumes than those in the No Substrate treatments but were fully absorbed on the same day. The larvae in tanks without substrate had no opportunity for refuge and thus were noticeably more active than larvae that had substrate for refuge. It is therefore likely that the absence of substrate lead to a more rapid use of internal resources and faster use of the energy stored in the yolksacs and their smaller size at 4-6 and 10 dph. Similar results have previously been reported in White Sturgeon, where yolksac larvae reared in tanks with substrate had higher yolksac absorption and growth rates (Boucher et al. 2014).

During the yolksac stage, there were significant differences in body length and mass between incubation treatments. From 4 dph until full yolksac absorption, GenPop fish had significantly smaller body lengths and from 10 dph (beginning of body mass measurements) until full yolksac absorption, GenPop fish had significantly smaller mass values. The GenPop fish (incubated in tumbling jars) were smaller on average and took longer to absorb their yolksacs than the Hatchery fish which used their yolksacs more quickly but grew larger and were therefore likely investing the energy from their yolksacs into growth more efficiently than the GenPop fish. A previous study comparing egg incubation methods in Green Sturgeon, *A. medirostris*, showed that tumbling jars can negatively affect survival due to high impact rolling action damaging the embryos (Van Eenennaam et al. 2008). If Lake Sturgeon embryos were also negatively impacted by this incubation method, it could impede growth immediately after hatch.

There were also significant differences in body length and mass for larvae that were reared in different substrate treatments. Fish in Substrate treatments were significantly larger in body length in the first week of the yolksac stage (4-6 dph), then becoming smaller in body length and/or

mass towards the end of yolksac absorption (10-15 dph). The larger size of Substrate fish at the start of the yolksac stage could be from an advantage of having substrate to use as shelter from the flow of water in the tanks which allowed them to use more energy from their yolksacs toward growth instead of being expended on increased swimming activity. The smaller sizes of Substrate fish towards the end of yolksac absorption align with the pattern in incubation treatments, where the fish also had larger yolksacs and were likely using less energy for growth.

Pre-winter

During the summer and fall growing stage, significant differences in body length and mass were present between incubation treatments. For the first month after yolksac absorption, GenPop fish remained significantly smaller than Hatchery fish, differences in size disappeared at 2 mph, then GenPop fish became significantly larger than Hatchery fish for the rest of the experiment. This is most likely an effect of high stocking density in the MSU which limited their growth despite similar temperature and feeding regimes. GenPop fish were reared in larger Hatchery troughs, while Hatchery fish were reared in 9L MSU tanks with higher stocking densities which have been shown to negatively impact growth rates in several sturgeon species such as Amur Sturgeon, *A. schrenckii*, (Yang et al. 2011), and Atlantic Sturgeon (Szczepkowski et al. 2011).

The only significant differences in body length and mass between substrate treatments occurred in the first month after yolksac absorption, when Substrate fish remained significantly smaller than No Substrate fish. After this point, the high stocking density in MSU tanks could have prevented any further differences that may have been detected had the fish been allowed more room to grow.

The significant differences in body length and mass between temperature treatments occurred in the 3 months after yolksac absorption. At 1 mph, Hatchery fish were significantly larger in body length and mass than Ambient and Stocking fish. And at 3 mph, Ambient fish were

significantly larger body length and mass than Stocking fish. This is likely an effect of temperature because at this time, the temperature in the Ambient treatment (20˚C) was higher than those of the Stocking and Hatchery treatments which were kept at 16˚C.

Winter

There were no significant differences in body length and mass between temperature treatments during the fall and overwintering period as the fish appeared to stop growing. Reduced environmental temperature is known to be closely related to metabolic rate in fishes, as seen in juvenile Shortnose Sturgeon, *A. Brevirostrum*, where the most prominent effects of temperature on reducing metabolic rates occurred at the lowest temperatures tested (10˚C) (Kieffer et al. 2014). Further it is perhaps no surprise growth will stop and body mass can decline along with changing body composition when there is a lack of available resources (Xiao et al. 2011). Therefore, the lack of growth during the overwintering period of cold (~3.5˚C) water and food deprivation was expected.

Post-winter

After the overwintering period, significant differences in body length and mass were present again with Hatchery fish becoming significantly larger in body length and mass than Ambient and Stocking fish at 8, 9 and 10 mph. and Ambient fish were significantly larger in body length than Stocking fish at 9 mph. At this stage, Hatchery fish had been kept at 16˚C and fed daily during the overwintering period, so they were able to convert the exogenous food into energy for growth while the Stocking and Ambient fish experienced an overwintering event with food deprivation and did not have an exogenous energy source for growth. At 8, 9, and 10 mph, there was also a significant effect of the interaction between substrate and temperature treatments which manifested as Substrate fish being larger in body length and mass for the Ambient treatment but No Substrate fish being larger for the Hatchery treatment and the substrate treatments switching

in rank for the Stocking treatment.

Summary

In summary, for the 2016-2017 cohort, GenPop fish were consistently larger in body length and mass compared to the Hatchery No Substrate treatment that experienced the same feeding and temperature regimes, likely due to the confounding factor of stocking density in the MSU. Within the treatments in the MSU, by the end of the experiment, the Hatchery treatment had outperformed the Ambient and Stocking treatments likely due to the sustained higher temperatures and feeding regimes in the Hatchery treatment. Interestingly, prior to the confounding effects of stocking density on growth rate, there were distinct differences in growth rate between incubation, substrate and temperature treatments during the first few weeks post hatch. So the hypothesis that different rearing environments would influence the growth rate of Lake Sturgeon can be accepted prior to the confounding factor of density masking results. To explore this further and to better understand the consequences of early life growth rates on growth later in life, a common garden experimental approach was undertaken in 2017-2018 and densities in all treatments were kept equivalent and at levels that did not inhibit growth rate.

2017-2018 cohort

Yolksac larvae

Yolksac volumes of larvae that hatched from eggs incubated in tumbling jars (Hatchery Transfer/GenPop) were significantly larger than those that adhered to surfaces (Hatchery No Substrate) during the second half of the yolksac stage and were fully absorbed a day after those of Hatchery fish. This means that the Hatchery Transfer fish absorbed their yolksacs more slowly, likely related to incubation method as seen in the first year. There were also significant differences in yolksac volumes of larvae that were reared in different temperature treatments. These temperature differences influenced the absorption rate of the yolksacs. Larvae from the

Hatchery+4°C treatment completely using up their yolksacs at 12 dph, followed by larvae from the Hatchery+2°C treatment at 14 dph, then larvae from the Hatchery treatment at 15 dph. During the second half of the yolksac stage, Hatchery fish had larger yolksacs at each time point and absorbed them the slowest and Hatchery+4˚C fish had smaller yolksacs and absorbed them the fastest. This is consistent with yolksac depletion occurring at the fastest rate at the highest temperature (20 ˚C) treatment in White Sturgeon (Wang et al. 1987).

Prior to transfer to the common garden setup, there were significant differences in body length and mass between incubation treatments. During the first week, Hatchery fish had significantly larger body lengths and for most of the first 2 weeks, Hatchery fish had significantly larger wet mass values. This is also consistent with the pattern seen in the first year growth experiment with Hatchery fish using their yolksacs more quickly but investing the energy into growth more quickly.

There were also significant differences in body length and mass for larvae that were reared in different temperature treatments with Hatchery fish being significantly smaller in body length than Hatchery+2˚C and/or Hatchery+4˚C from 8 dph until the transfer. Hatchery fish were also significantly smaller in body mass than Hatchery+2˚C and/or Hatchery+4˚C from 13 dph until the transfer, and Hatchery+4˚C had the largest body size. The Hatchery+4˚C fish used up their yolksacs the fastest and grew the fastest, and the Hatchery fish used up their yolksacs the slowest and also grew the slowest, indicating the energy from yolksacs was mobilized and used for growth more readily in the higher temperature treatments.

Pre-winter

After transfer to the common garden setup, during the summer and fall, significant differences in body length and mass were present between GenPop and Hatchery Transfer

treatments. When difference in body length and mass were present, Hatchery Transfer fish had larger lengths and wet mass values. The GenPop fish experienced lower temperatures in the summer compared to the river temperatures experienced by Hatchery Transfer (up to 22˚C), they also experienced a disease outbreak around 3 mph, and due to a miscommunication, the density in the experimental trough was dropped to ~75% the level of the GenPop density (and remained unequal for the remainder of the experiment), so the combination these factors likely contributed to the size differences observed.

There were no significant differences in body length between incubation treatments during the summer and fall but body mass was significantly different at 6 mph when Hatchery fish had significantly larger wet mass values than Hatchery Transfer. The effects of incubation method appear to be limited to the pre-transfer stage, with no significant differences in body length or mass between incubation treatments for the remainder of the experiment.

The significant differences in body length and mass between temperature treatments during this period, occurred at 5 and 6 mph, just prior to winter. At 5 mph, Hatchery fish were significantly smaller in body length and mass than the higher temperature treatments. And at 6 mph, Hatchery+4˚C fish were significantly larger in body mass than the lower temperature treatments. At this time, the temperatures in all treatments had only been different for 2 weeks between hatching and transfer, so the differences in size are due to the effects from the temperature differences experienced a few months prior. This suggests there may be a particular developmental timeframe in which growth rate phenotype is set and once the fish has developed beyond this time point the faster growth phenotype persists throughout at least the first year of life.

Winter

During the overwintering period, significant differences in total length and/or mass were present between incubation treatments for the entire duration of the overwintering period (7-11

mph). At this point, GenPop fish were significantly larger in body length and mass than Hatchery Transfer fish due to the Hatchery Transfer fish experiencing an overwintering event with 1˚C water and food deprivation for 4.5 months while the GenPop fish were fed to satiation daily in 16˚C water, allowing them to convert the energy obtained from feeding into growth of body size.

The significant differences in body length and/or mass between temperature treatments during this period, occurred at 7 and 8 mph, at the beginning of winter, then no significant differences were observed until after winter. At 7 mph, Hatchery fish were significantly smaller in body length and mass than the higher temperature treatments and at 8 mph, Hatchery fish were again significantly smaller in body length than the higher temperature treatments. The decrease in food deprivation and cold temperatures, experienced by all temperature treatments, impeded any growth during the overwintering period. Hatchery+4˚C had the largest decrease in body mass during winter. This is likely due to larger fish having higher energetic costs to maintaining their size (Cargnelli and Gross 1997) and without any exogenous source of food to put towards that maintenance, a decrease in mass was seen and the fish may have catabolized fat and muscle fibers (Kim and Lovel, 1995). The smaller fish may have been able to maintain their lower starting body mass because of their smaller body size having lower metabolic costs for maintenance (Xiao-Jun and Ruyung, 1992) at the start of the overwintering period and therefore had lower metabolic costs throughout the overwintering period when their metabolic rates were further suppressed (Deslauriers et al. 2018).

Post-winter

After recovery from the overwintering period, significant differences in total length and/or mass were again present between temperature treatments at both 12 and 13 mph. Hatchery fish had the smallest body length and mass values and Hatchery+2˚C and/or Hatchery+4˚C fish had the largest body sizes. This shows the continued effect of temperature differences from the first

month manifesting as size differences 1 year later, suggesting a critical period during early life-history that will influence long-term growth trajectory and ultimately fitness of the individual. Previously, MacQueen et al. (2008) showed the effect of rearing temperature on Atlantic Salmon embryos and larvae during early life, where a persistent effect on growth rates as adults was found with fish reared in colder water during early life having the lowest growth rate, throughout life. Similarly, in this study, fish from the coldest treatment were smallest and had lower growth a year after the initial temperature manipulation. However, fish initially raised in higher temperatures persisted their accelerated growth rate for at least two months after recovery from overwintering when an exogenous energy source was reintroduced and higher temperatures may have led to higher efficiencies for converting the energy to growth in the Hatchery+2 and Hatchery+4 treated fish (Xiao-Sun and Ruyung, 1992). Sogard and Olla (2000) found Walleye Pollock, *Theragra chalcogramma,* experienced nearly full recovery of lipid stores within 90 days of recovery from overwintering and no negative effects on future growth rates. Further, it has been shown that high growth rates in early life can lead to larger size, resulting in better energetic condition and higher fitness after the overwintering period (Cargnelli and Gross 1997). Thus, the present study supports an increase in growing temperature during early life history that will result in development of a fast-growing phenotype post the first winter of life.

Summary

My prediction that Lake Sturgeon reared in tanks without substrate would have lower growth rates than those reared with substrate was only clearly supported in the second week of development. After yolksac absorption, the confounding factors with density likely prevented significant developmental differences between substrate treatments. The prediction that Lake Sturgeon reared in colder water would have lower growth rates than those reared in warmer water was supported by the differences seen in the 2016-2017 cohort where final differences in size of

the MSU fish correlated with the differences in overall temperature experienced by the fish throughout the experiment. The differences seen in the 2017-2018 cohort where immediate effects of temperatures were seen in the first month as well as at the end of the experiment with larger fish resulting from exposure to higher temperature treatments prior to transfer. The predictions that fish exposed to a prolonged drop in temperature and deprivation of food (simulating an overwintering event) would exhibit a cessation in growth was supported by the growth rates in both cohorts. When water temperature was increased, and food was returned after winter, the prediction that fish in the 2017-2018 cohort that displayed a higher growth rate prior to winter (as a result of increased temperature during early rearing) would continue to exhibit that phenotype following winter was supported. Overall, the significant differences between incubation and temperature treatments suggests a fixed growth phenotype develops in early life and is temperature dependent.

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Chapter 3. Muscle development

Introduction

Although little is known about sturgeon muscle development, they do share some muscle characteristics with teleosts as shown in studies on Atlantic Sturgeon, *Acipenser baerii* and Sterlet Sturgeon, *A. ruthenus* and *A. ruthenus* · *A. gueldenstaedti* hybrids (Aidos et al. 2007; Steinbacher et al. 2006). In teleosts, myogenesis begins in early embryonic stages with fibres developing in late embryonic stages as shown in Atlantic Salmon, *Salmo salar* L. (Stickland et al. 1988). In teleost larvae, muscle tissue is organized into myotomes which run parallel to each other down the length of the trunk, divided laterally by the myoseptum (Johnston et al. 2011). Myotomes contain bundles of slow and fast multinucleate muscle fibres, mononuclear precursor cells (satellite cells), connective tissue, and adipocytes (Johnston et al. 2011). Fast muscle fibres (white muscle) are located closest to the fish's core with a thinner layer of slow muscle fibres (red muscle) located between the fast muscle and the skin (Kryvi et al. 1980), as seen in figure 3.1.

The fast muscle contains larger fibres with a higher density of myofibrils that provide more power at higher tailbeat frequencies, making it useful for high velocity swimming and functions through anaerobic metabolism (Johnston 1999). The slow muscle contains smaller fibres with a higher density of mitochondria and is the dominant muscle type used at lower tailbeat frequencies, making it useful for regular activity such as foraging and migration and functions through aerobic metabolism (Johnston 1999). The proportion of red and white muscle in fish is important for their swimming performance, higher red muscle content is necessary for sustained swimming, while white muscle is necessary for bursts of speed as shown in Southern Smelt, *Retropinna retropinna* (Meyer-Rochow et al. 1993). Temperature can influence swimming performance, for example in

common Carp, *Cyprinus carpio*, 50% more muscle tissue is recruited at 10˚C to generate the same force as compared to Carp acclimated to 20˚C (Rome et al. 1990).

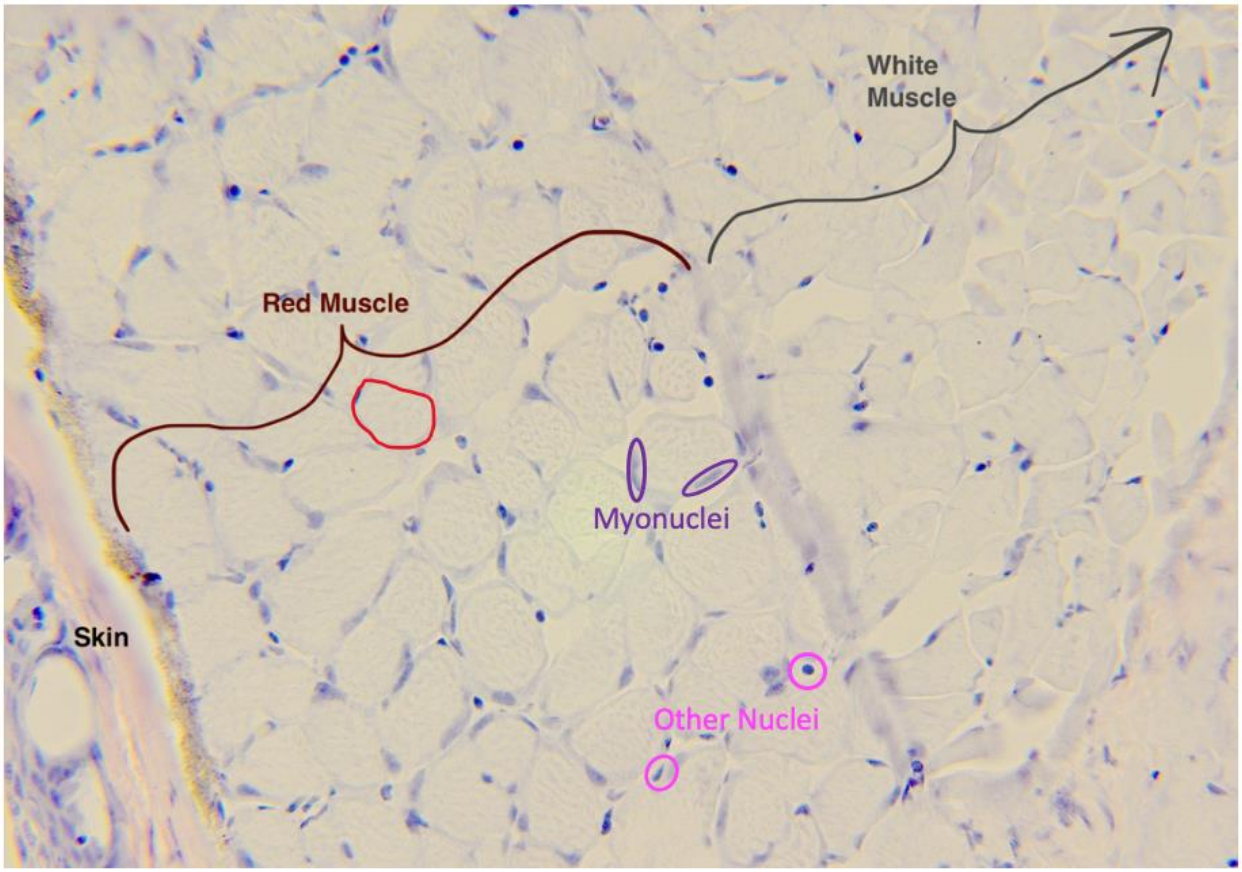

Figure 3.1. Cross section of Lake Sturgeon muscle showing red and white muscle tissue and skin at X20 magnification. Slide stained with hematoxylin and eosin. The white muscle tissue is labelled in grey. The red muscle tissue is labelled in dark red with a single fibre traced in lighter red. Myonuclei are circled in purple. Other (non-myonuclei) are circled in pink.

For both muscle fibre types, the number of nuclei associated with a fibre is linearly related to fibre diameter as shown in Atlantic Salmon (Johnston et al. 2003). Fibre size and number is also linearly related to growth rate and body size as shown in Southern Smelt and other species of freshwater teleosts from the Cyprinidae, Centrarchidae, Percidae, Salmonidae, and Esocidae families (Meyer-Rochow et al. 1993; Weatherley et al. 1988). At hatch, Atlantic Salmon have one layer of red muscle cells visible around their trunk which increases to 5-6 layers by first feeding with fibres having doubled in size (Johnston and McLay 1997). During development, there is an

increase in muscle mass at the onset of exogenous feeding due to muscle fibre hyperplasia (production or recruitment of new fibres) and hypertrophy (enlargement or elongation or existing fibres) as shown in Pacu, *Piaractus mesopotamicus* (Leitão et al. 2011). There is a limit to how much muscle fibres can grow through hypertrophy which is related to the maximal diameter of individual fibres as shown in Atlantic Salmon, and Sea Bass, *Dicentrarchus labrax* L. (Johnston et al. 2003; Ayala et al. 2001). This maximal fibre diameter is reached when the surface area to volume ratio reaches a threshold after which nutrient assimilation would be insufficient to support the fibre function (Weatherley et al. 1988). When this limit is reached, hyperplasia is necessary for further muscle growth which is aided by the proliferation of satellite cells (Weatherley et al. 1988; Johnston et al. 1998).

Satellite cells are myogenic cells that are located between the sarcolemma and the basement membrane of muscle fibres. They divide and proliferate to produce more small muscle fibres through hyperplasia (Johnston et al. 1998), fusing with existing muscle fibres to create the myonuclei involved in muscle growth (Koumans and Akster 1995; Johnston et al. 2000). Satellite cells play a role in the increase of muscle tissue when they are activated at the end of the yolksac stage as shown in Atlantic Herring, *Clupea harengus* (Johnston 1999). Immunohistochemistry staining can determine the difference between normal myonuclei that are associated with muscle fibres, which appear inside the basement membrane and the sarcolemma, and satellite cells, which appear inside basement membrane but outside the sarcolemma. This is done by staining for all nuclei as well as staining for laminin; a protein found in the basal lamina outside of the myofibre membrane (Steinbacher et al. 2006) and dystrophin; a protein found in the plasma membrane of differentiated myofibres and therefore should not be present between satellite cells and the basal lamina (Marti et al. 2013). Satellite cell populations as well as myonuclear counts and other

characteristics of muscle fibers can be affected by environmental factors in teleosts (Johnston 1999; Johnston 2006).

Early life history environment is very important for muscle growth and can determine fitness throughout the life of an individual having large impacts on muscle development and phenotype. Early environment determines the rate of myogenesis, and the size and number of muscle fibres (Johnston 2006). During early growth, increased temperature has been shown to increase muscle fibre number (hyperplasia) in Rainbow Trout, *Oncorhynchus mykiss*, (Kiessling et al. 1991) and Danube Bleak, *Chalcalburnus chalcoides mento*, (Stoiber et al., 2002). Increased temperature can also increase hypertrophy as shown in sea bass, Atlantic Herring, Atlantic Salmon, and Southern Smelt (Ayala et al. 2001; Johnston et al. 1998; Johnston et al. 2003; Meyer-Rochow et al. 1993). However, in larval Atlantic Salmon, colder temperatures also resulted in more muscle fibres which had larger cross-sectional areas at hatch compared to those reared in warmer temperatures (Johnston et al. 2000).

Food availability and temperature can also affect muscle development. A combination of increased temperature and food availability resulted in increased myobfibre number in Atlantic Salmon larvae (Nathanailides et al. 1995) and myobfibre number and myonuclear counts in juvenile European Sea Bass, *Dicentrarchus labrax* (Nathanailides et al. 1996). Temperature and food deprivation can also influence fish muscle fibre growth later in life particularly during periods of depressed environmental temperature and resources such as winter as seen in Marine Flatfish, *Pleuronectes platessa*, and Channel Catfish, *Ictarulus punctatus*, starved over winter in cold water which showed smaller average fibre sizes and a decrease in the abundance of large fibres (Johnston et al. 1981; Kim and Lovell, 1995). Overall, acute colder temperature rearing environments and

food deprivation lead to smaller fibre sizes with less myonuclei which correlates with smaller body size and these effects can last for a number of years.

In Atlantic Salmon, environmental conditions during the development of embryos had an effect on muscle phenotype displayed by the fish three years after hatching (Macqueen et al. 2008). Plasticity in muscle growth usually involves structural changes in the organelles of muscle cells (Johnston and Maitland 1980; Penney and Goldspink 1980), and in teleost fish embryos and larvae is usually irreversible due to the rapid development occurring in early life stages (Johnston 2006).

It is evident therefore that fish incubated and/or reared in environments with colder temperatures and less food availability display reduced muscle development. This chapter describes the effect of embryo incubation, temperature and age on muscle development during the first year of life in Lake Sturgeon, *Acipenser fulvescens*. Lake Sturgeon were reared from embryo in different temperature profiles with two incubation methods. I hypothesize that different rearing environments will influence the muscle development of Lake Sturgeon. Lake Sturgeon reared in colder water will have smaller fibres with fewer associated myonuclei than those reared in warmer water. Further, fish exposed to a prolonged drop in temperature and deprivation of food (simulating an overwintering event) will exhibit a cessation in hypertrophy and hyperplasia of muscle fibres. When water temperatures are increased, and food is returned after winter, fish will display a hyperplasia and hypertrophic response in muscle fibre growth and muscle growth patterns established in early development will persist as the fish are warmed and fed after the overwinter period.

Methods

The fish used in the muscle development experiments were taken from the experimental populations described in chapter 2 at GRFH (Hatchery Transfer, Hatchery, Hatchery+2, and Hatchery+4). Due to a disease outbreak among the GenPop treatment, samples were not taken for

muscle analysis from that treatment group. Muscle growth was assessed by histological means by sacrificing 10 fish randomly selected from each treatment at 3, 6, 8 and 13 months post-hatch. Fish were exposed to a prolonged over-wintering period when they were deprived of food, and water temperatures were maintained at approximately 1.5°C between six and eleven months post-hatch.

Fish were euthanized by immersion in an overdose of Tricaine methyl sulfonate (MS-222). A cross-section of the tail region was taken by making an incision at the anus and 5 mm posterior to the anus. Muscle samples were placed in 4% PFA ((4 g paraformaldehyde in 100 mL of 0.01M Phosphate Buffered Saline (PBS: 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4, dissolved in 800 mL ddH_2O, pH 7.4, total volume brought to 1 L with ddH_2O heated to 65 ºC; pH 7.0)) and stored for 24 hours at 4 ºC. Samples were then rinsed three times with 0.1M PBS and transferred to a cryoprotectant solution (150 g sucrose in 200 mL PBS) where they remained in the cryoprotectant solution until embedding 3-4 weeks later. Samples were prepared for cryosectioning by blotting off the cryoprotectant solution, then frozen in OCT, positioned perpendicularly on small cork strips by placing them in isopentane cooled with blocks of dry ice (-80 ºC) for 5-10 seconds (until solid white). Samples were kept frozen (-20 ºC) until sectioning. Frozen sections were cut at 7 μm thickness in a cryostat (-25 ºC) and mounted on glass slides coated with a gelatin solution (5.0 g gelatin, 0.5 g $CrK(SO_4)\cdot 12H_2O$ in 1 L H_2O heated to 45 ºC and filtered). Sections were air dried for an hour to remove moisture then stored at -20 ºC until staining. Red muscle fibres were stained with hematoxylin and eosin and by immunohistochemistry.

Hematoxylin and eosin staining was used to examine muscle fibre cross sectional area and myonuclear count by light microscopy. Slides were removed from the freezer and left at room temperature for 20 minutes followed by 20 minutes warming on a hotplate at 45 ºC to ensure

sections stuck to slides during staining. Slides were allowed to cool down to room temp then rehydrated by immersion in 100% ethanol for 5 minutes twice, then 95% ethanol for 2 minutes, then distilled water for 5 minutes. For hematoxylin staining, slides were placed in Harris Modified Method Hematoxylin with Acetic Acid (2%) (Fisher Scientific) for 4.5 minutes, then distilled water for 5 minutes, then 1% acid alcohol (0.7ml HCl with 70mL 70% ethanol) for 30 seconds, then distilled water for 5 minutes, then saturated lithium carbonate (1.54 g lithium carbonate in 100 mL distilled water) for 2 minutes, then finally washed in distilled water for 5 minutes. For eosin staining, slides were placed in eosin (1 g eosin Y, 100 mL distilled water and 0.1 mL acetic acid) at a 1:8 dilution in distilled water for 1 minute 30 seconds. After the stains were applied, slides were dehydrated from water to 100% ethanol by placing them for 30 seconds in 70% ethanol, 45 seconds in 95% ethanol, and 2 minutes in 100% ethanol twice. Slides were then placed in slide bright for 4 minutes mounted with a cover slip and dried. The stained sections were digitized using analySIS getIT software with an Olympus BH-2 microscope at x40 magnification with an Olypmus Soft Imaging Solutions camera model UC50. The images were analyzed, and fibre area was measured, for fibres with clear borders, using the freehand selection tool in Fiji by ImageJ with accompanying nuclei counts for 100-120 red muscle fibres for every fish sampled. Only red muscle was able to be analyzed due to issues with sectioning damaging white muscle tissue.

A subset of approximately 20% of samples from each treatment group and each time point were also stained with immunohistochemistry to add fluorescent tags with antibodies for laminin and dystrophin, and with DAPI blue. Slides were dried at room temperature for 30 minutes. Water repellant borders around sections were drawn with an IHC PAP pen (Enzo Life Sciences). Slides were washed 3 times in 0.1M PBS (8g NaCl, 0.2g KCl, 2.68g $Na_2HPO_4.7H_2O$, 0.24g $KH_2PO_4^-$ in 1L milli-Q, pH 7.4) for 2 minutes. Excess liquid was removed and slides were laid flat and

incubated with normal goat serum blocking solution (2% goat serum, 0.1% cold fish gelatin, 0.1% triton-100, 0.05% Tween-20, 0.05% sodium azide in 0.01M PBS, pH to 7.2) : 2M glycine : Goat anti-rabbit (AffiniPure Fab Fragment Goat anti-rabbit IgG (H+L), Jackson ImmunoResearch) (20:1:1) for 1 hour at room temperature, covered. Excess liquid was removed, and slides were washed 3 times in TBST (6.1g Tris base, 9g NCl, 1ml tween-20 in 1L milli-Q, pH to 8.4) for 2 minutes. Excess liquid was removed, and slides were laid flat and incubated with 0.001% Avidin (in 0.01M PBS) for 15 minutes at room temperature, covered. Excess liquid was removed, and slides were washed once in PBS for 2 minutes. Excess liquid was removed, and slides were laid flat and incubated with 0.001% Biotin (in 0.01M PBS) for 15 minutes at room temperature, covered. Excess liquid was removed, and slides were washed 3 times in TBST for 2 minutes. Excess liquid was removed and slides were laid flat and incubated with 0.125 mg/ml anti-laminin antibody produced in rabbit (Sigma Aldrich) in primary antibody dilution buffer (0.1% fish skin gelatin, 0.5% triton X-100, 0.05% sodium azide in 0.01M PBS, pH to 7.2) overnight at 4°C, covered. Excess liquid was removed, and slides were washed 3 times in TBST for 2 minutes. At this point, lights were turned off to protect the fluorescence of the stains. Excess liquid was removed, and slides were laid flat and incubated with 1µg/ml DAPI (4',6-Diamidino-2-phenylindole dihydrochloride) (Santa Cruz Biotechnology) for 30 minutes at room temperature. Excess liquid was removed, and slides were washed 3 times in TBST for 2 minutes. Excess liquid was removed, and slides were laid flat and incubated with the secondary antibody solutions for 2 hours at room temperature, covered. The solution was anti-rabbit-IgG conjugated to Mega 485 (Sigma Aldrich): secondary antibody dilution buffer (0.05% tween-20 in 0.01M PBS, pH to 7.2) (1:200). Excess liquid was removed, and slides were washed 3 times in TBST for 2 minutes, then once in 0.1M PBS for 5 minutes. Slides were covered in citrate buffer (0.0189g 10mM citric

acid·H_2O, 0.1468g sodium citrate in 500mL milli-Q, pH 6) and microwaved for 2 minutes. Slides were allowed to cool in the buffer solution for 30 minutes. Slides were washed 3 times in 0.1M PBS for 4 minutes. Excess liquid was removed, and slides were laid flat and incubated with triton X-100 (sigma aldrich) for 30 minutes at room temperature, covered. Excess liquid was removed and slides were laid flat and incubated with normal goat serum blocking solution : 2M glycine : Goat anti-mouse (AffiniPure Goat anti-mouse IgG (H+L), Jackson ImmunoResearch) (20:1:1) for 1 hour at room temperature, covered. Excess liquid was removed, and slides were washed 3 times in TBST for 2 minutes. Excess liquid was removed, and slides were laid flat and incubated with 0.001% Avidin (in 0.01M PBS) for 15 minutes at room temperature, covered. Excess liquid was removed, and slides were washed once in PBS for 2 minutes. Excess liquid was removed, and slides were laid flat and incubated with 0.001% Biotin (in 0.01M PBS) for 15 minutes at room temperature, covered. Excess liquid was removed, and slides were washed 3 times in TBST for 2 minutes. Excess liquid was removed, and slides were laid flat and incubated with 40 µg/ml dystrophin antibody raised in mouse (monoclonal mouse IgG1 κ (kappa light chain)) (Santa Cruz Biotechnology) in primary antibody dilution buffer overnight at 4°C, covered. Excess liquid was removed, and slides were washed 3 times in TBST for 2 minutes. Excess liquid was removed, and slides were laid flat and incubated with the secondary antibody solution for 2 hours at room temperature, covered. The solution mouse IgG kappa binding protein (m-IgGκ BP) conjugated to CruzFluor™ 594 (Santa Cruz Biotechnology) : secondary antibody dilution buffer (1:200). Excess liquid was removed, and slides were washed 3 times in TBST for 2 minutes. Excess liquid was removed, and slides were mounted with Vectashield mounting medium. Slides were stored in 4°C in the dark until visualization and digitization at 40X magnification using oil immersion with a

fluorescent microscope with ApoTome attachment (Zeiss Axio Imager Z1) within 1 week of staining.

The images of sections were analyzed using the multi-point tool in Fiji by ImageJ to identify the first 300 nuclei (approximately the number of nuclei present in the area of 100-120 fibres used for the previous analysis) in red muscle which were classified as myonuclei, satellite cells, or other nuclei. Raw counts were transformed to the percentage of satellite cell nuclei to total myonuclei. The nuclei of satellite cells were identified by the blue stained areas between the laminin and dystrophin stained areas. Myonuclei were identified in the immunohistochemistry stained slides as nuclei (DNA stained by DAPI) that were found within the laminin (basal lamina) and dystrophin (cytoplasmic side of cell membrane) border of a cell with cytoplasmic area (see figure 3.2a). Nuclei of satellite cells were identified as nuclei located between the laminin and dystrophin borders (see figure 3.2b). Nuclei identified as other were not associated with muscle fibres, either appearing between, beside or not touching any fibres i.e. appearing outside the laminin sheath of the fibres (see figure 3.2c).

Statistical analysis

Between treatment analysis for the proportion of satellite cells, nuclear counts and fibre area of fish in the Hatchery Transfer and Hatchery treatments were compared at each time point to examine differences that could have arisen during embryogenesis when embryos were incubated in a MacDonald tumbling jar or allowed to adhere to substrate. To compare the two treatments at each time point, data were analyzed using a Students t-test with n=7-10, results with significance of $p<0.05$ are reported.

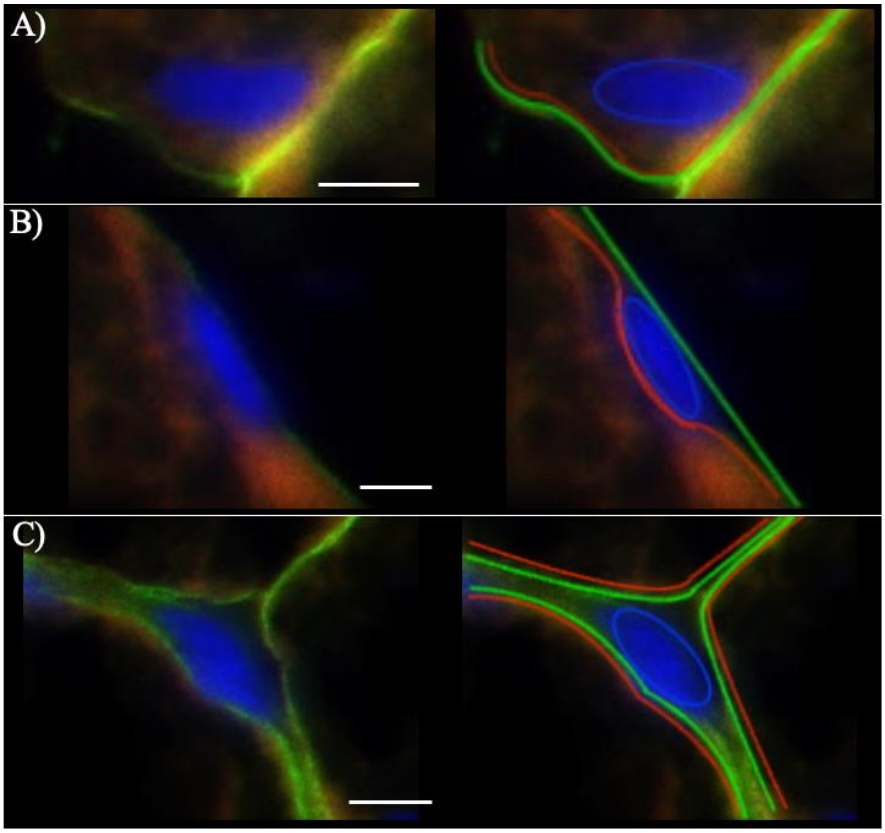

Figure 3.2. Cross section of Lake Sturgeon red muscle fibres at 6mph showing laminin in green, dystrophin in red, and nuclei in blue. Imaged with fluorescent microscopy at X40 magnification. Second column shows colored tracings of borders and nuclei. A) Myonucleus. B) Satellite cell. C) Other nucleus. White scale bars represent 10 µm.

Between treatment analysis for proportion of satellite cells, nuclear counts and fibre area of fish in the Hatchery, Hatchery+2°C, and Hatchery+4°C were compared at each time point to examine differences that could have arisen from early rearing temperature differences before the transfer to the common garden setup. To compare the three treatments for each time point and to compare the time points for each treatment, data were analyzed using a one-way ANOVA with n=7-10 and Tukey's LSD post hoc tests were used to determine which groups differed, results with significance of $p<0.05$ are reported.

Between treatment and sampling time analysis of pooled data for raw counts of the types of nuclei were analyzed using a one-way ANOVA with n=8-10 and Tukey's LSD post hoc tests were used to determine which groups differed, results with significance of $p<0.05$ are reported.

Within treatment analysis for proportion of satellite cells, nuclear counts and fibre area of fish were compared separately for each treatment to examine differences at between time points. Data were analyzed using a one-way ANOVA with n=7-10 and Tukey's LSD post hoc tests were used to determine which groups differed, results with significance of $p<0.05$ are reported.

Results

Satellite Cells

Figure 3.3 shows a representative image of the same sample section stained with hematoxylin and eosin, and immunohistochemistry. Raw counts of nuclei were different between categories in each and at each time point (figure 3.4). But when analyzed separately, none of the types of nuclei had different means between treatments or time points. The proportion of satellite cells vs myonuclei was not significantly different between or within treatments either. The average satellite cell population compared to the overall myonuclei count was 4.34±0.17%.

Myonuclei

Within treatment analysis showed that Hatchery Transfer fish had the lowest myonuclear counts at 3 mph (1.7±0.1 nuclei), increased values at 6 mph (2.5±0.1 nuclei), and highest values at 8 mph (2.1±0.1 nuclei), and with values at 13 mph (2.6±0.2 nuclei) not being significantly different from 6 or 8 mph (figure 3.5a). Hatchery fish had lowest myonuclear counts at 3 mph (1.8±0.1 nuclei), higher values at 6 mph (2.3±0.1 nuclei) and highest values at 13 mph (3.0±0.1 nuclei), with values at 8 mph (2.0±0.1 nuclei) not being significantly different from 3 or 6 mph (figure 3.5b). Hatchery+2˚C fish had lowest fibre areas and at 3 mph (1.8±0.1 nuclei), 6 mph (2.1±0.1 nuclei) and 8 mph (1.9±0.1 nuclei) which increased at 13 mph (2.7±0.1 nuclei) (figure 3.5b). Hatchery+4˚C fish had lowest fibre areas at 3 mph (1.8±0.1 nuclei) and highest values at 13 mph

(2.5±0.2 nuclei), with values at 6 mph (2.1±0.1 nuclei) and 8 mph (2.1±0.1 nuclei) not being significantly different from 3 or 13 mph (figure 3.5b).

Between treatment analysis did not reveal any significant difference for myonuclear counts within incubation treatments or temperature treatments.

Fibres

Within treatment analysis showed that Hatchery Transfer and Hatchery fish had lowest fibre areas 3 mph (872±108 and 925±108 μm^2, respectively) and higher fibre areas at 6 mph (1785±152 and 1428±117 μm^2, respectively), 8 mph (1601±85 and 1418±117 μm^2, respectively) and 13 mph (1427±109 and 1648±97 μm^2, respectively) (figure 3.6a). Hatchery+2˚C and Hatchery+4˚C fish had lowest fibre areas at 3 mph (883±86 and 840±48 μm^2, respectively) and highest fibre areas at 6 mph (1381±105 and 1337±87 μm^2, respectively) and 13 mph (1599±185 and 1285±147 μm^2, respectively) with values at 8 mph (1161±98 and 1165±75 μm^2, respectively) not being significantly different from 3 or 13 mph (figure 3.6b).

Between treatment analysis did not reveal any significant difference for individual fibre area within incubation treatments or temperature treatments.

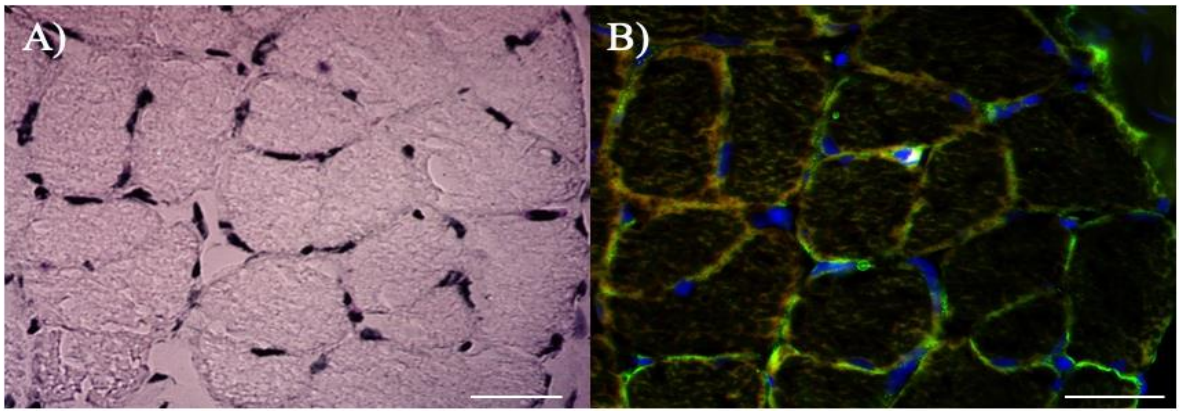

Figure 3.3. Cross section of Lake Sturgeon red muscle fibres at 6mph at X40 magnification. A) Slides stained with hematoxylin and eosin, showing nuclei in purple with pink counterstain. Imaged with light microscopy. B) Slides stained by immunohistochemistry, showing laminin in green, dystrophin in red, and nuclei in blue. Imaged with fluorescent microscopy. White scale bars represent 50 µm.

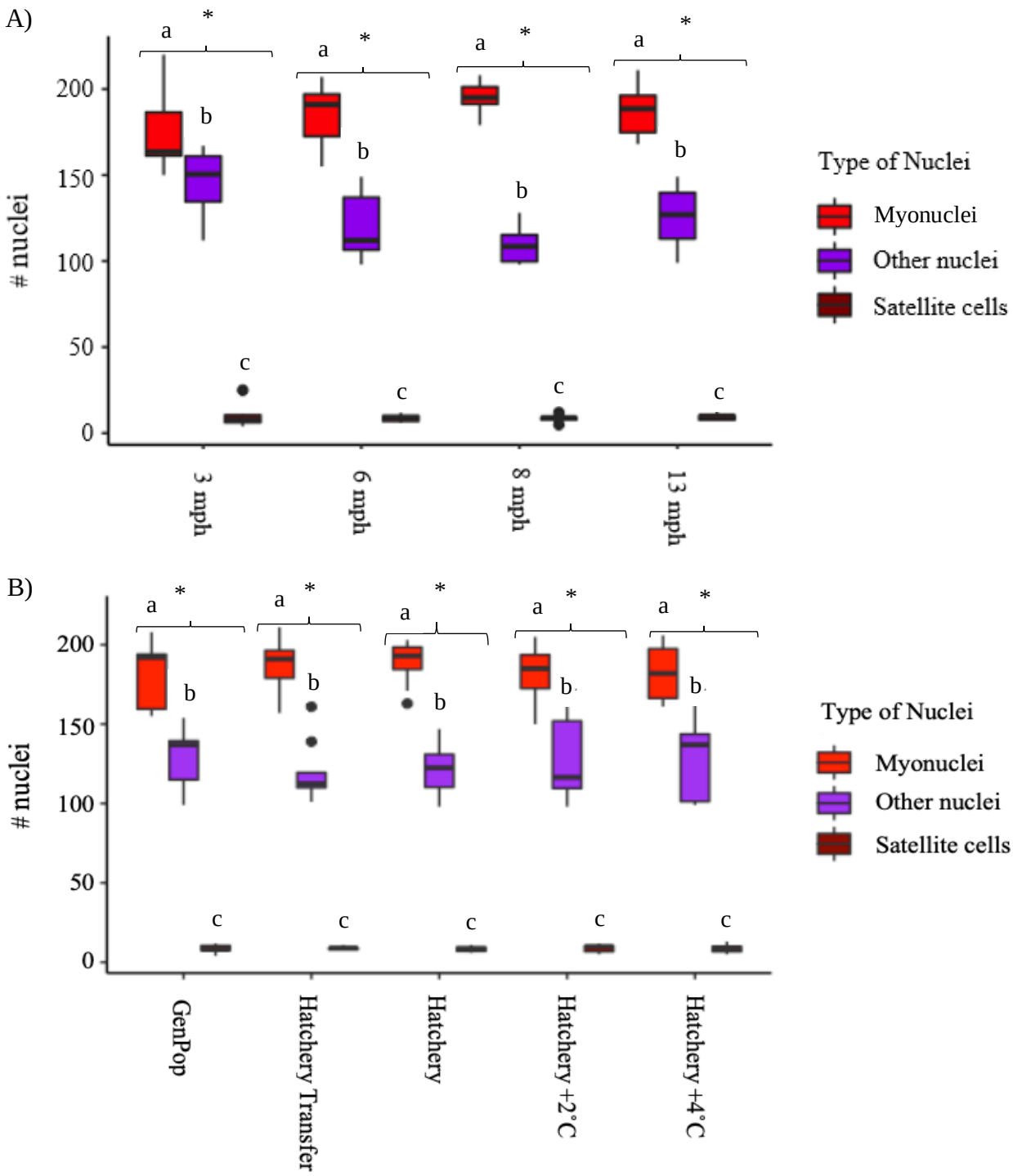

Figure 3.4. Raw counts of myonuclei, other nuclei and satellite cells in red muscle for Lake Sturgeon over the first year of life (2017-2018). Differences for values within category (time points or treatment) between nuclei types are marked by asterisks with further group differentiation indicated by lowercase letters. A) Pooled nuclear counts at measured time points. B) Pooled nuclear counts in each treatment. n=8-10.

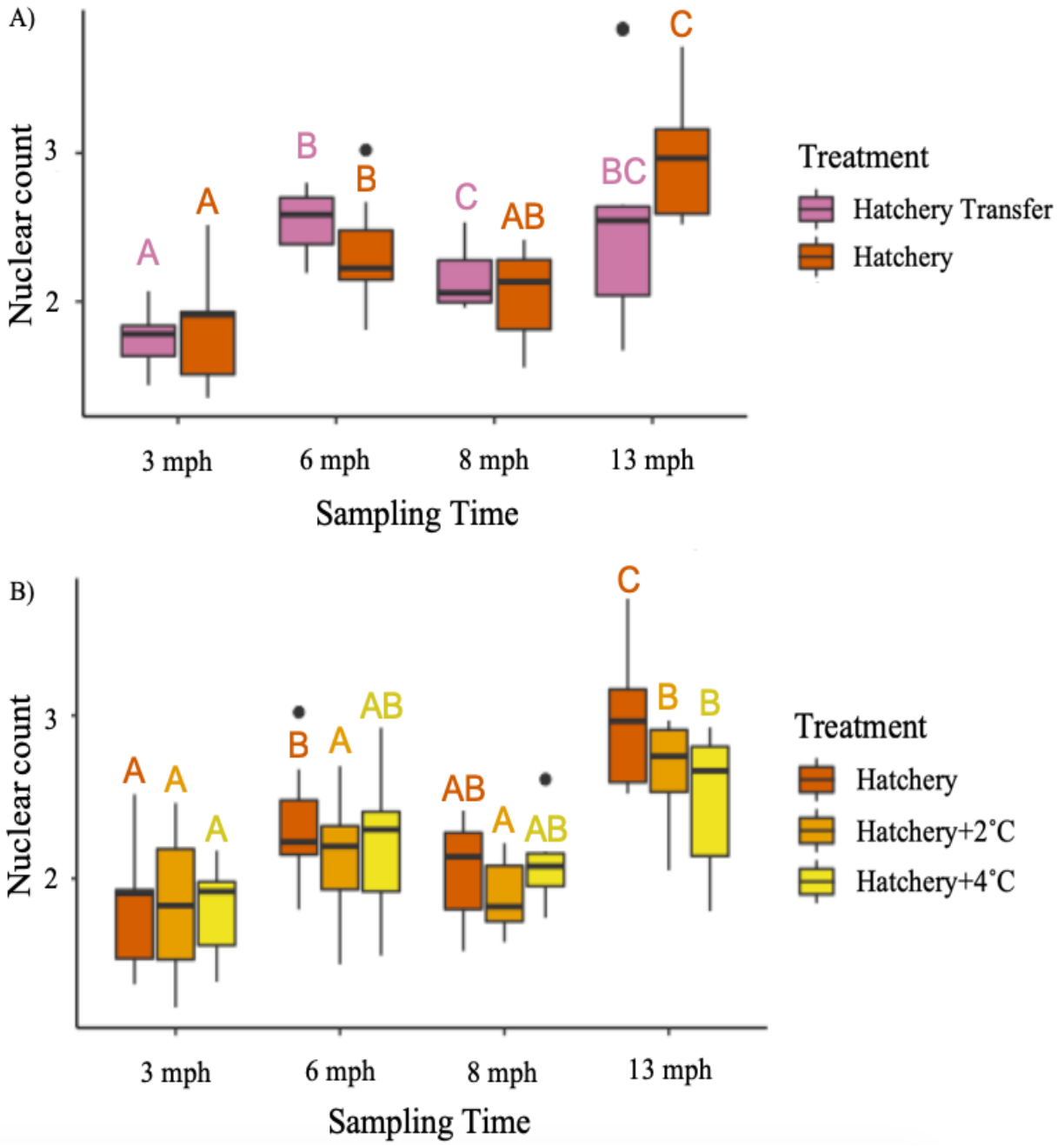

Figure 3.5. Differences in average nuclear (myonuclei and satellite cells) counts in red muscle for Lake Sturgeon over the first year of life (2017-2018). Differences for within treatments are indicated by uppercase letters with the colour associated with each treatment. Hatchery Transfer data is shown in purple. Hatchery data is shown in red. Hatchery+2˚C data is shown in orange. Hatchery+4˚C data is shown in yellow. A) Differences between Hatchery Transfer and Hatchery. B) Differences between Hatchery, Hatchery+2˚C, and Hatchery+4˚C. n= 7-10.

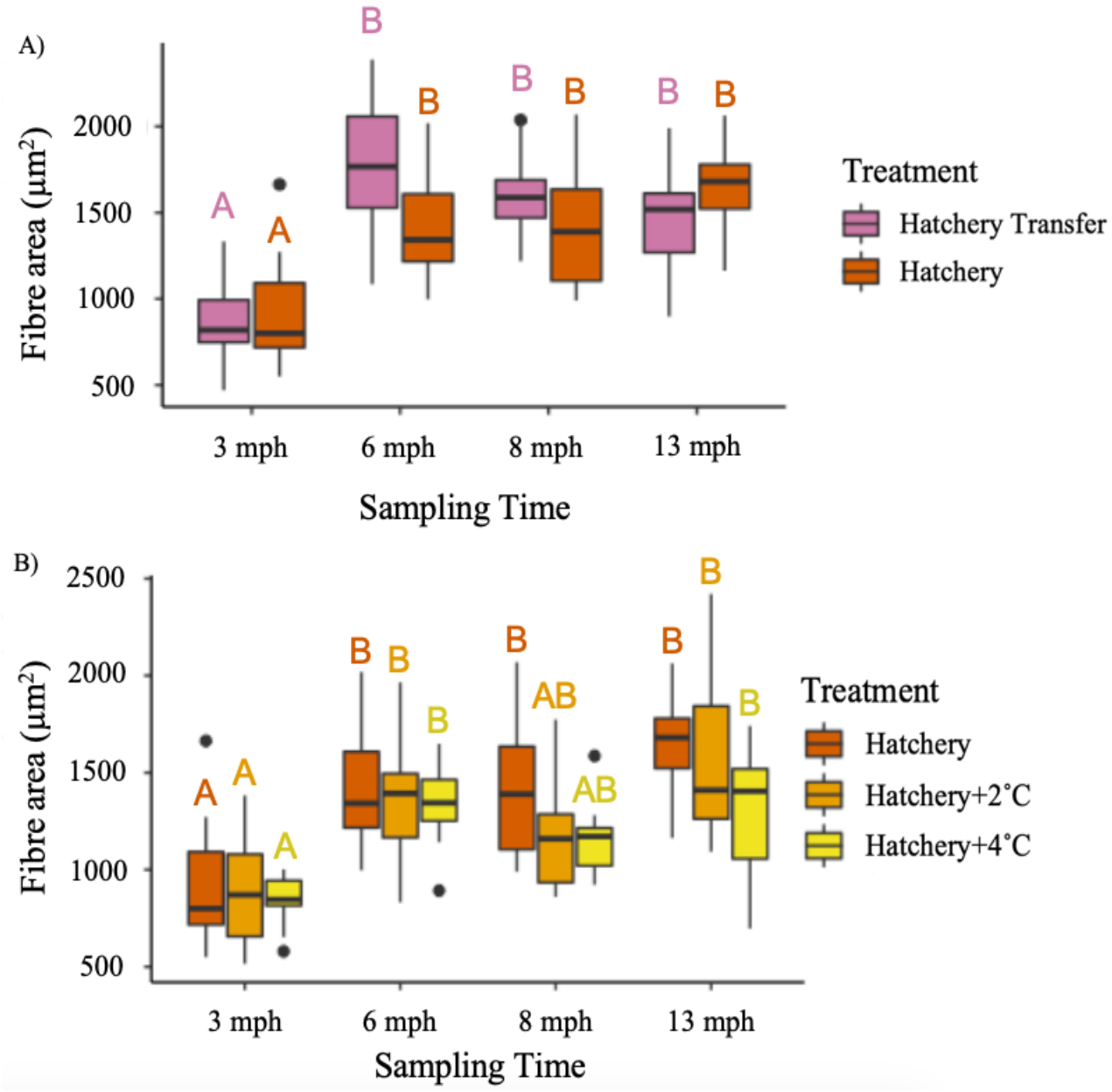

Figure 3.6. Differences in fibre area (µm^2) of red muscle for Lake Sturgeon over the first year of life (2017-2018). Differences for within treatments are indicated by uppercase letters with the colour associated with each treatment. Hatchery Transfer data is shown in purple. Hatchery data is shown in red. Hatchery+2˚C data is shown in orange. Hatchery+4˚C data is shown in yellow. A) Differences between Hatchery Transfer and Hatchery. B) Differences between Hatchery, Hatchery+2˚C, and Hatchery+4˚C. n= 7-10.

Discussion

Satellite cells

There were no significant differences in the proportion of satellite cells compared to myonuclei between or within any treatments. The percentage of satellite cells averaged 4.34±0.17% and is comparable to other studies which found the satellite cells comprised 5.8-6.6% of the total nuclei count in muscle tissue of sub-Antarctic fish, *Hapagifer bispinis*, (Brodeur et al. 2003), 2-5% of the total nuclei count in red muscle tissue in Carp (Koumans et al. 1994), and satellite cells were extremely rare in red muscle tissue of another sub-Antarctic species - Nybelin, *Notothenia neglecta* (Battram and Johnston 1991).

Fibre area and myonuclear count

Between treatment analysis did not reveal any significant difference for any metric analyzed between incubation treatments or temperature treatments. However, there were significant differences for both myonuclear counts and muscle fibre area within treatments.

Pre-winter

At 3 mph, the end of summer, myonuclear counts and muscle fibre areas were significantly lower than the other time points across treatments. Previous studies in freshwater teleost species have shown that fibre area increases as fish grow (Weatherley et al 1979), so the smaller fibre area at the first time point is expected as part of normal development.

Winter

At 6 mph, the beginning of the overwintering/food deprivation period, myonuclear count results were significantly higher than 3 mph for Hatchery and Hatchery Transfer fish but did not change for Hatchery+2˚C or Hatchery+4˚C fish. This increase is consistent with previous Lake Sturgeon myonuclei research which showed a general trend of increasing myonuclei per fibre with age (Heibert 2018). This is explained by the linear relationship of myonuclear count with general

somatic growth of the fish as it prepares for the first winter of life as shown in Atlantic Salmon and Southern Smelt (Johnston et al. 2003; Meyer-Rochow et al. 1993).

Muscle fibre area was significantly higher than the 3 mph values across all treatments. The lack of increase in fibre size from 6 to 8 mph is likely due to the decrease in nutrient and energy intake during this period which limits tissue growth. A reduction in muscle fibre growth in fish exposed to cold water has been shown in Northeast Arctic Cod, *Boreogadus saida*, and Carp (Galloway et al. 1998; Rome et al. 1990).

At 8 mph, the middle of the overwintering period, myonuclear count results were significantly higher than 3 mph but significantly lower than 6 mph for Hatchery Transfer fish but did not change for the other treatments. This maintenance (lack of increase with age) of myonuclei per fibre is to be expected with the food deprivation and low overwintering temperature and is similar to the previously reported fewer myonuclei and muscle fibers found in juvenile European Sea Bass held in suboptimal temperature and feeding conditions (Nathanailides et al. 1996). The decrease of Hatchery Transfer fish correlates with mass (see chapter 2), as fish are known to utilize fat and protein (predominantly muscle tissue) as an energy source during prolonged bouts of food deprivation as shown in juvenile Chinese Sturgeon, *A. sinesis* (Xiao et al. 2011), the decrease in myonuclear count could be a product of reabsorbing more muscle tissue as an energy source to sustain themselves due to their higher starting mass.

Muscle fibre area remained significantly higher than the 3 mph values in Hatchery and Hatchery Transfer fish but not significantly different from 3 or 6 mph values in Hatchery+2˚C or Hatchery+4˚C fish. The lack of increase in fibre size with age is again likely due to the food deprivation and low overwintering temperature.

Post-winter

At 13 mph, after 2 months of recovery from the overwintering period, myonuclear count results were still significantly higher than 3 mph for Hatchery Transfer fish, and values were significantly higher than all other time points for Hatchery fish and Hatchery+2˚C fish and values became higher than 3 mph for Hatchery+4˚C fish. Again, maintaining or further increasing the myonuclei per fibre with age seen at the previous time point for lower temperature treatments. The increased myonuclear counts coincided with increased growth and nuclear division at this time which would have been induced by the extra available energy from the reintroduction of food promoting somatic growth.

Muscle fibre area was again significantly higher than the 3 mph values across all treatments. It appears that the 2 month recovery period in the present study was insufficient time for the average fibre area to increase significantly through hypertrophy. Therefore, the increased growth observed in these fish during recovery (see chapter 2) is most likely the result of hyperplasia with satellite cells dividing and proliferating, creating more nuclei and muscle fibres without significantly increasing the size of fibres. Alternatively, a study on Southern Smelt found that fibre growth can have a sigmoidal pattern (Meyer-Rochow et al. 1993) which could also contribute to the lack of expected growth in muscle fibre area after recovery from overwintering.

The maximum average diameter of a red muscle fibre for any individual was 61 µm in a sturgeon with a wet mass of 14.5 g and total length of 164 mm. This is comparable to the maximum fibre diameter found in Nybelin, which was 50 µm in fish weighing ~12 g at 100 mm in length (Battram and Johnston, 1991). However, the diameter and area of fibres would be expected to increase with age and size up to the maximal diameter for the species (Weatherley et al. 1988).

It is important to note that the data were collected only for red muscle which may not be representative of white muscle development that might have occurred.

Summary

In summary, while fish in all treatments experienced the same patterns in significant increases in fibre area through the winter and recovery, fish in different treatments experienced increases and decreases in myonuclear counts at different times despite there being no diffrences between treatments. This could be because the fish were investing more in hyperplasia or hypertrophy at different times depending on their early rearing environments which had effects on the size of fish at several time points (see chapter 2). Slower growing fish tend to have fewer nuclear divisions resulting in fewer new fibres forming and instead there is an investment in hypertrophy to grow existing fibres (Nathanailides et al. 1995). In faster growing individuals, an investment in hyperplasia is prioritized which is associated with more nuclear divisions to create new small fibres (Nathanailides et al. 1995).

The prediction that Lake Sturgeon reared in colder water would have smaller fibres with fewer associated myonuclei than those reared in warmer water was not supported by data. However, the prediction that fish exposed to a prolonged drop in temperature and deprivation of food over winter would exhibit a cessation in hypertrophy and hyperplasia was supported by the lack of increase in fibre area and myonuclear counts. When water temperatures were increased, and food was returned after winter, the prediction that fish would display a hyperplasia and hypertrophic response in muscle fibre growth was partially supported by evidence of hyperplasia with fish size increasing along with myonuclear counts but not fiber area, indicating the addition of new muscle fibres instead of or in addition to existing fiber expansion, which is keeping the average fiber area from increasing. The overall absence of significant differences between incubation and temperature treatments and presence of significant differences between time points suggests a strong evolutionary pressure to maintain plasticity in muscle structure.

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Chapter 4. Larval Swimming

Introduction

As with all fishes, Lake Sturgeon, *Acipenser fulvescens*, rely on swimming to forage, escape predation, seek cover and survive. However, at a young age Lake Sturgeon are considered to be comparatively poor swimmers (Peterson et al. 2007) and may drift up to 60 km downstream from the spawning site to find suitable habitat (Auer and Baker 2002). Nonetheless when suitable habitat is found there is a requirement for volitional swimming to remain in that habitat. For example, at hatch Shortnose Sturgeon, *A. brevirostrum*, are positively rheotactic, photonegative, benthic and display strong cover seeking behaviour or will exhibit a swim-up and drift behaviour if no substrate suitable for cover and/or foraging is present (Richmond and Kynard 1995) and such behaviour continues later in life as juvenile fish forage on the benthos (Chiasson et al. 1997). Similarly, the negative phototactic response observed in Shortnose Sturgeon was used to develop a swimming performance assay for juvenile Lake Sturgeon (Deslauriers et al. 2018a). Importantly this type of swimming requires the use of red muscle fibres and relies predominantly on aerobic metabolism.

Conversely fast start responses involve the rapid acceleration away from perceived threats, utilize white muscle fibres and predominantly anaerobic metabolism (Wakeling 2001; Walker et al. 2005). Lake Sturgeon demonstrate c-start escape responses to predation threats. The c-start response occurs in phases which are activated by Mauthner cells (paired neurons either side of the vertebrae) (Eaton et al. 2001). These neurons reflexively initiate the preparatory stage, wherein reciprocal inhibition occurs with muscle fibres on one side of the fish are inhibited from contracting and the muscle fibres on the other side contract in a wave travelling towards the caudal fin resulting in the fish bending to form a “C” shape (Wakeling 2001). This is followed by the propulsive stage, wherein a wave of muscle contraction on the contralateral side of the fish results

in an explosive movement out of the "C" shape, moving away from the stimulus that initiated the response.

The efficacy of the escape response depends on several kinematic (acceleration, velocity, distance covered, rotation rate and angle) and behavioural (responsiveness and response latency) factors. Fish that detect predators at farther distances, react sooner, accelerate faster and have better odds of surviving the encounter as seen in Birchir, *Polypterus senegalys,* and Guppies (Tytell and Lauder 2002; Walker et al. 2005). Better swimming performance in escape responses can come from more effective transfer of power into hydrodynamic force, longer muscle activity duration and differences in body bending/rotation (Tytell and Lauder 2002; Wakeling 2001). This swimming motion can also be affected by the position of the stimulus (perceived predation threat) in relation to the animal which affects the turning angle and burst velocity as described in the birchir (Tytell and Lauder 2002). Importantly, like many performance metrics in fishes there is significant variation in responses between species and individuals (Eaton et al. 2001; Marras et al. 2011; Tytell and Lauder 2002). In Lake Sturgeon and White Sturgeon, *A. transmontanus,* larger individuals have been shown to have stronger escape responses due to their more developed caudal fins allowing for more powerful thrust (Boysen and Hoover 2009; Wishingrad et al. 2014b). As young fish the reduced escape response may be compensated for with a strong cover seeking behaviour when exposed to predation threats (Wishingrad et al. 2014b) and development of 5 rows of sharp scutes on the ventral, lateral and dorsal surface as body armour against potential predation (Peterson et al. 2007). Interestingly, development of body armour results in a trade-off as observed when comparing behaviour in responses to predation threat between Brook Stickleback, *Culea inconsta,* and Fathead Minnow, *Pimephales promelas,* where the stickleback, as the more armoured species, exhibited less behavioural adaptations to predation threats (Abrahams 1995).

Environmental conditions such as nutrient availability, hypoxia and habitat type have been shown to influence swimming behaviour (Claireaux and Lefrançois 2007; Domenici et al. 2007; Wishingrad et al. 2014a). Additionally, inter-individual variation over time in swimming performance has been demonstrated in European Sea Bass, *Dicentrarchus labrax* (Marras et al. 2011). In White Sturgeon there was no effect of temperature on startle response reaction time (Baker et al. 2014). However, swimming behaviours of the larvae may be species specific for sturgeon depending on the preferred substrate (Nguyen and Crocker 2006) and the effects of temperature on swimming behaviour have not been well studied in this species. Differences across seasons (size and temperature variations) have also been shown in juvenile Green Sturgeon, *A. medirostris*, and Shortnose Sturgeon critical swimming velocity performance (Allen et al. 2006; Deslauriers and Kieffer, 2012). In larval and juvenile white sturgeon, volitional, horizontal swimming performance increased with fish size, developmental stage and warmer temperatures (Crocker and Cech 1997).

Early environmental conditions can determine the range of any given physiological trait later in life, however, there can also be phenotypic plasticity determining how those traits present within that range when individuals encounter different environmental conditions later in life (Auer 2010). In the case of swimming performance, the success of individuals is dependent upon their ability to respond to environmental conditions by most effectively adjusting different metrics of their swimming performance without affecting their fitness (Pörtner and Farrell 2008). The evolution of phenotypic plasticity of swimming performance is influenced by the variability of conditions experienced by populations which can favor different behaviour and faster or slower responses and velocities at different times (Seebacher et al. 2012).

This chapter describes the effect of embryo incubation, temperature and age on voluntary and forced swimming performance during the first year of life in Lake Sturgeon. It is hypothesized that different rearing environments will influence the swimming performance of Lake Sturgeon throughout the first year of life. It is predicted that Lake Sturgeon reared in colder water will have slower escape responses in the pre-winter experiments due to lower growth rates leading to less developed bodies with smaller caudal fins. Further, fish exposed to a prolonged drop in temperature and deprivation of food (simulating an overwintering event) will exhibit slower swimming speeds as a result of reduced environmental temperature, or a lack of response to stimuli due to low energy resources available to the fish at the time of experimentation. When water temperatures increased following winter, and food was returned it is predicted that fish in all treatments will display faster swimming speeds and reaction times and that fish in warmer temperature treatments during early development will display faster swimming speeds due to larger body sizes/caudal fins.

Methods

In 2017-2018, volitional downward swimming and escape response trials were assessed. Experimental groups were as described in chapter 2. Briefly, eggs were fertilized and allowed to adhere to the tank bottom or substrate provided. Embryos were allowed to develop in the presence of substrate at hatch under ambient light conditions at 12°C. At hatch, yolksac larvae were exposed to 16 (Hatchery), 18 (Hatchery+2°C) or 20°C (Hatchery+4°C) water. In each treatment water temperature was increased by 0.5°C.day^{-1} increments. At 24 days post-hatch water temperature was reduced to 15.5°C by 0.5°C.day^{-1} and all fish were transferred to a common garden setup and allowed to on-grow for the following year. A fourth treatment group was transferred from the hatchery into the common garden setup and is described henceforth as Hatchery Transfer. This group differed in rearing environment during embryogenesis as they were fertilized and

immediately de-adhesed by gentle rolling in Fullers earth and added to a MacDonald hatching jar to complete embryogenesis. A total of eight (four duplicate tanks) were used in the common garden setup. Tanks were a flow-through design with water supplied from the Saskatchewan river and therefore were subject to ambient environmental temperatures. Light levels were also ambient except during winter months when opaque plastic lids were placed on the tanks to mimic low light levels due to ice and snow cover.

Both swimming performance tests were carried out on 10 fish in each of the four treatments at the following time points:

1) Hatch: early June 2017 when environmental temperature was equivalent across treatments
2) One month post-hatch: July 2017, when fish were transferred to the common garden setup following described temperature differences during early rearing and water temperature was on average 15.5°C
3) Three months post-hatch: September 2017, at the end of summer when water temperature was on average 18°C
4) Six months post-hatch: December 2017, just prior to over-wintering when water temperature was on average 2°C
5) Eight months post-hatch: February 2018, mid-winter when water temperature was on average 1.3°C
6) Thirteen months post-hatch: July 2018, when fish had recovered from over-wintering for two months and water temperature was on average 18°C

Volitional Swim down test

To test downward swimming performance, a protocol for volitional swimming following Deslauriers et al. (2018a) was used. Briefly, larvae from each treatment were placed in a clear 95 cm cylinder with an inner diameter of 12cm, filled with water from their respective tank reservoirs 85 cm in depth between the surface and the bottom. A sand substrate was placed on the bottom of the tube. To accommodate the increase in size, juvenile fish were placed in a clear acrylic, square tube 30cm x 30cm, filled to 85 cm with water and sand was again used in the bottom of the tube. For all time points after hatch, a food cue (artemia or blood worm juice depending on current feed) was added to the tube. A light shone from above (40W flashlight) and fish were released at the top of the vertical column of water and their swimming behaviour was recorded until they reached the bottom of the tube. Video recordings were analyzed to measure the following metrics:

1) Average swimming velocity: calculated from the time fish began to descend the entire length of the tube to when they reached the bottom
2) Burst velocity: calculated as the velocity of the longest descent without rest provided it was greater than 20cm of direct descent.

If fish spent ten minutes or greater in the tube without swimming to the bottom, they were excluded from further trials and new fish were chosen.

Escape response

To test escape responses, larvae were placed in a circular area (petri dish with 9 cm diameter) filled with water from their tanks (changed every 10-15 minutes to maintain water quality and temperature). Petri dishes were placed over a grid of known dimensions (1 mm x 1 mm) on white paper for contrast. Larger juvenile fish were placed in a rectangular arena (36 cm x 22 cm) with a similar grid. The escape response was then stimulated by a gentle prod to the tail of

the fish (approximately ¾ down the length of the body from the head) which was recorded at 250 frames per second (fps) by a high-speed camera (Fastec Imaging InLine model IN500M1GB) positioned directly over top of the experimental chamber. Three escape response trials were run for each individual when possible, as some individuals were unresponsive to the stimulus. The videos were analyzed to determine the following performance metrics:

1) Responsiveness: number of stimuli (stimuli) needed to initiate an escape response
2) Latency: time between stimulus and reaction
3) Tail beat frequency: number of complete undulations per second (hz)
4) Displacement: distance travelled by the center of mass of the fish during the first complete tail beat
5) Average and maximum velocity (body length/s)
6) Acceleration (body length/s^2).

Videos were converted from raw format to AVI format using MiDAS (Motion and integrated Data Acquisition System software), then uploaded to ImageJ for analysis. The multipoint tracking tool were used to mark fish in every second frame from the stimulus to the end of the first tail beat after the 3rd change in head direction. Marks were made to track the coordinates of the tip of the snout, center of mass (CoM) (for reliable marking, a spot at the posterior end of the yolksac was used in larvae and a spot in the center of the body between the tips of the pectoral fins was used in juveniles) and the end of the caudal fin. The coordinate data were separated into head, CoM and tail files and smoothed in Rstudio using spline smoothing with an order of 4. The coordinates were then transferred to excel to calculate reaction times and swimming performance metrics as described. The acceleration data were only used to determine which burst velocity corresponded to incidents of true escape response behaviour as opposed to false starts (fish not

completing a proper escape response with a full tail beat) which resulted in negative acceleration values.

Statistical analysis

The volitional and escape response swimming performance metrics of fish in the Hatchery Transfer and Hatchery treatments were compared at each time point to examine behavioural or physiological differences that could have arisen during embryogenesis when embryos were incubated in a MacDonald tumbling jar or allowed to adhere to substrate. To compare the two treatments at each time point, data were analyzed using a Students t-test with n=4-10; results with significance of $p<0.05$ are reported. Where data were not normally distributed, data were rank transformed to meet the assumptions of a normal distribution.

The swimming performance of fish in the Hatchery, Hatchery+2°C, and Hatchery+4°C treatments were compared at each time point to examine differences that could have arisen from early rearing temperature differences before the transfer to the common garden setup. To compare the three treatments for each time point and to compare the time points for each treatment, data were analyzed using a one-way ANOVA with n=4-10 and Tukey's LSD post hoc tests were used to determine which groups differed, results with significance of $p<0.05$ are reported. Where data were not normally distributed it was rank transformed to meet the assumptions of a normal distribution. Where data could not be transformed to meet the assumptions of a normal distribution, data were analyzed using a non-parametric Kruskal Wallis test followed by a Dunn post-hoc analysis to determine which groups differed.

Within treatment analysis for the swimming performance metrics of fish was compared separately for each treatment to examine differences at between time points. Data were analyzed using a one-way ANOVA with n=7-10 and Tukey's LSD post hoc tests were used to determine

which groups differed, results with significance of $p<0.05$ are reported.

Results

Volitional Swim Down Test - Average Swimming Velocity

No swim down data is presented from hatch or 1 mph due to the larvae being too small to appear consistently in video recordings making accurate movement tracking impossible.

Within treatment analysis showed that Hatchery Transfer fish had slower average swimming velocity at 8 mph (0.22±0.02 $BL \cdot s^{-1}$) when compared to 3 mph (0.45±0.17 $BL \cdot s^{-1}$) (figure 4.1a). Hatchery fish did not have significantly different average swimming velocity at different time points. Hatchery+2˚C fish had slower average swimming velocity at 13 mph (0.15±0.04 $BL \cdot s^{-1}$) when compared to 3 mph (0.74±0.19 $BL \cdot s^{-1}$). Hatchery+4˚C fish had slower average swimming velocity at 8 mph (0.18±0.02 $BL \cdot s^{-1}$) when compared to 3 mph (0.49±0.08 $BL \cdot s^{-1}$) (figure 4.1b).

Between treatment analysis did not indicate any significant differences for average swimming velocity in swim down tests between Hatchery and Hatchery Transfer fish at any time point (figure 4.1a). At 8 mph Hatchery fish (0.22±0.02 $BL \cdot s^{-1}$) had faster average swimming velocity than Hatchery+2˚C fish (0.13±0.01 $BL \cdot s^{-1}$), however, there were no differences at any later time point (figure 4.1b).

Volitional Swim Down Test - Burst Velocity

Within treatment analysis showed that Hatchery Transfer fish had slower burst velocity at 6 mph (0.26±0.02 $BL \cdot s^{-1}$), when compared to 3 mph (0.75±0.17 $BL \cdot s^{-1}$) and 13 mph (0.55±0.07 $BL \cdot s^{-1}$) (figure 4.2a). Hatchery fish had lower burst velocity at 6 mph (0.26±0.03 $BL \cdot s^{-1}$) and 8 mph (0.26±0.02 $BL \cdot s^{-1}$) when compared to 3 mph (0.62±0.11 $BL \cdot s^{-1}$) and 13 mph (0.57±0.09 $BL \cdot s^{-1}$). Hatchery+2˚C had lower burst velocity at 8 mph (0.18±0.02 $BL \cdot s^{-1}$) when compared to 3 mph

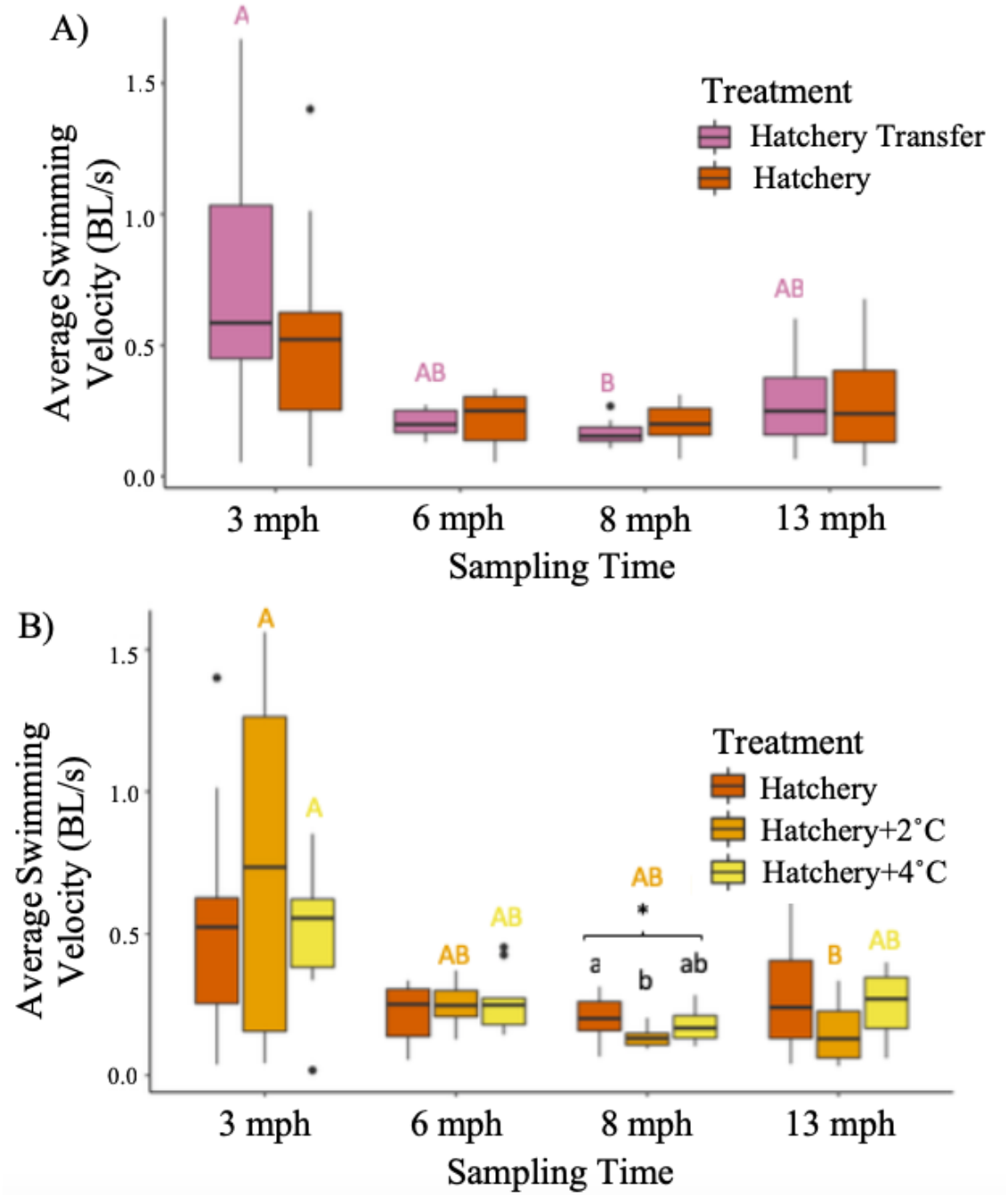

Figure 4.1. Differences in average swimming velocity (body length·s^{-1}) during the swim down test for Lake Sturgeon over the first year of life (2017-2018). Differences within time points but between treatments are marked by asterisks with further group differentiation indicated by lowercase letters. Differences for values across time points within treatments are indicated by uppercase letters. A) Differences between Hatchery Transfer and Hatchery. n= 8-10. B) Differences between Hatchery, Hatchery+2˚C, and Hatchery+4˚C. n= 9-10.

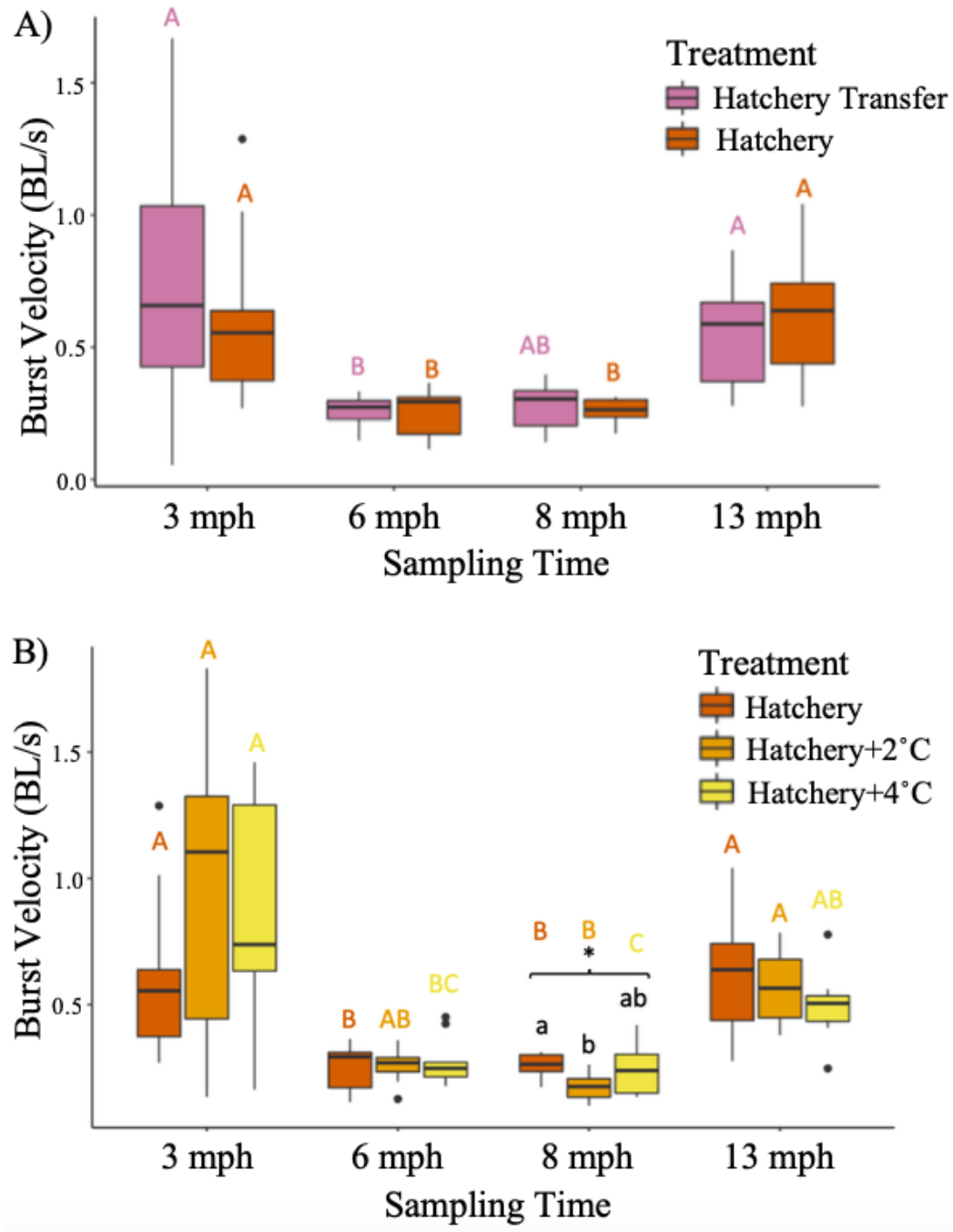

Figure 4.2. Differences in burst velocity (body length·s^{-1}) during the swim down test for Lake Sturgeon over the first year of life (2017-2018). Differences for values within time points between treatments are marked by asterisks with further group differentiation indicated by lowercase letters. Differences for values across time points within treatments are indicated by uppercase letters. A) Differences between Hatchery Transfer and Hatchery. n= 8-10. B) Differences between Hatchery, Hatchery+2˚C, and Hatchery+4˚C. n= 9-10.

(0.97±0.19 BL·s^{-1}) and 13 mph (0.55±0.05 BL·s^{-1}). Hatchery+4˚C fish had lower burst velocity at 6 mph (0.27±0.03 BL·s^{-1}) and 8 mph (0.24±0.03 BL·s^{-1}) when compared to 3 mph (0.80±0.13 BL·s^{-1}) and they had slower burst velocity at 8 mph when compared to 13 mph (0.46±0.04 BL·s^{-1}) (figure 4.2b).

Between treatment analysis showed that at 8 mph (middle of overwintering period) Hatchery fish (0.26±0.02 BL·s^{-1}) had faster burst velocity than Hatchery+2˚C fish (0.18±0.02 BL·s^{-1}), however, there were no differences at any later time points (figure 4.2b).

Escape Response - Responsiveness

Within treatment analysis showed that Hatchery Transfer fish had higher responsiveness at 3 mph (1±0 stimulus) when compared to 1 mph (2±0.3 stimuli), 8 mph (1.5±0.1 stimuli) and 13 mph (2.2±0.5 stimuli) (figure 4.3a). Hatchery fish had higher responsiveness at hatch (1±0 stimulus) and 3 mph (1±0 stimulus) when compared to 6 mph (2.4 ±0.5 stimuli) and 13 mph (2.2±0.4 stimuli). Hatchery+2˚C fish had higher responsiveness at 3 mph (1.3±0.1 stimuli) and 6 mph (1.3±0.1 stimuli) when compared to 13 mph (2.3±0.2 stimuli). Hatchery+4˚C fish had higher responsiveness at 3 mph (1±0 stimulus) when compared to hatch (2.5±0.5 stimuli) and 8 mph (1.9±0.2 stimuli) (figure 4.3b).

Between treatment analysis showed that at 6 mph, Hatchery Transfer fish (1.6±0.1 stimuli) had higher responsiveness than Hatchery fish (2.4±0.5 stimuli), however there were no differences at any later time points (figure 4.3a). At 3 mph, Hatchery (1±0 stimulus) and Hatchery+4˚C fish (1±0 stimulus) had higher responsiveness than Hatchery+2˚C fish (1.3±0.1 stimuli) and at 6 mph Hatchery+2˚C fish (1.3±0.1 stimuli) had higher responsiveness than Hatchery fish (2.4±0.5 stimuli) there were no differences at any later time points (figure 4.3b).

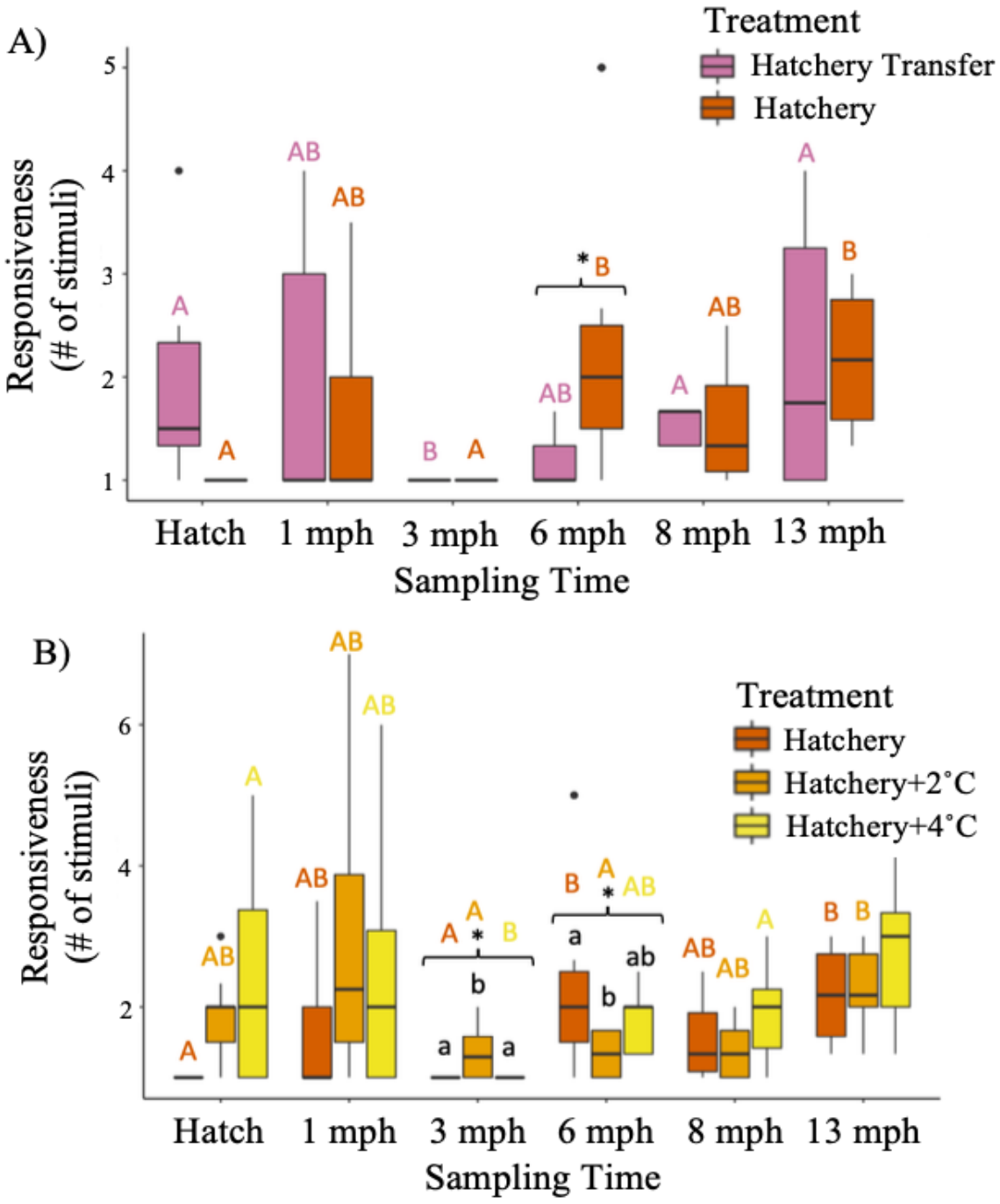

Figure 4.3. Differences in responsiveness (number of stimuli needed to elicit escape response) during the escape response test for Lake Sturgeon over the first year of life (2017-2018). Differences for values within time points between treatments are marked by asterisks with further group differentiation indicated by lowercase letters. Differences for values across time points within treatments are indicated by uppercase letters. A) Differences between Hatchery Transfer and Hatchery. n= 4-10. B) Differences between Hatchery, Hatchery+2˚C, Hatchery+4˚C. n= 4-10.

Escape Response - Latency

Within treatment analysis showed that Hatchery Transfer fish had faster reaction times at 3 mph (38.7±2.5 ms), 6 mph (76.8±8.1 ms) and 8 mph (80.4±5.3 ms) when compared to hatch (98.8±11 ms) (figure 4.4a). Hatchery fish had faster reaction times at 3 mph (26.8±2.5 ms) when compared to hatch (108±16.7 ms), 1 mph (111±27.5 ms), 6 mph (76.5±8.8 ms) and 8 mph (69.2±6.2 ms). Hatchery+2˚C fish had faster reaction times at 3 mph (34.6±3.5 ms) when compared to hatch (189±38.2 ms), 1 mph (100±14.9 ms) and 6 mph (79.6±9.8 ms). Hatchery+2˚C fish had slower reaction times at hatch when compared to 3 mph, 8 mph and 13 mph. Hatchery+4˚C fish had faster reaction times at 3 mph (41.0±4.5 ms) when compared to hatch (94.2±17.7 ms) and 1 mph (132±28.6 ms) (figure 4.4b).

Between treatment analysis showed that at 3mph, the latency was different for Hatchery Transfer fish (38.7±2.5 ms) and Hatchery fish (26.8±2.5 ms) with Hatchery fish having faster reaction times (figure 4.4a). At 3mph, the latency was different for Hatchery (26.8±2.5 ms) and Hatchery+4˚C fish (41.0±4.5 ms) with Hatchery fish having faster reaction times and at 8mph, the latency was different for Hatchery (69.2±6.2 ms) and Hatchery+2˚C fish (48.4±3.2 ms) with Hatchery fish having faster reaction times (figure 4.4b).

Escape Response - Tail Beat Frequency

No kinematic data is presented from 13 mph due to the juvenile fish not initiating proper bursts of swimming away from the stimulus after the initial reaction at this time point for any treatment.

Within treatment analysis showed that Hatchery Transfer fish had faster tail beat frequencies at 6 mph (3.93±0.34 hz) and 8 mph (4.79±0.39 hz) when compared to hatch (11.3±0.82 hz) and 3 mph (9.71±0.77 hz) (figure 4.5a). Hatchery fish had faster tail beat frequencies at 6 mph

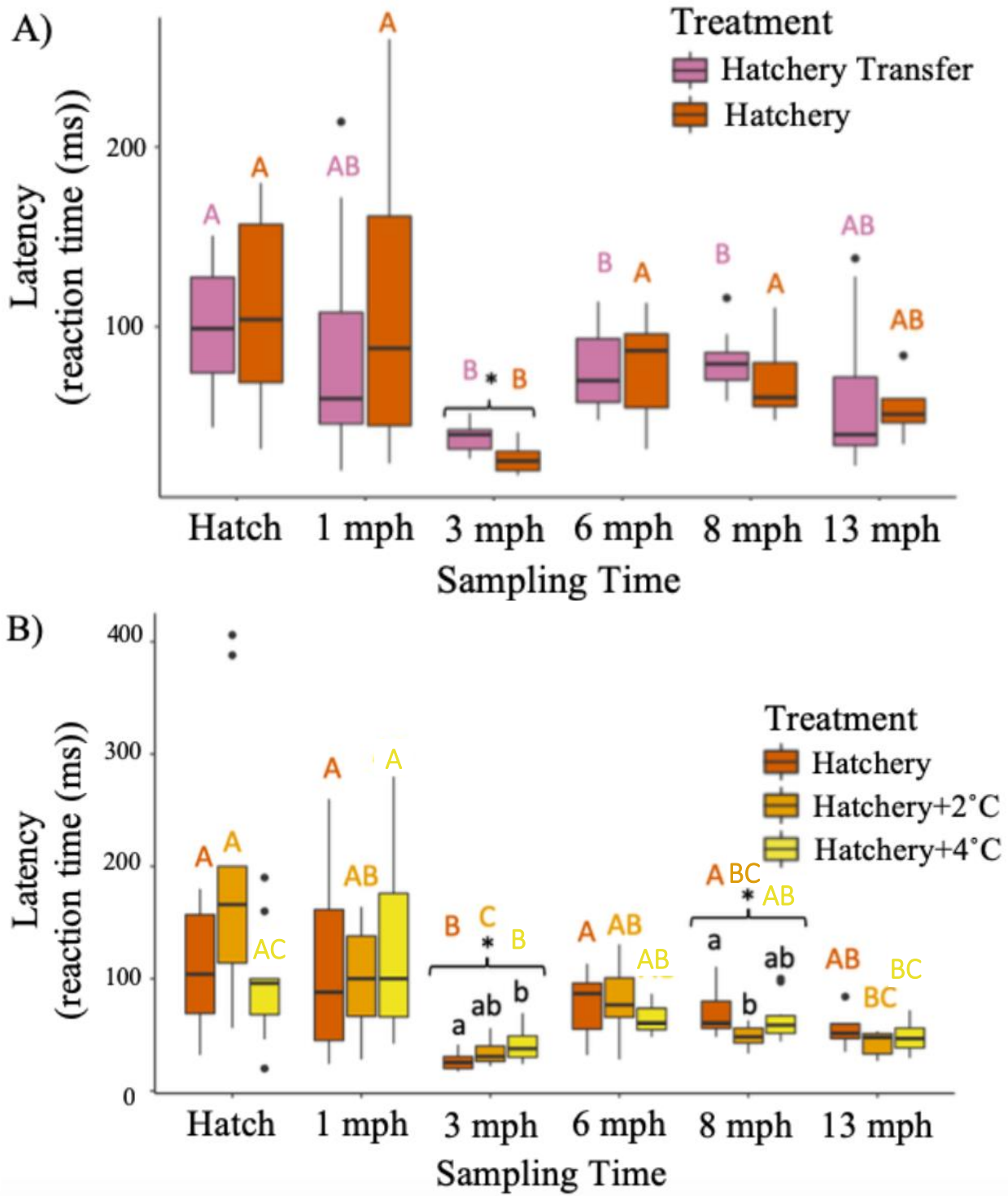

Figure 4.4. Differences in latency (reaction time (ms)) during the escape response test for Lake Sturgeon over the first year of life (2017-2018). Differences for values within time points between treatments are marked by asterisks with further group differentiation indicated by lowercase letters. Differences for values across time points within treatments are indicated by uppercase letters. A) Differences between Hatchery Transfer and Hatchery. n= 4-10. B) Differences between Hatchery, Hatchery+2˚C, and Hatchery+4˚C. n= 4-10.

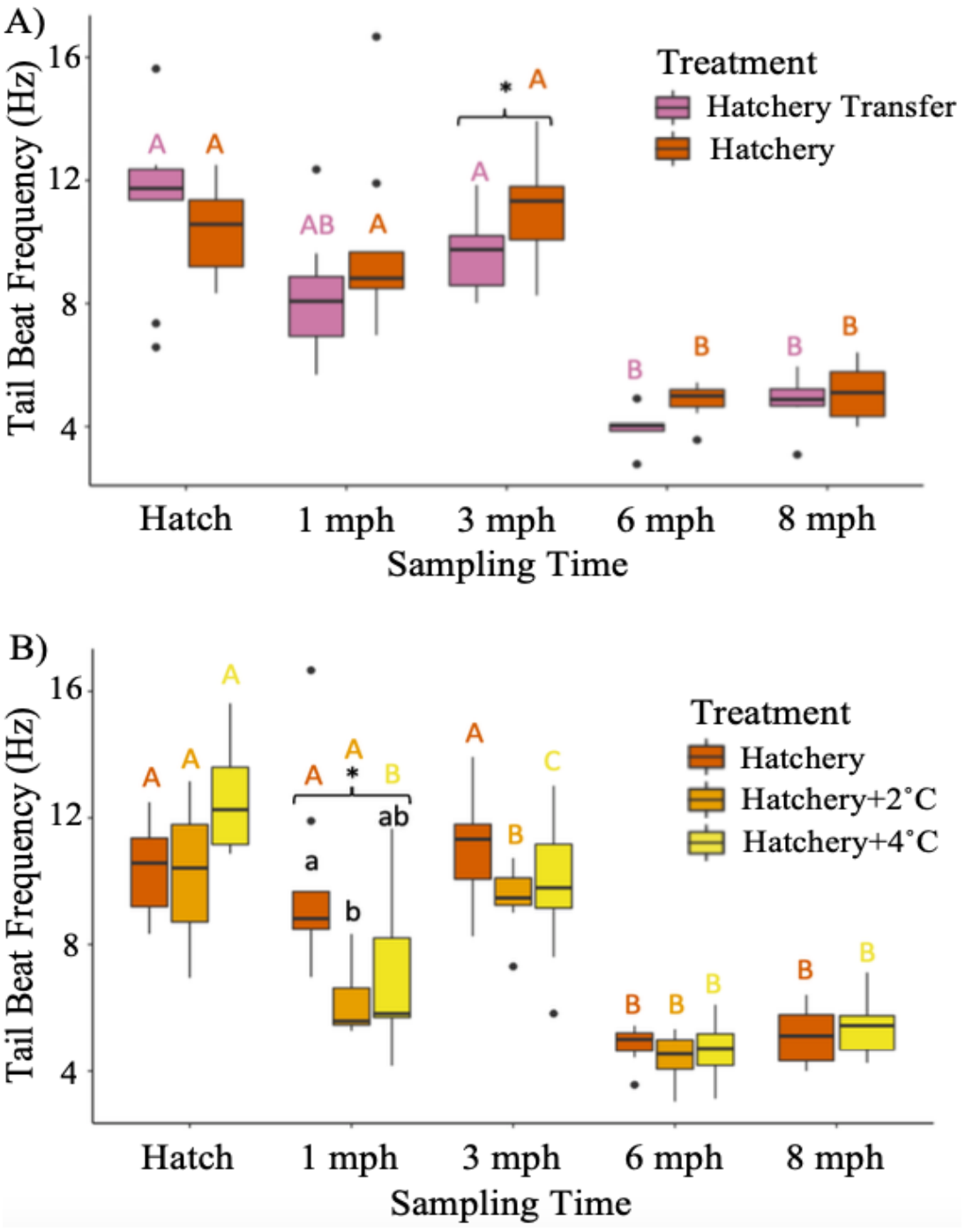

Figure 4.5. Differences in tail beat frequency (Hz) during the escape response test for Lake Sturgeon over the first year of life (2017-2018). Differences for values within time points between treatments are marked by asterisks with further group differentiation indicated by lowercase letters. Differences for values across time points within treatments are indicated by uppercase letters. A) Differences between Hatchery Transfer and Hatchery. n= 5-10. B) Differences between Hatchery, Hatchery+2˚C, and Hatchery+4˚C. n= 6-10.

(4.81±0.24 hz) and 8 mph (5.10±0.35 hz) when compared to hatch (10.3±0.44 hz), 1 mph (9.87±1.1 hz) and 3 mph (11.1±0.50 hz). Hatchery+2˚C fish had faster tail beat frequencies at 1 mph (6.15±0.39 hz) and 6 mph (4.41±0.34 hz) when compared to hatch (10.3±0.64 hz) and 3 mph (6.15±0.39 hz). Hatchery+4˚C fish had faster tail beat frequencies at 1 mph (6.82±0.78 hz), 6 mph (4.65±0.39 hz), and 8 mph (5.42±0.42 hz) when compared to hatch (12.7±0.58 hz) and 3 mph (9.91±0.68 hz) and the fish had faster tail beat frequencies 3 mph when compared to hatch (figure 4.5b).

Between treatment analysis showed that at 3 mph, the tailbeat frequencies were different for Hatchery Transfer fish (9.71±0.41 hz) and Hatchery fish, with Hatchery fish (11.1±0.50 hz) having faster tail beat frequencies (figure 4.5a). At 1 mph Hatchery fish (9.87±0.44 hz) had faster tail beat frequencies than Hatchery+2˚C fish (6.15±0.39 hz) (figure 4.5b).

Escape Response - Displacement

At hatch and 1 mph, the larvae swam in random directions, often in circular motion during the burst swimming after the stimulus while at 3mph and after, the juveniles swam in linear directions, most often in directions directly away from the stimulus.

Within treatment analysis showed that Hatchery Transfer and Hatchery fish travelled less distance at hatch (0.52±0.05 BL and 0.48±0.03 BL, respectively), 1 mph (0.43±0.04 BL and 0.38±0.05 BL, respectively) and 8 mph (0.38±0.03 BL and 0.43±0.04 BL, respectively) when compared to 3 mph (0.84±0.04 BL and 0.71±0.04 BL, respectively) and 6 mph (1.03±0.08 BL and 0.87±0.06 BL, respectively) (figure 4.6a). Hatchery+2˚C fish travelled less distance at 1 mph (0.29±0.03 BL) when compared to 3 mph (0.43±0.02 BL) and 6 mph (0.65±0.01 BL).

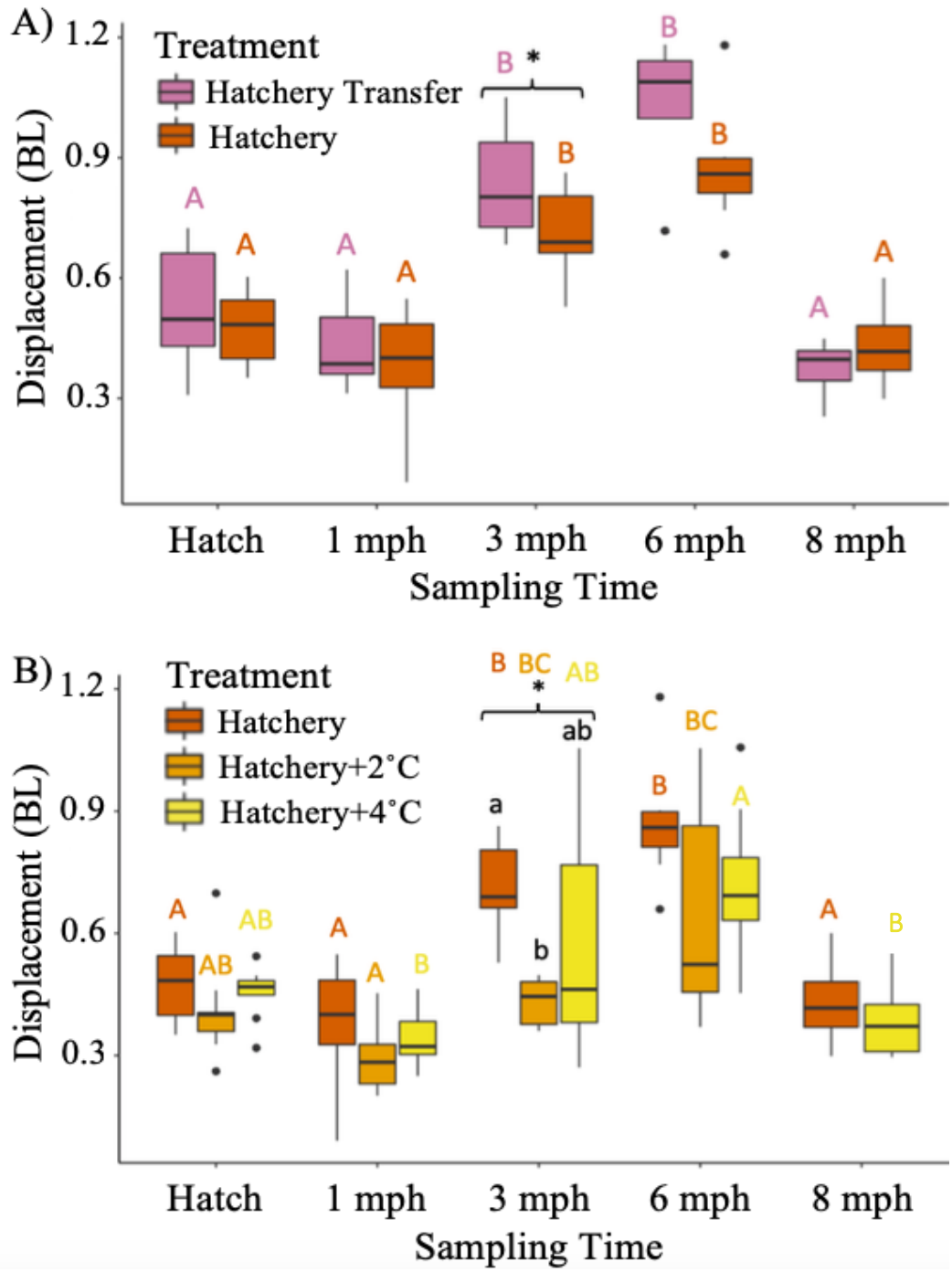

Figure 4.6. Differences in distance travelled (body length) during fist tail beat of the escape response test for Lake Sturgeon over the first year of life (2017-2018). Differences for values within time points between treatments are marked by asterisks with further group differentiation indicated by lowercase letters. Differences for values across time points within treatments are indicated by uppercase letters. A) Differences between Hatchery Transfer and Hatchery. n= 5-10. B) Differences between Hatchery, Hatchery+2˚C, and Hatchery+4˚C. n= 5-10.

Hatchery+4˚C fish travelled less distance at 1 mph (0.34±0.03 BL) and 8 mph (0.39±0.05 BL) when compared to 6 mph (0.43±0.07 BL) (figure 4.6b).

Between treatment analysis showed that at 3 mph, Hatchery fish (0.71±0.04 BL) travelled less distance than Hatchery Transfer fish (0.84±0.04 BL) (figure 4.6a). At 3 mph, Hatchery+2˚C fish (0.43±0.02 BL) travelled less distance than Hatchery fish (0.71±0.04 BL) (figure 4.6b).

Escape Response - Average Velocity

Within treatment analysis showed that Hatchery Transfer fish had slower average velocity at 8 mph (2.40±0.10 $BL{\cdot}s^{-1}$) when compared to hatch (5.67±0.58 $BL{\cdot}s^{-1}$) and 3 mph (7.94±0.31 $BL{\cdot}s^{-1}$) and faster average velocity at 3 mph when compared to 1 mph (3.00±0.17 $BL{\cdot}s^{-1}$) and 8 mph (figure 4.7a). Hatchery fish had slower average velocity at 1 mph (3.41±0.48 $BL{\cdot}s^{-1}$) and 8 mph (2.12±0.25 $BL{\cdot}s^{-1}$) when compared to hatch (5.04±0.40 $BL{\cdot}s^{-1}$) and 3 mph (7.72±0.36 $BL{\cdot}s^{-1}$) and faster average velocity at 3 mph when compared to the other time points. Hatchery+2˚C fish had faster average velocity at hatch (4.89±0.85 $BL{\cdot}s^{-1}$) and 3 mph (3.84±0.25 $BL{\cdot}s^{-1}$) when compared to 1 mph (1.79±0.20 $BL{\cdot}s^{-1}$) and faster average velocity at hatch when compared to 1 mph and 6 mph (2.803.84±0.25 $BL{\cdot}s^{-1}$). Hatchery+4˚C fish had faster average velocity at hatch (5.41±0.60 $BL{\cdot}s^{-1}$) and 3 mph (4.96±0.45 $BL{\cdot}s^{-1}$) when compared to 1 mph (1.97±0.15 $BL{\cdot}s^{-1}$), 6 mph (3.26±0.30 $BL{\cdot}s^{-1}$) and 8 mph (1.96±0.19 $BL{\cdot}s^{-1}$) and faster average velocity at hatch and 3 mph when compared to 6 mph (figure 4.7b).

Between treatment analysis did not indicate any significant differences for average velocity in the escape response tests between Hatchery and Hatchery Transfer at any time point (figure 4.7a). At 1 mph and 3 mph Hatchery fish (3.41±0.48 $BL{\cdot}s^{-1}$ and 7.72±0.36 $BL{\cdot}s^{-1}$, respectively) had faster average velocity than Hatchery+2˚C (1.79±0.85 $BL{\cdot}s^{-1}$ and 3.84±0.20 $BL{\cdot}s^{-1}$,

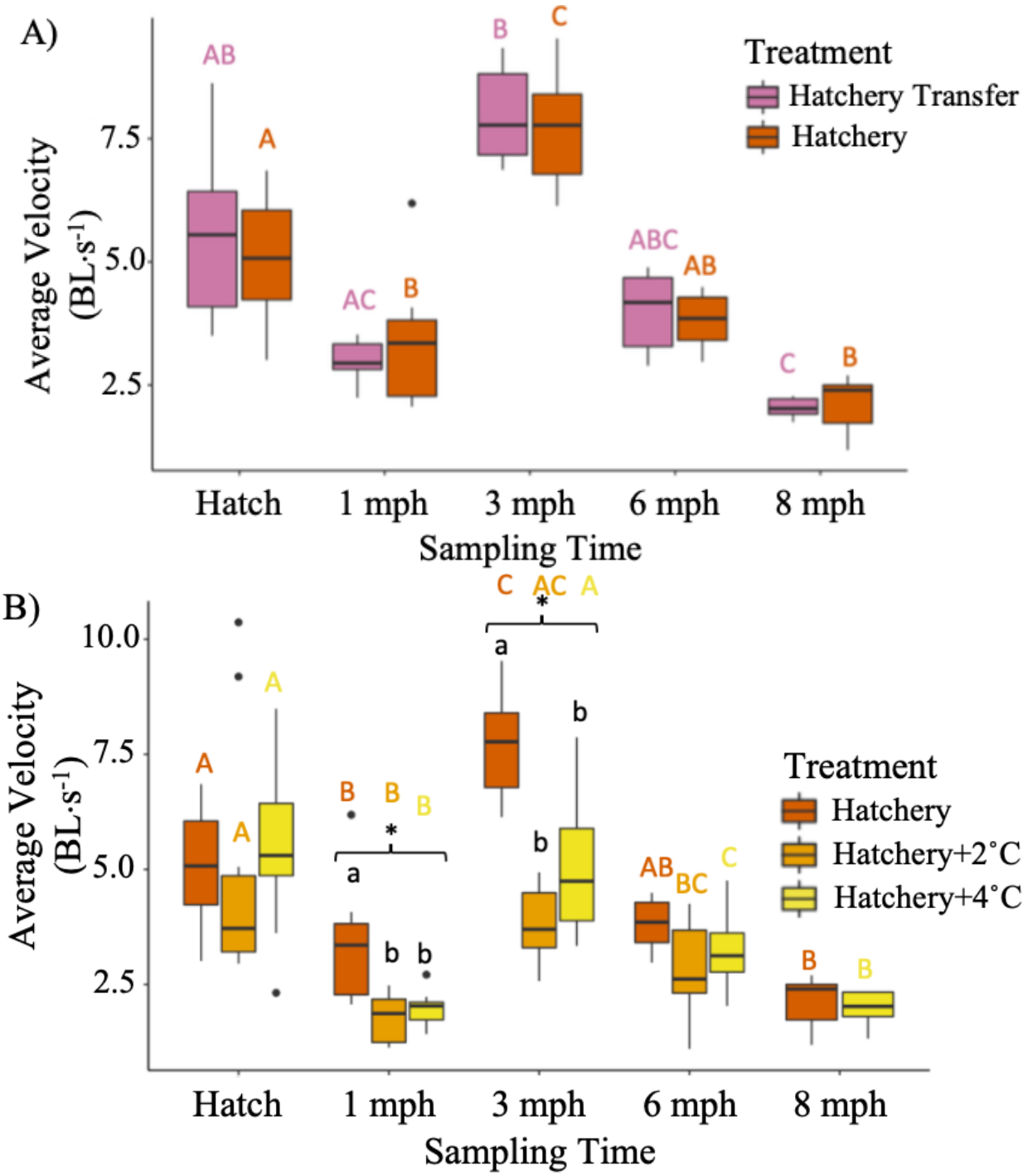

Figure 4.7. Differences in average velocity of first tail beat (body length·s^{-1}) during the escape response test for Lake Sturgeon over the first year of life (2017-2018). Differences for values within time points between treatments are marked by asterisks with further group differentiation indicated by lowercase letters. Differences for values across time points within treatments are indicated by uppercase letters. A) Differences between Hatchery Transfer and Hatchery. n= 5-10. B) Differences between Hatchery, Hatchery+2˚C, and Hatchery+4˚C. n= 5-10.

respectively) and Hatchery+4˚C fish (4.96±0.60 BL·s^{-1} and 4.96±0.45 BL·s^{-1}, respectively) (figure 4.7b).

Escape Response - Maximum Velocity

Within treatment analysis showed that Hatchery Transfer fish had slower maximum velocity at 8 mph (2.72±0.08 BL·s^{-1}) when compared to hatch (8.50±0.73 BL·s^{-1}) and 3 mph (10.06±0.40 BL·s^{-1}) and faster maximum velocity at 3 mph when compared to 1 mph (4.74±0.39 BL·s^{-1}) and 8 mph (figure 4.8a). Hatchery fish had slower maximum velocity at 8 mph (2.68±0.30 BL·s^{-1}) when compared to the other time points, they had faster maximum velocity at 3 mph (9.81±0.39 BL·s^{-1}) when compared to the other time points and slower maximum velocity at 1 mph (5.63±0.41 BL·s^{-1}) when compared to hatch (7.76±0.65 BL·s^{-1}). Hatchery+2˚C fish had faster maximum velocity at hatch when compared to 1 mph (2.95±0.31 BL·s^{-1}) and 6 mph (3.88±0.73 BL·s^{-1}) and faster maximum velocity at 3 mph (4.91±0.33 BL·s^{-1}) when compared to 1 mph. Hatchery+4˚C fish had faster maximum velocity at hatch (8.96±1.19 BL·s^{-1}) and 3 mph (6.76±0.54 BL·s^{-1}) when compared to the other time points and faster maximum velocity at 6 mph (4.47±0.40 BL·s^{-1}) when compared to 1 mph (3.24±0.25 BL·s^{-1}) and 8 mph (2.63±0.24 BL·s^{-1}) (figure 4.8b).

Between treatment analysis did not indicate any significant differences for maximum velocity in the escape response tests between Hatchery and Hatchery Transfer at any time point (figure 4.8a). At 1 mph, Hatchery fish (5.63±0.41 BL·s^{-1}) had faster maximum velocity than Hatchery+2˚C (2.95±0.31 BL·s^{-1}) and Hatchery+4˚C fish. (3.24±0.25 BL·s^{-1}). At 3 mph, Hatchery fish (9.81 BL·s^{-1}) had faster maximum velocity than Hatchery+2˚C (4.91±0.33 BL·s^{-1}) and Hatchery+4˚C (6.76±0.54 BL·s^{-1}) fish and Hatchery+4˚C fish had faster maximum velocity than

Hatchery+2˚C. At 6 mph Hatchery fish (6.07±0.63 BL·s^{-1}) had faster maximum velocity than Hatchery+2˚C fish (3.88±0.73 BL·s^{-1}) (figure 4.8b).

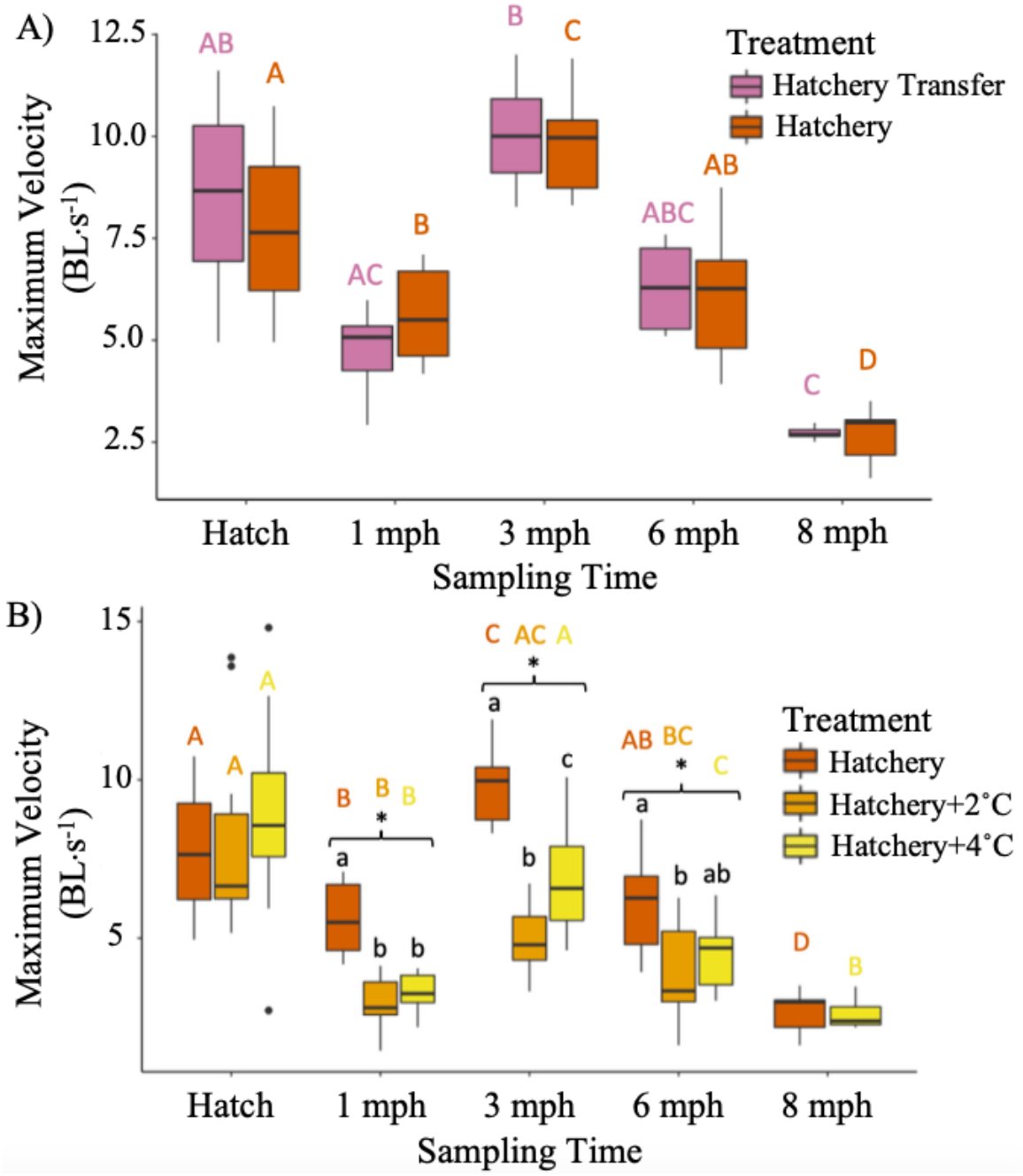

Figure 4.8. Differences in maximum velocity of first tail beat (body length·s^{-1}) during the escape response test for Lake Sturgeon over the first year of life (2017-2018). Differences for values within time points between treatments are marked by asterisks with further group differentiation indicated by lowercase letters. Differences for values across time points within treatments are indicated by uppercase letters. A) Differences between Hatchery Transfer and Hatchery. n= 5-10. B) Differences between Hatchery, Hatchery+2˚C, and Hatchery+4˚C. n= 5-10.

Discussion

The strongest patterns in swimming performance and variation were seen across time points. While there were some significant differences between treatments for volitional swimming, behavioural and kinematic metrics of the escape response, there was no clear and obvious pattern to these across the experimental groups. This suggests that the measured behavioural and physiological traits were sufficiently plastic (able to change with later environmental changes) at the time of exposure to the different incubation (embryogenesis) and temperature treatments (yolksac larvae) to not have a measurable impact with a clear pattern as the fish developed over the first year. The responses in the swimming metrics that were displayed throughout the development time were more consistent and will be the focus of this discussion.

Volitional Swim Down

In addition to morphological defenses (body armour and coloration), Lake Sturgeon utilize cover seeking and escape behaviours which function as adaptive responses to predation threats (Wishingrad et al. 2014a). Volitional downward swimming, primarily an oxidative mode of locomotion, is important for Lake Sturgeon for foraging and cover seeking and may be a less energy demanding behaviour compared to the high energy anaerobic mode of locomotion exhibited in escape response behaviour (Wishingrad et al. 2014a; Tytell and Lauder 2002). The need for cover in suitable substrate or to forage along the bottom of rivers and lakes is present throughout the life of Lake Sturgeon, making downward swimming an ecologically relevant performance metric (Richmond and Kynard 1995; Chiasson et al. 1997).

Pre-winter

At 3 mph, the end of summer, Hatchery Transfer, Hatchery +2˚C, and Hatchery +4˚C fish had the highest average swimming velocity, and all treatments had significantly highest burst velocity in the swim down trials. This is not surprising because at this stage, the fish had been

feeding for approximately two months and would have developed the behaviour and ability to swim down in search of food at this stage. Previous work on Lake Sturgeon behaviour showed that fish close to the 5 mph developmental stage did not change foraging behaviour in the presence of alarm cue suggesting a risky foraging strategy in favour of feeding and growing (Bjornson 2017). The starting peak in volitional swim down metrics is likely also due to fish being fed to satiation, giving them sufficient energy to travel more after their growth peak in summer, as well as higher temperature water, and better ability to swim as juveniles in the absence of yolk sacs which adds significant drag to locomotion (Müller et al. 2006).

Winter

At 6 mph, the beginning of the overwintering/food deprivation period, there was a significant decrease in burst velocity for Hatchery Transfer, Hatchery, and Hatchery +4˚C fish. This decrease is likely due to lower energy levels from no longer being fed to satiation (Cai et al. 2016; Pang et al. 2016) as well as being tested in colder water (~2˚C) compared to summer temperatures (~18˚C) (Allen et al. 2006). Importantly, it has been shown in plaice, *Pleuronectes platessa*, that changes in myosin sub-unit composition and muscle fibre size can vary with temperature and developmental stage (Brooks and Jonhston; 1993), and this would undoubtedly influence the swimming ability of fish in the present study. Furthermore, in warmer temperatures variation in performance was high compared to lower temperatures. Interestingly, temperature explained 30% of the variation in critical swimming velocities in Sockeye Salmon, *Oncorhynchus nerka* (MacNutt et al. 2006). Variation could also be due high mortality rates in the first few months of life which was shown to exceed 99.9% from egg stage to age-0 juvenile for Lake Sturgeon in the Peshtigo River, Wisconsin, USA (Carofino et al. 2010). The large mortalities would presumably include those with poor swimming ability, making them unable to obtain food

or escape predators in the wild, leading to only the strongest swimmers surviving, thereby improving overall swimming performance of the remaining fish and reducing variation.

At 8 mph, the middle of the overwintering period, Hatchery Transfer and Hatchery +4˚C fish had significantly lower average swimming velocity, furthermore, Hatchery, Hatchery +2˚C, and Hatchery +4˚C had significantly lower burst velocity. The fish had been in cold water during the tests at 6 mph with reduced feeding, the continuation of these conditions would have led to lower metabolic rate, reduced energy levels, and possibly impaired muscle activation time and likely a switch to more cold tolerant myosin subunit isoforms (Brooks and Jonhston 1993; Deslauriers et al. 2018b; Moran et al. 2019) all resulting in slower locomotion.

Post-winter

At 13 mph, after 2 months of recovery from the overwintering period, average swimming velocity remained low in all treatments, however, burst velocity increased to pre-winter levels in all treatments. The lack of increase in average swimming velocity could also be due to the fish having reached a size where they were less likely to swim down on instinct as increased body size is likely to result in a decreased predation risk. Previous critical swimming velocity measurement in green sturgeon showed a decrease in velocity with increasing total body length when total fish length exceeded 267 mm, which could also be impacting the downward swimming in this study despite Lake Sturgeon only reaching 190 mm (Allen et al. 2006). As mentioned, this may be a behavioural adaption as at this point the fish are of a large enough size and have well developed body armour to deter potential predators (Peterson et al. 2007). The increased sprint velocity in the post-winter time point is likely a function of increased temperature and also resources given the fish were being fed at that time (Pang et al. 2016).

Escape Response - Behavioural

Escape responses in sturgeon as with all fishes are an important adaptive response to predation pressure. Escape responses have both behavioural (responsiveness and latency) and kinematic (tail beat, velocity, distance travelled) components.

Pre-winter

At hatch and 1 mph, responsiveness and latency were highly variable across treatments, however, at 3 mph variation was significantly reduced and fish typically were most responsive and demonstrated some of the fastest reaction times across developmental time. The average reaction time of the different treatments in the present study ranged from 26.8-41 ms at this stage which is similar to the values found by Baker et al. (2014) of 35-50 ms for larval white sturgeon. Free-swimming and feeding very young fish at 3 mph would be more vulnerable to predation (Gadomski and Parsley 2005) and would need to rely on faster escape responses to survive. Further, shorter distances for neural networks and/or neural pattern generators in the fish at this stage would likely influence muscle activation time allowing for faster reaction (Voesenek et al. 2018).

Winter

At 6 mph, the beginning of the overwintering/food deprivation period, Hatchery fish showed slower responsiveness (needing more stimuli to react) and Hatchery and Hatchery+2˚C fish had significantly slower reaction times. At 8 mph, the middle of the overwintering period, there were no significant differences from the previous time point, however, Hatchery Transfer and Hatchery+4˚C fish showed significantly slower responsiveness (needing more stimuli to react) and Hatchery fish continued to have significantly slower reaction times when compared to the most responsive time point (3 mph) As with downward swimming, the decrease in behavioural metrics at the beginning of and during the overwinter period is most likely due to cold temperature

and/or and reduced feeding, leading to lower metabolic rate, reduced energy levels and impaired muscle activation time.

Post-winter

At 13 mph, after 2 months of recovery from the overwintering period, there were no significant differences from the previous time point, however all treatments showed significantly slower responsiveness (needing more stimuli to react) when compared to the most responsive time point (3 mph). This could be due to the investment in morphological defences allowing the fish to not need to react to potential predation threats because their size is a deterrent. At 13 mph, there are also 1 mph larvae in the wild that could be better targets for predators which could further deter the 13 mph fish from deciding to react to every potential predation threat.

Escape Response - Kinematic

At 1 mph, fish are still be developing the ability to perform escape responses effectively in the first month. Indeed, considerable changes in neural development and brain structure have been shown in the Adriatic Sturgeon, *A. naccarii*, between the 40 dph stage and juveniles (Vázquez et al. 2002). Generally, larval fish tend to have higher tail beat frequencies (Voesenek et al. 2018) and this is seen in the fish at hatch and 1mph in the present study. However, what might be gained by faster tailbeat frequencies is lost in power from a fast start sequence leading to a significant reduction in distance covered and slower velocities. Lake Sturgeon are known to be poor swimmers at this stage and their main focus could be utilize cover seeking behaviour to evade predators instead of being in open water where there would be a requirement to effectively perform escape responses (Peterson et al. 2007; Richmond and Kynard 1995). At 3 mph, there is a peak in kinematic components of the escape response with typically high tailbeat frequencies resulting in greater velocity and distance moved. This improvement is likely the result of a combination of increased available energy from feeding to fuel the fast start response alongside the absence of a

yolksac which would result in increased drag during a forward motion reducing velocity and distance moved (Müller et al. 2006).

Winter time points showed slower velocities and less distance travelled and the variation in these metrics was lower compared to previous time points. The reduction in temperature will limit enzyme rates that are the basis for muscle contraction and excitation of motor units powering a swimming stroke reducing variation in contraction rate between individuals.

Summary

The behavioural and kinematic metrics discussed for the escape response mostly showed similar patterns in swimming performance and variation across time points as seen with the downward swimming. Responsiveness, swimming velocities and variation were all generally high from hatch until 3 mph, which is at the end of the summer when fish were growing and learning to swim better in order to forage effectively and build up energy store for the coming winter, then a decrease at 6 and 8 mph, through the overwintering period which entailed cold temperatures, food deprivation and a need to conserve the energy they had accumulated over summer. After 2 months of recovery, however, a return to pre-summer values was not necessary for all swimming performance metrics as morphological defenses (larger body size and armour) had been developed to possibly deter predators. Temperature is likely the most important factor contributing to the patterns seen in the swimming metrics and individual variation of those metrics, with lower temperatures influencing changes in myosin sub-unit composition, muscle fibre size, metabolic enzyme activity and gene expression (Brooks and Johnston 1994; Nathanailides et al. 1996; McClelland et al. 2006). The overall absence of significant differences between incubation and temperature treatments and presence of significant differences between time points indicates a strong evolutionary pressure to maintain plasticity in swimming performance. Therefore, in

changing environmental conditions, it is essential that there be plasticity in behavioural and physiological responses for the survival at a young age.

The prediction that Lake Sturgeon reared in colder water would have slower escape responses in the pre-winter experiments was not supported by the data. However, the most significant differences in swimming performance metrics were observed at 1 and 3 mph. The prediction that fish exposed to a prolonged drop in temperature and deprivation of food (simulating an overwintering event) would exhibit slower swimming speeds or a lack of response to stimuli was supported by the data which showed all treatments exhibited general decreases in velocities and responsiveness. When water temperatures increased following winter, and food was returned, the prediction that fish in all treatments would display faster swimming speeds and reaction times and that fish in warmer temperature treatments during early development would display faster swimming speeds was not supported by the data. This finding was likely due to changes in morphology creating greater friction and therefore reducing swimming performance of the individual.

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Chapter 5. Final Discussion

Growth rate, muscle development and swimming performance are all important for various reasons throughout the first year of life in Lake Sturgeon. The size of fish is important for growing large enough to reduce the risk of predation as well as the development of physical defenses (scutes and colouration) and the accumulation of energy reserves to survive the first winter of life to ensure subsequent fitness (Cargnelli and Gross 1997). Growth rate will impact muscle development by influencing the rate of nuclear divisions which are necessary for new fibres. Slower growth tends to result in an investment in existing fibres (hypertrophy) while faster growth leads to an investment in new fibres (hyperplasia) (Nathanailides et al. 1995). Development of red and white muscle fibers is important for slow and fast swimming, respectively, and each has different implications on locomotion. The slow, red muscle, fibres are the dominant muscle type used for regular activity such as foraging and migration and function through aerobic metabolism while fast, white muscle, fibres are used for bursts of high-velocity swimming (Johnston 1999). The proportion of red and white muscle is important for their swimming performance at different velocities as shown in Southern Smelt, *Retropinna retropinna* (Meyer-Rochow et al. 1993). Recruitment of muscle fibres is also influenced by temperature which can result in a change in swimming performance as shown in Carp, *Cyprinus carpio* (Rome et al. 1990). Volitional downward swimming is important for foraging and cover seeking and would rely predominantly on red muscle while white muscle is mostly used for escape responses that are utilised when predation threats are more immediate as it is more energy efficient to seek out suitable habitat than to escape encounters by utilizing bursts of speed (Wishingrad et al. 2014a; Tytell and Lauder 2002).

Yolksac larvae

In the present study, fish at hatch tended to have higher responsiveness, slower reaction times, higher tail beat frequency but lower displacement. At 1 mph, before transfer to the common garden setup, body length and mass had increased from at hatch. The fish again tended to have higher responsiveness and slower reaction times compared to later developmental times. Furthermore, average and maximum velocities in escape response trials were low. At these stages fish are growing quickly and may still be developing the ability to swim properly and control their developing bodies and brain structure (Vázquez et al. 2002). They are very vulnerable to predators at this stage, so it is perhaps not surprising that responsiveness is high, however, reaction times were low. The low reaction times may be explained by the rapid muscle development at this stage and incomplete coordination between afferent input and muscle contraction in response to the stimuli (Voesenek et al. 2018). The high tail beat frequency but low displacement at these early life stages is best explained by the inability of the fish to produce sufficient power to counteract the drag created by the yolksac as the fish moves in a forward motion (Voesenek et al. 2018).

Pre-winter

At 3 mph, after transfer to the common garden setup, body length and mass continued to increase but myonuclear counts and muscle fibre areas were significantly lower than the earlier time points. The fish had the highest average swimming and burst velocities in the swim down test; relatively fast reaction times, no change in tail beat frequency compared to earlier time points but increased displacement alongside increased average and maximum velocities in escape response trials. The increasing body size compared to earlier time points but typically lower fibre area compared to later time points, is suggestive of muscle growth occurring primarily through hyperplasia ie: an increase in number of small fibers. At this stage, the fish had been feeding to

satiation for two months, thus energy to invest in growth and activity was not a limiting factor (Sogard and Olla 2000). Further, they had developed improved swimming performance for foraging (swimming down) and escaping predators (escape response) which also relies on the faster reaction times seen here.

Winter

At 6 mph, the beginning of the overwintering/food deprivation period, body length and mass continued to increase but at a slower rate than during the first summer of life, in addition, myonuclear counts and average red muscle fibre area also increased. However, fish had decreased burst velocity in the swim down test, and some fish were less responsive and had slower reaction times, decreased tail beat frequencies, and decreased average and maximum velocities in escape response trials compared to earlier time points. In Southern smelt, *Retropinna retropinna* Richardson, the proportion of red myotome in muscle (hyperplasia) was correlated to sustained swimming ability (Meyer-Rochow et al. 1993). The reduced feeding and lower temperatures likely played the largest roles in reducing growth rates as well as decreasing the swimming performance metrics for both tests as previously discussed (Cai et al. 2016; Pang et al. 2016; Allen et al. 2006). These decreases were seen along with an increase in muscle fibre area and myonuclear counts, this could be evidence to suggest if any growth in muscle fibres was occurring during the winter months, it was most likely accomplished through hypertrophy which occurs in fish as they age and due to the linear relationship between myonuclear count both parameters would increase (Johnston et al. 2003; Meyer-Rochow et al. 1993).

The effects of lowered growth and swimming performance that were beginning to emerge at the start of winter were exaggerated in the middle of the overwintering period. Body length and mass plateaued and myonuclear counts and muscle fibre areas remained largely similar to the

previous time point. Fish tended to have low average velocities and burst velocities in the swim down test. Some reaction times were slow and despite no change in tailbeat frequencies compared to earlier time points there was a decrease in displacement, maximum and average velocities in the escape response trials. These data are most likely explained by the effects of temperature on enzymatic rates and ability of the fish to generate power in a forward motion at low environmental temperatures.

Post-winter

At 13 mph, after 2 months of recovery from the overwintering period, body length and mass and myonuclear counts increased and were among the higher values in comparison to all other time points while muscle fibre area remained the same as 8 mph. However, while burst velocities might have increased in the swim down test, average velocities were low compared to other time points. With warming temperatures and an exogenous food source reintroduced to the fish there was a significant period of growth prior to conducting any of these trials. However, there was no change in the muscle fibre area but a suggestion of an increase in myonuclear counts compared to earlier time points suggesting that the fish were adopting a hyperplasia approach to muscle growth post-winter. Interestingly, the behavioural components of the two swim trials did not change compared to the earlier time points, suggesting that while fish had the ability to move faster, they chose not to. This could be due to the fish perceiving the predation threats as less serious at 13mph when scutes were fully developed and coloration of the skin would aid in camouflage from predators (Wishingrad et al. 2014b).

General discussion

In the second year of this study (2017-2018 cohort), significant differences in growth were found between incubation and temperature treatments during the first few weeks of life, at the start

of winter (6mph) and after recovery (13 mph) which suggests that the growth rates are somewhat fixed at these key developmental stages. At 13 mph, there were no differences between treatments for muscle or swimming performance so the manifestation of size differences 1 year after exposure to different temperatures suggests a critical period during early life-history that will influence long-term trajectory of growth which could affect the ultimate fitness of the fish. Curiously, at 3 mph, there were no differences between the body length or mass of fish but there were multiple significant differences in swimming performance metrics between treatments. Data produced from the swimming trials suggest that the performance and muscle growth traits examined in this study were sufficiently plastic at the time of exposure to the different incubation (embryogenesis) and temperature treatments (yolksac larvae) to not have a measurable impact with a clear pattern at later times in development. The environmental conditions and stage of development also had an effect on variation of results as previously seen in growth of larval White Sturgeon, *Acipenser transmontanus* (Boucher et al. 2014), composition of muscle fibre type in the Plaice, *Pleuronectes platessa* (Brooks and Jonhston 1993), and swimming performance of juvenile Green Sturgeon, *A. medirostris* (Deslauriers and Kieffer 2012). In the present study, there was very little variation seen in the muscle within treatments but clear variation in swimming performance metrics, with lower variation in the winter for volitional swimming and some aspects of forced escape responses.

In conclusion, growth of the fish was most strongly influenced by the temperature and food availability in the 2017-2018 study and these effects were likely confounded by differences in stocking density in the 2016/2017 cohort. There were differences in growth between the temperature treatments in 2017/2018 cohort before and after winter suggesting that growth trajectory may be fixed early in life. Muscle development of the fish was also strongly influenced by the temperature and food availability, but no significant differences were found between

treatments, indicating this trait may be more plastic during early life history alongside the swimming performance metrics used in the present study. Hatcheries could use this information to increase the yolksac utilization and growth rate of their Lake Sturgeon by increasing the temperature during the first month.

The growth studies were limited by density issues which was partially solved in the second year, with a similar setup of an experimental trough design, future directions for this study could include similar treatment differences at different periods in early life to narrow down the most effective window of change for increasing growth in Lake Sturgeon later in the first year. The muscle study was impacted by the white muscle fibres breaking (likely during sectioning), with different embedding, sectioning and/or staining techniques, both muscle tissues could have been examined, potentially at a more anterior section of the trunk in addition to the caudal section used in this study. This would allow for a more complete picture of the muscle development. The swimming performance studies could have been affected by inconsistencies in running experiments with one vs two people and the escape response experiments were potentially affected by the physical limitations of the high speed camera setup which could only record a small area leading to the fish being kept in swimming arenas that could have been too small and limited movement, leading to fish not swimming away from stimuli as fast as they could in order to not swim into the sides of the arenas. Finally, a more direct link between swimming and muscle development could be established through muscle fibre contraction experiments.

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