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## Environmental calcium and variation in yolk sac size influence swimming performance in larval lake sturgeon (*Acipenser fulvescens*)

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## Summary statement

This study shows that low environmental calcium concentration and large yolk sac volume reduce spontaneous and sprint swimming performances of fish larvae. The study also presents a novel methodology to examine volitional swimming performance.

## Abstract

In many animal species, performance in the early life stages strongly affects recruitment to the adult population; however, factors that influence early life history stages are often the least understood. This is particularly relevant for lake sturgeon, *Acipenser fulvescens*, living in areas where environmental calcium concentrations are declining, partly due to anthropogenic activity. As calcium is important for muscle contraction and fatigue resistance, declining calcium levels could constrain swimming performance. Similarly, swimming performance could be influenced by variation in yolk sac volume, because the yolk sac is likely to affect drag forces during swimming. Testing swimming performance of larval *A. fulvescens* reared in four different calcium treatments spanning the range of 4-132 mg l<sup>-1</sup> [Ca<sup>2+</sup>], this study found no treatment effects on the sprint swimming speed. A novel test of volitional swimming performance, however, revealed reduced swimming performance in the low calcium environment. Specifically, volitionally swimming larvae covered a shorter distance before swimming cessation in the low calcium environment compared to the other treatments. Moreover, sprint swimming speed in larvae with a large yolk sac was significantly slower than in larvae with a small yolk sac, regardless of body length variation. Thus, elevated maternal allocation (i.e., more yolk) was associated with reduced swimming performance. Data suggest that larvae in low calcium environments or with a large yolk sac exhibit reduced swimming performance and could be more susceptible to predation or premature downstream drift. Our study reveals how environmental factors and phenotypic variation influence locomotor performance in a larval fish.

## Introduction

Recent studies have revealed that calcium contents in aquatic environments are declining in many softwater lakes in boreal North America (Jeziorski et al., 2008; Jeziorski and Smol, 2016). Low calcium content has been coupled with the near extirpations of important crustacean species, including calcium-rich daphnia and amphipod species (Cairns and Yan, 2009), freshwater gastropods (Dalesman and Lukowiak, 2010) as well as keystone benthic predators such as the eastern crayfish (*Cambarus bartonii*; Hadley et al., 2015). As a result of these food web alterations, algal production may rise (Korosi et al., 2012), and there is concern that zooplanktivorous fish populations inhabiting low calcium environments (e.g., Canadian Shield) may decline (Jeziorski et al., 2008). Moreover, adequate calcium levels may be critical for fish from a physiological standpoint during early life stages when demand is highest (Genz et al., 2014). One species with a native range including the Canadian Shield is the lake sturgeon (*Acipenser fulvescens*), considered a species at risk under the assessment of the Committee on the Status of Endangered Wildlife in Canada (COSEWIC, 2006).

Previous studies on Chondrosteans, an Actinopterygian subclass that includes sturgeon species, have demonstrated that these organisms are likely to be more prone to fluctuations in environmental calcium due to their low plasma calcium levels and their lack of a bony skeleton or scales (which can act as a calcium buffer in teleost fish), particularly among species that are constrained to a freshwater life cycle (Allen et al., 2009). Studies have demonstrated that calcium uptake in juvenile and adult fish is primarily achieved across the gills (Perry and Wood, 1985; Flik et al., 1995), but in larval *A. fulvescens* maternal provisioning and uptake by the yolk epithelium are significant sources of calcium (Genz et al., 2014), and align well with similar observations in teleost fishes (Chen et al., 2003).

Lake sturgeon, unlike other species from the Acipenseridae family in North America, spend their entire life cycle in freshwater lakes and rivers. Spawning, which typically occurs in late spring, takes place over gravel beds in fast flowing river sections (McKinley et al., 1998). Upon hatch, larvae settle in the sediment where they rely on their yolk reserves for 3-19 days depending on water temperature (Wang et al., 1985; Auer and Baker, 2002; Peterson et al., 2007). During this life stage, larvae are especially vulnerable to benthic predators due to the absence of scutes (Caroffino et al., 2010) and presumably rely on their swimming abilities to escape and seek cover (Peterson et al., 2007; Wishingrad et al., 2014). Upon yolk absorption, larvae begin drifting downstream until suitable habitat is encountered (Smith and King, 2005; Pollock et al., 2015). While the specific

mechanisms leading to settlement are still unknown, swimming performance could play a major role (Barth and Anderson, 2015).

Calcium availability plays a key part in muscle contraction and fatigue resistance during exercise (Berchtold et al., 2000; Anttila et al., 2008) and can thus play an important role for larval fish where swimming activity is often highest (Verhille et al., 2014). In turn, larval swimming ability plays a major role in survival as it facilitates foraging (once yolk is absorbed), escaping from predators, and avoiding premature downstream drift to suboptimal habitats and potentially energy costly environments (e.g., high water velocity or temperature). Swimming performance therefore has potential significant fitness-related consequences (Plaut, 2001; Johnson et al., 2015; Pimental et al., 2016).

Body shape and size metrics are often used to explain swimming variation in fish (Ojanguren and Braña, 2003; Verhille et al., 2014; Baktoft et al., 2016). Of particular interest for larval fish is the effect of the yolk sac, because it is maternally derived and crucial for larval survival. Mothers allocating resources to offspring face a trade-off between 1) allocating more energy to fewer offspring and 2) allocating less energy to many offspring (Smith and Fretwell, 1974). Selection should favour mothers that find an optimal solution to the trade-off, particularly in unpredictable environments (Fisher et al., 2011; Segers and Taborsky, 2011). While several lines of evidence suggest elevated survival among larvae with large yolk sacs (Miller et al., 1988; Rideout et al., 2005; Ussi-Heikkilä et al., 2010), few studies have identified factors favouring larvae with small yolk sacs (Gagliano and McCormick, 2007). Riverine piscivores may, however, select prey with large yolk sacs (Fresh and Schroder, 1987), perhaps indicating elevated mortality of larvae with large yolk sacs. The mechanistic basis could be related to reduced swimming performance in larvae with large yolk sacs, as hypothesized by previous studies (Louhi et al., 2011; Fresh and Schroder, 1987), but further study is warranted (Rollinson and Hutchings, 2011; Kopf et al., 2014).

The objective of this study was to evaluate the effects of environmental calcium concentration and variation in yolk sac volume on the swimming performance of larval lake sturgeon. To this end, lake sturgeon eggs and larvae were reared in environmentally-relevant calcium concentrations and assessed for their swimming performance using two different approaches 1) a novel measure of volitional vertical swimming speed and distance to swimming cessation, and 2) horizontal sprint swimming speed and body kinematics measured using high speed video recordings.

## Materials and methods

### *Animal Husbandry*

Adult *A. fulvescens* were caught using 8-12 h gill net sets in the Winnipeg River, Canada, assessed to confirm sexual maturity and transported to the University of Manitoba. *A. fulvescens* were acclimated to the laboratory environment for 48 h and then given an intraperitoneal injection (0.5 ml kg<sup>-1</sup>) containing a combination of salmonid GnRH and a dopamine inhibitor (Ovaprim™, Syndel Laboratories Ltd., Qualicum Beach, BC, Canada) to stimulate ovulation or spermiation (Goncharov et al., 1991; Genz et al., 2014). Gametes were collected 20 h post-injection. Eggs from 6 females and milt from 4 males were mixed manually, and fertilized eggs were placed in freshwater containing Fuller's earth for 30 min to prevent adhesive clumping and thus increase resistance to fungal growth (Genz et al., 2014). Parentage was equally distributed and replicated across treatments. Thus, once hatched, all fish were the same age, were held under identical conditions, and any developmental differences between treatments would be attributed to ambient [Ca<sup>2+</sup>] (see below).

Incubation of eggs and rearing of larval *A. fulvescens* followed previously described procedures for Acipenseridae (Doroshov et al., 1983; Aloisi et al., 2006). Fertilized eggs were placed in MacDonald jars (Bates et al., 2014), which provided continuous water mixing at 12.0 ± 0.4°C. Holding tanks containing three different environmental [Ca<sup>2+</sup>] treatments (low: nominally 0.1 mmol l<sup>-1</sup> or 4 mg l<sup>-1</sup>; medium: 0.4 mmol l<sup>-1</sup> or 16 mg l<sup>-1</sup>, and high: 3.3 mmol l<sup>-1</sup> or 132 mg l<sup>-1</sup> [Ca<sup>2+</sup>]) and a control tank reflecting water in the Winnipeg River (0.35 mmol l<sup>-1</sup> or 14 mg l<sup>-1</sup> [Ca<sup>2+</sup>]; Allen et al., 2009) were used to supply water to the jars (Table 1). The three [Ca<sup>2+</sup>] treatments were selected based on previous studies on *A. fulvescens* (Allen et al., 2011; Genz et al., 2013 & 2014). Calcium levels found in the Winnipeg River are similar to those found in Lake Superior (~13 mg l<sup>-1</sup>) but lower than those found in lakes Ontario and Erie (~32-33 mg l<sup>-1</sup>) (Chapra et al., 2012). Calcium treatments were made by adding salts (Fluka; Sigma) to deionized water using (in mmol l<sup>-1</sup>): 0.11 NaCl, 0.022 KCl, 0.16 MgSO<sub>4</sub>·7H<sub>2</sub>O, 170 nM Na<sub>2</sub>HPO<sub>4</sub> and 0.1, 0.4 or 3.3 CaCl<sub>2</sub>·2H<sub>2</sub>O. Water pH was adjusted to 7.69 ± 0.05 using NaHCO<sub>3</sub>. Ionic composition of the treatment water was measured via ion exchange chromatography (Table 1). The control treatment was supplied with dechlorinated tap water standard to the animal holding facility, and thus exhibited different concentrations of measured cations in addition to Ca<sup>2+</sup> compared to the other three groups. Water was continually recirculated between MacDonald jars and reservoir tanks (160 l) with the appropriate treatment water using pumps equipped with biofilters. Aeration of the water was

ensured using air stones. Half of the water in each reservoir was replaced every other day during incubation.

Dead eggs were removed daily to avoid fungal infections. After 5-7 days, larvae began hatching and were transferred from the MacDonald jars to opaque, partly covered 5 l tanks with the corresponding treatment water inflow. Larvae were maintained under these conditions for 1-4 days post-hatch (dph) at which point they were used for experimentation (total length:  $13.1 \pm 0.1$  mm; body mass:  $0.0174 \pm 0.0001$  g (mean  $\pm$  S.E.)). At the time of sampling, fish were 4-8 dph, approximated to be developmental stage 36 (Dettlaff, 1993). All larvae were transferred without air exposure (Poulsen et al., 2010) between the 5 l holding tanks and the tanks used for data collection. Specifically, individual larvae were guided into a small container (0.3 l) without air exposure and released in the tank used for data collection. For the release, the container was tilted  $90^\circ$  and submerged about 1 cm. If the larva remained in the container, the container was tilted slightly more and drained by lifting the container above the water surface. All experiments were carried out in a temperature controlled room adjusted to  $12^\circ\text{C}$ , equivalent to the holding temperature. All procedures followed the University of Manitoba Animal Care and Use Protocol (F09-039/1/2) approved for the study.

#### *Vertical volitional swimming performance: a novel methodology*

Previous studies have used small swim tunnels to measure the swimming performance of larval fish (Poulsen et al., 2012; Baker et al., 2014; Deslauriers et al., 2017) and other small organisms (Hata et al., 2017). Preliminary tests indicated that *A. fulvescens* yolk sac larvae do not exhibit consistent positive rheotactic behaviour (i.e., swimming against the water flow) in a swim tunnel until they have nearly absorbed all their yolk. Therefore, an alternative protocol based the negative phototactic behaviour, swimming away from light typically toward the benthos, of larval Lake Sturgeon was developed to test larval swimming performance for volitional swimming duration and speed.

Tests were carried out using a vertical transparent cylinder that was 111 cm tall with an inner diameter of 12 cm. The acrylic cylinder was sealed at the lower end and the bottom was covered with 10 cm of gravel (5-10 mm diameter). Intervals of 5 cm were marked on the cylinder from the water surface to the gravel for behavioural observations. Water was added to the cylinder to ensure 100 cm of water above the gravel bottom. A 40 W bulb provided light 40 cm above the water surface, and water temperature was kept at  $12.1 \pm 0.6^\circ\text{C}$ .

*A. fulvescens* were sampled randomly from the treatment tanks (control, low, medium and high calcium;  $n = 42$  per treatment) and released individually at the water surface of the cylinder. Water chemistry in the cylinder reflected the corresponding calcium treatment. All larvae started sprinting towards the bottom immediately after release and swimming performance was recorded. Specifically, travel speed ( $\text{mm s}^{-1}$ ) was recorded by dividing 1000 mm (i.e., cylinder length) by the time interval (to nearest 0.1 s) it took the larvae to move from the water surface to the bottom of the cylinder. In addition, observations were carried out to record the distance swum (to nearest 5 cm) from the water surface until cessation of active swimming movements (i.e., absence of tail beats). These observations were included because many larvae ceased to actively swim before reaching the bottom of the cylinder. After ceasing tail beat, larvae would sink passively towards the bottom. Due to significant image distortion of the circular cylinder and because larvae were difficult to retrieve from the gravel after testing, kinematic (i.e., tail beat frequency, tail beat amplitude) and morphological (i.e., body length, body mass and yolk sac volume) metrics were not quantified in these fish; rather such variables were assessed from fish utilised in the horizontal sprint swimming trials as outlined below.

#### *Horizontal sprint swimming trials*

Individual *A. fulvescens* were sampled randomly from the treatment tanks (control, low, medium and high calcium;  $n = 20-25$  per treatment) and transferred to a circular arena ( $4 \times 35$  cm; depth  $\times$  diameter) for high-speed video recordings. Tested larvae differed from those used in the vertical swim trials. A high speed camera (HiSpec1 (125 Hz); Fastec Imaging, San Diego, CA, USA) was positioned above the arena for dorsal recordings of fish swimming. Similar to Shepherd et al. (2000), water depth in the arena was set at 4 cm, and a dim light underneath the arena was used to enhance image contrast. A  $10 \text{ mm} \times 10 \text{ mm}$  grid was placed in the field of view to calibrate distances. Sprint swimming was elicited by touching the caudal region of the larva with a thin needle, similar to previous studies on larval fish (Batty and Blaxter, 1992). All larvae responded to the stimuli by rapid undulations of the tail and swimming along the bottom of the dish to escape the pressure placed on the tail region. A total of 1-6 video swimming sequences were recorded per individual larvae. Each sequence included at least two complete tail beats.

Analysis of the video recordings was restricted to sequences in which larvae swam in a steady and linear fashion (Fuiman and Batty, 1997) and at least 2 cm away from the sides of the arena (Fish et al., 1991; Svendsen et al., 2003) to minimize any wall effects (Webb, 1993). The

software Tracker (version 4.82; available from <http://www.cabrillo.edu/~dbrown/tracker/>) was employed to track the tip of the tail and the centre of the yolk sac. The latter was assumed to approximate the centre of mass (COM) of the larva. Video sequences were analysed frame by frame and provided three biomechanical variables: 1) swimming speed ( $\text{mm s}^{-1}$ ) calculated as the total accumulated distance (mm) covered by the COM divided by the duration (s). The distance ( $d$ ) between two pairs of  $x,y$  coordinates was calculated using:

$$d = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2} \quad (1)$$

where  $x_1$  and  $x_2$  and  $y_1$  and  $y_2$  indicate the coordinates of two sequential positions; 2) tail beat frequency defined as a full oscillation of the tail (i.e., a complete cycle of left and right lateral displacement; Hunter and Zweifel, 1971; Svendsen et al., 2010); 3) tail beat amplitude calculated as the perpendicular distance (mm) between the trajectory of COM and the most lateral  $x, y$  coordinates of the tip of the tail. Measurements of COM and tail tip were synchronized and the COM trajectory was extrapolated posteriorly in a linear fashion to the tail tip coordinates to estimate amplitude. A full tail oscillation around the COM trajectory was used as the measure of amplitude. Each analysed video sequence included at least two consecutive tail beats (Fuiman and Batty, 1997) and provided one measure of speed, tail beat frequency and tail beat amplitude. The latter two variables were estimated as the average of consecutive tail beats.

Body mass, body length and yolk sac volume were quantified after each sprint swim trial. Individual larvae were removed from the circular arena using a wide mouth pipette (Shepherd et al., 2000) and transferred to 25 mL vials, where larvae were euthanized with ~250 parts per million tricaine methanesulfonate (MS222; Syndel Labs, Nanaimo, BC, Canada) following previous studies (Hale, 1999). Vials contained a limited volume (2-3 mL) of treatment water to prevent dehydration and were kept on ice immediately after finishing the high speed video recordings. Next, larvae were carefully blotted dry using soft paper towel and transferred to a microscope (Nikon, YS100) connected to a digital camera (Sony 3CCD colour camera) to take lateral (dexter; i.e., right side) and dorsal images of each larva. Vials were kept on ice for no more than 6 h prior to taking the images. Image pixels were converted to mm using a 10 mm  $\times$  10 mm grid included in the field of view. Wet larva body mass ( $M$ ; g) was determined (to nearest 0.0001 g) using a Mettler AE163 analytical balance (Mettler-Toledo, Columbus, OH, USA).

Images were analysed using the software Vernier Logger Pro (version 3.6.; Vernier Software & Technology, Beaverton, OR, USA). The analysis provided a measure of total body

length in addition to yolk sac length, height and width (all in mm) for each larva. The yolk sac was assumed to be an ellipsoid (Kamler, 2008) with the volume calculated as:

$$V = \frac{4}{3} \cdot \pi \cdot \frac{l}{2} \cdot \frac{h}{2} \cdot \frac{w}{2} \quad (2)$$

where  $V$  is yolk sac volume ( $\text{mm}^3$ ), while  $l$ ,  $h$  and  $w$  represent yolk sac length, height and width (mm), respectively. By assuming that the yolk specific gravity is 1 (Kamler, 2008), the proportion ( $P$ ; %) of the total body mass allocated to yolk sac was calculated as:

$$P = \frac{V}{(M \cdot 1000)} \cdot 100 \quad (3)$$

### Statistical analyses

Effects of environmental calcium on travel speeds ( $\text{mm s}^{-1}$ ) tested in the vertical cylinder were analysed using a one-way ANOVA. The same analysis was applied to examine effects of environmental calcium on the distance swum (cm) before swimming cessation. Standard transformations of data [e.g.,  $\ln(x + 1)$ ] prior to statistical analysis were employed to meet assumptions of normal distribution of data and homogeneity of variance. If assumptions were met, the tests were followed by Holm-Šidák pairwise multiple comparison procedures. If data transformations did not permit the use of parametric testing, an ANOVA on ranks was employed, followed by pairwise multiple comparisons procedures involving the Student-Newman-Keuls method.

To test the effects of environmental calcium and yolk sac volume on sprint swimming speed in the circular arena, a linear mixed effects model (LMM) approach was applied following Zuur et al. (2009). Larva ID was included as a random effect, thereby inducing a compound correlation structure to accommodate the repeated measures on each larva (up to six measures on individual larva). Yolk sac volume, total body length and calcium treatment of individual larvae, as well as tail beat frequency and amplitude, were included as covariates in the model to account for potential effects of these variables. Body mass was not included as a covariate in the model as it is highly correlated with total body length. The initial full model was written as:

$$\text{Speed}_{ij} = \alpha + V_j + L_j + \text{Ca}^{2+}_j + f_{ij} + \text{amp}_{ij} + a_j + \varepsilon_{ij}$$

$$a_j \sim N(0, \sigma_a^2)$$

$$\varepsilon_{ij} \sim N(0, \sigma^2)$$

in which the swimming speed of individual  $j$  in sequence  $i$  is a function of a common intercept ( $\alpha$ ), yolk sac volume ( $V$ ), body length ( $L$ ) and calcium treatment ( $\text{Ca}^{2+}$ ) of individual  $j$ , tail beat frequency ( $f$ ) and amplitude ( $\text{amp}$ ) of individual  $j$  measured in sequence  $i$ , all two-way interactions

including yolk sac volume of individual  $j$ , a random intercept ( $a_j$ ) and residual variation ( $\varepsilon_{ij}$ ). The model assumed that  $a_j$  was normally distributed with mean zero and variance  $\sigma_a^2$  and  $\varepsilon_{ij}$  was normally distributed with mean zero and variance  $\sigma^2$ .

Significance of model terms were evaluated using likelihood ratio tests comparing nested models fitted using maximum likelihood estimation. Non-significant ( $P > 0.05$ ) interactions were removed to obtain a final model including all significant main effects. Parameter estimates of the final model were obtained using restricted maximum likelihood estimation. The final model was validated using qq-plots of model residuals and by plotting model residuals as functions of fitted values and all explanatory variables. There were no indications that model assumptions of normality and variance homogeneity were violated. The model output was correlated with the empirical data using least square linear regression to assess the predictive ability of the developed model.

The free statistical software R (R Development Core Team, 2014) and SigmaPlot 11.0 (Systat Software, Erkrath, Germany) was used for statistical analyses and graphing. The R package nlme (Pinheiro et al., 2017) was employed to fit models. Results were considered significant at  $P < 0.05$ . All values are reported as means  $\pm$  SE unless otherwise noted.

## Results

### *Vertical swimming performance*

All tested larvae began swimming towards the bottom immediately after transfer to the vertical cylinder. The results revealed that travel speed was reduced significantly ( $P < 0.001$ ) by 19% in the low calcium treatment compared to the other treatments. Travel speeds in the control, medium and high calcium treatments were similar; fish travelled at speeds ranging between 62.7 - 66.7 mm s<sup>-1</sup>.

Although all larvae started swimming downwards after release in the cylinder, not all larvae reached the bottom while actively swimming. Instead, active swimming frequently stopped before the larvae reached the bottom. Results showed that larvae conditioned in low calcium environments ceased swimming after a significantly ( $P < 0.001$ ) shorter distance compared to the other treatments (Fig. 1). Following cessation, larvae sank passively towards the bottom, which decreased the average travel speed. This observation indicated that the slower travel speeds associated with fish reared in the low calcium environments were a product of early swimming cessation in the vertical cylinder rather than slower swimming speeds per se.

### *Horizontal sprint swimming trials*

A total of 250 high-speed video sequences were analysed for sprint swimming speed ( $\text{mm s}^{-1}$ ) using 92 different larvae with an average total length of  $13.1 \pm 0.1$  mm (total length) and wet body mass of  $0.0174 \pm 0.0001$  g. The average yolk sac volume was  $7.4 \pm 0.1$   $\text{mm}^3$  (range: 5.4 - 10.3  $\text{mm}^3$ ). The percentage of the total larval body mass allocated to the yolk sac was  $42.6 \pm 0.5\%$  (range: 30.6 - 53.5%) as calculated using Equation 3. Using a linear mixed effects model (LMM) analysis, there was no evidence that the calcium treatments influenced the sprint swimming speed ( $P > 0.05$ ). Thus, this covariate was removed, and the LMM approach continued to test for effects of body length, body kinematics (i.e., tail beat frequency and tail beat amplitude) as well as yolk sac volume on the sprint swimming speed. This test provided evidence ( $P < 0.05$ ) that sprint swimming speed correlates positively with body length, tail beat frequency and amplitude, and negatively with yolk sac volume (Fig. 2; Table 2). On average, sprint swimming speed was 10.3% faster in larvae with a small yolk sac compared to larvae with a large yolk sac.

Using the explanatory variables (i.e., yolk sac volume, body length, tail beat frequency and amplitude), the model output was correlated with the empirical data to examine whether the parameters used to construct the model allow for good precision in the prediction of sprint swimming speeds. This test revealed a strong correlation between modelled and empirical data ( $P < 0.0001$ ;  $r^2 > 0.91$ ) showing that the model captured the vast majority of the variation in sprint swimming speed.

### **Discussion**

Examining locomotor performances in larval *A. fulvescens*, this study revealed 1) that low environmental calcium concentrations ( $\leq 4$   $\text{mg l}^{-1}$  [ $\text{Ca}^{2+}$ ]) reduce volitional swimming performance, and 2) that elevated maternal allocation, as indicated by a large yolk sac volume, is associated with reduced sprint swimming speed. These findings are important because variation in locomotor performance may have fitness implications. For example, swimming performance predicts survival in tadpoles of the Pacific tree frog (*Pseudacris regilla*) (Watkins, 1996) and the Trinidadian guppy (*Poecilia reticulata*) (Walker 2005) as well as foraging efficiency in larval Atlantic cod (*Gadus morhua*) (Hunt von Herbing and Gallager, 2000). Moreover, swimming performance predicts the outcomes of cannibalistic interactions in tadpoles of green poison frog (*Dendrobates auratus*) (Wilcox and Lappin, 2013) and predator avoidance in spadefoot toad (*Spea multiplicata*) (Arendt,

2009). Therefore, *A. fulvescens* larvae in low calcium environments or with a large yolk sac could be more susceptible to predation or premature downstream drift. Furthermore, reduced swimming performance in response to environmental changes has been recently demonstrated to have fitness-related consequences in the larvae of two marine teleost species (Pimental et al., 2016).

Larval swimming performance and behaviour can be affected by many factors (Voesenek et al., 2018), including temperature (Batty and Blaxter, 1992; Green and Fisher, 2004; Hunt von Herbing, 2002), hypoxia (Kaufmann and Wieser, 1992), ontogenetic development (Fuiman and Webb, 1988; Hunter, 1972), drag coefficients (Sagnes et al., 2000), Reynolds number (i.e., viscous versus inertial forces) (Weihs, 1980; Fuiman and Webb, 1988; Hunt von Herbing, 2002; Voesenek et al., 2018;), prey density (Dabrowski et al., 1988), growth rate (Wieser et al., 1988) and fish species (Faillettaz et al., 2018). To date, no study has examined the effects of  $[Ca^{2+}]$  on the swimming performance of larval fish, despite reports of widespread declining environmental calcium concentration (Jeziorski et al., 2008; Jeziorski and Smol, 2016) and field-based relationships between environmental calcium concentration and fish production (Jonsson et al., 2011). The present study examined the effects of discrete calcium environments on the swimming performance of larval *A. fulvescens*. To this end, we employed two different swimming tests: 1) vertical swimming was used to investigate the effects of  $[Ca^{2+}]$  on travel speed and time to swimming cessation, while 2) horizontal swimming was used to investigate the interactive effects of  $[Ca^{2+}]$ , body kinematics and morphology (e.g., yolk sac volume) on sprint swimming speed. Except for the low  $[Ca^{2+}]$  treatment, average larvae covered 90-95% of the vertical cylinder prior to swimming cessation. This differed significantly from the low  $[Ca^{2+}]$  treatment where larvae only covered about 80% of the vertical cylinder prior to swimming cessation. Importantly, the effects of the low  $[Ca^{2+}]$  were only observed in the vertical swimming test where many larvae stopped swimming during the test. Data revealed no effects of  $[Ca^{2+}]$  in the horizontal swimming test where the full swimming duration was not quantified. Thus, we found no evidence that  $[Ca^{2+}]$  affects swimming speed during ongoing locomotion, but low  $[Ca^{2+}]$  reduced the time that *A. fulvescens* larvae were capable of swimming actively. These findings indicate that low  $[Ca^{2+}]$  affects swimming towards the end of a continuous swimming bout.

The fact that low  $[Ca^{2+}]$  larvae stopped swimming, or perhaps fatigued faster than the other  $[Ca^{2+}]$  treatments may indicate that the low  $[Ca^{2+}]$  modulated fatigue resistance. Although we use the term fatigue here, we acknowledge the limitations of our data, and we recommend further physiological and behavioural studies to investigate if volitionally swimming larvae are truly

fatigued when they cease swimming in a vertical cylinder test. Approaching physiological fatigue commonly involves decreasing levels of intracellular pH (Lurman et al., 2007) and muscle glycogen (Peake and Farrell, 2004) and increasing levels of lactate (Martínez et al., 2004; Peake and Farrell, 2004), inorganic phosphate (Lurman et al., 2007) and excess post exercise oxygen consumption (Svendsen et al., 2010, 2015). Studies of such variables are required before the vertical cylinder test can be recognized as a tool to examine fatigue resistance in aquatic locomotion. Likewise, it would be relevant to examine if data from vertical cylinder tests are repeatable within individual fish (Killen et al., 2016; Merrick and Koprowski, 2017; Svendsen et al., 2014). Tests of repeatability reveal if a measure is consistent across time and context within individuals. Several studies have reported that tests of aerobic and anaerobic swimming performances are repeatable within individual fish (Claireaux et al., 2007; Handelsman et al., 2010; Kolok, 1992; Kolok et al., 1998; Marras et al., 2010; Oufiero and Garland, 2009; Reidy et al., 2000), hinting that the swimming performance revealed by the vertical cylinder test could be repeatable as well, but the hypothesis remains to be tested.

Larvae from the low  $[Ca^{2+}]$  environment could be more prone to disruption of the mechanisms regulating calcium homeostasis. Internal  $[Ca^{2+}]$  is involved in many important physiological processes and will be likely influenced by available environmental  $Ca^{2+}$ . For example, the fundamental mechanism of excitation–secretion coupling involves  $Ca^{2+}$  triggering synaptic vesicle exocytosis, thereby releasing neurotransmitters contained in the vesicles and initiating synaptic transmission and neuronal connectivity (Mintz et al., 1995; Petersen and Verkhratsky, 2016; Südhof, 2012). Moreover,  $Ca^{2+}$  handling by the sarcoplasmic reticulum (SR) mediates the muscle excitation–contraction–relaxation coupling and determines contraction and relaxation rates (Seebacher and Walter, 2012). Specifically,  $Ca^{2+}$  released from the SR increases  $Ca^{2+}$  content in the cytosol and binds to troponin on the actin filaments (Berchtold et al., 2000; Frontera and Ochala, 2015) exposing attachment sites for myosin on the actin filaments and binding of myosin on actin causing cross-bridge formation and thereby muscle contraction. Muscle relaxation is facilitated by the SR calcium-ATPase actively pumping  $Ca^{2+}$  back into the SR (Frontera and Ochala, 2015; Mosca et al., 2016). When  $Ca^{2+}$  content approaches resting levels, the force declines and relaxation occurs (Berchtold et al., 2000). The discrete steps of  $Ca^{2+}$  release and resequestration as well as the availability of ATP may all constrain muscle function and swimming performance (Seebacher and Walter, 2012). In the present study, larvae from the low  $[Ca^{2+}]$  environment were therefore hypothesized to exhibit reduced swimming performance. Surprisingly, the startle horizontal sprint

swimming speed was unaffected by the  $[Ca^{2+}]$  environment, indicating that larvae maintained calcium dependent functions during swimming, even in a low  $[Ca^{2+}]$  environment. In contrast, the volitional vertical cylinder test showed that the low  $[Ca^{2+}]$  larvae stopped swimming after a shorter distance than the larvae exposed to higher  $[Ca^{2+}]$ . Studies have uncovered several mechanisms relating swimming cessation or more specifically fatigue to SR  $Ca^{2+}$  release and resequestration (Allen et al., 2008; Hostrup and Bangsbo, 2017). The SR can accumulate substantial amounts of additional  $Ca^{2+}$  without evident effect, whereas reduction of the amount of releasable  $Ca^{2+}$  in the SR adversely affects  $Ca^{2+}$  release and force production (Allen et al., 2008). Indeed, recent studies have linked muscle fatigue and  $Ca^{2+}$  release from the SR (Ørtenblad et al., 2013; Nielsen et al., 2014). The present study did not include any internal or flux  $Ca^{2+}$  measurements needed to identify the exact mechanism, however, on a whole-animal scale, *A. fulvescens* at this developmental stage have been shown to increase overall calcium influx in low calcium environments, suggesting an increased demand (Genz et al., 2014), although the distribution of absorbed calcium and the exact nature of the presumed demand remains unknown. However, we speculate that the low  $[Ca^{2+}]$  environment might constrain SR  $Ca^{2+}$  handling, or perhaps the myosin binding, such that the low  $[Ca^{2+}]$  larvae stopped swimming after covering a relatively short distance.

Body morphology also affects swimming performance of larval fishes. For example, propulsive area (Fisher et al., 2000), fineness ratio (Fisher et al., 2005) and caudal peduncle depth (Fisher and Hogan, 2007) may influence larval swimming performance. To date, few studies have examined the influence of yolk sac size on swimming performance, leading researchers to encourage further areas of study (Kopf et al., 2014; Louhi et al., 2011). The studies so far have provided inconsistent results (Kekäläinen et al., 2010; Shepherd et al., 2000), perhaps because morphological measurements were limited to two-dimensional measurements of the yolk sac. Specifically, Kekäläinen et al. (2010) estimated yolk sac volume from yolk sac length and height (i.e., no width) and reported a weak negative correlation between yolk sac volume and swimming performance. In contrast, Shepherd et al. (2000) reported a positive correlation between yolk sac area (i.e., 2D) and swimming performance. To our knowledge, the present study is the first to relate swimming performance to yolk sac volume estimated using measures of yolk sac length, height and width (i.e., 3D; equation (2)). Our data revealed a significant negative correlation between yolk sac volume and sprint swimming speed, indicating that elevated yolk sac volume constrains sprint swimming performance in larval *Acipenser fulvescens*. Thus, elevated maternal allocation (i.e., more yolk) was associated with reduced swimming performance. This is because a larger yolk sac

volume will most likely increase the cross sectional area of the organism, leading to an increase in drag forces and faster energy loss (Vogel, 1996) compared to larvae of similar sizes but with smaller yolk sacs. For example, in many coral reef fishes, the maximum swimming speed is reduced by isopod ectoparasites, because the ectoparasites elevate drag coefficients (Östlund-Nilsson et al., 2005; Binning et al., 2013). Similarly, highly pregnant Trinidadian guppies (*P. reticulata*) often exhibit reduced sprint swimming speeds, presumably because of increased drag associated with pregnancy (Banet et al., 2016; Ghalambor et al., 2004). Although these organisms are much larger than the larvae tested in the present study, we suggest that the reduced swimming speed, observed in larval *A. fulvescens* carrying a large yolk sac, is induced by elevated drag forces experienced while swimming. It is unknown if larvae with large yolk sacs, and reduced swimming performance, modify behaviours (e.g. elevated shelter use) to offset the locomotor constraints associated with increased maternal allocation. In teleosts, highly pregnant individuals may modify behaviours (i.e., elevated use of slow current habitats) to offset the locomotor constraints associated with pregnancy (Banet et al., 2016).

An evolutionary compromise or trade-off (Elgar, 1990; Ghalambor et al., 2004; Stearns, 1989) may be at play in *A. fulvescens*, where the benefits of maternal investment in elevated offspring body condition (i.e., more yolk) are partly countered by the cost of reduced swimming performance. In a similar example, Oufiero et al. (2012) suggested that a superior physiological condition in sword tails (*Xiphophorus hellerii*) with an unusually long sword compensates for the negative effect of the sword on swimming performance. Resource allocation trade-offs play an important role in life history theory (Stearns, 1989; Zera and Harshman, 2001) such that fish with larger yolk sac could spend a longer period of time prior to emergence (i.e., longer yolk absorption period), thus resulting in a bigger body size at emergence. This, in turn, might increase the probability of survival as smaller individuals are often selected against (Rosenburg and Haugen, 1982; Einum and Fleming, 2000). On the other hand, in more harsh environments (Fischer et al., 2011; Segers and Taborsky, 2011), where conditions in the substrate might deteriorate and larvae are forced to leave (Louhi et al., 2011), a large yolk sac may decrease swimming performance and perhaps elevate mortality until favourable substrate is located.

How the interaction between large yolk sac and low calcium environments might influence *A. fulvescens*, or any other fish species, is currently unknown. More studies are needed to understand the phenotypic plasticity of early ontogeny under inherent biological attributes and induced environmental changes because different phenotypes could influence the demographics of

various populations and species. This remains particularly important to understand for imperiled species, including many members of the Acipenseridae family.

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## Tables

**Table 1**

Water quality parameters of treatment waters used for rearing of larval lake sturgeon (*Acipenser fulvescens*) in control, low-, medium-, and high-environmental-[Ca<sup>2+</sup>] treatments.

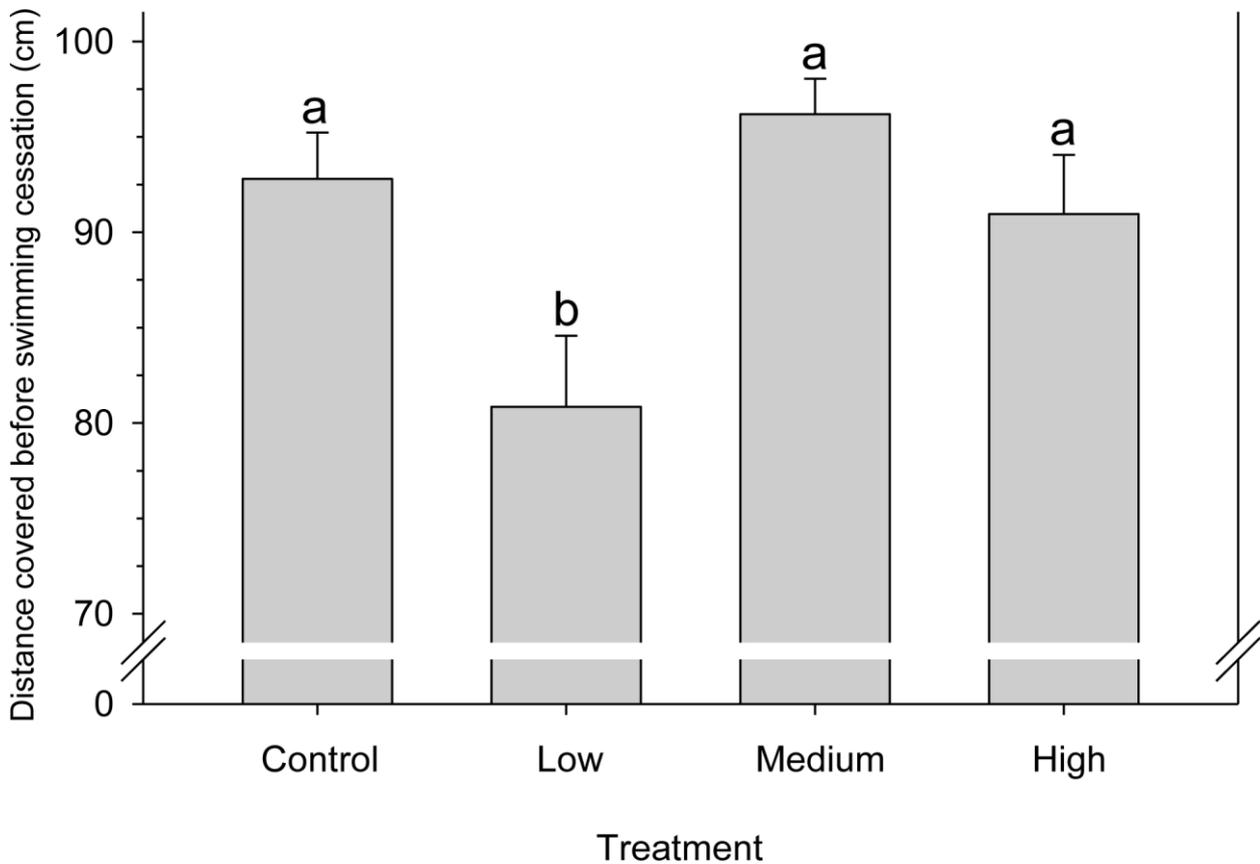
Parameter	Control	Low [Ca <sup>2+</sup> ]	Medium [Ca <sup>2+</sup> ]	High [Ca <sup>2+</sup> ]
Total CO <sub>2</sub> (mmol l <sup>-1</sup> )	0.67 ± 0.09	0.31 ± 0.08	0.41 ± 0.1	0.41 ± 0.11
Osmolality (mOsm)	3.86 ± 1.25	2.29 ± 0.86	2.43 ± 0.93	8.00 ± 1.16
pH	7.75 ± 0.05	7.71 ± 0.04	7.68 ± 0.04	7.56 ± 0.04
Temperature (°C)	11.19 ± 0.55	11.35 ± 0.54	11.58 ± 0.48	11.59 ± 0.49
Na <sup>+</sup> (mmol l <sup>-1</sup> )	1.682 ± 0.244	0.655 ± 0.070	0.632 ± 0.086	1.031 ± 0.180
K <sup>+</sup> (mmol l <sup>-1</sup> )	0.313 ± 0.064	0.165 ± 0.031	0.108 ± 0.026	0.184 ± 0.161
Ca <sup>2+</sup> (mmol l <sup>-1</sup> )	0.345 ± 0.009	0.174 ± 0.020	0.223 ± 0.009	1.709 ± 0.327

**Table 2**

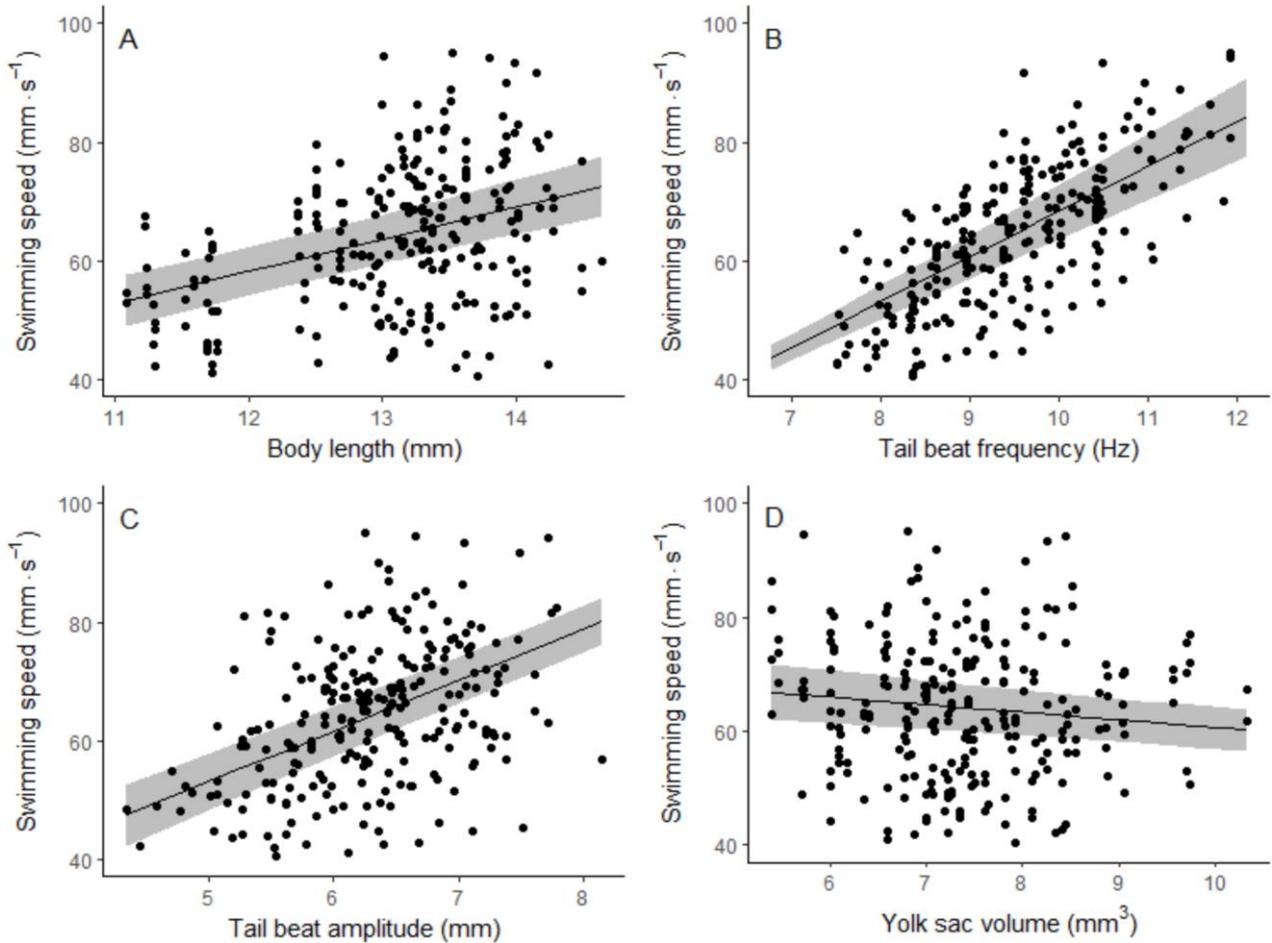
Linear mixed model parameter estimates for terms that were found significant using likelihood ratio tests. Swimming speed of individuals was a function of a common intercept ( $\alpha$ ), yolk sac volume (V), body length (L), tail beat frequency (fq) and tail beat amplitude (amp).  $\sigma_a^2$  and  $\sigma^2$  represent the variance associated with the random intercept and the residuals, respectively.

Parameter	Estimate	S.E.	Likelihood ratio	P-value
$\alpha$	-122.72398	9.724401	103.2131	<0.0001
V	-1.34766	0.539637	6.263945	0.0123
L	5.41273	0.679113	50.62374	<0.0001
fq	7.60075	0.344439	270.5962	<0.0001
amp	8.56544	0.539862	174.7058	<0.0001
$\sigma_a^2$	4.161507	NA	NA	NA
$\sigma^2$	4.410238	NA	NA	NA

## Figures



**Figure 1.** Vertical swimming behaviour (mean  $\pm$  s.e.m.) of larval lake sturgeon *Acipenser fulvescens* is affected by environmental calcium. *A. fulvescens* were tested in four different calcium treatments: control, low, medium and high environmental calcium. Using a vertical distance of 100 cm, the figure shows the distance swum actively (i.e., beating the tail) before swimming cessation in *A. fulvescens*. Larval *A. fulvescens* reared in low calcium swam a significantly shorter distance before swimming cessation compared to the other treatments (n = 42 per treatment). This result shows that the low calcium environment was associated with reduced volitional swimming performance. Different letters indicate significant ( $P < 0.05$ ) differences between treatments.



**Figure 2.** Raw data points superimposed on model visualization showing the effects of each covariate on sprint swimming speed. Data show that sprint swimming speed in larval lake sturgeon *Acipenser fulvescens* is affected by body length and body kinematics as well as yolk sac volume ( $P < 0.05$ ). Sprint swimming speed correlated positively with body length (A), tail beat frequency (B) and tail beat amplitude (C) and negatively with yolk sac volume (D) ( $n = 20-25$  per treatment). The grey area associated with each model indicates the 95% confidence interval. Data revealed no effects of the environmental calcium treatments. Note that each regression line is conditional on the other covariates being at their respective mean values.