Assessment of family-derived metabolic traits for the conservation of an ancient fish

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Abstract

Physiological and behavioral traits of aquatic organisms are often highly dependent on environmental conditions, but genetic (family) effects often contribute to phenotypic variation. In this study, a series of physiological indices were used to assess the variability that exists amongst progeny of lake sturgeon (*Acipenser fulvescens* Rafinesque, 1817) produced from eight different families. We designed a controlled experiment aimed to evaluate metabolic performance of age-0 lake sturgeon where growth, energy density, survival, metabolic rate, volitional swimming performance, and critical thermal maxima were quantified for fish reared under the same environmental conditions. We found a strong family effect for most metrics that were quantified, and primarily influenced by the female. Furthermore, poor growth and survival within families were strongly correlated to low energy density levels and depressed routine metabolic rates at the yolk-sac stage. Lastly, the quantification of energy density at the onset of exogenous feeding appeared to be an excellent predictor of future growth and survival. Our results suggest that the choice of female for production of progeny in conservation hatcheries will have significant impacts on the success of stock enhancement as a conservation strategy for lake sturgeon.

Keywords: Lake sturgeon, acipenseridae, *Acipenser fulvescens*, physiology, respirometry, early-life history, conservation aquaculture, critical thermal maxima, swimming performance

Introduction

Acipenseridae (i.e., sturgeons) are an ancient family of fish that are considered threatened or endangered throughout their native range primarily due to over-harvesting and habitat alterations (Pikitch et al. 2005; Pedersen et al. 2008). To remediate stock depletion, conservation stocking practices have been adopted to restore or enhance historical abundances (Chebanov et al. 2002; Ireland et al. 2002; Jackson et al. 2002). The basis of these conservation programs is to rear fish within facilities during critical early life stages when mortality is high and release them into the wild as young-of-year or as yearlings when mortality rates are lower (Anderson et al. 2022). Rearing success within the facilities, characterized as fast growth and low mortality, is believed to be indicative of long-term success for juvenile sturgeon following release into the wild (Justice et al. 2009). While monitoring efforts indicate that these stocking programs can lead to population recovery for sturgeon (Jackson et al. 2002; McDougall et al. 2014; Buszkiewicz et al. 2016; McDougall et al. 2020), no physiological assessment tool has yet been developed to determine the short- and long-term success of these fish once released into the wild. Indeed, assessing the physical condition of sturgeon before release traditionally relies on growth metrics (i.e., length and mass), which often relay limited information pertaining to the physiological ability of a fish to survive during the first winter of life (Deslauriers et al. 2018*a*).

Maintaining high levels of genetic diversity is also critically important for these restoration attempts to be successful (Pikitch et al. 2005; Drauch & Rhodes 2007) given the small sizes of most sturgeon populations. However, conservation programs are often logistically dependent on a small number of broodstock adults to produce the offspring

79 that will be released, which has been hypothesized to lead to long-term loss of genetic
80 diversity (Schueller & Hayes 2011; Welsh & Jackson 2014). Additionally, a loss of
81 physiological diversity can often result from this practice, thus compromising the survival
82 of stocked early sturgeon life stages under a natural environment and potentially affecting
83 long-term population dynamics.

84 Although several previous studies have shown that the abiotic environment during
85 early life is particularly important for phenotypic development of some sturgeon species
86 (Gessner et al. 2014; Yoon et al. 2022*a*; Yoon et al. 2022*b*), recent studies have
87 suggested that there may be a wide array of phenotypic variability that exists within any
88 given year-class or potentially across families (Bugg et al. 2021) . As genotype can often
89 dictate patterns of phenotypic development in lake sturgeon (*Acipenser fulvescens*
90 Rafinesque, 1817; Dammerman et al. 2015), novel assessment criteria are necessary to
91 fully understand environment-genotype-phenotype interactions on metabolism, such as
92 energy storage and metabolic rates (Régnier et al. 2010) that have been found to favor
93 fish differently in a captive or wild setting (Van Leeuwen et al. 2011).

94 The focus on fish physiology in the context of conservation stocking programs is
95 a novel but important approach as many physiological traits have been linked to not only
96 growth and survival but also long-term fitness (Burton et al. 2011). For example, standard
97 metabolic rate (SMR) is the minimum energy expenditure to support vital processes such
98 as ion regulation, substrate cycling and maintenance of organismal integrity at a specific
99 temperature (Rolfe and Brown 1997). SMR sets the pace of basal biological processes,
100 which is a reflection of physiological adaptation to the environment and has been shown
101 to be a heritable trait that can be linked to somatic growth (Auer et al. 2015). SMR can

also act as an indicator of the fundamental ecological niche of fish (Farrell 2016), thus setting the boundaries for which habitat will be suitable for long-term survival. In terms of immediate post-release survival of juvenile fish, swimming performance and thermal tolerance could also be extremely important (McDonald et al. 1998, Pedersen et al. 2008; O'Donnell et al. 2020), but studies often only focus on a single performance metric rather than assessing an array of morphological and physiological performance traits (but see Claireaux et al. 2013).

In this study, we focus on a lake sturgeon stocking program in Manitoba, Canada, for which the metabolic performance of age-0 fish from different families was monitored over time. The main objectives of the work were to tease apart how genotype influences phenotypic traits and identify which of these phenotypic traits may favour growth and survival of age-0 lake sturgeon during their first year of life. To further refine our understanding of paternal and maternal effects, we used a half-sibling breeding design to quantify indices of physiological performance such as standard metabolic rate, critical thermal maxima, and swimming performance assessed over the course of a 5-month period. Results of this study will allow us to understand family effect contributions towards a range of physiological performance metrics and will help refine assessment methods of fish condition prior to stocking.

Methods

Broodstock origin and spawning

Eight different male (n=4) x female (n=4) combinations were generated (Table 1) from adult fish captured during their spawning migration in the Nelson River in Northern

Manitoba, Canada. Adult fish were caught using gill nets and artificially induced to spawn between May 27-31st 2016 by intra-peritoneal injection of Gonadotropin-releasing hormone (GnRH; 1.5-3 $\mu g.Kg^{-1}$ followed by 5-13 $\mu g.Kg^{-1}$; Genz et al. 2014). Fish were held in streamside tanks, and gametes were collected and fertilized on site in ambient river water before being gently rolled in Fullers earth for 40 minutes to prevent egg adhesion. Following this deadhesion procedure, fertilized eggs were transported to the Grand Rapids Fish Hatchery (GRFH) in Grand Rapids, Manitoba, Canada. Fish originated from either the Birthday Rapids (Lower Nelson River; 2 families) or Landing River (Upper Nelson River; 6 families) populations, which have been shown to be genetically distinct (Gosselin et al. 2015). Eggs from each male x female cross were incubated separately in McDonald tumbling jars within a 10.7-15.0°C temperature range. Fertilization success (%) was estimated five days post-fertilization (DPF) by determining the average percentage of eggs that had a visible notochord. A total of three egg samples (~100 eggs/sample) were collected from each incubation jar (six jars each for the two Birthday Rapids families and two jars each for the six Landing River families) to determine fertilization success (see Table 1).

Fish husbandry

Upon hatch, fish from each male x female cross were reared in separate 200 L flow-through raceways at the GRFH until 27 to 31 DPF. Length, body mass, energy density, and routine metabolic rate were quantified (see specific methods below) at or before 25 DPF on endogenous (i.e., fish feeding on their yolk reserves) fish at the GRFH. After traits were quantified, a total of 4800 yolk-sac larvae (600 per family) were

148 transported to the University of Manitoba's Animal Holding Facility where fish from
149 each family were distributed across three 10L tanks at rearing densities of 200 larvae per
150 tank (~1g of biomass·L^{-1}). Water temperature (16±1°C) and photoperiod (12h light:12h
151 dark) were maintained throughout the duration of the experiment. Exogenous (i.e., once
152 yolk-sac had been fully absorbed) fish were fed three times a day (7h, 14h, 21h) on *ad*
153 *libitum* rations of artemia (Artemia International LLC, Texas, USA) before being
154 gradually transitioned to a diet of bloodworm (Hikari USA, California, USA) where the
155 ratio of bloodworm:artemia began at 1:10 and fish were fully transitioned to a diet of
156 bloodworm within 10 days. At each feeding, water flow and aeration for each tank were
157 turned off for a minimum of 30 minutes prior to excess food being removed by siphon
158 and flow and aeration turned back on. All experimental procedures described below were
159 performed under the animal use protocol F15-007 approved by the University of
160 Manitoba's Protocol Management Review Committee under the guidelines of the
161 Canadian Council for Animal Care.
162
163 *Growth, energy density and survival*
164 Fifteen fish from each family (five fish from each rearing tank) were sampled for
165 body mass (g), length (mm), and energy density (J·g^{-1}) on six separate occasions to
166 capture ontogenetic shifts and family effects. During each sampling event (hatch, yolk
167 absorption, 50 DPF, 75 DPF, 110 DPF, and 146 DPF), fish were randomly selected from
168 each tank and placed in an overdose of anesthetic (MS-222, 200 mg·L^{-1}), patted dry,
169 measured and weighed before being placed in a drying oven at 60°C for 48h or until
170 constant dry mass had been achieved. Energy density was estimated using the dry to wet

mass ratio as an input into the linear regression equation developed by Yoon et al. (2019a). Mortalities were removed and recorded daily prior to feeding. Rearing densities were balanced across all tanks twice during the study (50 and 110 DPF) by removing excess individuals so that density did not become a confounding factor for treatments where survival remained high.

Respirometry

Whole-body oxygen consumption rates (MO_2) were measured using an intermittent flow respirometry system (Loligo Systems, Viborg, Denmark) to assess routine metabolic rate (RMR) at 25 DPF (yolk sac larvae), and standard and maximum metabolic rates (SMR and MMR, respectively) at 125 DPF. At each timepoint, eight fish per family treatment were used for respirometry trials following the protocols outlined by Yoon et al. (2021) with some modifications. Measurement cycles consisted of the following parameters: 360 s flushing, 60 s waiting, and 300 s measurement. Background microbial respiration was obtained by measuring oxygen consumption without fish for one measurement cycle in each respirometry chamber before and after each trial. Mass and length were measured on each fish following each respirometry trial. Black curtains surrounded the respirometry setup to avoid disturbance during trials. At 25 DPF, we chose to measure RMR as a proxy for SMR because we measured MO_2 for a 6-h period due to logistical constraints while MMR was not quantified. This 6-h measurement was necessary due to the fragility of the yolk-sac larvae as preliminary trials had shown that longer measurement periods and manual chasing to quantify MMR could lead to mortality of the larvae in the respirometer. At 125 DPF, following a 6-h acclimation

period, MO_2 was measured for a 16-h period to estimate SMR after which the fish were removed from the chambers and chased for 15 min using a plastic pipette to induce MMR. Fish were then returned within 60 s to the same respirometry chamber, and oxygen consumption was measured for three additional measurement cycles. Assuming a linear increase, background respiration throughout the trial was quantified by linearly interpolating the initial and final measurements and all MO_2 values were corrected by subtracting background respiration from the MO_2 measurements. Slopes of declining oxygen concentration with coefficients of determination (r^2) values above 0.9 were used for analysis. The q0.1 method (10% quantile; Chabot et al. 2016) was used to calculate SMR values. MMR was determined by choosing the highest oxygen consumption rate value among three measurements immediately following a 15-min standardized chasing protocol. Aerobic scope (AS) was calculated by subtracting SMR from MMR. Additional details on the respirometry setup can be found in Table S1 according to recommendations from Killen et al. (2021).

Thermal tolerance

A modified critical thermal maxima (CTM) challenge was used to assess temperature tolerance and provide information on the upper temperature threshold of individuals (Elliott and Elliott 1995). Fish from all families were assessed for thermal tolerance at 100 DPF following a protocol used by Yoon et al. (2019b). Six fish from each family treatment (two fish randomly selected from each tank; n = 3 tanks per family) were placed in individual containers (250 mL) with a screened mesh surrounding them allowing for water to move in and out of the container. Each container was placed

in a random order in a water bath where water temperature was controlled using a thermostat (Fisher, Massachusetts, USA). The water bath temperature was initially set at 16°C (i.e., acclimation temperature) and temperature was increased at a rate of 2°C·hour^{-1} following the protocol described in Deslauriers et al. (2016). Air stones were placed throughout the water bath to avoid oxygen depletion in any single container and ensure adequate mixing such that each chamber was heated and aerated at an equivalent rate. When the fish could not maintain equilibrium following the third attempt at straightening itself with gentle prodding, temperature to the nearest 0.01°C was measured inside the container using a probe equipped with a Witrox 4 Oxygen Meter (Loligo Systems, Viborg, Denmark). Fish were then removed from the experimental setup and allowed to recover for a 15 min period before being placed in an overdose of MS-222 after which length and body mass were recorded.

Volitional swimming test

Volitional swimming tests were carried out at 140 DPF following the methods described in Deslauriers et al. (2018*b*). Tests were performed using a vertical transparent acrylic cylinder that was 111 cm tall with an inner diameter of 12 cm. The cylinder contained a PVC elbow fitting at the bottom with a screw-on cap allowing for easy drainage of the water. Intervals of five cm were marked on the cylinder from the water surface to the PVC fitting to allow for the determination of where in the vertical tube the fish was at the end of each trial. Water height was a minimum of 100 cm above the PVC fitting at the base, and water temperature was maintained at 16°C.

239 Fish were sampled randomly from each family treatment (n = 8 per treatment; 2-3
240 fish per tank) and placed in meshed dip nets for one minute at the surface of the cylinder.
241 Following this short acclimation period, five drops of bloodworm extract were released
242 near the fish while the net was removed to induce a feeding behavior response where all
243 fish start swimming toward the bottom of the tube. The time (to nearest 1 s) it took each
244 fish to move from the water surface to the bottom of the cylinder was calculated. Once
245 the fish reached the bottom, the cylinder was drained of all water and rinsed thoroughly,
246 and we recorded the length and mass of the fish before starting a new trial with clean
247 water. For the analysis, we divided the length of the cylinder (100 cm) with the time it
248 took to reach the bottom and the length of the fish to report the swimming speed data as
249 body lengths per second ($BL \cdot s^{-1}$).
250
251 *Statistical analysis*
252 Our main analysis was intended to demonstrate how family effects can explain
253 phenotypic responses and thus a multivariate statistical approach was employed using all
254 performance metrics. We performed a non-metric multidimensional scaling analysis
255 (NMDS) using the vegan package (Oksanen et al. 2019) in R (version 3.5.1). This
256 analysis allowed us to infer how dissimilar families were to each other and how strong
257 each phenotype was associated with a given family. Specifically, we used a permutation
258 test ("envfit" function with 999 permutations) to test the significance individual female
259 and male combinations had on shaping the phenotypic responses. We did this by creating
260 a loop function systematically removing one of the 8 traits (Table 2) at a time and re-
261 running the permutation test while recording the ensuing goodness of fit parameters (r^2

and p-values). Because some metrics such as length, mass, and energy density were not always quantified on the same day for each family, we developed locally estimated scatterplot smoothing (LOESS) interpolations (Hedger et al. 2008) to estimate these metrics at the same time period. This approach was intended to capture the impact of the temporal resolution; hence 25, 50, 75, 100, and 125 DPF. Similarly, we used mortality rates at 50, 75, 100, 125, and 150 DPF to capture the impact of the temporal resolution on this metric. Because we adjusted fish density throughout the experiment, survival was calculated as:

$$S_i = S_{i-1} - \left({}^{M_i}\!/_{D_{init}} \cdot S_{i-1} \right) \quad (1)$$

where S represents survival in %, M is for the number of dead fish observed on day i, and D_{init} represents the number of fish found in each tank once density had been adjusted. All tanks started with 200 fish, and were adjusted to 80 fish on day 27, and 20 fish on day 57.

Finally, SMR, MMR, AS, thermal tolerance, and swimming performance data were all analyzed using a one-way ANOVA with Tukeys post-hoc test. All analyses were performed using the R statistical software (v. 4.1.1, R Core Team 2021).

Results

Family effects were detected early on in ontogeny and persisted throughout the experimental period. Families that demonstrated lower energy density at the egg (5-10 DPF) and feed transition (endogenous vs. exogenous feeding; 50-100 DPF) stages exhibited slower growth rates (Fig. 1) and lower survival rates (Fig. 2 and Fig. S1). Fish from the two Birthday Rapids families had slower RMR (mg $O_2 \cdot kg^{-1} \cdot h^{-1}$) at 25 DPF while SMR for these families were higher at 150 DPF (Fig. 3). The opposite trend was found

for the Landing River families; however, this did not lead to observable differences with regards to MMR and AS (Fig. 4). Similarly, CTM did not demonstrate a family effect, suggesting a highly conserved mechanism to cope with acute thermal stress (Fig. 5; $P = 0.454$). Lastly, two out of the three Landing River families generated with female #2 (LR1B and LR3B) produced the highest volitional swimming speeds (Fig. 6; $P < 0.001$). Because mortality rates were higher for the LR3A family, fish were not available for length, mass, energy density, metabolic rate measurements near the end of the study. To prevent our models from generating errors (NMDS) for the LR3A family for each one of these six metrics, we attributed values to this family based on mean values from the two other Landing River families that were generated with female #1.

Combining the suite of traits into the NMDS analysis demonstrated that families generated from the same population (Landing River or Birthday Rapids) were clustered closer together while the individual female explained most of the clustering within a population (Fig. 7). It was found that the female effect was highly significant ($r^2 = 0.92$; $P = 0.004$) compared to the male effect ($r^2 = 0.44$; $P = 0.417$) in shaping the response of the metabolic traits. Length, energy density, and CTM parameters were associated more closely with the Landing River families generated from female #1 while Landing River families generated from female #2 were more closely associated with higher body mass at 25 and 50 DPF and survival parameters at 100 and 125 DPF. The Birthday Rapids families were both associated with higher SMR, MMR, and AS. Interestingly, fertilization success, swimming performance, and RMR at 25 DPF were not closely associated with any of the families. When looking at the influence of each metabolic traits separately (n = 8 separate traits; Fig. 8), fit parameters from the permutation tests

ranged between 0.90-1.00 and 0.37-0.55 for r^2 while P values ranged between 0.002-0.007 and 0.357-0.492 for females and males respectively. The removal of any one trait in the analysis impacted the fit of the NMDS analysis by a maximum of 7% for females and 11% for males. Females and males responded similarly towards removing length and energy density but opposite trends were observed for the influence of mass, survival, metabolic rate, CTM, swimming performance, and fertilization success. Lastly, the mass and fertilization success (reduced fit when removed) along with survival (improved fit when removed) parameters were the most influential metrics (>5% fit change) explaining model fit for males while only the swimming performance metric, when removed from the analysis, improved the model fit for females.

Discussion

While both environmental conditions and parental influences have been shown to affect aspects of early ontogeny in lake sturgeon (Duong et al. 2011; Dammerman et al. 2015), our work has demonstrated that families are highly variable in their metabolic responses where results are generally driven by the female used as broodstock. Although we acknowledge that our breeding scheme did not replicate every female with every male (and vice versa) for both the Landing River and Birthday Rapids populations, results do point to a strong maternal influence in shaping the physiological response during early life stages of lake sturgeon. These results are consistent with findings of another lake sturgeon study where yolk sac reserves of hatched larvae were under a strong maternal influence (Hastings et al. 2013). These findings are important to consider, especially in the context of conservation, as the outcome of premature release into the wild at an early

life stage can have repercussions on the drift distances (i.e., the distance required before larvae settle following yolk-sac absorption) and limit access to suitable nursery habitat. Furthermore, this genetic-dependent effect can also lead to long-term consequences for the recovery of the species (Araki et al. 2008) assuming that a very small proportion of individuals from an already small genetic pool will survive and potentially reproduce (i.e., selection effect). Simulation work on lake sturgeon breeding designs has shown that genetic diversity (i.e., allele retention, inbreeding) is directly proportional to the number of adults contributing to the progeny (Schueller and Hayes 2011). While it has been shown here and elsewhere that female influences early on in ontogeny is one of the main drivers (Van Leeuwen et al. 2016), fertilization success can be mostly attributed to males in this study. This would suggest that differences in either milt quality or egg and sperm compatibility is also to be considered when developing breeding designs (Butts et al. 2007).

The optimization of traditional metrics quantified in hatchery settings such as length, body mass, and survival have often been assumed to lead to higher survival rates and increased fitness upon fish release. However, traits favored under artificial rearing regimes can be maladaptive in the wild and in fact decrease overall fitness (Ford 2002; Thompson et al. 2018). This can be caused by a lack of complexity in environmental variables that are found in the wild. Specifically, artificial fertilization often uses limited numbers of female and male fish that may lead to reduced phenotypic variation (Crossman et al. 2014; Dammerman et al. 2015). In our study, energy density, which is often considered as a condition index, was shown to be positively correlated with growth but negatively correlated with survival, indicative of a potential trade-off between

somatic growth and energy storage during early life. While rapid growth is important early on in ontogeny to avoid predation, the accumulation of energy reserves before the first over-wintering period is a crucial physiological adaptation that has direct implications for survival (Post and Parkinson 2001; Deslauriers et al. 2018*a*).

Interestingly, removing energy density as a metabolic trait in the permutation test to explain male and female variability leads to a better model fit for both sexes. This result is indicative of energy assimilation response not being uniform across families and populations and potentially explaining why some families tend to do better than others under given environmental conditions. This result also contrasts from a physiological performance trait such as critical thermal maxima, which was found to be well conserved within lake sturgeon in this study and in previous research that has focused on other sturgeon species (Ziegeweid et al. 2008; Zhang & Kieffer 2014; Deslauriers et al. 2016; Yoon et al. 2019*b*). This thermal tolerance is contrary to the family response observed for metabolic rates, which have been shown in this study to have a strong genetic basis. This may suggest that energy density and SMR could be intrinsically linked and a potential trade-off can exist between them as higher metabolic costs for basal maintenance (SMR) can lead to decreased energy densities when food source is limited. One potential explanation for this could be that energy content is regulated at the individual level while SMR varies as a function of family (Burton et al. 2011). Studies attempting to quantify the metabolic rates of egg and larval stages of brown trout (*Salmo trutta* Linnaeus, 1758) found that not only significant inter-family differences occurred but that the intra-family variance was linked to maternal investment in the eggs (Régnier et al. 2010, 2012). While some metabolic traits have been shown to have a strong genetic basis, care must be taken

when extrapolating these results once the fish are released as SMR becomes reduced under captive settings in salmonids (Van Leeuwen et al. 2011). The same result does not always hold true for swimming performance, where the net energetic costs have been shown to be similar between wild and farmed Atlantic Salmon (*Salmo salar* Linnaeus, 1758; Enders et al. 2004), supporting the idea that the results shown here might translate to a similar performance once the fish are released into the wild.

In summary, we have shown in this study that metabolic traits could be strongly influenced by maternal differences during early life stages in age-0 lake sturgeon. The implications of this work are significant as family effects are still not well understood for conservation stocking programs. As the environmental conditions experienced by females prior to spawning has also been shown to affect offspring phenotypes (Gagliano and McCormick 2007; Sopinka et al. 2016), subsequent work should focus on quantifying reliable indicators of physiological condition (e.g., sperm cell viability and egg energy density prior to fertilization; fish energy density prior to stocking) to aid in the design of an improved stocking strategy that accounts for genetic diversity and enhances post-release survival rates. Furthermore, metrics quantified on energy density, SMR, and CTM will serve as the starting point for the development of a bioenergetics model for age-0 lake sturgeon, which would be useful to understand optimal rearing conditions as well as to predict population energetic demands once stocked in their natural habitat.

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Competing interests

The authors declare there are no competing interests.

Author contribution

Conceptualization: DD, GY, KM, CK, WGA

Formal analysis: DD, GY, KM

Funding acquisition: WGA

Investigation: DD, GY

Project administration: CK, WGA

Resources: CK, WGA

Writing-original draft: DD

Writing-review & editing: DD, GY, KM, CK, WGA

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Data availability

Data files and scripts used for this study are publicly available from the Université du Québec à Rimouski's Dataverse Collection within the Borealis Repository (https://doi.org/10.5683/SP3/I01IG6).

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Figure captions

Figure 1: Growth (length [L] and mass [M]; top and middle panel) and condition (energy density [ED]; bottom panel) metrics for 8 lake sturgeon (*Acipenser fulvescens*; *n* = 8 per family within 3 replicate tanks) families spanning 146 days following egg fertilization. Length and mass were only measured on hatched larvae while energy density was also measured on fertilized eggs.

Figure 2: Survival (%) for 8 lake sturgeon (*Acipenser fulvescens*; *n* = 3 tanks per family) families relative to the start of the growth trial, which occurred at 36 and 40 DPF for the Birthday Rapids (BR) and Landing River (LR) populations, respectively. Survival only started being quantified once the fish were brought back to the University of Manitoba's Animal Holding Facility. Points on the plot only occur when the mortality of at least one individual within a tank was observed on any given day.

Figure 3: Routine and standard metabolic rate (RMR/SMR at 25 and 150 days post-fertilization, respectively) of 8 lake sturgeon (*Acipenser fulvescens*) families (*n* = 8 per family) from the Birthday Rapids (BR) and Landing River (LR) populations over time at 16±1°C. An insufficient number of fish were left at the end of the experiment for the Landing River 3A family to allow for a second SMR trial.

Figure 4: Metabolic rate (MR, 16±1°C) as it relates to the difference between maximum metabolic rate (MMR) and standard metabolic rate (SMR) to give the aerobic scope (AS) for 7 lake sturgeon (*Acipenser fulvescens*) families (*n* = 8 per family). The Landing River 3A family is not depicted here due to the lack of individuals (i.e., high mortality rates) near the end of the experiment when metabolic rates were quantified.

Figure 5: Critical thermal maxima for 8 lake sturgeon (*Acipenser fulvescens*) families from the Birthday Rapids (BR) and Landing River (LR) populations.

Figure 6: Relative volitional swimming speed for 7 lake sturgeon (*Acipenser fulvescens*) families from the Birthday Rapids (BR) and Landing River (LR) populations. The Landing River 3A family is not depicted here due to the lack of individuals (i.e., high mortality rates) near the end of the experiment when swimming trials were conducted.

Figure 7: NMDS plot indicating relative distances between lake sturgeon (*Acipenser fulvescens*) families and the physiological traits that were quantified over time. Different symbol shape represents females while different symbol colours represent males. The closer families are together on the plot, the more similar the traits quantified from the offspring during the different experiments. Similarly, the closer the traits are to a given family, the more that family was associated with that trait (i.e., lower or higher response compared to the other families). Acronyms stand for L: Length, M: Mass, ED: Energy density, S: Survival, R/S/MMR: Routine/Standard/Maximum Metabolic rate, AS: Aerobic scope, CTM: Critical thermal maxima, Swim: Volitional swimming speed, and Fert:

Fertilization success. The number following the trait represents the number of days post-fertilization when the trait was quantified. Same traits quantified at different times are displayed using the same colour.

Figure 8: Influence of removing 8 different physiological traits on the overall multivariate model fit for both female and male lake sturgeon (*Acipenser fulvescens*) used in the analysis. Most traits include more than one temporal data point as seen in Table 2. A positive value indicates that the model fit is improved (i.e., a larger proportion of the variability is explained) when including a given trait in the analysis. Acronyms stand for ED: Energy density, MR: Metabolic rate, CTM: Critical thermal maxima, and Fert: Fertilization success.

Figure 1

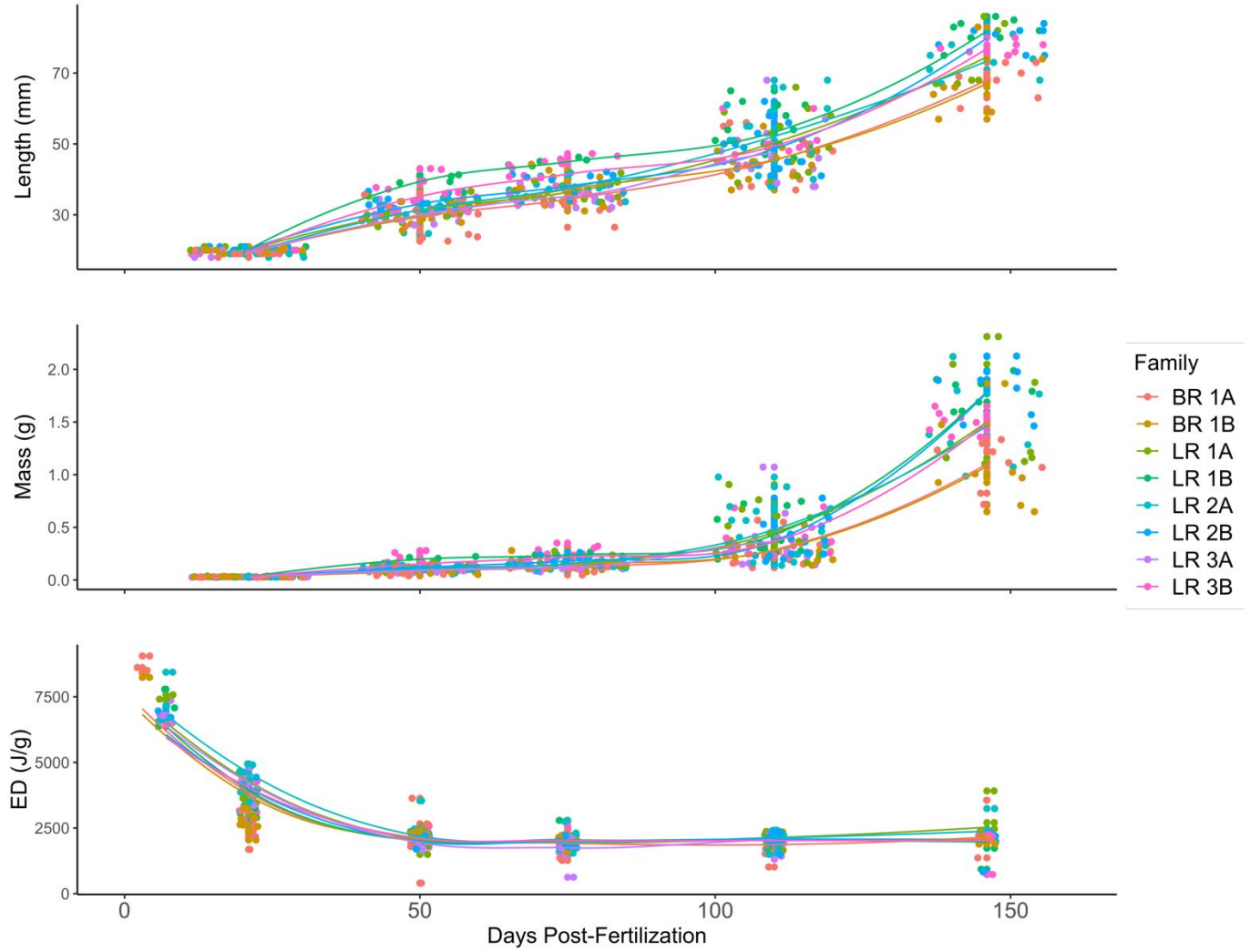
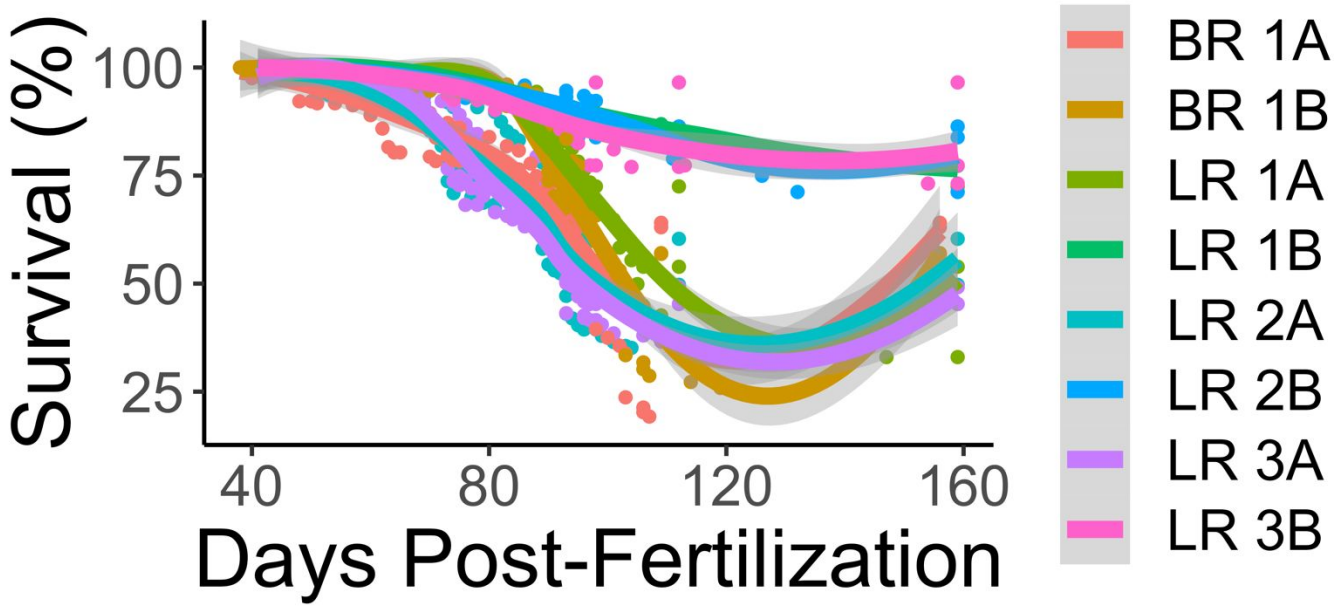


Figure 2



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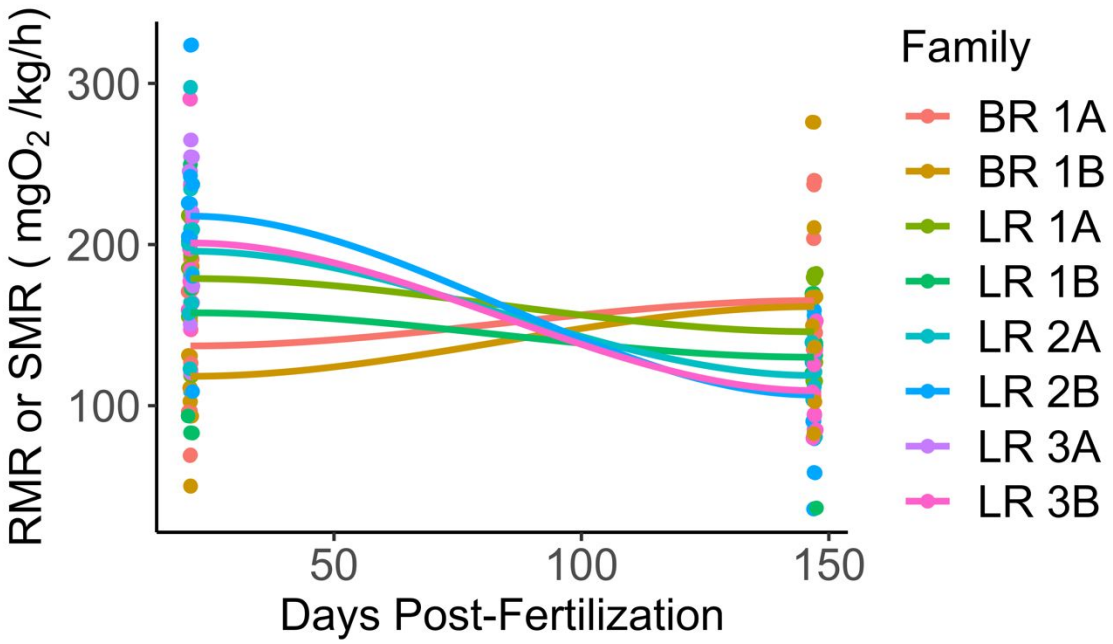

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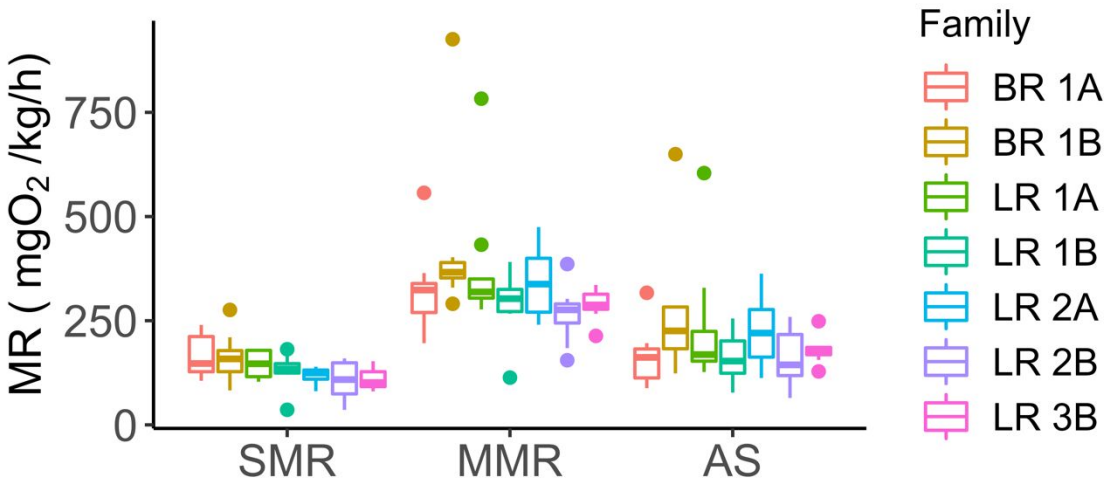
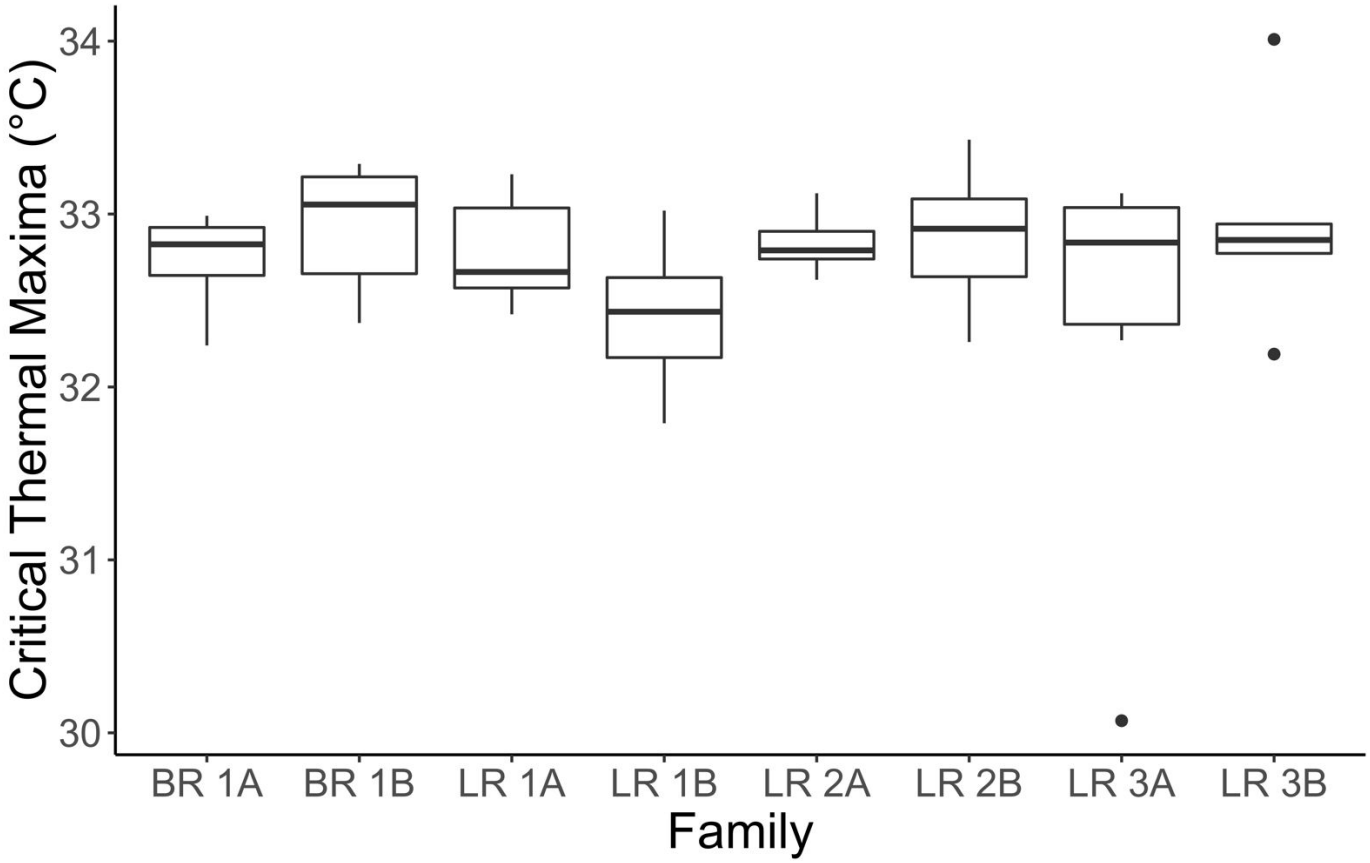


Figure 5



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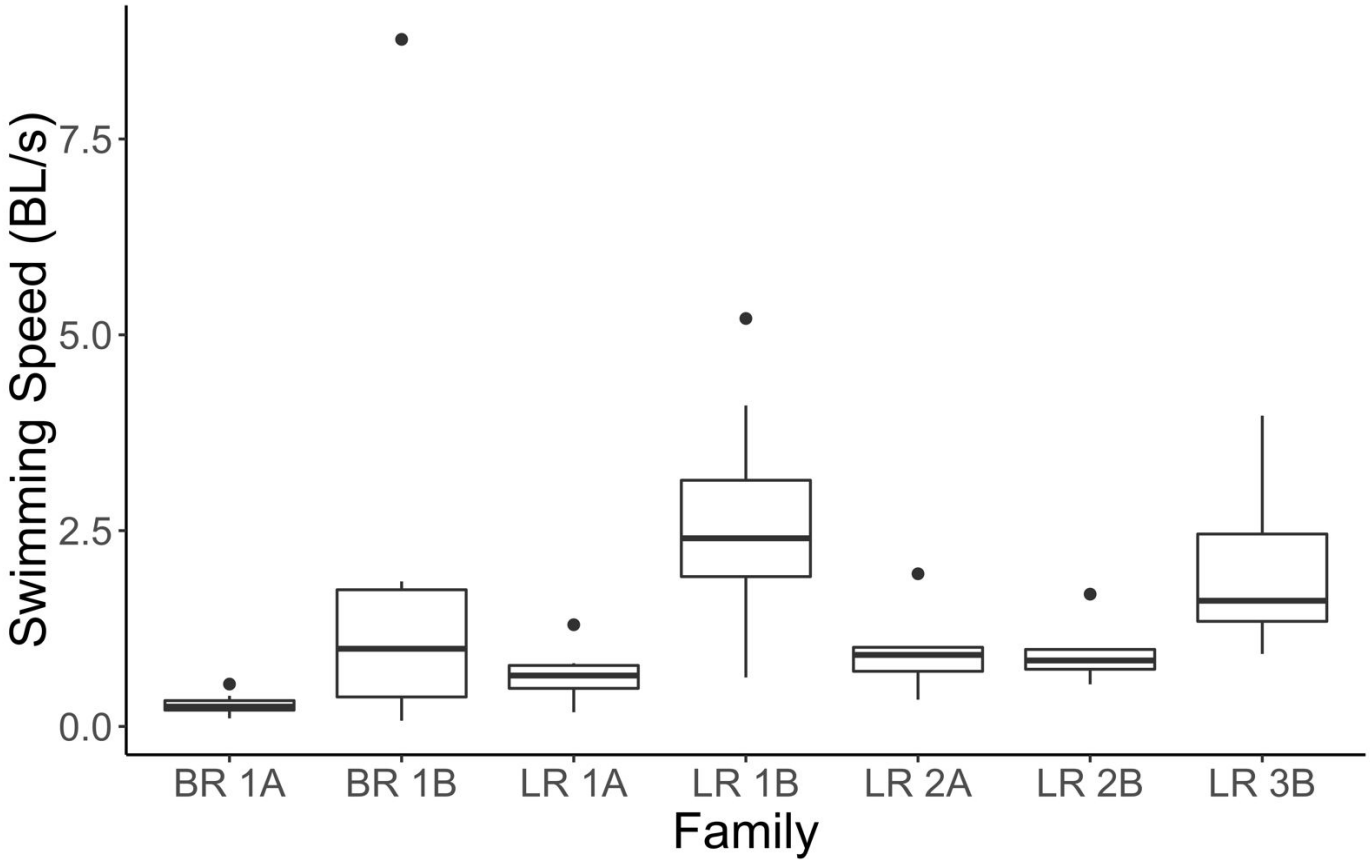


Figure 7

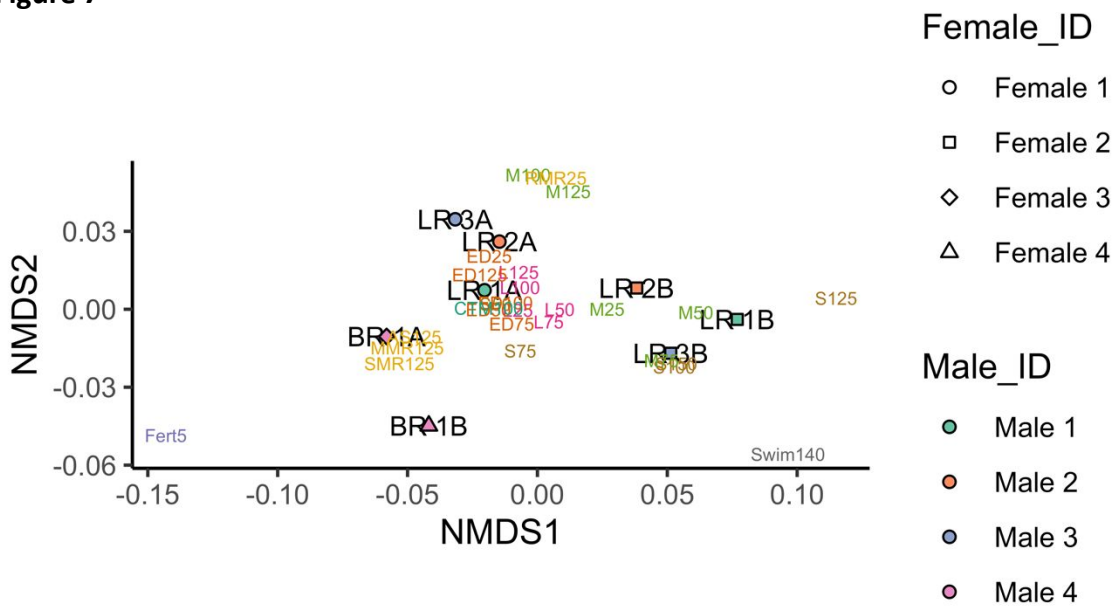
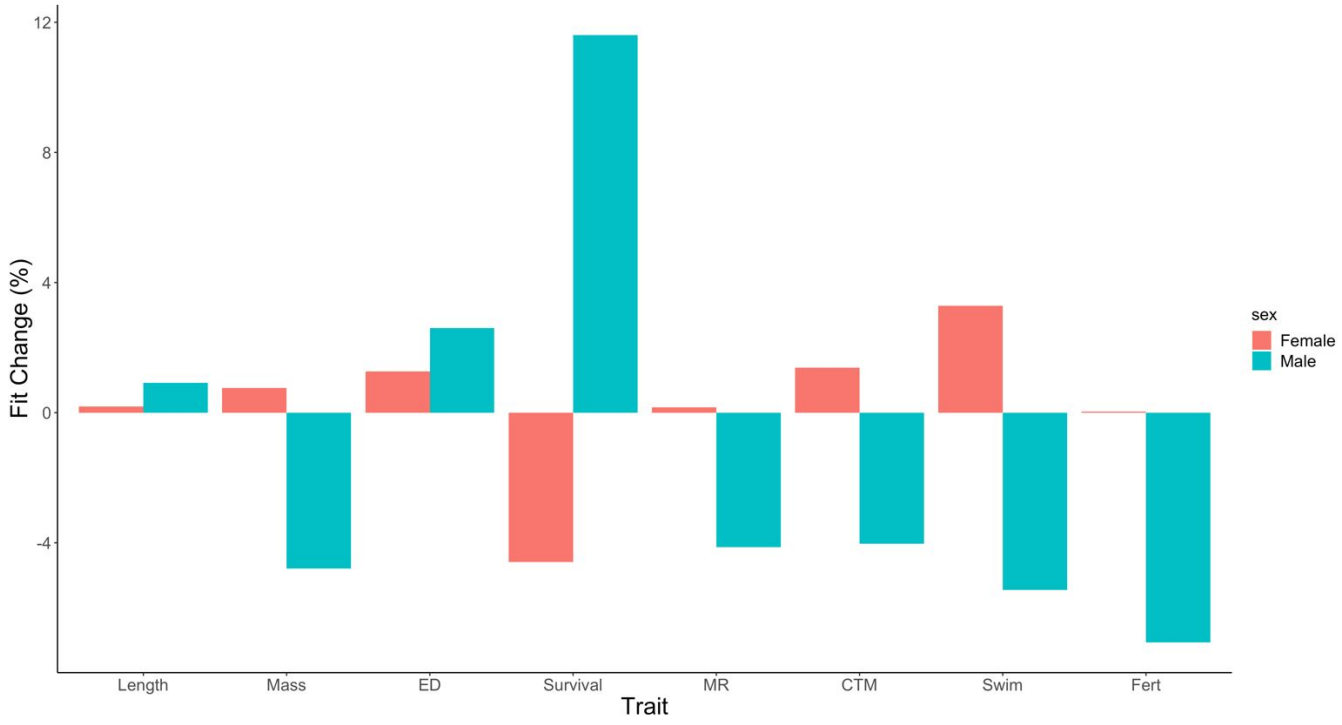


Figure 8



Tables

Table 1. Spawning dates and broodstock characteristics that were used to produce lake sturgeon (*Acipenser fulvescens*) for the metabolic phenotype experiments. Males and females originated from the Upper (Landing River) and Lower (Birthday Rapids) Nelson River in the province of Manitoba, Canada.

Origin	Family	Female ID	Female mass (kg)	Male ID	Male mass (kg)	Fertilization date	Fertilization success (%)
Landing River	1A	Female 1	28.1	Male 1	10.0	May 27th 2016	53.9
	2A	Female 1	28.1	Male 2	11.8	May 27th 2016	48.8
	3A	Female 1	28.1	Male 3	9.5	May 27th 2016	51.3
	1B	Female 2	28.1	Male 1	10.0	May 27th 2016	30.8
	2B	Female 2	28.1	Male 2	11.8	May 27th 2016	34.1
	3B	Female 2	28.1	Male 3	9.5	May 27th 2016	19.3
Birthday Rapids	1A	Female 3	19.5	Male 4	10.0	May 31st 2016	83.0
	1B	Female 4	29.0	Male 4	10.0	May 31st 2016	92.7

Table 2. Description of the different lake sturgeon (*Acipenser fulvescens*) physiological traits (n = 8) and their respective units that were quantified during this experiment.

Phenotype	Acronym	Definition
Length	L25	Length (mm) at 25 days post-fertilization
	L50	Length (mm) at 50 days post-fertilization
	L75	Length (mm) at 75 days post-fertilization
	L100	Length (mm) at 100 days post-fertilization
	L125	Length (mm) at 125 days post-fertilization
Mass	M25	Mass (g) at 25 days post-fertilization
	M50	Mass (g) at 50 days post-fertilization
	M75	Mass (g) at 75 days post-fertilization
	M100	Mass (g) at 100 days post-fertilization
	M125	Mass (g) at 125 days post-fertilization
Energy density	ED25	Energy density (J/g) at 25 days post-fertilization
	ED50	Energy density (J/g) at 50 days post-fertilization
	ED75	Energy density (J/g) at 75 days post-fertilization
	ED100	Energy density (J/g) at 100 days post-fertilization
	ED125	Energy density (J/g) at 125 days post-fertilization
Survival	S50	Survival (%) at 50 days post-fertilization
	S75	Survival (%) at 75 days post-fertilization
	S100	Survival (%) at 100 days post-fertilization
	S125	Survival (%) at 125 days post-fertilization
	S150	Survival (%) at 150 days post-fertilization
Metabolic rate	RMR25	Routine metabolic rate (mg O_2/kg/h) at 25 days post-fertilization
	SMR125	Standard metabolic rate (mg O2/kg/h) at 125 days post-fertilization
	MMR125	Maximum metabolic rate (mg O2/kg/h) at 125 days post-fertilization
	AS125	Aerobic scope (mg O2/kg/h) at 125 days post-fertilization
Critical thermal maxima	CTM100	Critical thermal maxima (°C) at 100 days post-fertilization
Swimming speed	Swim140	Swimming (BL/s) at 140 days post-fertilization
Fertilization success	Fert5	Fertilization success (%) at 5 days post-fertilization