

# Over-wintering physiology of age-0 lake sturgeon (*Acipenser fulvescens*) and its implications for conservation stocking programs

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**Abstract** Lake sturgeon (*Acipenser fulvescens*) occupy some of the most northerly distributions of any sturgeon species and experience extended overwintering periods when resources may be limited. Conservation stocking is currently used as a management tool to enhance lake sturgeon populations that are at risk or endangered. One of the most limiting components of the conservation effort is our understanding of energy requirements that allow age-0 lake sturgeon to survive their first winter. In this study, age-0 fish (mean mass  $5.6 \text{ g} \pm 0.5 \text{ S.E.}$ ; mean total length  $12.7 \text{ cm} \pm 0.4 \text{ S.E.}$ ) were held in groups of 12 individuals (10 total groups) and starved for a period of four weeks while being held at  $1 \pm 1 \text{ }^\circ\text{C}$ . This setting was intended to simulate winter conditions that occur in the Winnipeg River, MB, Canada. Post-winter fish condition and physiology were compared to pre-winter fish using survival, energy density, metabolic rate, glucose, triglyceride, protein, and cortisol production as metrics. While mortality was high (42%) during the experiment,

results indicated that fish with total water content below 90% and energy density above 2000 J/g were more likely to survive. Whole body triglyceride, plasma triglyceride, plasma glucose levels, and standard metabolic rates were also found to significantly decline over time while whole body cortisol concentration increased. Understanding these thresholds will help in future refinements of rearing conditions, which look to improve the survival of age-0 lake sturgeon released into the wild pre-winter.

**Keywords** Sturgeon · Overwintering · Physiology · Conservation · Age-0 · Survival

## Introduction

A number of factors influence year-class strength among fish species including the number of participating spawners, environmental conditions during egg incubation, summer growing conditions and winter severity (Hurst and Conover 1998; Shaw et al. 2012). Among north-temperate fish populations, considerable efforts have focused on overwinter survival and on the effect that size has on the ability of individuals to endure periods of low water temperature and reduced feeding opportunities (Post and Parkinson 2001). Beamish and Mahnken (2001) proposed the ‘critical size and period hypothesis’, which states that larger individuals have a greater chance of surviving to spring than smaller conspecifics and size-selective overwintering mortality studies within a limited number of fish populations

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have confirmed the hypothesis that bigger is better (Henderson et al. 1988; Hurst and Conover 1998; Biro et al. 2004; Sutton and Ney 2001; Pangle et al. 2004).

These outcomes are primarily attributed to smaller individuals exhausting energy reserves faster than larger individuals due to the combined effect of reduced capacity for energy storage and higher mass-specific basal metabolic rate (Xiaojun and Ruyung 1990; Cargnelli and Gross 1997; Biro et al. 2004). Starvation is one of several mechanisms, which include thermal stress, predation, and pathogens, that can cause winter mortality and which tend to select against the smallest members of a cohort (reviewed in Hurst 2007). However, some studies have reported an advantage for the smaller individuals of a cohort under unusually warm winter conditions and reduced food availability (Schultz and Conover 1999; Connolly and Petersen 2003), as smaller individuals require lower resource levels than larger individuals to sustain themselves (Werner 1994). Despite the seemingly disproportionate attention that body size receives in overwintering studies, it is more likely that survival depends on a number of interacting factors including temperature, food, predation, latitude (Connolly and Petersen 2003; Hurst 2007) and physiological state.

Ectotherms have a number of physiological and behavioral adaptations for coping with low water temperatures and/or starvation. Fish often enter into a torpor-like state (i.e., dormancy) where activity, metabolic rates, and subsequent energy expenditure are dramatically reduced (Lemons and Crawshaw 1985; McCue 2010). When feeding activity ceases, individuals mobilize endogenous reserves through three distinct phases. This initially includes the acute mobilization of glycogen and fat/protein reserves (mobilization source can vary by species during the initial phase), followed by a longer phase where lipids are mobilized until reaching a critical threshold, and ending with the final phase where protein is the main source of energy (Bar and Radde 2009; Bar 2014). This last phase has also been associated with increases in circulating levels of cortisol in many taxa (Cherel et al. 1988; Groscolas and Robin 2001) including fish (Dave et al. 1975). While this pattern has generally been accepted for teleost fish (Bar 2014), it has not yet been determined for primitive fish like the threatened Acipenseridae species (i.e., sturgeons).

Sturgeon species are restricted to the temperate waters of the Northern Hemisphere (Billard and Lecointre

2001; Auer 2004) and lake sturgeon (*Acipenser fulvescens*) together with Siberian sturgeon (*A. baerii*) inhabit the most northerly distribution and coldest waters of all sturgeon species (Artyuklin 1995). Lake sturgeon numbers are currently below historic levels and most populations are listed as rare or threatened (Peterson et al. 2003). Increasing population numbers depends on the recruitment of strong year-classes, which can be influenced by the ability of young-of-the-year (YOY) to survive their first winter (Post and Evans 1989; Hurst and Conover 1998).

Conservation stocking programs have been developed as a means to supplement lake sturgeon populations in many areas of the species' native range including the province of Manitoba, Canada (McDougall et al. 2014a). However, due to economic (i.e., cost of food, heat) and spatial constraints (i.e., sturgeon outgrowing rearing tanks), it is often necessary to release groups of age-0 fish in the fall of their first year when they are believed to be most vulnerable to overwintering conditions (Brown and Day 2002; Pikitch et al. 2005). Age at stocking, and hence size, has been shown to be positively correlated with recapture rates of lake sturgeon in the Upper Black River, Michigan, United States (Crossman et al. 2011). Crossman et al. (2009) also found that lake sturgeon that reached at least 100 g (6 months post-hatch) prior to being stocked had a 40% minimum overwintering survival rate. Similar to this, lake sturgeon conservation efforts in Manitoba have found that fish stocked as age-1 were 130 times more likely to recruit to the population than fish stocked as age-0 (McDougall et al. 2014a). To date, research has focused on the performance of sturgeon in response to low temperatures (Kappenman et al. 2009) and following periods of starvation (Gillis and Ballantyne 1996). However, a mechanistic understanding of how these two factors interact and influence sturgeon physiology and survival over winter is lacking. The main objective of the current work was to describe the physiological responses of age-0 lake sturgeon when challenged with reduced water temperatures (i.e., 1 °C) and feedings. Lake sturgeon overwinter in the Winnipeg river in water temperatures approaching 0 °C in Manitoba (McDougall et al. 2014a). We also aimed to determine physiological thresholds that can act as predictors of over-winter mortality during a period of prolonged starvation and cold water conditions in order to provide information that will lead to better stocking practices of lake sturgeon throughout their native range.

## Methods

### Fish husbandry

Fish used in the experiments came from two different sources produced in 2015 and 2016. Fish used in 2015 were the progeny of lake sturgeon broodstock originating from the Winnipeg River while those used in 2016 originated from Nelson River broodstock; both rivers are found in the Hudson's Bay watershed in the province of Manitoba, Canada. In 2015, eggs and sperm were brought back to the Animal Holding Facility at the University of Manitoba and fertilization occurred in June 2015. In 2016, eggs were fertilized in the field and brought back to Manitoba Hydro's Grand Rapids Fish Hatchery (GRFH) where they were incubated until hatch, which occurred in early June. In both years, de-adhersed eggs were incubated in McDonald jars at 12 °C. At 18 days post-hatch, approximately 10,000 larvae were brought from the GRFH to the Animal Holding Facility at the University of Manitoba. During both years larval fish were raised at 15 °C until the beginning of experimentation (December in 2015 and November in 2016). Fish produced in 2016 were used in the respirometry and cortisol experiments only (see below). Tanks were supplied with flow-through dechlorinated tap water and mortalities were removed daily. A few days prior to full yolk absorption, fish were provided with live artemia and were gradually transitioned to chironomidae larvae once they reached 14 days post yolk absorption.

### 2015 Cohort. Energy density, protein, triglyceride and glucose measurements

Using the 2015 cohort, 120 fish at 134 d post-fertilization (dpf) were randomly selected from a 60 L holding tank and equally distributed across ten aquaria (10 L) two weeks prior to the experimentation. Water temperature was kept at  $15 \pm 1$  °C during the 2-week acclimation period by controlling the room temperature. Light followed the cycle found at Pointe du Bois, Manitoba ( $50^{\circ}18'03''\text{N}$   $95^{\circ}33'05''\text{W}$ ). Fish were fed ad libitum rations of chironomidae larvae once a day during this time. During this period 50% of the water volume was exchanged every other day. Following this period, water temperature (i.e., room temperature) was decreased at a rate of 1 °C/day until it reached 1 °C. At the end of this 14 d period fish were deprived of food for 28 d to simulate

starvation. Ammonia (monitored every other day) and dissolved oxygen (monitored daily) levels were always observed below 0.01 mg/L and above 90%, respectively.

Sampling occurred once at the end of the acclimation period (day 0), once when the temperature reached 1 °C (day 14), and then on days 28 and 42 (Fig. 1, upper panel). At the time of sampling, one fish per tank ( $n = 10$  total) was processed at a time. The fish were euthanized in MS-222 (200 mg/L) before being patted dry, weighed (0.001 g) and measured (1 mm). The tail was then cut off behind the anal fin and blood was collected from the caudal vein using ammonium-heparin micro-hematocrit tube. The blood was then transferred to a 1.5 mL tube and centrifuged for 4 min at 10000 rpm. Resulting plasma was collected using a Pasteur pipette and placed in a new 1.5 mL centrifuge tube, before being transferred to a  $-80$  °C freezer. The remainder of the fish was weighed again and dried at 60 °C until constant dry mass was obtained ( $\geq 48$  h). Dried carcasses were weighed and homogenized for whole body analyses (see below). Fish that died during the course of the experiment went through the same sampling procedures. However, plasma from these fish was not used for analysis because of potential degradation of macronutrients associated with the mortality.

### Energy density

Whole-body energy density was quantified using previously established relationships (Yoon 2017,  $r^2 = 0.96$ ) allowing for the conversion of fish dry to wet mass ratio to energy density. Thus, the following equation was used:

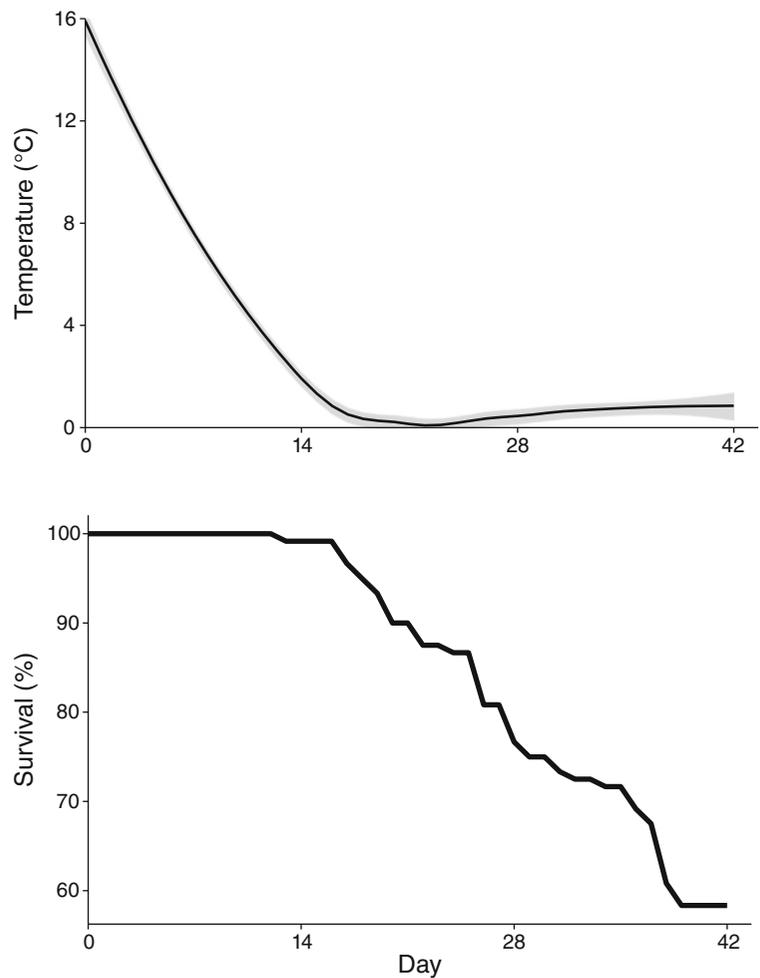
$$ED = -433.1 + 23965.6 \cdot DWR \quad (1)$$

where *ED* is energy density (in J/g of wet mass), and *DWR* is the dry to wet mass ratio.

### Biochemical assays

Dried carcasses were used for whole body protein and triglyceride analyses. Samples were homogenized using a TissueLyser II (QIAGEN, Cat No./ID: 85,300) set at 30 RPS for 10 min and by using a 1:14 ratio (i.e., 0.01 g of tissue in 0.14 mL of assay buffer) of dried sample to a 50 mM Tris buffer solution (pH 7.4). The samples were then transferred to a 1.5 mL tube and centrifuged for 10 min at 10000 RPM. The supernatant was then used for whole body protein and triglyceride assays.

**Fig. 1** Average temperature profile throughout the study period (upper panel) and percentage (%) of fish that survived over time (lower panel) for the 2015 Cohort. The onset of starvation occurred on day 14. Fish were sampled on days 0, 14, 28, and 42. The 2016 Cohort experienced a similar temperature regime except for a 3-week period between the end of the acclimation period and the beginning of the starvation period when fish were fed. A single fish died during the whole experiment with the 2016 Cohort



### Protein

Total protein concentrations were determined using the Pierce Coomassie (Bradford method) Protein Assay Kit (Thermo Fischer; Catalog No. 23200). Standard curves were generated using varying concentrations of BSA combined with 150  $\mu$ L of 50 mM Tris buffer solution. The supernatant was then diluted 299X with the Tris solution for the absorbance readings to fall within the range provided by the standard curve. Samples and standards were placed in triplicate into 96-well microplates and absorbance was quantified at 595 nm and 37 °C. Absorbance values were determined using a PowerWave HT Microplate Spectrophotometer (BioTek; Model No. XS2) and Gen5 (v. 1.11) software.

### Triglyceride

Whole body triglyceride levels were obtained using the GPO DAOS method (LabAssay Triglyceride by Wako, Code No. 290–63,701). Triglyceride samples were not diluted further from the supernatant. All standards and samples were placed in a 96-well microplate where absorbance values were taken in triplicate at 505 nm and 37 °C using the same instrumentation and software as described above.

### Plasma analysis

Plasma samples for protein and triglyceride were quantified using the same methods as for the whole body analyses. Plasma glucose concentrations were determined using the Mutarose-GOD method (LabAssay

Glucose by Wako, Code No. 298–65,701). Plasma glucose samples were not diluted further from the supernatant. All standards and samples were placed in a 96-well microplate where absorbance values were measured in triplicate at 505 nm and 37 °C.

### 2016 Cohort. Respirometry and whole body cortisol measurements

Using the 2016 cohort, 150 fish at 158 dpf were randomly selected from a 60 L holding tank and transferred to another 60 L tank located inside a temperature-controlled environmental chamber. The tank was fitted with a filtration pump and water temperature was controlled by the room temperature, which was kept at  $15 \pm 1$  °C during a 2-week acclimation period. Fish were fed once a day during this time. Following this period, water temperature (i.e., room temperature) was decreased at a rate of 1 °C/day until it reached 1 °C following the procedure used with the 2015 cohort. Fish were also fed once daily during this period. Fish were maintained at this temperature for an additional 45 d while being fed small food rations (< 1 g) every three days to simulate the unproductive winter season. This additional period differed from that with the 2015 cohort and was intended to minimize over-winter mortality (i.e., improve fish condition ahead of the overwintering period). At the end of the 45 d period fish were deprived of food for 28 d to simulate starvation as described for the 2015 Cohort.

#### Respirometry

At the onset of the starvation period, eight fish were removed from the tank for respirometry trials following the protocol outlined by Yoon (2017). The same trials were conducted with eight different fish at the end of the starvation period 28 d later. Standard metabolic rate (SMR) was assessed as whole body oxygen consumption rates ( $MO_2$ ) using intermittent flow respirometry (Loligo Systems, Viborg, Denmark) with the following parameters: 360 s flushing, 60 s waiting, and 300 s measurement. A pre-experiment calibration value quantifying biological oxygen demand (BOD) was obtained by measuring oxygen consumption without fish for fifteen minutes in each respirometry chamber (30 mm diameter; 90 mm length; 45 mL effective volume). Mass and length were measured on each fish prior to placing

them into the resting chambers. Black curtains surrounded the respirometry setup to avoid disturbance during trials. SMR was measured for a 24-h period after which the fish were removed from the chambers and chased for 15 min using a plastic pipette to induce forced maximum metabolic rate (FMR). Fish were then returned immediately to the same respirometry chamber, and oxygen consumption was measured for three additional measurement cycles. Oxygen consumption was measured one last time for fifteen minutes without fish to determine post-experiment BOD. BOD throughout the trial was quantified by linearly interpolating the initial and final BOD measurements. All  $MO_2$  values were corrected by accounting for BOD.

Slopes of declining oxygen concentration with coefficients of determination ( $r^2$ ) values above 0.9 were used for analysis. The  $q_{0.1}$  method (10% quantile; Chabot et al. 2016) was used to calculate SMR values. FMR was determined by choosing the highest oxygen consumption rate value among three measurements immediately following the 15-min standardized tail-chasing period. Metabolic scope (MS) was calculated by subtracting SMR from FMR.

#### Cortisol

Fish were sampled first for whole body cortisol when the water temperature reached 1 °C, then at the beginning and end of the starvation period. Each time, 8–14 fish were sampled for whole-body cortisol production under basal conditions. Sturgeon were sacrificed in an overdose of 500 mg/L of MS-222 immediately after removal from the tank. Whole body samples were homogenized in PBS and cortisol was extracted from tissue using solid phase extraction techniques using C18 SEP-Paks cartridges (Waters Corporation; Milford, MA, USA) as previously described (Armour et al. 1993; Zubair et al. 2012). The samples were eluted with 3 mL of ethanol and dried down in a Savant Speed Vac (Fisher Scientific). The dried-down eluents were individually reconstituted, on ice, in 250  $\mu$ L of radioimmunoassay (RIA) buffer (20 ml PBS, 180 ml Milli-Q water, 150 mM NaCl and 0.5% bovine serum albumin). Duplicates of each sample were measured for whole body cortisol by RIA (Van Anholt et al. 2003; Zubair et al. 2012). Once the sample was reconstituted it was split into two 100  $\mu$ L replicates. One hundred  $\mu$ L titrated cortisol (Perkin Elmer; 5000 disintegrations per minute

(DPM)) and 100  $\mu\text{L}$  of cortisol antibody (rabbit anti-cortisol polyclonal antibody; Fitzgerald Industries) were then added to each sample tube. After briefly vortexing, samples were left to incubate at room temperature for 60 min and then overnight at 4  $^{\circ}\text{C}$ . The assay was stopped the following day by the addition of a charcoal separation buffer (1.25 g of charcoal and 0.125 g of dextran in 25 mL RIA buffer), vortexing and sitting on ice for 15 min. The samples were then centrifuged at 4  $^{\circ}\text{C}$  for 30 min at 2500 RPMs. The supernatant from each sample was added to a 6 mL scintillation vial with 4 mL of Ultima Gold scintillation fluid (Perkin Elmer). Samples were counted on a scintillation counter for 5 min. Unknown cortisol values from the samples were interpolated against a standard curve, generated for each assay. Inter-assay variation and intra-assay variation were determined to be 18% and 7% respectively and extraction efficiency was  $99.5 \pm 1.32\%$ .

#### Statistical analysis

For the 2015 cohort, we used a linear mixed effect modeling approach to determine the effects of sampling time, survival (live or dead), and fish size on the different physiological parameters we tested for. Linear mixed models are useful when unbalanced designs are used and when repeated measures are taken on the same unit (Zuur et al. 2009). In this study, the model used fish mass (g), sampling time (d), and survival as fixed effects while tank ID (1–10) was used as a random effect. We also included interaction terms between fish mass and time/survival. These interaction terms allowed us to account for decreasing fish mass over time due to starvation or mortality. The model was thus written as:

$$R_{ij} = \alpha_0 + M_{ij} + T_i + S_{ij} + M_{ij} * T_i + M_{ij} * S_{ij} + a_j + \varepsilon_{ij} \quad (2)$$

where the response variable (R), which denotes either energy density ( $\text{J g}^{-1}$  of sample mass), whole body protein and triglyceride concentrations ( $\text{mg g}^{-1}$  of sample mass), and plasma protein, triglyceride, and glucose concentrations ( $\text{mg mL}^{-1}$  of plasma), as a function of fish  $i$  sampled in tank  $j$ . Fish mass is represented by  $M$ , sampling time is  $T$ , survival is given by  $S$  (note: survival was not included in the plasma models),  $a_j$  is the random intercept associated with tank ID and  $\varepsilon_{ij}$  is the residual variation. Box-Cox data transformations prior to statistical analysis were employed when assumptions of

normality and homoscedasticity were not met. Likelihood ratio test was performed to determine the significance of model terms, and model terms were sequentially removed and the ensuing model was tested against the full model.

Again with the 2015 cohort, we performed a logistic regression to determine which one of the factors we quantified was a better predictor of mortality such as:

$$S = \alpha_0 + T + Tank + TL + M + K + ED + BP + BT \quad (3)$$

where  $S$  is a binomial variable distinguishing between fish that were sampled while alive or dead,  $\alpha_0$  is the intercept,  $T$  is the time (d), tank is the tank number that the fish was sampled from,  $TL$  is total length (mm),  $M$  is fish mass (g),  $K$  is the condition factor measured as  $M \text{ TL}^{-3} \cdot 10^5$ ,  $ED$  is the energy density ( $\text{J g}^{-1}$ ), and  $BP$  and  $BT$  stand for body protein and body triglyceride concentrations ( $\text{mg g}^{-1}$ ), respectively.

Lastly, we performed a Students T-Test on the 2016 cohort to determine if standard/maximum metabolic rates or metabolic scopes differed between fish at the beginning and the end of the 30-day starvation period. We also performed a one-way ANOVA to determine the effect of time (240, 255, 285 dpf) on the cortisol response. All terms were deemed not significant when the  $p\text{-value} > 0.05$ . All statistical analyses and figures were developed using R (R Core Team 2014).

## Results

### Mortality

A total of 90 fish (out of 120) were sampled during the course of the experiment with the 2015 Cohort. Of those 120, 50 died, which represents a 42% mortality rate. All mortalities except for a single fish came after the temperature reached 1  $^{\circ}\text{C}$  (day 14; Fig. 1). Average wet body mass  $\pm$  S.E. of fish was  $5.59 \pm 0.48$  g ( $0.82 \pm 0.09$  g dry body mass),  $4.97 \pm 0.74$  g ( $0.68 \pm 0.13$  g dry body mass),  $5.33 \pm 0.76$  g ( $0.67 \pm 0.12$  g dry body mass), and  $6.00 \pm 0.70$  g ( $0.77 \pm 0.11$  g dry body mass) at the 0, 14, 28, and 42 day sampling periods, respectively. Dead fish averaged  $4.92 \pm 0.31$  g ( $0.52 \pm 0.04$  g dry body mass) throughout the duration of the study. A single fish died (out of 150) during the course of the experiments with the 2016 Cohort.

## 2015 Cohort

**Energy density** Energy density was found to decline over time (Fig. 2a) and was significantly affected by both fish body mass and whether the fish were alive or dead at the time of sampling. By the 28th day of the experiment, live fish sampled had lost on average 17.5% of their initial energy density. Average energy density for dead fish was substantially lower ( $2042 \pm 35 \text{ J g}^{-1}$  throughout the study). The interaction between body mass and time or status were both found to be significant ( $\chi^2 = 6.51$  and  $6.65$ , respectively;  $p$ -value = 0.01; Fig. 2a; Table 1).

**Protein** Whole body protein concentrations did not decline over time (Table 1). However, protein levels were seen to negatively correlate with body mass, with smaller fish showing higher concentrations per unit of body mass (Fig. 2b). In addition to this, fish that were sampled dead, on average, had 11% less protein content than fish that were sampled live (Fig. 2b).

**Triglycerides** Whole body triglycerides showed a significant decline over time and levels were also affected by the status of fish at the time of sampling (Table 1, Fig. 2c). Triglyceride levels for dead fish were found to be extremely low averaging only  $1.91 \text{ mg g}^{-1}$  throughout the study compared to  $29.2 \text{ mg g}^{-1}$  for fish sampled live. However, triglyceride levels for fish sampled live showed a high variance at each one of the four time points with coefficients of variation ranging from 31, 97, 124, and 116% on days 0, 14, 28, and 42, respectively.

## Plasma analyses

**Protein** Plasma protein levels remained constant over time and were not affected by body mass (Table 1, Fig. 3a). Average values of  $5.16 \pm 0.44 \text{ mg mL}^{-1}$  were observed throughout the experiment.

**Triglycerides** Plasma triglyceride concentrations significantly declined over time and were also affected by body mass (Table 1, Fig. 3b). As such, fish during the last sampling event ( $0.61 \pm 0.35 \text{ mg mL}^{-1}$ ) showed a 72% decline compared to fish at the beginning of the acclimation period ( $2.15 \pm 0.62 \text{ mg mL}^{-1}$ ). Similar to the trend observed with the energy density, larger fish, on average, demonstrated higher plasma triglyceride concentrations regardless of the time period from which

they were sampled (Fig. 3b). Similar to whole body triglycerides, plasma triglyceride levels for fish sampled live showed a high variance at each one of the four time points with coefficients of variation ranging from 71, 84, 140, and 182% on days 0, 14, 28, and 42, respectively.

**Glucose** Similar to plasma triglyceride levels, plasma glucose concentrations declined significantly over time and with decreasing body mass (Table 1, Fig. 3c). Values at the beginning of the acclimation period ( $0.50 \pm 0.1 \text{ mg mL}^{-1}$ ) were 86% higher than those measured on day 42 ( $0.07 \pm 0.03 \text{ mg mL}^{-1}$ ).

## Mortality predictors

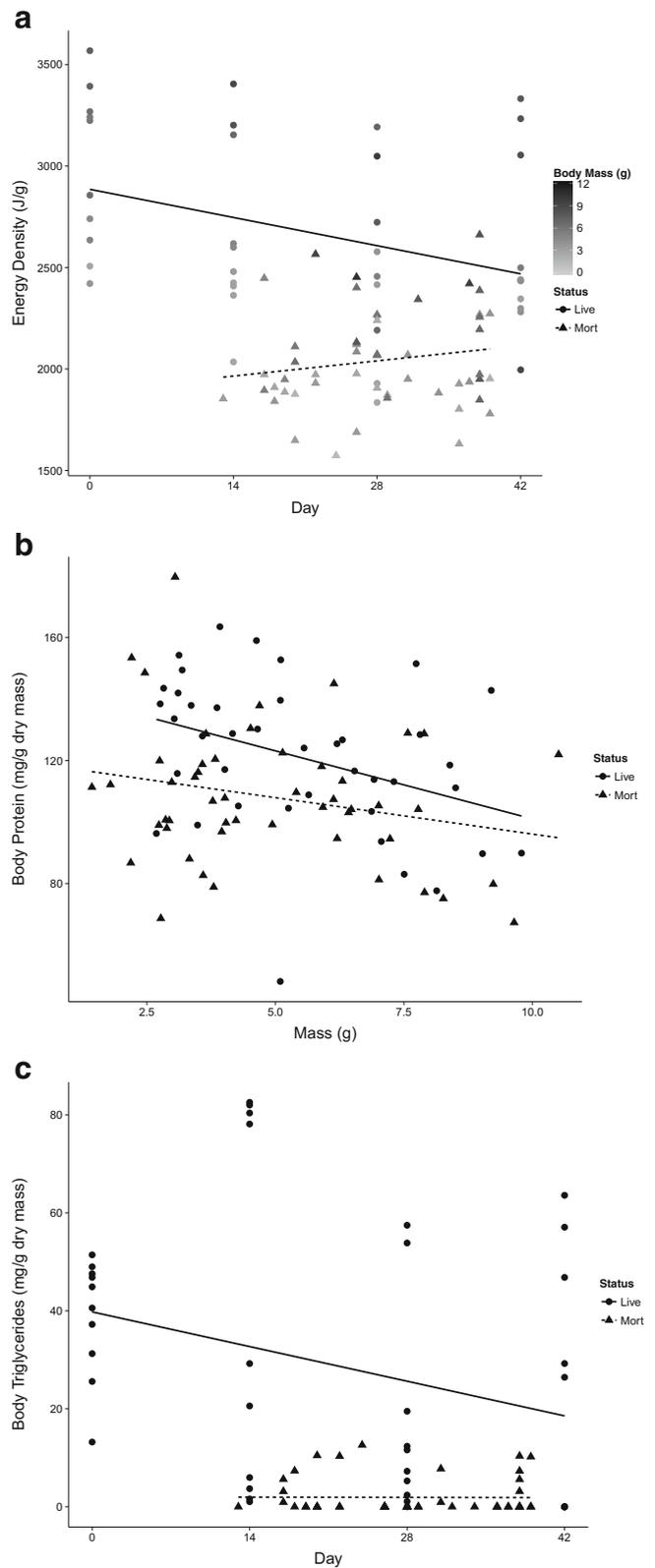
The logistic regression model was developed to estimate factors that can be used to predict if a fish was alive or dead at the time of sampling and yielded significant results ( $p$ -value < 0.05) for energy density ( $p$ -value = 0.003) and whole body triglycerides ( $p$ -value = 0.019; Table 2). Total length ( $p$ -value = 0.095) and body mass ( $p$ -value = 0.056) were close to significance while condition factor barely explained any of the variance ( $p$ -value = 0.409). Lastly, both the day of sampling ( $p$ -value = 0.927) and the tank the fish were sampled from ( $p$ -value = 0.883) did not play a significant role in determining the status of the fish.

## 2016 Cohort

**Metabolic rates** Fish mass averaged  $1.75 \pm 0.11$  and  $2.86 \pm 0.23 \text{ g}$  ( $81 \pm 2$  and  $95 \pm 3 \text{ mm}$  in length) prior and following the 28-day simulated overwintering period, respectively. SMR (in  $\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ) was found to be significantly lower on day 28 compared to day 0 ( $p$ -value = 0.008; Fig. 4). SMR output variability associated with pre-starvation conditions was more than double that observed for fish under overwintering conditions (coefficient of variation of 41.4 vs. 16.5%). Conversely, FMR and MS were not significantly different between the two time points ( $p$ -value = 0.531 and 0.386, respectively; Fig. 4).

**Whole body cortisol concentration** Fish mass averaged  $3.28 \pm 0.67$ ,  $1.75 \pm 0.22$ , and  $1.39 \pm 0.09 \text{ g}$  on days 0, 15, and 45, respectively. A Tukey's post-hoc test revealed that starvation time (day 45) led to a significant increase in cortisol production compared to days 0 and 15 (both  $p$ -values < 0.001; Fig. 5). Cortisol concentrations were similar on days 0 and 15 ( $p$ -value = 0.876).

**Fig. 2** **a** Fish energy density over time depending on status (i.e., live or dead at the time of sampling). Closed circles represent fish that were alive when sampled and closed triangles represent fish that were dead when sampled. Body mass (g) at time of sampling is represented on a grey scale. **b** Whole body protein content as a function of fish mass (g) and status at the time of sampling. **c** Whole body triglyceride concentration as a function of time and whether the status of the fish at the time of sampling. Note: Data were not transformed for plotting purposes



**Table 1** Full model coefficient estimates and standard errors associated with each physiological metric quantified. The  $\chi^2$  statistics and associated *p-values* result from the maximum likelihood comparisons between the full model and the reduced model (i.e., without the specific effect). All models were run using the tank

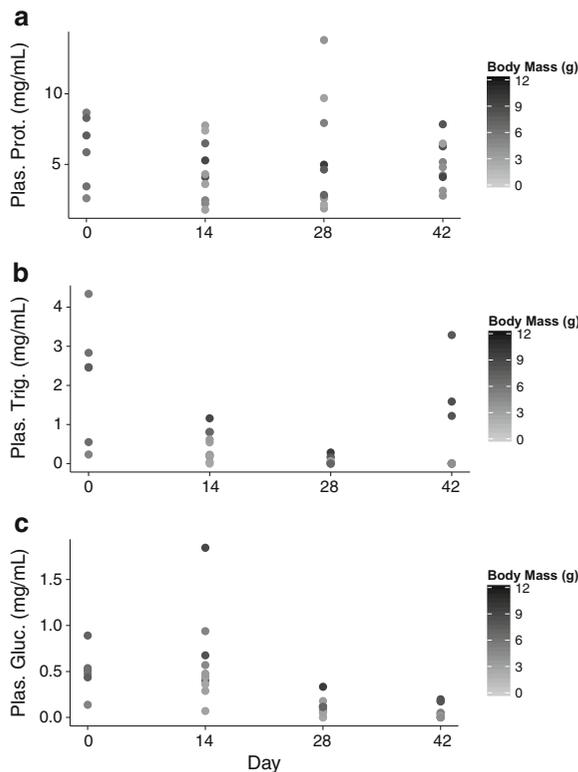
where fish were sampled from as a random effect.  $\alpha_0$  is the model intercept, *M* is for fish mass, *T* is for time, and *S* is for survival (i.e., live or dead). Models for body triglycerides and all plasma parameters were ran on box-cox transformed data. **Note:** Plasma samples were only analyzed for fish sampled alive

Measurement	Effect	Estimate	St. Error	$\chi^2$	<i>p-value</i>
Energy density	$\alpha_0$	1654.013	177.662	NA	NA
	<i>M</i>	222.498	31.573	72.44	<0.001
	<i>T</i>	6.521	6.274	22.35	<0.001
	<i>S</i>	-184.306	133.727	68.73	<0.001
	<i>M x T</i>	-2.814	1.083	6.51	0.011
	<i>M x S</i>	-61.854	23.551	6.65	0.010
Body protein	$\alpha_0$	153.786	16.784	NA	NA
	<i>M</i>	-4.994	2.967	8.65	0.034
	<i>T</i>	-0.441	0.588	1.95	0.378
	<i>S</i>	-22.544	12.506	7.50	0.024
	<i>M x T</i>	0.033	0.101	0.10	0.748
	<i>M x S</i>	1.870	2.216	0.71	0.400
Body triglyceride	$\alpha_0$	0.428	0.134	NA	NA
	<i>M</i>	0.010	0.024	3.97	0.264
	<i>T</i>	0.011	0.005	11.78	0.003
	<i>S</i>	0.125	0.101	41.89	<0.001
	<i>M x T</i>	-0.001	0.001	1.97	0.161
	<i>M x S</i>	0.029	0.018	2.67	0.102
Plasma protein	$\alpha_0$	0.700	0.076	NA	NA
	<i>M</i>	-0.011	0.012	1.71	0.426
	<i>T</i>	-0.001	0.003	0.17	0.919
	<i>M x T</i>	0.000	0.000	0.15	0.695
Plasma triglyceride	$\alpha_0$	0.732	0.280	NA	NA
	<i>M</i>	-0.068	0.046	16.49	<0.001
	<i>T</i>	0.017	0.010	15.92	<0.001
	<i>M x T</i>	-0.001	0.002	0.24	0.627
Plasma glucose	$\alpha_0$	0.626	0.167	NA	NA
	<i>M</i>	-0.040	0.027	10.36	0.006
	<i>T</i>	0.012	0.006	32.90	<0.001
	<i>M x T</i>	0.000	0.001	0.00	0.995

**Discussion**

This study has shown that age-0 lake sturgeon experience important shifts in energy balance and use during the over-wintering acclimation phase as well as within the first few weeks of over-wintering. Observations of mortality began at the onset of the overwintering period, when temperature reached 1 °C, and remained constant throughout the experiment for the 2015 Cohort. This

result prompted us to modify the overwintering acclimation procedure for the 2016 cohort to limit the number of mortalities. While high mortality rates have also been reported among brook trout, *Salvelinus fontinalis*, early in the over-winter period (Cunjak et al. 1987), the severity of the response can likely be linked to the duration of the cold temperature acclimation period, which was short (14 days) in the current study. A lack of physiological adaptation in this type of situation can



**Fig. 3** Plasma protein (a), triglyceride (b), and glucose (c) concentrations over time and as a function of body mass (g) represented on a grey scale. Note: Data were not transformed for plotting purposes

lead to an osmoregulatory dysfunction, which occurs when fish approach their lower thermal limit (Hurst 2007), and can be a cause of mortality.

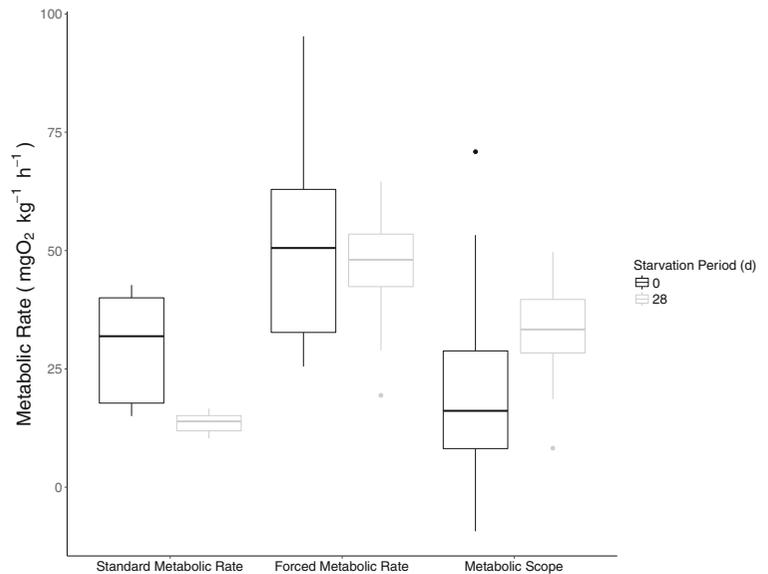
**Table 2** Logistic regression coefficients predictive of fish mortality. *T* is for time, *Tank* is the tank ID number where fish were sampled from, *TL* is for total length, *M* is fish mass, *K* is the condition factor, *ED* is the energy density, and *BP* and *BT* stand for whole body protein and triglyceride concentrations, respectively

Measurement	Estimate	St. Error	z-value	p-value
$\alpha_0$	63.638	31.544	2.017	0.044
<i>T</i>	-0.006	0.067	-0.092	0.927
<i>Tank</i>	0.026	0.179	0.147	0.883
<i>TL</i>	-0.417	0.249	-1.671	0.095
<i>M</i>	4.145	2.170	1.910	0.056
<i>K</i>	-26.824	32.493	-0.826	0.409
<i>ED</i>	-0.010	0.004	-2.947	0.003
<i>BP</i>	-0.016	0.025	-0.631	0.528
<i>BT</i>	-0.336	0.143	-2.352	0.019

Another source of mortality often associated with young fish in temperate climates is the depletion of energy reserves (Garvey et al. 2004), which has clearly been shown in the current study. Similar to teleost fish during initial starvation periods (Bar 2014), we have shown that triglycerides decline substantially in both whole body and blood, and significantly affected the energy density of the fish thus indicating that triglycerides likely act as a primary energy source during starvation. Plasma triglyceride levels have also been reported to decline over time in starved sub-yearling white sturgeon (*Acipenser transmontanus*; Hung et al. 1997) and European seabass (*Dicentrarchus labrax*; Pérez-Jiménez et al. 2007). Similar to our study, Congleton and Wagner (2006) reported high variability of plasma triglyceride values among starved and fed juvenile salmonids, and concluded that it was not a particularly useful index of nutritional condition. However, this high variability could be indicative of the transition phase between lipid and protein mobilization (Bar 2014). This assumption is reinforced by results of whole body and plasma protein concentrations remaining constant throughout the experiment, and by the increase in whole body cortisol during the same period. Protein concentrations could be expected to decline under a longer experimental overwintering period, especially where cold water and starvation conditions can last up to 3 months in the northern range of the species (McDougall et al. 2014b). While survival under winter-like conditions is often correlated to large fish sizes (Henderson et al. 1988; Schultz et al. 1998; Pangle et al. 2004), the current study has shown that energy depletion can explain the majority of observed mortalities, which supports the notion that morphometric indices are not always the sole predictors of fish condition (Congleton and Wagner 2006; McCue 2010).

Very few studies have quantified energy density depletion under over-wintering conditions even though this metric, combined with the knowledge of metabolic costs, is important to understanding energy requirements. Age-0 Pacific herring (*Clupea pallasii*) have been shown to decrease their somatic energy reserves significantly over the course of the winter season even though feeding still occurred (Foy and Paul 1999). In the current study, a clear threshold ( $2042 \pm 35 \text{ J g}^{-1}$ ) was seen between the energy density of fish that survived compared to those that died. While no similar dataset exist for sturgeon until now, the few studies quantifying energy density have never reported values considerably

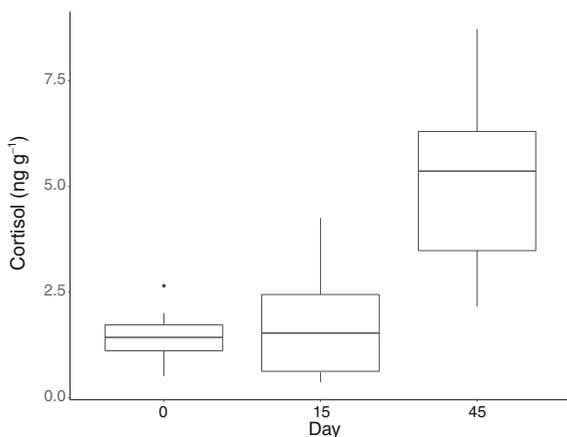
**Fig. 4** Boxplots of metabolic rate performance metrics for fish at the beginning (black outline) (avg. mass  $1.75 \pm 0.11$  g) and the end (grey outline) (avg. mass  $2.86 \pm 0.23$  g) of a 28-day starvation period at  $1^\circ\text{C}$



lower than  $2000 \text{ J g}^{-1}$ , regardless of the life stage (Beamish et al. 1996; Chipps et al. 2009; Deslauriers et al. 2016). Larval lake sturgeon transitioning from endogenous to exogenous feeding can experience short periods where the energy density will go below this threshold (i.e.,  $2000 \text{ J g}^{-1}$ ), which often leads to mortality early on in development (Yoon 2017). This dichotomy between the energetic condition of dead and live fish has also been documented for gizzard shad (*Dorosoma cepedianum*) following a stressful overwintering period in the Watts Bar Reservoir, Tennessee, USA (Adams

et al. 1985). However, it still remains unclear as to why some individuals were more prone to rapid declines in energy while others were able to maintain these high levels that allowed them to survive.

Fish in this study adopted a primarily sedentary existence at water temperatures below  $10^\circ\text{C}$  and practically ceased feeding below  $5^\circ\text{C}$ . Negative correlations between water temperature and activity have been reported for juvenile lake sturgeon (Peake 1999), white sturgeon (Crocker and Cech 1997) and green sturgeon (*Acipenser medirostris*; Mayfield and Cech 2004). However, this is the first study to describe metabolic rate under winter conditions for age-0 sturgeon. Results showed that these fish are capable of significantly suppressing their standard metabolic rate in response to rigorous environmental conditions and thus minimize the energetic expenditure to sustain life. A ten-fold reduction in SMR was observed when compared with fish of similar size at  $16^\circ\text{C}$  (Yoon 2017) with net losses of  $\sim 4 \text{ J g}^{-1} \text{ d}^{-1}$  assuming the minimal effects of egestion, excretion and specific dynamic action under overwintering conditions. Without going as low as  $1^\circ\text{C}$ , Kieffer et al. (2014) showed that the differences in routine metabolic rates of juvenile shortnose sturgeon (*Acipenser brevirostrum*) were more pronounced at low temperatures ( $10^\circ\text{C}$ ) relative to  $15$ ,  $20$ , or  $25^\circ\text{C}$  but that the absolute metabolic scope was not influenced by temperature (Zhang and Kieffer 2017). Similarly, the SMR of over-wintering lake sturgeon are comparable to those displayed by some polar fish species



**Fig. 5** Basal whole body cortisol concentration ( $\text{ng g}^{-1}$  body mass) over time. Whole body cortisol was found to be significantly higher at day 45 compared to days 0 and 15 ( $p\text{-value} < 0.001$ ). All measurements were taken at  $1^\circ\text{C}$  while the onset of starvation began on day 15

(Steffensen 2002). Even though SMR experienced a radical decline, our study showed that age-0 lake sturgeon were still capable of maintaining aerobic capacity and appropriately responded to requirements for increased activity as FMR and MS did not differ from the beginning to the end of the cold acclimatization/starvation period.

The quantification of blood-borne metabolites such as glucose are known to reflect net production and utilization by tissues in fish (Gillis and Ballantyne 1996). Indeed, Costas et al. (2011) has suggested that, due to the importance of glucose as a fuel for a number of tissues, animals often have the ability to maintain glucose levels, even when starved, through a number of mechanisms including: reducing the rate of glucose utilization, increasing gluconeogenic and glycogenolytic potentials and (or) enhancing liver glucose exporting capacity. Circulating concentrations of glucose can also become elevated during stressful events as a result of released catecholamines and corticosteroids which initiates glycogenolysis (Mazeaud et al. 1977) and thus increases in plasma glucose levels are often reported in studies examining stress in sturgeon (Cataldi et al. 1998; Barton et al. 2000; Allen et al. 2009; Falahatkar et al. 2009). Conversely, sharp declines in glycogen levels have been reported shortly after the onset of starvation in fish (Bar 2014). In accordance with these results, plasma glucose levels decreased significantly over the course of the 42-day experiment in this study. Similarly, sub-yearling white sturgeon starved over 10 weeks had significantly lower plasma glucose levels at the end of the study than at the start (Hung et al. 1997). However, Gillis and Ballantyne (1996) reported that two year old lake sturgeon (~500 g body mass) starved over an 8-week period did not show significantly different levels of plasma glucose concentrations in comparison to fed conspecifics, with the exception of one sampling point 10 days following the start of the study. The lack of consistency amongst these results might suggest a synergistic effect of starvation and temperature on glycogen depletion and glucose utilization.

The role cortisol plays throughout overwintering has not been well documented in fish species, particularly chondrosteian fishes. There is indirect evidence in other vertebrates supporting cortisol having a role in protein catabolism once lipid stores are depleted (Bar 2014). In the European eel (*Anguilla anguilla*) for example, plasma cortisol levels almost doubled during a 70-day starvation period while whole body lipids decreased

throughout and muscle protein began to decrease at the end of the experiment (Dave et al. 1975). Similarly, cortisol has been directly correlated to protein catabolism during migration and smoltification in teleost fish (Mommensen et al. 1999). Further, when exposed to cortisol during a starvation period fish consistently lose mass at a faster rate than fish not exposed to cortisol (Storer 1967; Pickford et al. 1970). These studies suggest cortisol could play a similar internal role in mobilizing energy reserves during the overwintering period.

Evidence from our work indicates that traditional morphometrics fail to give a complete representation of the physiological condition of the fish during starvation and overwintering. Instead, energy density and whole body triglycerides proved to be reliable indicators of fish condition at the time of sampling. While the quantification of triglycerides requires specialized equipment, energy density can be easily measured using an established linear model of dry:wet mass against energy density. In light of this, we encourage managers of lake sturgeon rearing facilities to gain a better understanding of fish condition prior to them being stocked. In addition, further studies are suggested here to understand the changes and allocations of energy reserves (i.e., lipid, carbohydrate, protein) during a prolonged starvation period and their role in overwintering survival. It could be that the limited presence of seasonal temperature cues (i.e., stable vs. fluctuating) in hatchery settings lead to a different strategy for somatic growth and energy storage (Post and Parkinson 2001), which compromises the survival of stocked age-0 lake sturgeon.

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## References

- Adams M, Breck J, McLean R (1985) Cumulative stress-induced mortality of gizzard shad in a southeastern U.S. reservoir. *Environ Biol Fish* 13:103–112

- Allen PJ, Barth CC, Peake SJ, Abrahams MV, Anderson WG (2009) Cohesive social behaviour shortens the stress response: the effects of conspecifics on the stress response in lake sturgeon *Acipenser fulvescens*. *J Fish Biol* 74(1):90–104. <https://doi.org/10.1111/j.1095-8649.2008.02112.x>
- Armour KJ, O'Toole LB, Hazon N (1993) The effect of dietary protein restriction on the secretory dynamics of  $1\alpha$ -hydroxycorticosterone and urea in the dogfish, *Scyliorhinus canicula*: a possible role for  $1\alpha$ -hydroxycorticosterone in sodium retention. *J Endo* 138:275–282, 2, DOI: <https://doi.org/10.1677/joe.0.1380275>
- Artyuklin EN (1995) Contributions on biogeography and relationships within the genus *Acipenser*. *Sturg Quart* 3:6–8
- Auer NA (2004) Conservation. In: Sturgeons and Paddlefish of North America. Kluwer Academic Publishers Dordrecht The Netherlands, pp 252–276
- Bar N (2014) Physiological and hormonal changes during prolonged starvation in fish. *Can J Fish Aquat Sci* 71(10):1447–1458. <https://doi.org/10.1139/cjfas-2013-0175>
- Bar NS, Radde N (2009) Long-term prediction of fish growth under varying ambient temperature using multiscale dynamic model. *BMC Syst Biol* 3(1):107. <https://doi.org/10.1186/1752-0509-3-107>
- Barton BA, Bollig H, Hauskins BL, Jansen CR (2000) Juvenile pallid (*Scaphirhynchus albus*) and hybrid pallid x shovelnose (*S. albus x platyrhynchus*) sturgeons exhibit low physiological responses to acute handling and severe confinement. *Comp Biochem Physiol Part A* 126(1):125–134. [https://doi.org/10.1016/S1095-6433\(00\)00192-6](https://doi.org/10.1016/S1095-6433(00)00192-6)
- Beamish RJ, Mahnken C (2001) A critical size and period hypothesis to explain natural regulation of salmon abundance and the linkage to climate and climate change. *Progr Ocean* 49(1–4):423–437. [https://doi.org/10.1016/S0079-6611\(01\)00034-9](https://doi.org/10.1016/S0079-6611(01)00034-9)
- Beamish FWH, Jebbink J-A, Rossiter A, Noakes DLG (1996) Growth strategy of juvenile lake sturgeon (*Acipenser fulvescens*) in a northern river. *Can J Fish Aquat Sci* 53(3):481–489. <https://doi.org/10.1139/f95-222>
- Billard R, Lecointre G (2001) Biology and conservation of sturgeon and paddlefish. *Rev Fish Biol Fish* 10:355–392
- Biro PA, Abrahams MV, Post JR, Parkinson EA (2004) Predators select against high growth rates and risk-taking behaviour in domestic trout populations. *Proc Royal Soc London B* 271(1554):2233–2237. <https://doi.org/10.1098/rspb.2004.2861>
- Brown C, Day RL (2002) The future of stock enhancements: lessons for hatchery practice from conservation biology. *Fish Fish* 3(2):79–94. <https://doi.org/10.1046/j.1467-2979.2002.00077.x>
- Cargnelli LM, Gross MR (1997) Fish energetics: larger individuals emerge from winter in better condition. *Trans Am Fish Soc* 126(1):153–156. [https://doi.org/10.1577/1548-8659\(1997\)126<0153:NFELIE>2.3.CO;2](https://doi.org/10.1577/1548-8659(1997)126<0153:NFELIE>2.3.CO;2)
- Cataldi E, Di Marco P, Mandich A, Cataudella S (1998) Serum parameters of Adriatic sturgeon *Acipenser naccarii* (Pisces: Acipenseriformes): effects of temperature and stress. *Comp Biochem Physiol Part A* 121(4):351–354. [https://doi.org/10.1016/S1095-6433\(98\)10134-4](https://doi.org/10.1016/S1095-6433(98)10134-4)
- Chabot D, Steffensen JF, Farrell AP (2016) The determination of standard metabolic rate in fishes. *J Fish Biol* 88(1):81–121. <https://doi.org/10.1111/jfb.12845>
- Cherel Y, Robin J-P, Le Maho Y (1988) Physiology and biochemistry of long-term fasting in birds. *Can J Zool* 66(1):159–166. <https://doi.org/10.1139/z88-022>
- Chippis SR, Klumb RA, Wright EB (2009) Development and application of juvenile pallid sturgeon bioenergetics model. South Dakota Department of Game, Fish and Parks, Pierre South Dakota
- Congleton JL, Wagner T (2006) Blood-chemistry indicators of nutritional status in juvenile salmonids. *J Fish Biol* 69(2):473–490. <https://doi.org/10.1111/j.1095-8649.2006.01114.x>
- Connolly PJ, Petersen JH (2003) Bigger is not always better for overwintering young-of-the-year steelhead. *Trans Am Fish Soc* 132(2):262–274. [https://doi.org/10.1577/1548-8659\(2003\)132<0262:BINABF>2.0.CO;2](https://doi.org/10.1577/1548-8659(2003)132<0262:BINABF>2.0.CO;2)
- Costas B, Aragão C, Ruiz-Jarabo I, Vargas-Chacoff L, Arjona FJ, Dinis MT, Mancera JM, Conceição LE (2011) Feed deprivation in Senegalese sole (*Solea senegalensis* Kaup, 1858) juveniles: effects on blood plasma metabolites and free amino acid levels. *Fish Physiol Biochem* 37(3):495–504. <https://doi.org/10.1007/s10695-010-9451-2>
- Crocker CE, Cech JJ Jr (1997) Effects of environmental hypoxia on oxygen consumption rate and swimming activity in juvenile white sturgeon, *Acipenser transmontanus*, in relation to temperature and life intervals. *Environ Biol Fish* 50(4):383–389. <https://doi.org/10.1023/A:1007362018352>
- Crossman JA, Forsythe PS, Baker EA, Scribner KT (2009) Overwinter survival of stocked age-0 lake sturgeon. *J App Ichthyol* 25(5):516–521. <https://doi.org/10.1111/j.1439-0426.2009.01310.x>
- Crossman JA, Forsythe PS, Scribner KT, Baker EA (2011) Hatchery rearing environment and age affect survival and movements of stocked juvenile lake sturgeon. *Fish Man Ecol* 18(2):132–144. <https://doi.org/10.1111/j.1365-2400.2010.00762.x>
- Cunjak RA, Curry RA, Power G (1987) Seasonal energy budget of brook trout in streams: implications of a possible deficit in early winter. *Trans Am Fish Soc* 116(6):817–828. [https://doi.org/10.1577/1548-8659\(1987\)116<817:SEBOLT>2.0.CO;2](https://doi.org/10.1577/1548-8659(1987)116<817:SEBOLT>2.0.CO;2)
- Dave G, Johansson-Sjöbeck M-L, Larsson A, Lewander K, Lidman U (1975) Metabolic and hematological effects of starvation in the European eel, *Anguilla anguilla* L. I. Carbohydrate, lipid, protein and inorganic ion metabolism. *Comp Biochem Physiol Part A* 52(3):423–430. [https://doi.org/10.1016/S0300-9629\(75\)80059-4](https://doi.org/10.1016/S0300-9629(75)80059-4)
- Deslauriers D, Heironimus LB, Chippis SR (2016) Test of a foraging-bioenergetics model to evaluate growth dynamics of endangered pallid sturgeon (*Scaphirhynchus albus*). *Ecol Model* 336:1–12. <https://doi.org/10.1016/j.ecolmodel.2016.05.017>
- Falihatkar B, Poursaeid S, Shakoorian M, Barton B (2009) Responses to handling and confinement stressors in juvenile great sturgeon *Huso huso*. *J Fish Biol* 75(4):784–796. <https://doi.org/10.1111/j.1095-8649.2009.02334.x>
- Foy RJ, Paul AJ (1999) Winter feeding and changes in somatic energy content of age-0 Pacific herring in Prince William sound, Alaska. *Trans Am Fish Soc* 128(6):1193–1200. [https://doi.org/10.1577/1548-8659\(1999\)128<1193:WFACIS>2.0.CO;2](https://doi.org/10.1577/1548-8659(1999)128<1193:WFACIS>2.0.CO;2)
- Garvey JE, Ostrand KG, Wahl DH (2004) Energetics, predation, and ration affect size-dependent growth and mortality of fish during winter. *Ecology* 85(10):2860–2871. <https://doi.org/10.1890/03-0329>

- Gillis TE, Ballantyne JS (1996) The effects of starvation on plasma free amino acid and glucose concentrations in Lake sturgeon. *J Fish Biol* 49(6):1306–1316. <https://doi.org/10.1111/j.1095-8649.1996.tb01797.x>
- Groscolas R, Robin J-P (2001) Long-term fasting and re-feeding in penguins. *Comp Biochem Physiol Part A* 128:645–655
- Henderson PA, Holmes RHA, Bamber RN (1988) Size-selective overwintering mortality in the sand smelt, *Atherina boyeri* Risso, and its role in population regulation. *J Fish Biol* 33(2):221–233. <https://doi.org/10.1111/j.1095-8649.1988.tb05465.x>
- Hung SSO, Liu W, Li H, Storebakken T, Cui Y (1997) Effect of starvation on some morphological and biochemical parameters in white sturgeon, *Acipenser transmontanus*. *Aquaculture* 151:357–363
- Hurst TP (2007) Causes and consequences of winter mortality in fishes. *J Fish Biol* 71(2):315–345. <https://doi.org/10.1111/j.1095-8649.2007.01596.x>
- Hurst TP, Conover DO (1998) Winter mortality of young-of-the-year Hudson River striped bass (*Morone saxatilis*): size-dependent patterns and effects on recruitment. *Can J Fish Aquat Sci* 55(5):1122–1130. <https://doi.org/10.1139/f98-017>
- Kappenman KM, Fraser WC, Toner M, Dean J, Webb MAH (2009) Effect of temperature on growth, condition, and survival of juvenile shovelnose sturgeon. *Trans Am Fish Soc* 138(4):927–937. <https://doi.org/10.1577/T07-265.1>
- Kieffer JD, Penny FM, Papadopoulos V (2014) Temperature has a reduced effect on routine metabolic rates of juvenile shortnose sturgeon (*Acipenser brevirostrum*). *Fish Physiol Biochem* 40(2):551–559. <https://doi.org/10.1007/s10695-013-9865-8>
- Lemons DE, Crawshaw LI (1985) Behavioural and metabolic adjustments to low temperatures in the largemouth bass (*Micropterus salmoides*). *Physiol Zool* 58(2):175–180. <https://doi.org/10.1086/physzool.58.2.30158564>
- Mayfield RB, Cech JJ Jr (2004) Temperature effects on green sturgeon bioenergetics. *Trans Am Fish Soc* 133(4):961–970. <https://doi.org/10.1577/T02-144.1>
- Mazeaud MM, Mazeaud F, Donaldson EM (1977) Primary and secondary effects of stress in fish: some new data with a general review. *Trans Am Fish Soc* 106:201–212
- McCue MD (2010) Starvation physiology: reviewing the different strategies animals use to survive a common challenge. *Comp Biochem Physiol Part A* 156(1):1–18. <https://doi.org/10.1016/j.cbpa.2010.01.002>
- McDougall CA, Pisiak DJ, Barth CC, Blanchard MA, MacDonell DS, Macdonald D (2014a) Relative recruitment success of stocked age-1 vs age-0 lake sturgeon (*Acipenser fulvescens* Rafinesque, 1817) in the Nelson River, northern Canada. *J App Ichthyol* 30(6):1451–1460. <https://doi.org/10.1111/jai.12555>
- McDougall CA, Blanchfield PJ, Anderson WG (2014b) Linking movements of lake sturgeon (*Acipenser fulvescens* Rafinesque, 1817) in a small hydroelectric reservoir to abiotic variables. *J App Ichthyol* 30(6):1149–1159. <https://doi.org/10.1111/jai.12546>
- Mommsen TP, Vijayan MM, Moon TW (1999) Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. *Rev Fish Biol Fish* 9(3):211–268. <https://doi.org/10.1023/A:1008924418720>
- Pangle KL, Sutton TM, Kinnunen RE, Hoff MH (2004) Overwinter survival of juvenile Lake Herring in relation to body size, physiological condition, energy stores, and food ration. *Trans Am Fish Soc* 133(5):1235–1246. <https://doi.org/10.1577/T03-127.1>
- Peake S (1999) Substrate preferences of juvenile hatchery-reared Lake sturgeon, *Acipenser fulvescens*. *Environ Biol Fish* 56(4):367–374. <https://doi.org/10.1023/A:1007523607190>
- Pérez-Jiménez A, Guedes MJ, Morales AE, Oliva-Teles A (2007) Metabolic responses to short starvation and refeeding in *Dicentrarchus labrax*. Effect of dietary composition. *Aquaculture* 265(1–4):325–335. <https://doi.org/10.1016/j.aquaculture.2007.01.021>
- Peterson D, Vecsei P, Noakes LG (2003) Threatened fishes of the world: *Acipenser fulvescens* Rafinesque, 1817 (Acipenseridae). *Environ Biol Fish* 68:68–174
- Pickford GE, Pang PKT, Weinstein E, Toretti J, Hendler E, Epstein FH (1970) The response of the hypothsectomized cyprinodont, *Fundulus heteroclitus*, to replacement therapy with cortisol: effects on blood serum and sodium-potassium activated adenosine triphosphatase in the gills, kidney, and intestinal mucosa. *Gen Comp Endocrinol* 14(3):524–534. [https://doi.org/10.1016/0016-6480\(70\)90036-5](https://doi.org/10.1016/0016-6480(70)90036-5)
- Pikitch EK, Doukakis P, Lauck L, Charkrabarty P, Drickson DL (2005) Status, trends and management of sturgeon and paddlefish fisheries. *Fish. Fisheries* 6:233–265
- Post JR, Evans DO (1989) Size-dependent overwinter mortality of young-of-the-year yellow perch (*Perca flavescens*): laboratory, in situ enclosure, and field experiments. *Can J Fish Aquat Sci* 46(11):1958–1968. <https://doi.org/10.1139/f89-246>
- Post JR, Parkinson EA (2001) Energy allocation strategy in young fish: allometry and survival. *Ecology* 82(4):1040–1051.
- R Core Team (2014) R: a language and environment for statistical computing. R Foundation for Statistical Computing Vienna Austria
- Schultz ET, Conover DO (1999) The allometry of energy reserve depletion: test of a mechanism for size-dependent winter mortality. *Oecologia* 119(4):474–483. <https://doi.org/10.1007/s004420050810>
- Schultz ET, Conover DO, Ehtisham A (1998) The dead of winter: size-dependent variation and genetic differences in seasonal mortality among Atlantic silverside (Atherinidae: *Menidia menidia*) from different latitudes. *Can J Fish Aquat Sci* 55(5):1149–1157. <https://doi.org/10.1139/f97-320>
- Shaw SL, Chipps SR, Windels SK, Webb MA, McLeod DT, Willis DW (2012) Lake sturgeon population attributes and reproductive structure in the Namakan reservoir, Minnesota and Ontario. *J App Ichthyol* 28(2):168–175. <https://doi.org/10.1111/j.1439-0426.2011.01927.x>
- Steffensen JF (2002) Metabolic cold adaptation of polar fish based on measurements of aerobic oxygen consumption: fact or artefact? Artefact! *Comp Biochem Physiol Part A* 132(4):789–795. [https://doi.org/10.1016/S1095-6433\(02\)00048-X](https://doi.org/10.1016/S1095-6433(02)00048-X)
- Storer GH (1967) Starvation and the effects of cortisol in the goldfish (*Carassius auratus*). *Comp Biochem Physiol* 20(3):939–948. [https://doi.org/10.1016/0010-406X\(67\)90065-5](https://doi.org/10.1016/0010-406X(67)90065-5)
- Sutton TM, Ney JJ (2001) Size-dependent mechanisms influencing first-year growth and winter survival of stocked striped bass in a Virginia mainstream reservoir. *Trans Am Fish Soc*

- 130(1):1–17. [https://doi.org/10.1577/1548-8659\(2001\)130<0001:SDMIFY>2.0.CO;2](https://doi.org/10.1577/1548-8659(2001)130<0001:SDMIFY>2.0.CO;2)
- Van Anholt RD, Spanings T, Koven W, Wendelaar Bonga SE (2003) Effects of acetylsalicylic acid treatment on thyroid hormones, prolactins, and the stress response of tilapia. *Am J Phys* 285:R1098–R1106
- Werner EE (1994) Ontogenetic scaling of competitive relations: size-dependent effects and responses in two anuran larvae. *Ecology* 75(1):197–213. <https://doi.org/10.2307/1939394>
- Xiaojun X, Ruyung S (1990) The bioenergetics of the southern catfish (*Silurus meridionalis* Chen). I. Resting metabolic rate as a function of body weight and temperature. *Physiol Zool* 63(6):1181–1195. <https://doi.org/10.1086/physzool.63.6.30152639>
- Yoon G (2017) Effects of temperature, dissolved oxygen and substrate on the development of metabolic phenotypes in Lake sturgeon. Thesis, University of Manitoba, Winnipeg, Manitoba, *Acipenser fulvescens*, 113 pp
- Zhang Y, Kieffer JD (2017) The effects of temperature on the resting and post-exercise metabolic rates and aerobic metabolic scope in shortnose sturgeon *Acipenser brevirostrum*. *Fish Physiol Biochem* 43(5):1245–1252. <https://doi.org/10.1007/s10695-017-0368-x>
- Zubair SN, Peake SJ, Hare JF, Anderson WG (2012) The effect of temperature and substrate on the development of the cortisol stress response in the lake sturgeon, *Acipenser fulvescens*, Rafinesque (1817). *Environ Biol Fish* 93(4):577–587. <https://doi.org/10.1007/s10641-011-9951-7>
- Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009). Mixed effects models and extensions in ecology with R. Springer New York xxii + 574 pp