

Influence of a dynamic rearing environment on development of metabolic phenotypes in age-0 Lake Sturgeon, *Acipenser fulvescens*

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Environment–phenotype interactions are the most pronounced during early life stages and can strongly influence metabolism and ultimately ecological fitness. In the present study, we examined the effect of temperature [ambient river temperature (ART) vs ART+2°C], dissolved oxygen (DO; 100% vs 80%) and substrate (presence vs absence) on standard metabolic rate, forced maximum metabolic rate and metabolic scope with Fulton's condition factor (K), energy density (ED) and critical thermal maximum (CTmax) in age-0 Lake Sturgeon, *Acipenser fulvescens*, before and after a simulated overwintering event. We found that all the environmental variables strongly influenced survival, K, ED and CTmax. Fish reared in elevated temperature showed higher mortality and reduced K pre-winter at 127 days post-hatch (dph). Interestingly, we did not find any significant difference in terms of metabolic rate between treatments at both sampling points of pre- and post-winter. Long-term exposure to 80% DO reduced ED in Lake Sturgeon post-winter at 272 dph. Our data suggest that substrate should be removed at the onset of exogenous feeding to enhance the survival rate of age-0 Lake Sturgeon in the first year of life. Effects of early rearing environment during larval development on survival over winter are discussed with respect to successful recruitment of stock enhanced Lake Sturgeon, a species that is at risk throughout its natural range.

Key words: Conservation aquaculture, environmental matching hypothesis, lake sturgeon, metabolic phenotypes

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Introduction

Early phenotypic development is known to strongly influence growth trajectory and ecological fitness of individuals (Lindström, 1999). Thus, the importance of the environment during early development has been well studied across taxa, and a number of models have been proposed to understand

the long-term consequences (Monaghan, 2008). The environmental matching hypothesis suggests that thrifty phenotypes proliferate in a suboptimal environment, but they may become maladaptive when the environment becomes mismatched (Monaghan, 2008). When the mismatch is negligible, individual phenotypic plasticity should permit adaptation to the new environment; however, when the mismatch is greater

than their adaptive capability, individuals may show reduced fitness in the long term (Hendry *et al.*, 2011).

The environmental matching hypothesis has many implications for conservation biology in instances where captive-reared individuals are released into the wild to enhance the status of endangered or at-risk populations. Conservation fish hatcheries typically employ artificial rearing environments to facilitate hatchery management, but such environments lack the variability found in the wild and often result in lower phenotypic variation in progeny (Johnsson *et al.*, 2014). For instance, absence of complexity in rearing environments can result in naïve individuals towards natural selective pressures (Araki *et al.*, 2008). Practically it is impossible to implement all wild environmental variables in a hatchery, thus, understanding key environmental variables at critical life stages is important to produce more suitable individuals that are likely to increase post-release survival rates (Roberts *et al.*, 2011, 2014). Environmental enrichment in artificial settings has been used to mimic key factors from the natural environment to naturalize phenotypes and promote acquisition of vital skills as well as growth performance (McAdam, 2011; Lönnstedt *et al.*, 2012; Chivers and Ferrari, 2013). For example, previous studies have indicated that simple inclusion of substrate (e.g. gravel) at the appropriate time can promote growth performance in sturgeon species (Gessner *et al.*, 2009; Bates *et al.*, 2014). Thus, understanding how key abiotic factors shape phenotypic development and how these phenotypes will influence survival rates in the first year of life, particularly during the challenging first overwintering event, would directly benefit conservation programs (Johnsson *et al.*, 2014).

Environmental temperature is the key to biological phenomena as it dictates the rate of biochemical reactions and strongly influences physiological processes, locomotion and the geographical distribution of many fish species (Schulte *et al.*, 2011; Stewart and Allen, 2014). Standard metabolic rate (SMR) is defined as the minimum oxygen consumption rate to maintain vital processes such as cellular homeostasis and organismal integrity (Hochachka and Somero, 2001; Treberg *et al.*, 2016). SMR in fish may play an important role in ecological fitness because SMR is known to be correlated with growth trajectories, maximum aerobic performance and some behaviours (Frappell and Butler, 2004; Burton *et al.*, 2011; Norin and Clark, 2016). Maximum metabolic rate (MMR) is the maximum oxygen consumption rate required to maintain maximum activity. MMR is important in prey capture, predator escape, reproduction and habitat use as all these ecological factors are largely dependent on the fish's swimming ability (Kieffer, 2000). The difference between SMR and MMR is defined as metabolic scope (MS), which is the aerobic capacity for routine activities such as foraging, growth, locomotion and reproduction (Hochachka and Somero, 2001). The relationship between MS and temperature has been described by a unimodal curve with the upper limit known as critical thermal maximum (CT_{max}) beyond

which normal physiological function cannot meet the oxygen demand of tissues thus leading to metabolic suppression (Pörtner, 2010). Specifically, decreased MS due to increased temperature and hypoxia can be detrimental for fish because of the importance of MS for growth, reproduction and ecological fitness (Pörtner, 2010; Holt and Jørgensen, 2015).

Warmer aquatic environments decrease the solubility of oxygen, further, increased temperature results in an increased metabolic demand for most ectotherms leading to increased oxygen consumption rates. Studies have suggested that increased temperature and hypoxia may have a synergistic effect (Pörtner, 2010; McBryan *et al.*, 2013) as temperature increases metabolic rate in fish (Clarke and Johnston, 1999; Jo and Kim, 1999) and hypoxia can negatively impact locomotion, growth, development of the embryo and aerobic metabolism coupled with increased levels of glycolysis (Baker *et al.*, 2005; Behrens and Steffensen, 2007; Farrell and Richards, 2009; Richards, 2009; Dupont-Prinet *et al.*, 2013). Previous results have indicated that acute exposure to hypoxia down to 80% of air saturation did not affect aerobic metabolism or hypoxia avoidance behaviours in a number of species of fish (Poulsen *et al.*, 2011; McBryan *et al.*, 2013; Svendsen *et al.*, 2014); however, long-term exposure to 80% dissolved oxygen (DO) could result in increased mortality and loss of aerobic capacity following exposure to a stressful environment (i.e. low temperature and food deprivation; Yoon *et al.*, 2018). Previous studies have predominantly focused on teleost fish (Fernandes *et al.*, 1995; Hobbs and McDonald, 2010), but these relationships remain poorly understood in ancestral fishes such as sturgeons.

Fulton's condition factor (K) represents standardized body mass by total length, and K implies condition of individuals (Nash *et al.*, 2006). Many studies have reported that K is strongly influenced by extrinsic factors such as seasonality, food availability and geography (Sutton *et al.*, 2000; Leclercq *et al.*, 2010). Energy density (ED) is a unit of energy per volume or mass in animals (often expressed in J·g⁻¹), which directly shows the status of energy reserves in an individual. ED is known to change with geographical distribution (Schultz and Conover, 1997) and developmental stages (Wuenschel *et al.*, 2006). Specifically, energy allocation towards somatic growth and energy reserve during the first year of life is critical prior to winter, particularly in northern fishes where food resources become scarce and endogenous stores must be mobilized to sustain life (Post and Parkinson, 2001). Previous studies have shown that individuals with higher energy stores are more likely to survive prolonged winter conditions (Biro *et al.*, 2004; Houston *et al.*, 2014; Deslauriers *et al.*, 2018).

Lake Sturgeon, *Acipenser fulvescens*, Rafinesque 1817, is a large cartilaginous, benthic fish. They are native to North America and complete their full life history in freshwater. Lake Sturgeon were once widely distributed throughout North America (Peterson *et al.*, 2007); however, they are now recognized as an endangered or threatened species across

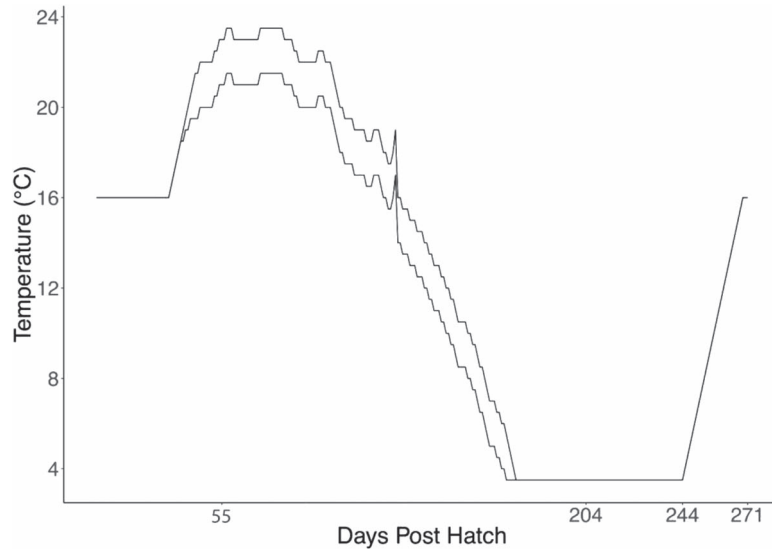


Figure 1: Temperature regimes used in this study. Days post hatch (dph) on the X-axis indicate when sampling occurred or there was a change in environmental parameters. ART denotes ambient river temperature. Two lines represent two different temperature regimes: (i) upper line: ART+2°C and (ii) lower line: ART. Dissolved oxygen remained constant within each experimental treatment. At 204 dph, food was deprived for 40 days following which temperature increased by 0.5°C per day. Temperature profiles used in this study were a reflection of natural temperature profiles collected from 2013 to 2016 at Slave Falls, on the Winnipeg River, MB, Canada. The average daily temperature was rounded to the nearest 0.5°C.

most of their natural range due to a variety of factors such as over-harvesting, hydroelectric dams and pollution. Despite conservation efforts for many decades, poor success has often been observed due in part to low recruitment rates of stocked fish. Specifically, a previous study showed that age-0 stocked Lake Sturgeon showed a relative recruitment rate 17.7 times lower than those stocked at age-1 (McDougall *et al.*, 2014), which implies that phenotypes of fish produced from the hatchery may not be best suited to survive the first winter of life. Thus, it is necessary to understand how abiotic factors (e.g. temperature and DO) might shape phenotypic development in age-0 Lake sturgeon promoting increased survival rates during the first winter.

In this study, we examined how development of metabolic phenotypes in age-0 Lake Sturgeon was dependent on early rearing environments. Specifically, we were interested in variable temperature profiles, DO and substrate. We chose ambient river temperature (ART) and ART+2°C to represent natural temperature profiles that age-0 Lake Sturgeon may experience in Manitoba, Canada, and 80% of air-saturation DO was chosen as mild hypoxia is often observed in aquaculture due to high biomass density, loss of flow or in nature due to eutrophication (Diaz and Breitburg, 2009). Further, we included substrate based on the previous findings regarding its impacts on phenotypic development in sturgeon species (Gessner *et al.*, 2009; McAdam, 2011; Zubair *et al.*, 2012; Boucher *et al.*, 2018; Yoon *et al.*, 2018). Specifically, we tested the environmental matching hypothesis and predicted that fish raised in ART 100% DO with substrate would show

the highest K, ED, MS and survival rate throughout the experiment. Furthermore, we predicted interactive effects of increased temperature and hypoxia would reduce forced maximum metabolic rate (FMR) and MS and that the presence of substrate would increase ED and K. Finally, assuming a fall stocking event, we examined how these phenotypes would influence differential survival rates during a simulated overwintering event.

Materials and methods

Animal husbandry and maintenance

Two females (28 ± 0.0 kg; mean \pm S.E.) and three males (10.4 ± 1.21 kg; mean \pm S.E.) were captured by gill net in the Nelson River, MB in May 2016. Upon fertilization, eggs were brought to Grand Rapids Fish Hatchery on 27 May 2016. Upon yolk absorption [8 days post-hatch (dph)] fish were fed to satiation three times a day with freshly hatched brine shrimp (*Artemia International LLC*, Texas, USA). Any uneaten brine shrimp was carefully removed by siphon after 15 min. At 20 dph, Lake Sturgeon larvae were transferred to the Animal Holding Facility at the University of Manitoba. Fish were distributed into 1 of 18 9L aquaria placed in a three-row multistressor unit (AquaBiotech, Quebec, Canada). Fish were acclimated to 16°C until 32 dph when temperature manipulation was initiated. ART was created based on the average daily temperature profile rounded up to the nearest 0.5°C recorded at Slave Falls on the Winnipeg River from 2013 to 2016 (Fig. 1), and 100% or 80% DO hereafter refers

to treatments where tank water was maintained at 100% or 80% DO relative to air saturation in holding water regulated by injecting air or nitrogen gas. Rearing environments were maintained as one of the following: ART and 100% DO; ART+2°C and 80% DO; and ART+2°C and 100% DO. In each rearing environment, rearing tanks were evenly split between three tanks with substrate and the other three tanks without substrate. Each tank was supplied with dechlorinated City of Winnipeg tap water on a semi-recirculating system with a replacement rate of 10% per day for the whole unit, and temperature and DO in the treatment tanks were regulated within 0.2°C and 5%, respectively. Ammonia levels were observed to always be below 0.1 ppm across tanks (Ammonia Test Kit, Fluval, Quebec, Canada). Substrate consisted of sinking plastic pall rings with 25 mm diameter (Rashig USA INC, Texas, USA). At 22 dph, a mixture of ground bloodworm (Hikari USA, California, USA) and brine shrimp was fed to the fish, and the proportion of bloodworm was increased by 10% per day as the fish grew until they were on a diet of 100% bloodworm at 32 dph. At 32 dph, the pall rings were replaced with sand with sufficient amounts (2 cm height) to just cover the base of the tank and mimic the preferred habitat at this life stage in the wild (Benson *et al.*, 2005). Photoperiod was set to correspond to the time of dawn and dusk at Pointe Du Bois on the Winnipeg River. Any mortalities were immediately removed, and daily survival in each treatment was calculated as the average survival across the three treatment tanks throughout the experimental period.

Overwintering

A simulated overwintering event was performed with changes in temperature and light intensity similar to what has previously been described (Yoon *et al.*, 2018) while DO remained constant within each treatment throughout the experimental period (Fig. 1). Temperature in all treatment tanks reached 3.5°C at 175 dph after which light intensity in all treatments was decreased gradually and remained at 10% of the initial brightness prior to the wintering period. At 204 dph, temperature and light intensity were maintained at 3.5°C and 10%, respectively, and food was deprived for 40 days to simulate an overwintering period. Following this period, temperature in all treatments was increased to 16°C at a rate of 0.5°C per day and light intensity was set to pre-winter levels. During this warming phase, fish in each treatment were fed bloodworm to satiation and were finally sampled within 4 days after all treatments reached 16°C.

Condition factor and Energy Density

At 127 and 271 dph, total length and body mass of fish were measured to the nearest 1 mm and 0.0001 g, respectively (Table 1). Due to higher mortality rates and limited numbers of fish reserved for the overwintering study, we were unable to sample from ART+2°C 100% DO with substrate at 127 dph and ART+2°C 80% DO with substrate at both 127 and

Table 1: Mean ± SE mass (g) and total length (TL, mm) of Lake Sturgeon, *A. fulvescens*, raised under different rearing environments

Treatment	Winter	n	Mass (g)	TL (mm)
ART 100% DO Sub	Pre	15	3.39 ± 0.46	94 ± 5
	Post	29	3.12 ± 0.3	83 ± 3
ART 100% DO NoS	Pre	14	2.51 ± 0.19	86 ± 3
	Post	26	2.39 ± 0.19	78 ± 2
ART+2°C 100% DO Sub	Pre	nd	nd	nd
	Post	21	4.41 ± 0.66	95 ± 5
ART+2°C 100% DO NoS	Pre	6	3.58 ± 0.66	99 ± 6
	Post	30	3.01 ± 0.23	87 ± 2
ART+2°C 80% DO NoS	Pre	14	2.7 ± 0.30	90 ± 3
	Post	26	2.49 ± 0.19	80 ± 3*

Mass and length were assessed at 127 and 272 dph representing pre- and post-winter. ART denotes ambient river temperature, and ART+2 represents 2°C increased regime. DO represents the dissolved oxygen as percent air saturation. Sub and NoS indicate substrate and no substrate. While '*' denotes a significant difference between pre- and post-winter values, nd denotes no data due to limited sample sizes. ART+2°C 80% DO Sub data were not collected due to limited sample sizes.

272 dph. From Table 1, K was calculated following the equation

$$K = \frac{\text{Weight (g)} \times 100}{\text{Total Length (cm)}^3}$$

Fish were sacrificed by immersion in an overdose of tricaine methanesulfonate (MS222; 250 mg·L⁻¹; Syndel Laboratories, British Columbia, Canada) and length and wet mass were recorded. Carcasses were then placed in a drying oven and desiccated at 60°C for 48 h or until a constant mass was recorded. Dry to wet mass ratio was then used to predict the ED (J·g⁻¹ of body mass) of individual fish using the model previously verified by Yoon *et al.* (2018).

Metabolic rate

At approximately 122 and 269 dph, metabolic rate was assessed as whole body oxygen consumption rate ($\dot{M}O_2$) using intermittent flow respirometry as previously described in Yoon *et al.* (2018). The following parameters were used to assess $\dot{M}O_2$: a 360 s flushing period, 60 s wait period and 300 s measurement period (Loligo Systems, Viborg, Denmark). Flow rate was set to minimize stress on individuals, but sufficient to provide water exchange for measurement of $\dot{M}O_2$ on an intermittent basis. Respirometry chambers were surrounded by black curtains to avoid visual disturbance for each trial.

Individual fish were fasted for 12 h prior to measurement of $\dot{M}O_2$. Individuals were lightly anesthetized by immersion in 50 mg·L⁻¹ of MS222 buffered with equal volumes of sodium bicarbonate for 10 s, and their mass and total

length were recorded before being placed in the respirometry chamber. Our pre-experiment trials revealed that at least 6 h of acclimation was required to obtain SMR in age-0 Lake Sturgeon, so $\dot{M}O_2$ data were collected for the following 18 h to estimate SMR. Following this period, FMR was assessed using a standardized chase protocol where individuals were chased for 15 min with gentle prodding of the tail region with a plastic pipette. Fish were then immediately returned to the same respirometry chamber and oxygen consumption was measured for three measurement periods. Biological oxygen demand (BOD) was quantified by measuring oxygen consumption without fish for 15 min in each metabolic chamber before measurement of SMR and following measurement of FMR to correct for any increases in BOD during the time frame of the experiment. It is important to note that $\dot{M}O_2$ prior to the overwintering event was measured at 16°C and 18°C because these were corresponding temperatures at 122 dph, whereas $\dot{M}O_2$ following the overwintering event was measured at 16°C. All $\dot{M}O_2$ were conducted at 100% DO.

Analysis of metabolic rate data was conducted following the protocol used previously (Yoon *et al.* 2018). Slopes of declining oxygen concentration were collected for each measurement, and only coefficients of determination (r^2) above 0.9 were used for analysis. Measured BODs in the beginning and end of each trial were then used to interpolate a linear slope over time to reflect BOD drifting, and all the data points were corrected by each corresponding BOD at each measurement time. The $q_{0.1}$ method (10th quantile) was used as previously described by Chabot *et al.* (2016) to estimate representative SMR values. FMR was determined by choosing the highest oxygen consumption rate among three measurements following the standardized chase protocol. MS was calculated by subtracting SMR from FMR.

Critical Thermal Maximum

At 55 dph and 271 dph, CTmax was assessed to the nearest 0.01°C for individuals as described previously by Deslauriers *et al.* (2016) with slight modifications. It is important to note that due to the limited numbers of fish reserved for the overwintering study, we were unable to assess CTmax at 127 dph (pre-winter). In brief, 48 small plastic containers with screen mesh were placed on a tray where the water temperature was increased by 2°C per hour. Initial water temperature was set to the same temperature of each rearing environment and was regulated by a thermostat (Fisher, Massachusetts, USA) while being well oxygenated. Once fish lost equilibrium and showed no response to gentle prodding, temperature was recorded in each container using a temperature probe equipped with a Witrox 4 Oxygen Meter (Loligo Systems, Viborg, Denmark). At the end of the trial fish were removed from their individual containers, measured and weighed before being euthanized by immersion in an overdose of MS222 (250 mg·L⁻¹). All the experimental protocols were performed as described under animal use protocol F15-007 approved by the University of

Manitoba Protocol Management Review Committee under the guidelines of the Canadian Council for Animal Care.

Statistical analysis

A generalized linear mixed model (GLMM) was employed to analyse the data to account for the random effect of rearing tanks as previously described (Yoon *et al.*, 2018). Temperature, DO and substrate are considered as discrete factor variables, and they are represented by T (ART to ART+2°C), DO (100% to 80%) and S (absence to presence), respectively. α_{ID} represents the intercept of random effects of rearing tanks, and ε indicates residuals errors. The full model was written as follows:

$$\hat{R} = \alpha_0 + \beta_t \cdot T + \beta_{do} \cdot DO + \beta_s \cdot S + \beta_{t*s} \cdot T \times S + \beta_{do*s} \cdot DO \times S + \alpha_{ID} + \varepsilon,$$

where the response variable (\hat{R}) represents either condition factor ($100 \times g \text{ cm}^{-3}$), ED ($J \cdot g^{-1}$), metabolic rate ($mgO_2 \cdot kg^{-1} \cdot h^{-1}$) or CTmax (°C). The model was used to assess all variables independently at each sampling date across all treatments, and a likelihood ratio test was performed to test significance of each variable using the ‘anova’ function in the R package lme4 with backward elimination (Winter, 2013). When results indicated significance, *post hoc* comparisons were performed for each sampling date using the ‘glht’ function in the R package ‘multcomp’ (Hothorn *et al.*, 2008; Allen *et al.*, 2016; Yoon *et al.*, 2018) with significance determined at $\alpha = 0.05$ (see Tables S1 and S2 for the full results of *post hoc* analysis for data). Student’s *t*-test was performed to examine changes in each measured phenotype after the simulated overwintering event within treatments, and the results are summarized in Table S3. All statistical analyses were conducted in R (R Core Team, 2018).

Results

Our results showed complex interactions between increased temperature, hypoxia and presence of substrate on the metabolic phenotypes in age-0 Lake Sturgeon.

Growth and condition factor

Our data indicate that there was no significant effect of environmental variables on body mass or total length of age-0 Lake Sturgeon at 127 and 272 dph (Table 1); however, higher temperature reduced K at both sampling times (Table 2; $P < 0.01$). Specifically, at 127 dph, comparison of the individual treatments revealed that Lake Sturgeon raised in ART 100% DO showed a significantly higher K than those raised in ART+2°C 100% DO without substrate and ART+2°C 80% DO without substrate (Fig. 2; $P < 0.05$). At 272 dph, we saw a similar trend where Lake Sturgeon raised in ART 100% DO with substrate showed higher K than those raised in ART+2°C 100% DO. Within treatment analysis

Table 2: Analysis of condition factor (K) and energy density (ED) at 127 and 272 dph in Lake Sturgeon, *A. fulvescens*, raised in different rearing environments by generalized linear mixed model

Measurement	Dph	Variables	Coefficient	Std. Error	χ^2	P
K	127	α_0	-0.97	0.02	na	na
		T (ART+2°C)	-0.10	0.03	9.514	<0.001
		DO (80%)	0.03	0.03	1.222	0.269
		S (Presence)	-0.00	0.02	0.005	0.944
	272	α_0	72.68	7.59	na	na
		T (ART+2°C)	-29.41	10.84	9.669	0.008
		DO (80%)	28.85	10.71	5.727	0.017
		S (Presence)	15.37	10.89	3.174	0.205
		T × S	-2.16	15.60	0.019	0.891
ED	127	α_0	2974.03	61.27	na	na
		T (ART+2°C)	-205.19	111.86	2.973	0.085
		DO (80%)	185.71	110.74	2.598	0.107
		S (Presence)	170.49	85.19	3.243	0.071
	272	α_0	2538.59	74.05	na	na
		T (ART+2°C)	197.96	100.93	4.021	0.134
		DO (80%)	-302.3	99.67	6.731	0.001
		S (Presence)	-33.12	101.43	0.950	0.622
		T × S	-63.81	145.22	0.173	0.678

Results were obtained by likelihood ratio test with an elimination of each variable. Three variables are described in the table below: α_0 , intercept; T, temperature (ART+2°C compared to ART); DO, dissolved oxygen (80% of air saturation compared to 100%); S, substrate presence compared with absence, while 'na' means 'not applicable'. χ^2 denotes Chi-square value from the likelihood ratio test between the full model and reduced model while P is probability that is significant when bolded. Dph represents days post hatch for sampling. All the environmental variables were regarded as factor variables. Tanks were used as the random effect. Log and rank transformations were performed on the data of K at 127 and 272 dph, respectively, for the analysis.

showed that K significantly increased in treatments of ART 100% DO and ART+2 °C 100% DO without substrate after the simulated overwintering event (Fig. 2).

Energy Density

There were no significant effects of environmental variables on ED at 127 dph. However, at 272 dph, DO had a significant effect on ED (Table 2; $P=0.001$), and comparison of the individual treatments showed that Lake Sturgeon raised in ART+2°C 100% DO without substrate showed higher ED than those raised in ART+2°C 80% DO without substrate (Fig. 3). Within treatment analysis indicated that Lake Sturgeon raised in ART+2°C 100% DO without substrate did not change in ED after the winter, whereas Lake Sturgeon raised in ART 100% DO and ART+2°C 80% DO without substrate showed decreased ED (Fig. 3; $P < 0.001$)

Metabolic rate (SMR, FMR and MS)

Interestingly, our results indicated no significant effects of environmental variables on SMR, FMR and MS between treatments at each sampling point (Fig. 4).

CTmax

At 55 dph, only substrate had effects on CTmax (Table 3; $P < 0.001$); however, comparisons of the individual treatments showed that this was limited in the ART+2°C 80% DO treatment where presence of substrate significantly reduced CTmax (Fig. 5). Although not consistent with our findings from CTmax, at 55 dph we also observed significant differences in body mass by treatment used for the CTmax experiment (Fig. 6). Within treatment analysis indicated that CTmax did not decrease after the winter in two treatments of ART+2°C 100% DO with substrate and ART+2°C 80% DO with substrate, whereas the remaining treatments showed significant decreases in CTmax post-winter (Fig. 5; $P < 0.001$).

Survival rate

Survival in all treatments decreased throughout development prior to the simulated overwintering (Fig. 7). The increased temperature appeared to have the greatest impairment on survival in all treatments, with hypoxia and presence of substrate also resulting in reduced survival rate prior to overwintering. Interestingly, no mortalities were observed across treatments during the simulated winter period.

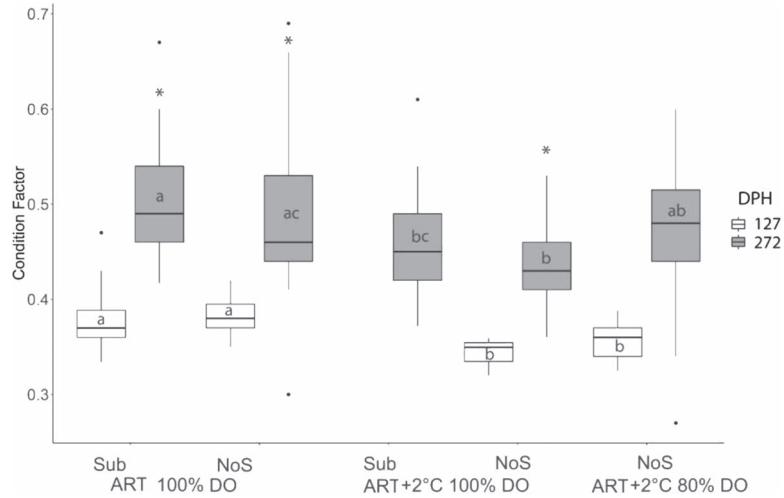


Figure 2: Condition factor of Lake Sturgeon, *A. fulvescens*, raised in different rearing environments DPH is days post-hatch for sampling. ART denotes ambient river temperature, and ART+2°C represents 2°C increased regime. DO represents the air saturation levels of dissolved oxygen. Sub and NoS indicate substrate and no substrate. Samples for ART+2°C 100% DO Sub at 127 dph and ART+2°C 80% DO Sub at both 127 and 272 dph were not collected due to limited sample sizes. Different letters represent significant difference between treatment at each sampling point. The ** denotes significant change after the simulated overwintering event.

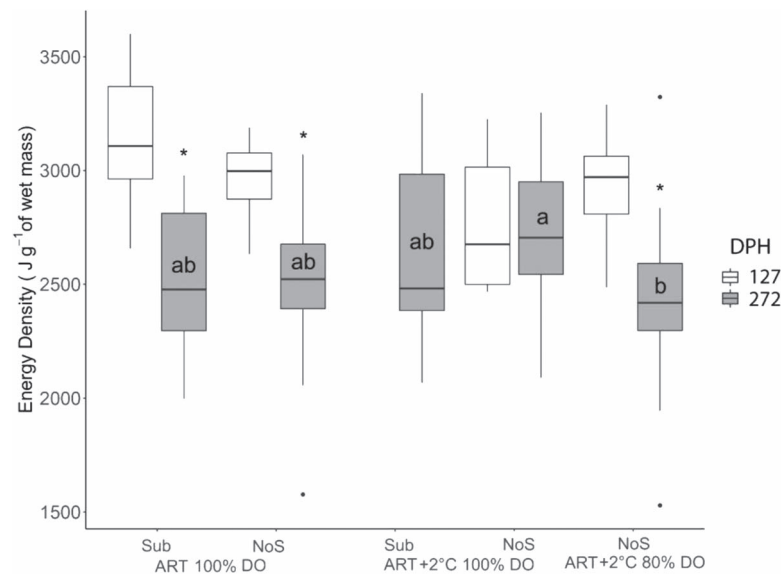


Figure 3: Energy density of Lake Sturgeon, *A. fulvescens*, raised in different rearing environments DPH is days post-hatch for sampling. ART denotes ambient river temperature, and ART+2 represents 2°C increased regime. DO represents the air saturation levels of dissolved oxygen. Sub and NoS indicate substrate and no substrate. Samples for ART+2°C 100% DO Sub at 127 dph and ART+2°C 80% DO Sub at both 127 and 272 dph were not collected due to limited sample sizes. Different letters represent significant difference between treatment at each sampling point. The ** denotes significant change after the simulated overwintering event.

Discussion

K and ED by environment

Early developing fish are known to be particularly sensitive to temperature as thermal stress can often result in developmental deformities and increases in oxidant stress during

key life history phases (Werner *et al.*, 2007; Simčič *et al.*, 2015). We found a decrease in K for Lake Sturgeon reared under increased temperature at both pre- and post-winter sampling points, which suggests that the increased thermal regime for Lake Sturgeon in the present study was not optimal for energy assimilation. A similar trend was reported in White

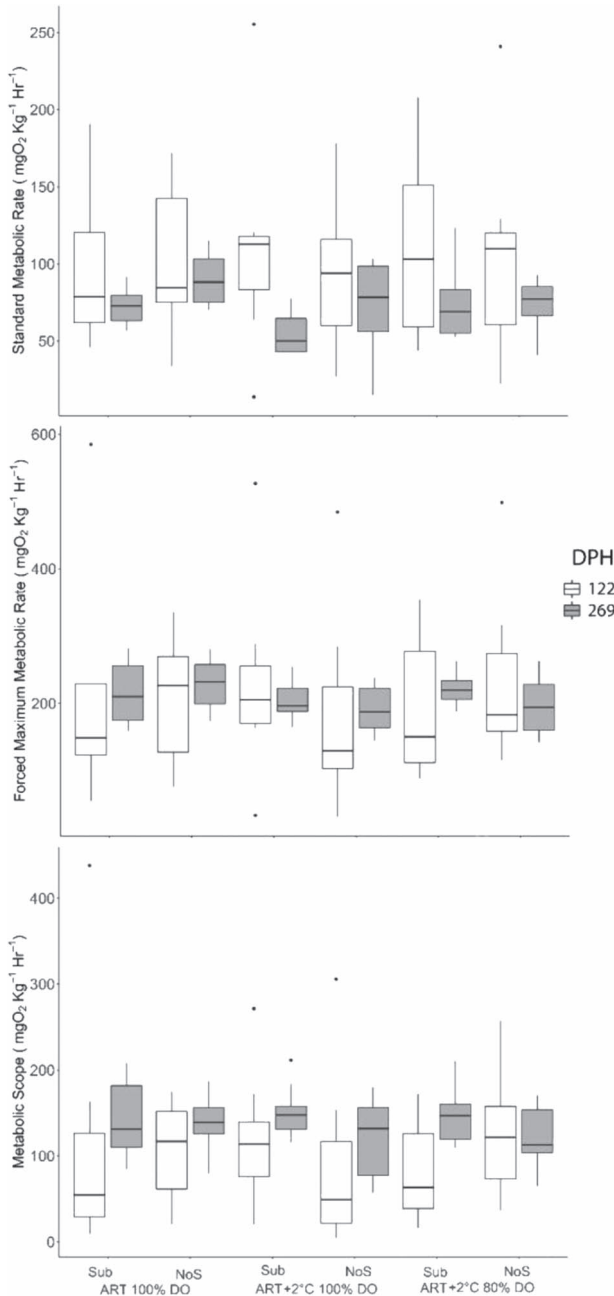


Figure 4: Metabolic rates and scopes of age-0 Lake Sturgeon, *A. fulvescens*, raised in different rearing environments Dph is approximate days post-hatch for sampling. ART denotes ambient river temperature, and ART+2 represents 2°C increased regime. DO represents the air saturation levels of dissolved oxygen. Sub and NoS indicate substrate and no substrate.

Sturgeon larvae that had a 7% reduction in condition factor when fish were reared at increased temperatures (14.5°C vs 17.5°C; Boucher *et al.*, (2014)). In Shovelnose Sturgeon, *Scaphirhynchus platyrhynchus*, maximum feed conversion rate

was found to be at 21.7°C, which was slightly lower than the optimal temperature for growth of 22.4°C. When environmental temperature exceeded 22.4°C for the same species, juveniles showed a reduced growth rate, feed efficiency and survival rate (Kappenman *et al.*, 2009). Thus, fish reared in higher temperatures in our present study may experience lowered efficiency of energy assimilation and thus reduced K, which may have led to increased mortality rates.

Our data also showed that the inclusion of substrate did not improve K, which disagrees with previous studies (Gessner *et al.* 2009; Boucher *et al.*, 2014; Yoon *et al.*, 2018). Importantly, Boucher *et al.* (2014, 2018) removed substrate at the onset of the exogenous feeding stage, whereas in this study substrate remained either as pall rings or sand throughout the study period. Anecdotally, during the onset of exogenous feeding, brine shrimp often fell into the space between the substrate, which may have reduced foraging efficiency for fish raised over substrate. It has been reported that high mortality in the first year of life is not unusual in sturgeon (Caroffino *et al.*, 2010) particularly at the onset of exogenous feeding (Gisbert *et al.*, 2000). Our observation of more challenging access to food may explain lower survival rate in the substrate treatment groups during the larval development prior to our overwintering phase in the present study. Interestingly, in all but one of the treatments K was significantly higher post-winter at 272 dph, which is in disagreement with Yoon *et al.* (2018) where K was significantly lower post-winter regardless of treatment. It is well documented that fish that experience starvation replace lipid reserves and proteins with water (Martinez *et al.*, 2003; Bar, 2014). Thus, it is possible that increases in water content during the overwintering event may have led to a perceived increase in K that was not supported by an increase in ED (see below).

We saw a general decreasing trend of ED following the wintering event, which agrees with previous studies (Hurst, 2007; Deslauriers *et al.*, 2018). It is thought that fish maximize their somatic growth as well as energy storage prior to winter such that they can survive when resources are likely to be scarce. Indeed, previous research showed that fish increase their lipid reserve from summer to fall (Booth and Keast, 1986; And and Kirkwood, 1995; Schultz and Conover, 1997). Specifically, energy allocation to lipid reserves may play an important role in overwintering as lipids were shown to be the critical energy source for fish exposed to periods of starvation (Byström *et al.*, 2006). Because ED in fish is known to be positively correlated with lipid content (Anthony *et al.*, 2000), our results emphasize that lipid reserves are important for surviving overwintering conditions.

Following the wintering event fish raised in increased temperature under hypoxia showed significantly lower ED than those raised under increased temperature regimes. It is unknown why ED was lower in this hypoxia group, but it is possible that metabolic adaptation to hypoxia at increased temperature may become detrimental during periods of starvation and reduced temperature.

Table 3: Analysis of Critical thermal maximum at 55 and 271 dph in Lake Sturgeon, *A. fulvescens*, raised in different rearing environments by generalized linear mixed model

Dph	Variables	Coefficient	Std. Error	χ^2	P
55	α_0	34.67	4.19	na	na
	T (ART+2°C)	-5.00	5.93	4.007	0.135
	DO (80%)	10.33	5.93	3.582	0.167
	S (Presence)	-5.50	5.93	17.240	<0.001
	T × S	-6.00	8.38	0.510	0.475
	DO × S	-15.17	8.38	3.178	0.075
271	α_0	32.64	0.24	na	na
	T (ART+2°C)	-0.05	0.34	0.417	0.812
	DO (80%)	0.60	0.34	3.171	0.205
	S (Presence)	-0.03	0.34	2.424	0.489
	T × S	0.26	0.48	0.302	0.583
	DO × S	-0.71	0.48	2.182	0.140

Results below were obtained by likelihood ratio test with an elimination of each variable. Dph represents days post hatch for sampling. Three variables are described in the table: α_0 , intercept; T, temperature (ART+2°C compared to ART°C); DO, dissolved oxygen (80% of air saturation presence compared with absence, while 'na' represents 'not available'. χ^2 denotes Chi-square value from the likelihood ratio test between the full model and reduced model while P is probability that is significant when bolded. All variables were regarded as factor variables, and replicate tanks were used as the random effect. Rank transformation was performed on the data at 55 dph to account for homoscedasticity in the data.

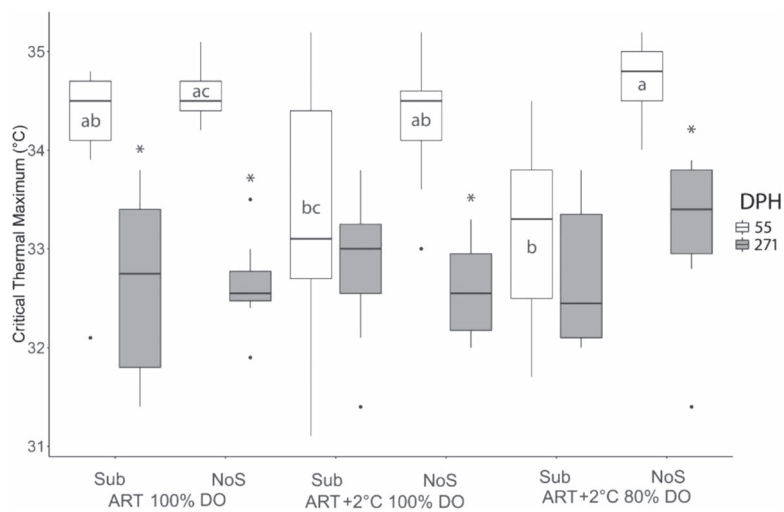


Figure 5: Critical thermal maximum of age-0 Lake Sturgeon, *A. fulvescens*, raised in different rearing environments DPH is days post-hatch for sampling. ART denotes ambient river temperature, and ART+2 represents 2°C increased regime. DO represents the air saturation levels of dissolved oxygen. Sub and NoS indicate substrate and no substrate. Different letters represent significant difference between treatment at each sampling point. The '*' denotes significant change after the simulated overwintering event.

Metabolic rate by environment

Our data showed that increased rearing temperature had no impact on SMR, FMR and MS of Lake Sturgeon, which disagrees with Yoon *et al.* (2018). The discrepancy between these studies could be explained by the temperature and light regimes used in each study. While Yoon *et al.* (2018) used fixed rearing environments with temperature, the present

study used a fluctuating temperature regime reflective of the natural environment. Cyclic changes in temperature are known to cause synchronism in many organisms across taxa such as cyanobacteria, algae, fungi and animals (Rensing and Ruoff, 2002; López-Olmeda, 2017). For example, reproductive cycles of adult fish are known to be regulated by annual photo-thermal cycles (Wang *et al.*, 2010), and in Zebrafish, *Danio rerio*, changes in environmental temperature act as an

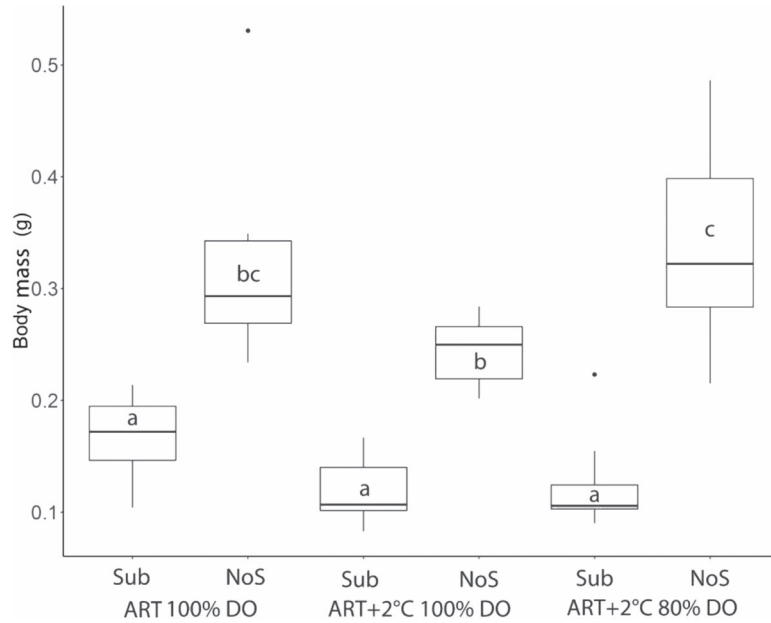


Figure 6: Body mass of age-0 Lake Sturgeon, *A. fulvescens*, used for CTmax experiment at 55 dph Fish were raised in different rearing environments. ART denotes ambient river temperature, and ART+2 represents 2°C increased regime. DO represents the air saturation of dissolved oxygen. Sub and NoS indicate substrate and no substrate. Different letters represent significant difference between treatments.

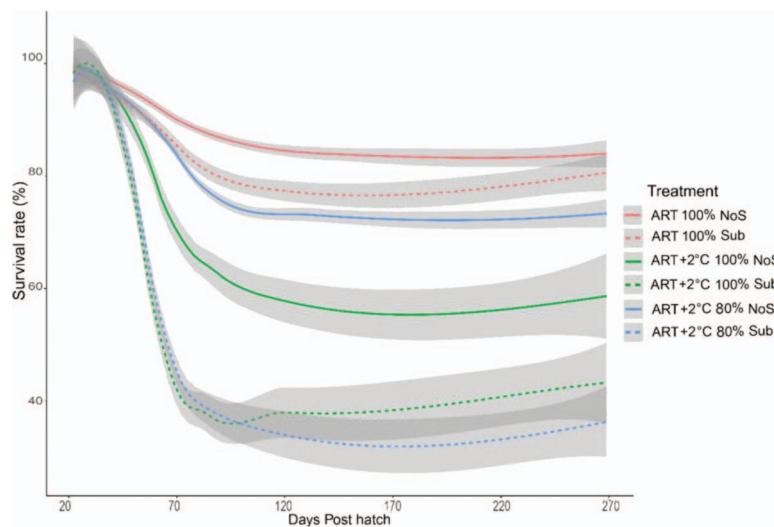


Figure 7: Survival rate of Lake Sturgeon, *A. fulvescens*, raised in different rearing environments Each treatment consists of three rearing tanks. Survival rate was calculated from the average at each date, and graph was generated with smooth function in 'ggplot' in R. ART denotes ambient river temperature, and ART+2 represents 2°C increased regime. DO represents the air saturation of dissolved oxygen. Sub and NoS indicate substrate and no substrate. Solid and dashed lines represent different treatments, and grey areas show the 95% confidence intervals.

important cue for the observed seasonal changes in swimming performance (Condon *et al.*, 2010). Moreover, previous studies demonstrated some evidence of circadian rhythm in hormonal stress responses of Green Sturgeon, *Acipenser medirostris* (Lankford *et al.*, 2003), metabolic rate in Lake Sturgeon (Svendsen *et al.*, 2014) and migrating behaviours

of adult Lake Sturgeon under full or new moon phases (Forsythe *et al.*, 2012). Thus, natural environmental cues of temperature regimes and photoperiod used in this study may have triggered seasonal synchronism in age-0 Lake Sturgeon that induces subtle changes in regulation of energy balance throughout the experimental period.

The lack of change in SMR, FMR and MS between pre- and post-winter in the present study is also counter to previous studies (Mehner and Wieser, 1994; O'Connor *et al.*, 2000; Yoon *et al.*, 2018) and may be explained by available energy. Deslauriers *et al.* (2018) demonstrated the critical level of endogenous energy reserves in age-0 Lake Sturgeon (~2000 J·g⁻¹) to survive 40 days starvation at 1°C. Given that we found zero mortality during the wintering period our data may imply that fish did not reach the critical threshold to initiate metabolic changes. Further, ED following the overwintering event in this study was higher than the reported threshold of ED for survival.

In the present study 80% of air-saturation DO did not compromise aerobic metabolism in Lake Sturgeon and this is supported by Svendsen *et al.* (2014), but contrary to Yoon *et al.* (2018) who demonstrated that Lake Sturgeon raised at 80% of air-saturation DO showed lower survival and reduced aerobic capacity during a simulated overwintering event. It remains undetermined why results from these studies differ, but it is important to note that the Lake Sturgeon used in this study were from the Nelson River while Lake Sturgeon in the previous study came from the Winnipeg River. Interestingly, intraspecific variation in SMR has been explained by geographical adaptation of populations in isopods, *Porcellio laevis* (Lardies and Bozinovic, 2008), as well as family effects in Lake Sturgeon (Deslauriers *et al.* unpublished). Further, Stewart and Allen (2014) demonstrated intraspecific variation in thermal tolerance in Channel Catfish, *Ictalurus punctatus* and Hybrid Catfish, *Ictalurus furcatus*. Alternatively, the discrepancy may also be explained by the two different temperature regimes used. Natural changes of temperature may trigger seasonal changes in metabolic rate, which may regulate the effect of hypoxia on metabolic rate in this species.

CTmax by environment

Our results showed that increased rearing temperature did not influence CTmax, which is in stark disagreement with previous studies where increases in acclimation temperature tend to increase CTmax (Beitinger and Bennett, 2000; Beitinger *et al.*, 2000). However, several studies have reported that body mass may also have an important role in thermal tolerance in fish. For example, a positive relationship between body mass and CTmax was found in Shortnose Sturgeon, *Acipenser brevirostrum*, Shovelnose Sturgeon, *S. platyrhynchus* and Pallid Sturgeon, *Scaphirhynchus albus* (Zhang and Kieffer, 2014; Deslauriers *et al.*, 2016). Therefore, the different body mass used in the CTmax experiments may in part explain our findings of reduced CTmax in the elevated temperature treatment group.

Many studies have reported that hypoxia could significantly reduce thermal tolerance of fish (Alabaster and Welcomme, 1962; Weatherley, 1970; Rutledge and Beitinger, 1989; Healy and Schulte, 2012). A positive correlation between CTmax and hypoxia tolerance was reported in

Atlantic Salmon, *Salmo salar* (Anttila *et al.*, 2013). Healy and Schulte (2012) reported that Killifish, *Fundulus heteroclitus*, exposed to 0.8 mgO₂·L⁻¹ for 4 weeks had a significant reduction in CTmax. However, our data showed that long-term exposure to hypoxia of 80% of air-saturation DO had no effect on CTmax of age-0 Lake Sturgeon.

Survival rate

Increased temperature and hypoxia significantly reduced survival rate prior to the winter period. This is likely due to failure to appropriately respond to thermal and hypoxic stress during early development especially during the onset of exogenous feeding. This trend of decrease in survival is similar to Boucher *et al.* (2014), who reported decreased survival rate during larval development at higher temperatures (17.5°C vs 13.5°C). Also, a previous study reported that increased temperature reduced survival in juvenile Lake Sturgeon (Wehrly, 1995). It remains unknown why the survival rates were lower in the treatments with substrate, but again it is possible that the limited food availability at the critical developmental stage could result in underdeveloped larvae, which can be vulnerable to hypoxic stressors.

Applications for conservation aquaculture

Increased temperature reduced condition factor and significantly reduced survival rate throughout the early development of Lake Sturgeon. 80% of air-saturation DO did not impact aerobic metabolism of Lake Sturgeon, but longer-term exposure to 80% of air-saturation DO significantly reduced their ED. In addition, our results suggest that substrate should be used for developing larval sturgeon for a short period during yolk sac absorption prior to emergence, to enhance survival rate in the first year. Further research is needed to understand what trade-offs allowed those fish to survive in the long term.

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Supplementary material

Supplementary material is available at *Conservation Physiology* online.

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