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The effect of temperature and substrate on the growth, development and survival of larval white sturgeon

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ABSTRACT

White sturgeon yolk sac larvae (YSL) were reared at 13.5 and 17.5 °C with and without gravel substrate. Larvae reared within the gravel emerged from the substrate after 11–14 days (depending on temperature), and all larvae were subsequently fed in bare tanks until 46 days post hatch (dph). Temperature and substrate significantly affected size; at 46 dph, fish reared in gravel at 17.5 °C were the largest (288 ± 19 mg), while fish reared at 13.5 °C without gravel were the smallest (107 ± 3 mg). Yolk absorption rate did not differ between substrate treatments but was greater at 17.5 °C than at 13.5 °C. In contrast, yolk absorption efficiency was independent of temperature but was significantly greater in gravel-reared larvae. YSL reared in gravel also had more lipid vacuoles in their liver. Substrate and temperature significantly affected survival. Greatest survival (84.6% ± 0.6%) was achieved when YSL were reared in gravel at 13.5 °C, and survival was lowest (46.6% ± 0.6%) when larvae were reared without gravel at 17.5 °C. Understanding factors that affect growth and survival during early life history provides insight into factors affecting wild recruitment and should improve hatchery production.

1. Introduction

White sturgeon (*Acipenser transmontanus*) are native to western North America and are found in the Sacramento, Columbia and Fraser Rivers. Within British Columbia, six populations have been recognized, four of which are listed as endangered under Canada's Species At Risk Act. The primary reason for population decline in three of these populations is recruitment failure (COSEWIC, 2013).The cause of low recruitment is not well understood, and the lack of a detailed knowledge of the behavior, ecology and habitat requirements for the early life history of these species continues to be a key limitation. Early life history mortality, prior to juvenile metamorphosis, is a major survival bottleneck for many species (Houde, 1987; May, 1974), and high larval mortality can be particularly acute during the transition between endogenous to exogenous feeding. This period is commonly viewed as critical, and survival can significantly affect recruitment (Houde, 1987). The size of fish when they begin exogenous feeding has also been cited as an important factor affecting survival and recruitment (Cushing, 1972). Based on the link between substrate changes and recruitment failure (McAdam et al., 2005), understanding the effect of environment on the growth and survival of larval sturgeon provides important information for understanding factors that affect recruitment.

Newly hatched sturgeon larvae have been described as exhibiting a swim-up and drift dispersal behavior (Conte et al., 1988; Kynard and Parker, 2005; Richmond and Kynard, 1995). Many prior studies, however, were conducted in the absence of suitable substrate or were gathered from drift net data from highly regulated rivers and may not reflect the behavior of larvae in a natural environment (McAdam, 2011). When cover has been provided, the sturgeon yolk sac larvae (YSL) of multiple species have been shown to use it. For example, Atlantic sturgeon (*Acipenser oxyrinchus*), shortnose sturgeon (*A. brevirostrum*) and white sturgeon (*A. transmontanus*) all seek cover shortly after hatch (Kynard and Horgan, 2002; McAdam, 2011). Comparison among substrates shows that both shortnose sturgeon and white sturgeon displayed drift behavior when YSL were prevented from seeking cover (McAdam, 2011; Richmond and Kynard, 1995). Gessner et al. (2009) not only found that larvae continued to swim until adequate substrate was found but also found that survival was higher when YSL were reared in gravel. Gravel substrate has also been shown to provide cover that reduces mortality due to piscine predation, including from benthic predators such as sculpin (Gadomski and Parsley, 2005; McAdam, 2011). Gravel substrates have long been known to be integral to the life histories of many riverine fish species, particularly for early life stages. Substrates have often been used in salmonid hatcheries in the culture of alevins, as this generally produces larger fry than rearing methods without substrate (Fuss and Johnson, 1982; Peterson and Martin-Robichaud,

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1995). Substrate may also play a significant role in the early life history of sturgeon, and a few studies have attempted to evaluate the potential effect of gravel rearing on sturgeon recruitment (Gadomski and Parsley, 2005; Gessner et al., 2009; McAdam, 2011; McAdam et al., 2005).

Temperature is also an important factor affecting the physiological function of ectotherms (Blaxter, 1992; Rombough, 1996), and changes in temperature will affect enzyme activity, metabolic rate, growth, development and even locomotory function (Fry, 1971). Little is known about the effects of temperature on the early life history of sturgeon, particularly larvae. Wang et al. (1987) found that egg incubation in Sacramento River white sturgeon was possible between 10 °C and 18 °C, with greatest survival to hatch between 14 °C and 16 °C. Van Eenennaam et al. (2005) found a similar thermal tolerance and optima for green sturgeon (*A. medirostris*) YSL and feeding larvae (FL). A study by Hardy and Litvak (2004) found that the survival of Atlantic and shortnose sturgeon FL was greater at lower temperatures, but growth increased with warmer temperature. As sturgeon generally hatch on a declining hydrograph after spring freshet, temperature can be expected to increase dramatically during their first month. The effect of temperature on growth and survival, therefore, may strongly influence year-class strength.

It is important to understand the effect of rearing environment on larval sturgeon development, as this information is vital for effective hatchery practices, particularly in conservation aquaculture, but also for habitat restoration to enhance natural propagation. Yet the interaction between substrate and temperature on white sturgeon YSL during incubation has not been examined previously. The objective of this study, therefore, was to determine how substrate and temperature influence growth, efficiency of endogenous energy use (rate of yolk absorption) and survival from hatch until 46 dph for the white sturgeon, a species listed as endangered in British Columbia, Canada.

2. Methods

2.1. White sturgeon larvae and broodstock

Broodstock from the Nechako River were caught in May 2009. To induce ovulation, female sturgeon were injected intramuscularly with mammalian GnRH analogue [d-Ala^6, Pro^9, NEthylamide]-mGnRH dissolved in physiological saline to induce ovulation using an initial dose of 5 μg/kg and a resolving dose of 45 μg/kg, 24 h in advance of the anticipated time of ovulation.

Eggs were collected and de-adheased as described by Conte et al. (1988). The eggs were evenly divided into three separate bowls and individually mixed with milt from three males (one male per bowl). Fertilized eggs from each cross were incubated separately at 15 °C in McDonald jars (J30, Aquatic Eco-Systems, Apopka, FL) in a streamside hatchery at Vanderhoof, British Columbia (operated by Freshwater Fisheries Society of British Columbia). All experiments were conducted in a separate field laboratory and maintained on a simulated natural photoperiod. YSL were transferred to experimental tanks at approximately 1 day post hatch (dph), which corresponded to 8 days post fertilization and 120 accumulated thermal units (ATU). Each tank contained 450 YSL, composed of 150 from each of three half-sibling family groups. Newly hatched fish were placed in plastic bags, floated on top of the experimental tanks and allowed to acclimate for 30 minutes before introducing the fish to the tanks.

2.2. Experimental tanks

Sturgeon were reared at two temperatures referred to as cool (13.5 °C ± 0.1 °C) and warm (17.5 °C ± 0.1 °C). These temperatures are representative of those experienced by sturgeon at hatch and shortly afterward in the Nechako River at Vanderhoof. Non-chlorinated municipal water (10.5 °C to 11.5 °C) was continuously added to each temperature system (680 L) to allow for partial exchange; 6%–9% of the water was exchanged per hour. Targeted temperatures were maintained in each head tank using multiple aquarium coil heaters (Hagen, Fluval Tronic, A-770, Montreal, QC). Temperature was measured hourly (Hobo water temperature pro V2, Onset, Bourne MA). A canister filter (Hagen Fluval 405) was placed in each header tank to maintain quality of recirculated water. Water quality measurements (dissolved oxygen, pH, temperature, ammonia and nitrates/nitrites) were also monitored daily throughout the experiment.

Experimental tanks were 36 × 25 × 20 cm (L × W × H) Rubbermaid tubs (Roughneck 2213, Oakville, ON). Water was supplied to each tank from a header and manifold system and drained from the tank sides through holes covered with 750 μm Nitex screening (Sefar, Heiden, Switzerland). Each tank was placed into a larger Rubbermaid tote (Roughneck 2547), and water was allowed to overflow into the tote. Water was pumped from the totes back into the header using a submersible pump (Little Giant 4E-34NR, Bluffton, IN). Two substrate treatments were used: gravel (3 cm depth) and no substrate (bare conditions). The gravel substrate and the grain size used were based in part on the findings of a white sturgeon YSL substrate preference study by Bennett et al. (2007) and from results of our previous work (Boucher and McAdam, unpublished). A mixture of gravel was used ranging in size from 12 to 22 mm on the longest axis. A preliminary study showed that deeper substrates sometimes trapped YSL; therefore, relatively shallow substrate depths (~3 cm) were used. Four replicate tanks were used per substrate treatment per temperature.

YSL (450) were introduced into each tank just after hatch (within 24 h) and monitored until 46 dph. Sturgeon YSL exhibit negative phototaxis (Conte et al., 1988; Loew and Sillman, 1998) and to minimize disturbance, tanks were partially covered with a dark plastic lid. All dead fish were removed immediately upon detection and recorded to calculate survival rates among treatments. Sturgeon reared in tanks with substrate emerged from the gravel to initiate exogenous feeding when the yolk was absorbed (approximately 330 ATU). After that time, FL from all treatments were transferred to bare tanks and fed a combination of equal parts EWOS zero (EWOS Canada Ltd., Surrey, BC), powdered krill and Cyclop-eeze (Argent Chemical Laboratories, Redmond, WA). All larvae were fed to satiation twice daily. All sampling procedures were approved by the University of Northern British Columbia Animal Care and Use Committee.

2.3. Size, condition factor and growth

Weight (mg) and total length (mm) were determined for 8 larvae from each tank every 4 days. All fish were terminally anesthetized in 200 mg·L^{-1} tricaine methane sulfonate buffered with 400 mg·L^{-1} sodium bicarbonate. Total length was measured for each fish using digital calipers viewed under a dissecting microscope and weight determined to 0.1 mg. All fish were patted dry using paper towel prior to being weighed. After length and weight measurements, fish were preserved in 10% phosphate buffered formalin for histological analysis. Data were not collected for weight and length from warm water treatments at 43 dph (872.5 ATU). Condition factor was calculated as $K = 100(\mathrm{W}\cdot\mathrm{L}^{-3})$. The difference in growth rate for the two treatments was estimated using the temperature coefficient, Q_{10}, calculated from specific growth rate estimates using the following equation:

$$Q_{10} = \left(\frac{SGR_2}{SGR_1}\right)^{10/(T_2 - T_1)}$$

where SGR is the specific growth rate and T is the temperature. Specific growth rate was calculated using the following equation:

$$\mathrm{SGR} = 100\left(\frac{\ln W_{t_2} - \ln W_{t_1}}{t_2 - t_1}\right)$$

where W is weight at 46 dph (t_2) and initial weight at hatch (t_1).

2.4. Yolk absorption

Yolk absorption rate (YAR) and yolk absorption efficiency (YAE) were assessed using a protocol similar to Hardy and Litvak (2004). Four to eight fish from each treatment were sampled for weight (mg) and yolk sac area (mm^2) every 4 days from hatch until yolk was completely absorbed. Hardy and Litvak (2004) measured YAR from changes in volume over time; instead, we calculated YAR from changes in yolk sac area (YSA) over time. YSA was used in place of volume, as it was a direct measurement. Images of yolk sacs were taken using a digital camera (Canon PowerShot G5, Lake Success, NY) at 100× magnification with a light microscope (Zeiss Axiostar Plus, Oberkochen, Germany). YSA was measured using ImageJ (Rasband, 2010, version 1.43r, Bethesda MD) by tracing the outside edge of the yolk sac. A slide micrometer was used at the same magnification to calibrate area calculations in ImageJ. YAE for each treatment was calculated by expressing the change in mean weight of larvae divided by YAR for each treatment. Mean values of YAR and YAE were calculated from hatch to 8 dph (260 ATU) for the warm temperature groups and from hatch to 8 and to 12 dph (228 and 282 ATU; mean 255 ATU) for the cool temperature groups. The mean of values from 8 and 12 dph were used in the calculations for the cool temperature groups as it achieved a similar ATU value to the warm temperature group.

2.5. Histology

Whole-body histology was used to examine presence or absence of yolk and liver structure (Wax-it Histology Services Inc., Vancouver BC). Fish were dehydrated in graded ethanol and embedded in paraffin, sectioned at 4 μm and stained using hematoxylin and eosin. For each fish, 10 sagittal sections were taken longitudinally about the center of the fish, five on either side. Slides were examined with a light microscope (Zeiss Axiostar Plus, Oberkochen, Germany) and photos taken at 1000× magnification using a digital camera (Canon PowerShot G5, Lake Success, NY).

Hepatic lipid was compared between treatments for four fish collected at 8, 12, 16, 24 and 32 dph. Areas for lipid vacuoles in 1000× magnification images were expressed as the percent of the total liver area using a calibrated grid overlay in ImageJ. Grids containing lipid vacuoles were counted and area expressed as a percentage of total area in each slide. If a grid square was partially filled with lipid, the square was further divided into quarters and each quarter was counted as lipid if >50% of the area was occupied by lipid.

2.6. Statistical analysis

We used a multilevel mixed-effects linear regression model (xtmixed; STATA version 12, College Station TX) with observations nested in tank to account for the change in each variable (weight, *K*, yolk area and hepatic lipid vacuole area) throughout the experiment using either days post hatch (dph) or accumulated thermal units (ATU). For weight, *K* and liver lipid, we modeled data for samples collected after fish had begun exogenous feeding (330 ATU). Days post hatch or ATU were modeled as linear or quadratic terms as appropriate dependent on distribution of residual plots. Two-way analysis of variance (ANOVA) was used to test for significant differences between substrate and temperature treatments on SGR, YAR and YAE. A *t*-test was used to assess differences between the substrate treatments for Q_{10}.

3. Results

3.1. Emergence

Larvae emerged from gravel between 9 and 11 dph at 17.5 °C (278–313 ATU) and between 13 and 15 dph at 13.5 °C (296–323 ATU). YSL in bare tanks were observed to swim near the surface until 5 dph at 17.5 °C (208 ATU) and 6 dph at 13.5 °C (201 ATU), before becoming more benthic oriented, when they rested on the bottom tightly grouped together in a dense cluster. When lights were turned out, YSL that were grouping together began to spread out and swim throughout the water column (at 295 ATU in both temperatures). Larvae in gravel treatments generally remained within the gravel and were rarely observed in the water column prior to emergence. Melanin plugs were shed between 11 and 12 dph (313 to 330 ATU) at 17.5 °C and between 15 and 16 dph (323 to 336 ATU) at 13.5 °C. Larvae in all tanks actively took feed starting at 12 dph at 17.5 °C (330 ATU) and 16 dph at 13.5 °C (336 ATU).

3.2. Size, condition factor and growth

The size of sturgeon was affected by substrate and temperature; fish reared in substrate were larger than fish reared in bare tanks and warm water reared fish were larger than cool water reared fish (Fig. 1). Differences in weight among the treatment groups occurred early and continued throughout the experiment. Our regression model indicated that there was a significant effect of temperature ($p < 0.001$) and substrate ($p < 0.001$) for weight, but the interaction was not significant ($p = 0.090$) when using a quadratic relationship to describe dph; both the linear and squared terms for dph were significant. The positive effect of substrate presence on weight was similar in magnitude to that of warm temperature, as fish reared in the gravel cool treatment were similar in size to the bare warm treatment (Fig. 2a). Weight plotted as a function of thermal experience (ATU), however, indicated little difference between fish reared with similar substrates (Fig. 2b). In order to satisfy the assumptions of equal variances and normally distributed errors, ATU was transformed using a quadratic function. The regression model for fish with ATU indicated that temperature was not significant ($p = 0.062$), but substrate ($p < 0.001$) and the interaction ($p < 0.05$) were significant. Both the linear and quadratic terms for ATU were also significant. The significant interaction between temperature and substrate may reflect the greater range of ATU values for the warm treatment groups (330 to 925 ATU for warm groups compared to 336 to 741 ATU for cool groups) and the possible change in pattern of growth at higher ATU. When we constrained the analysis to samples collected between 330 and 750 ATU (in order to more closely match the ATU range of the cool groups), the regression model indicated that temperature was not significant ($p = 0.816$), nor was the interaction ($p = 0.579$), just substrate ($p < 0.001$).

Condition factor (*K*) declined as the fish absorbed their yolk sac and then increased after the fish began to feed exogenously (~15 dph or 330 ATU). Fish from gravel treatments generally had higher *K* for their age compared to those without gravel (Fig. 3a), and by 46 dph, sturgeon reared in cool temperatures had 7% higher *K* than warm reared sturgeon. Regression analysis indicated that both substrate ($p < 0.001$) and temperature ($p = 0.045$) effects were significant, but their interaction was not significant ($p = 0.738$). Differences in *K* were less clear when plotted as a function of ATU (Fig. 3b), although there were significant differences for temperature ($p < 0.001$) and substrate ($p < 0.001$), but no interaction effect ($p = 0.756$), and the linear term for ATU was not significant, although the quadratic term was significant.

SGR for weight were 22% lower in the cool treatments than the warm treatment groups; $5.44 \pm 0.31\% \cdot d^{-1}$ and $4.42 \pm 0.21\% \cdot d^{-1}$ for warm treatments in gravel and bare tanks, respectively, and $4.37 \pm 0.08\% \cdot d^{-1}$ and $3.31 \pm 0.11\% \cdot d^{-1}$ for cool treatments in gravel and bare tanks, respectively. The effects of substrate and temperature were significant ($p < 0.01$), but there was no interaction effect ($p = 0.85$). Q_{10} calculated for weight SGR was not significantly different between treatments with and without gravel ($p = 0.16$); Q_{10} for larvae reared in bare and gravel treatments was 2.08 ± 0.17 and 1.74 ± 0.12, respectively.

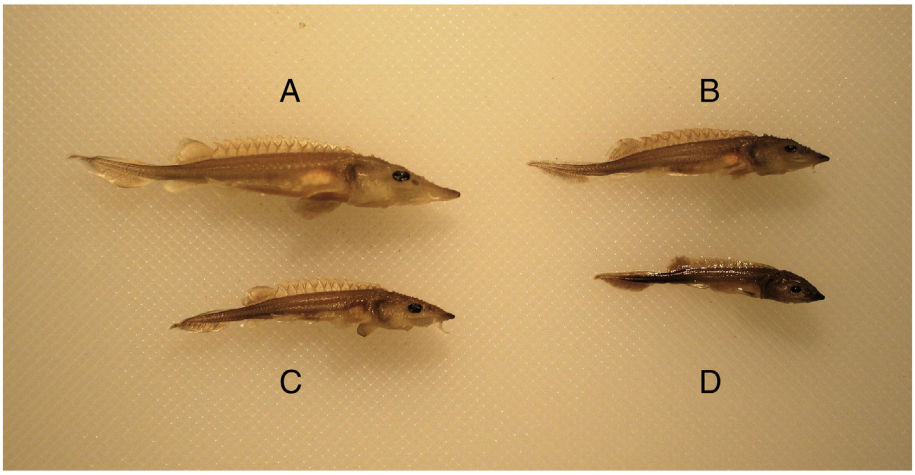

Fig. 1. Representative white sturgeon at 46 dph from all treatments. (A) Gravel 17.5 °C, (B) bare 17.5 °C, (C) gravel 13.5 °C and (D) bare 13.5 °C. (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)

3.3. Yolk absorption

Representative histological sections of yolk and gut from white sturgeon at 12 dph stained with hematoxylin and eosin are shown in Fig. 4. Yolk was absorbed at an earlier date for larvae reared at 17.5 °C compared to 13.5 °C. Yolk had completely disappeared in larvae from gravel and bare treatments at 17.5 °C by 12 dph, but it was not until 16 dph that yolk was absent in the 13.5 °C treatments (Fig. 5a). The date of emergence from substrate occurred following yolk depletion and consequently the date fish first showed interest in taking exogenous feed. Regression analysis for yolk area change with time (dph) indicated a significant effect of temperature ($p < 0.001$), but not substrate ($p = 0.371$), and there was no interaction ($p = 0.392$) when using a linear relationship to describe dph. When yolk area was plotted as a function of ATU, there was still a difference between the temperature groups (Fig. 5b). Our regression model showed that the difference in temperature was significant ($p < 0.005$), with no difference between gravel and bare substrate treatments ($p = 0.293$) and no interaction ($p = 0.325$); the linear term for ATU was also significant. We also calculated YAR at ~260 ATU, and our finding was consistent with the regression analysis; YAR was significantly greater for fish in the warm temperature groups than cool temperature groups ($p < 0.001$), and there was no difference between gravel and bare substrate treatments ($p = 0.787$; Table 1). In contrast, YAE was significantly greater in larvae reared in gravel substrate than bare tanks ($p < 0.001$), but not temperature ($p = 0.812$); however, the interaction term was significant ($p < 0.05$; Table 1).

3.4. Liver vacuole area

Fig. 6 shows longitudinal sections of livers from sturgeon reared at 17.5 °C sampled at different times during incubation. Lipid vacuoles decreased in size as the fish developed but consistently appeared larger for fish from the gravel substrate treatment; this pattern was similar for the sturgeon reared at 13.5 °C (images not shown). Quantitative analysis revealed that lipid vacuoles comprised a greater proportion of liver area from YSL white sturgeon reared in gravel compared to bare treatments. Sturgeon in warmer groups and with no substrate showed decreased

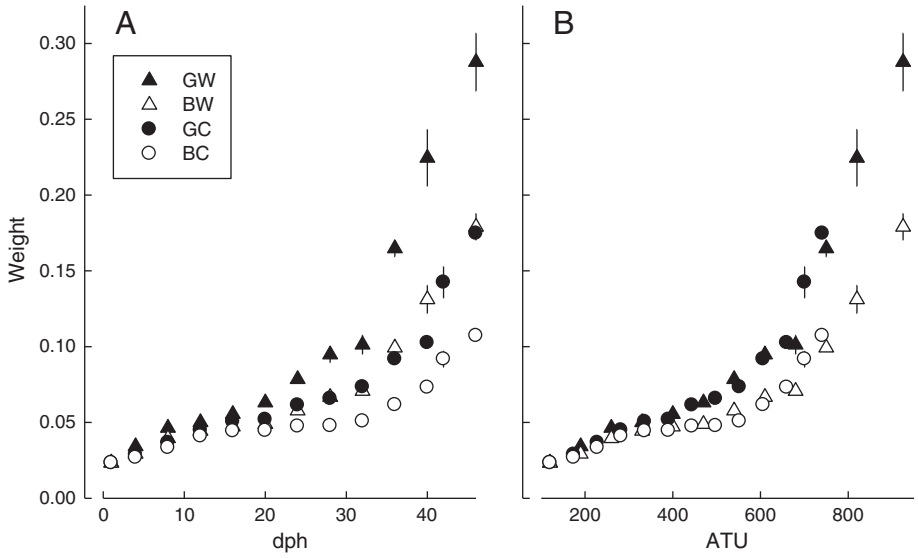

Fig. 2. Mean wet weight (mg) of white sturgeon plotted against (A) days post hatch (dph) and (B) accumulated thermal units (ATU), reared with and without gravel during the first ~300 ATU and at two different temperature regimes; 13.5 °C (cool) and 17.5 °C (warm). GW is gravel warm, BW is bare warm, GC is gravel cool and BC is bare cool. $N = 4$ replicates, 8 fish per replicate. Error bars represent ± 1 standard error.

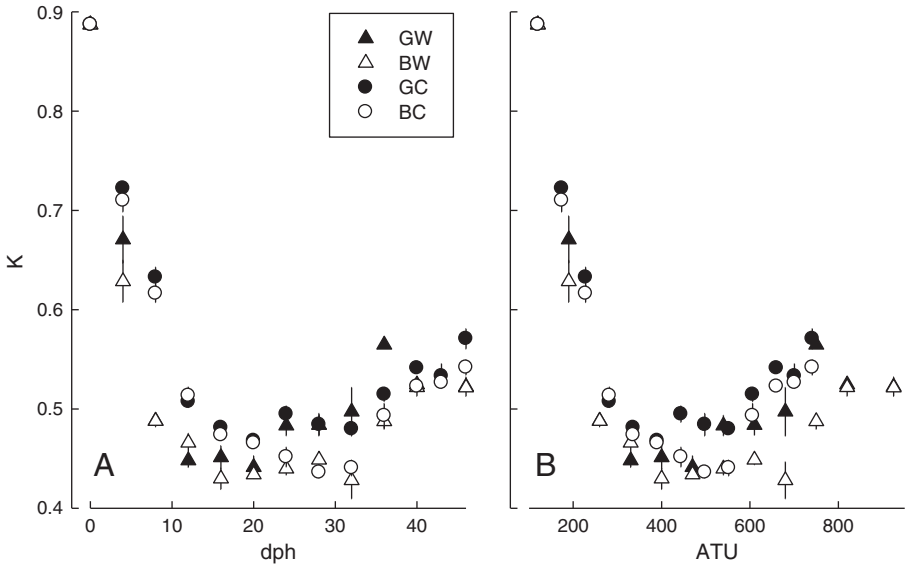

Fig. 3. Mean condition factor (K; mg·mm^{-3}) of white sturgeon plotted against (A) days post hatch (dph) and (B) accumulated thermal units (ATU), reared with and without gravel during the first ~300 ATU and at two different temperature regimes. Symbols described in Fig. 2. Error bars represent ± 1 standard error.

liver lipid vacuole area at an earlier development stage (Fig. 7a). A large decline (>50%) appeared coincident with the onset of exogenous feeding in all groups. Our regression model indicated that lipid area was significantly greater for the gravel treatment groups than bare ($p < 0.001$) but was not affected by temperature ($p = 0.243$), and there was no interaction ($p = 0.273$); the linear and quadratic terms for dph were both significant. Findings were similar in our regression model with ATU (Fig. 7b); substrate was significant ($p < 0.001$), but not temperature ($p = 0.085$) or the interaction ($p = 0.262$); the linear and quadratic terms for ATU were both significant.

3.5. Survival

Daily mortality was low in all treatments until approximately 15 dph (330 ATU; just after first feeding) (Fig. 8). Those reared at 17.5 °C showed a sharp increase in mortality at first feeding and emergence, a

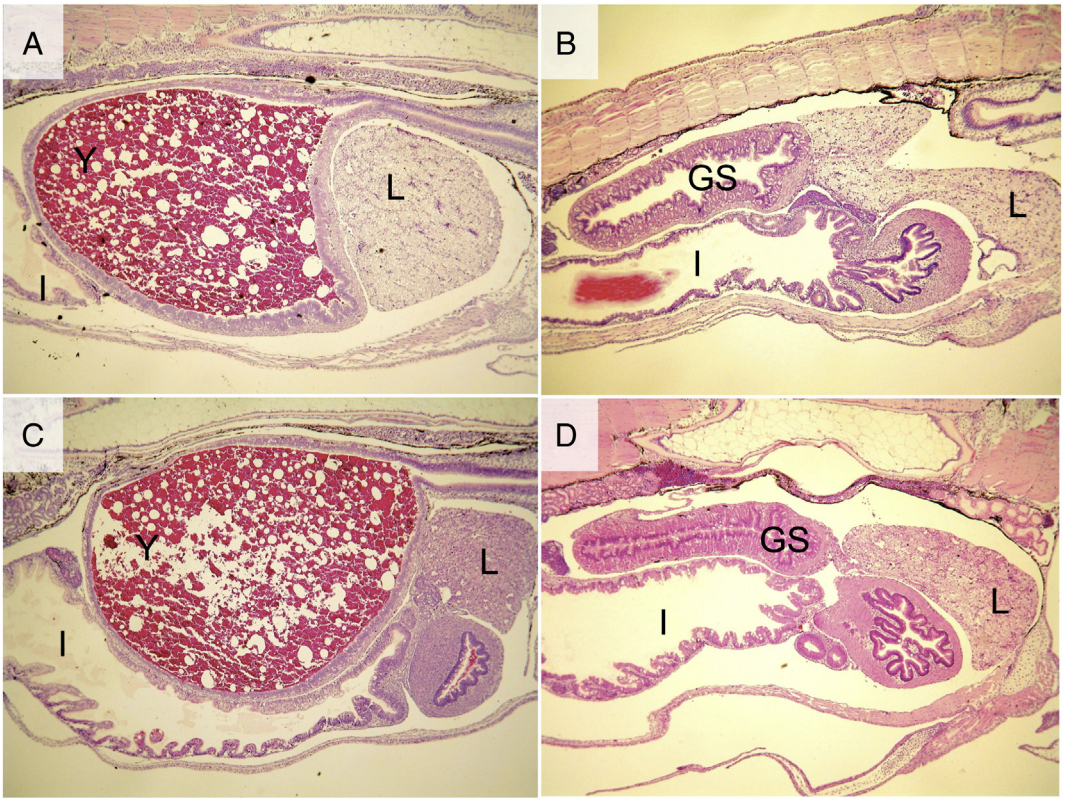

Fig. 4. Longitudinal histological sections of the gut of white sturgeon at 12 dph from all treatments stained with hematoxylin and eosin: (A) gravel at 13.5 °C, (B) gravel at 17.5 °C, (C) bare at 13.5 °C and (D) bare at 17.5 °C. L—liver, I—intestine, GS—gastric stomach, Y—yolk. All images are the same magnification and scale bar represents 500 μm. (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)

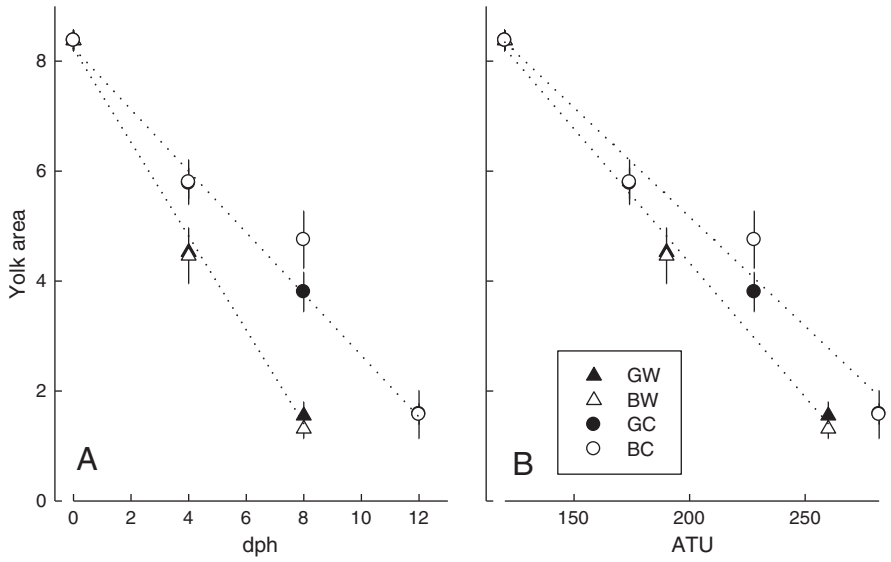

Fig. 5. (A) Yolk sac area (mm^2) over time (dph) of white sturgeon reared in gravel and without gravel at 13.5 °C and 17.5 °C from hatch to 12 dph. (B) Yolk sac area plotted against ATU. Symbols described in Fig. 2. Error bars represent ± 1 standard error.

change greater than seen for fish reared at 13.5 °C. While mortality was greater after emergence and first feeding in all groups, there was no discernable spike in mortality in the 13.5 °C treatments. Most mortality (61.8%–64.5% of total mortality) in warm treatments occurred between 11 and 18 dph. While temperature greatly affected daily mortality, YSL reared within substrate generally had lower daily mortality. Consequently, cumulative survival differed with temperature and substrate treatments. At 46 dph, survival was greatest (84.6%) for the gravel cool treatment and lowest (46.6%) when reared in the warm bare tanks (Fig. 8a). At the end of the experiment (46 dph), survival was similar between warm gravel tanks and cool bare tanks (66.1% and 66.9%). Unlike the growth data, however, the survival curves do not overlap when plotted by ATU (Fig. 8b).

4. Discussion

Provision of gravel as substrate for rearing larval white sturgeon from hatch until the onset of exogenous feeding was beneficial for all indicators examined. The presence of substrate during a relatively short time during incubation (11–14 days, depending on temperature) led to greater growth and survival, and differences persisted after transfer of larvae from the substrate treatments to bare tanks. Gravel rearing of salmonid alevins has long been known to produce larger "swim-up" fry than conventional culturing practices where no substrate was used (Bams, 1967; Marr, 1966; Peterson and Martin-Robichaud, 1995). The benefits of rearing in gravel substrate on growth and survival have generally been attributed to an observed (but not quantified) reduction in locomotory activity (swimming and moving) (Bams, 1967; Marr, 1966; Peterson and Martin-Robichaud, 1995). Greater swimming activity of larval sturgeon was also observed in bare tanks in the present study and by Gessner et al. (2009) for larval Atlantic sturgeon. Locomotor activity may explain the difference in the size of the fish between substrate treatments, as significant trade-offs between growth and activity can occur during the endogenous feeding period (Brett and Groves, 1979). As yolk reserves are finite and limited (Bams, 1967; Brett and Groves, 1979; Marr, 1966), increased movement of YSL in bare tanks would increase energy used to fuel locomotion rather than growth, compared to fish reared in gravel. Stress has been shown to significantly influence the growth of fish (McCormick et al., 1998) and may also contribute to the observed growth differences between treatments. Bates (2011) measured whole body cortisol levels in fish used in the present study and found that the bare treatment had higher cortisol than fish in gravel, which may offer an additional explanation for differences in length and weight between substrate treatments. Similarly, Zubair et al. (2012) found that with the onset of exogenous feeding, basal cortisol levels were greater in larval lake sturgeon reared without gravel than those reared with gravel.

The identification of temperature effects on growth is not surprising since temperature directly affects biochemical reaction rates, metabolic requirements for food and rate of food processing (Brett and Groves, 1979). A reduction in temperature of 4 °C reduced specific growth rates by 20%–25%, but for our study, Q_{10} for growth did not differ for fish reared in gravel compared to bare treatments. This type of extrapolation of a Q_{10} calculated at a whole organism level (growth) is difficult to make without a biochemical rate to support it; therefore, it may only be used as an indicator of temperature sensitivity of larval white sturgeon and cannot give any definitive information about biochemical rates (Chaui-Berlinck et al., 2004). The lack of a difference in weight within each substrate treatment for fish at the same ATU indicates that ATUs can be used to predict the growth of larval sturgeon (depending on rearing environment) similar to salmonids (Peterson and Martin-Robichaud, 1995) and marine teleosts (Pepin, 1991).

The large decrease in *K* from hatch to about 330 ATU occurs during the period of yolk absorption, reflecting a developmentally determined non-linear length-weight relationship. Although the effect of temperature on *K* was significant, there appeared to be little difference between fish from warm and cool tanks until about 600 ATU when cool groups appear to have higher *K* than warm groups. While the relationship between temperature and *K* appears to vary with developmental stage, the effect of substrate was much clearer, gravel-reared fish had significantly higher *K* than those reared in bare tanks. Similar effects have

Table 1
Yolk absorption rate (YAR; $mm^2 \cdot d^{-1}$) and yolk absorption efficiency (YAE; $mg \cdot mm^{-2}$) for white sturgeon reared in gravel and without gravel at 13.5 °C and 17.5 °C. Mean values were calculated for cool temperature groups at 8 and 12 dph (228 and 282 ATU; mean 255 ATU) and for warm temperature groups at 8 dph (260 ATU). Data are presented as mean ± SD. One to two fish were analyzed from four tanks for each treatment. Values with a common letter do not differ significantly.

Substrate	Temperature (°C)	YAR ($mm^2 \cdot d^{-1}$)	YAE ($mg \cdot mm^{-2}$)
Gravel	Warm	0.856 ± 0.075^{a}	33.9 ± 3.2^{a}
Bare	Warm	0.887 ± 0.059^{a}	22.9 ± 1.6^{b}
Gravel	Cool	0.572 ± 0.050^{b}	30.3 ± 2.9^{ab}
Bare	Cool	0.521 ± 0.127^{b}	27.2 ± 5.3^{ab}

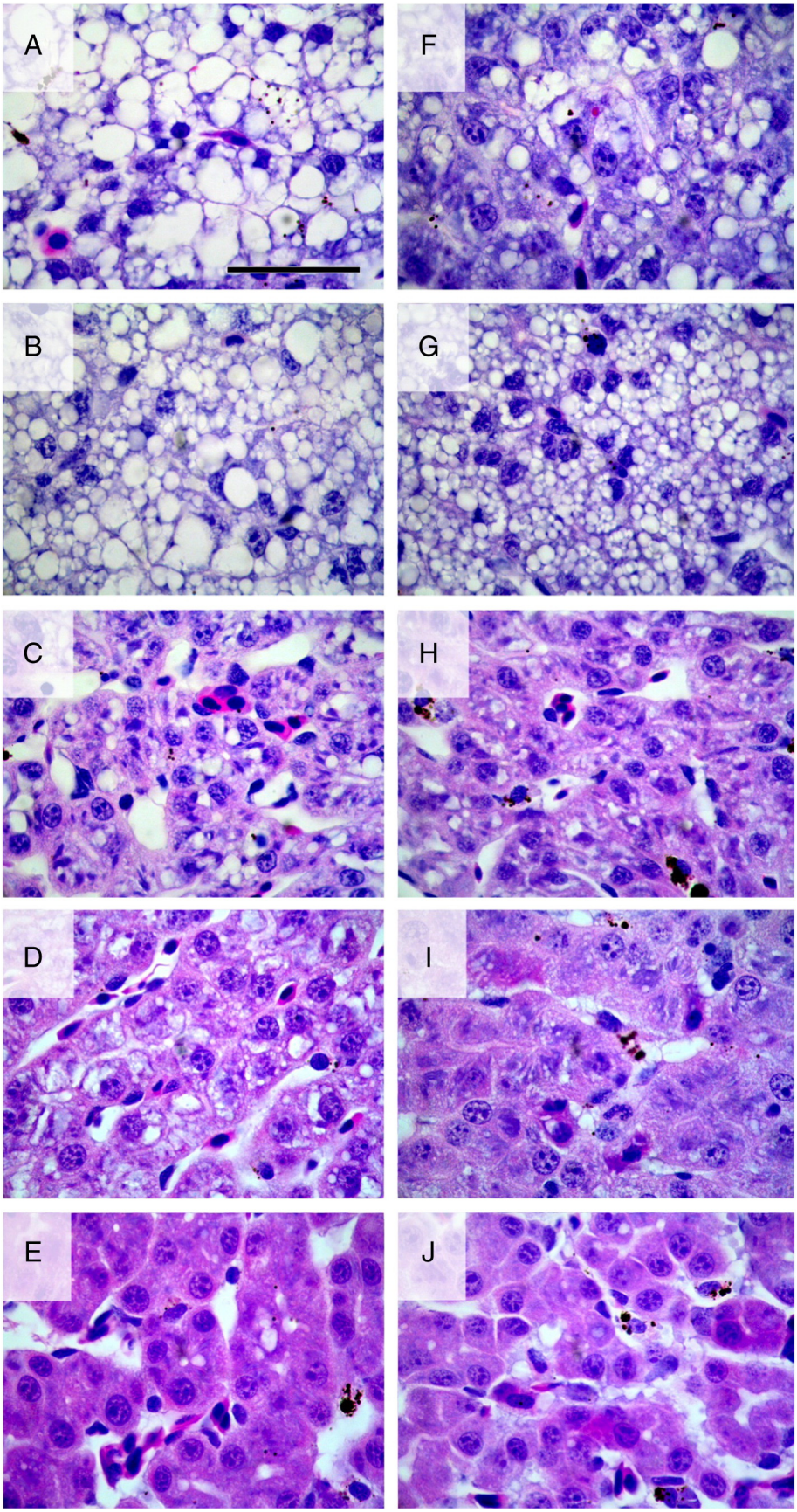

Fig. 6. Representative longitudinal histological sections of white sturgeon liver samples stained with hematoxylin and eosin for fish reared in warm water. Larval sturgeon were reared in gravel at 17.5 °C and sampled at (A) 8 dph, transferred to bare tanks at 11 dph and then sampled at (B) 12 dph, (C) 16 dph, (D) 24 dph and (E) 32 dph. Sturgeon were reared in bare tanks (no gravel) at 17.5 °C sampled at (F) 8 dph, (G) 12 dph, (H) 16 dph, (I) 24 dph and (J) 32 dph. Lipid vacuoles are the large clear structures that show no staining. All images are the same magnification and scale bar represents 50 μm. (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)

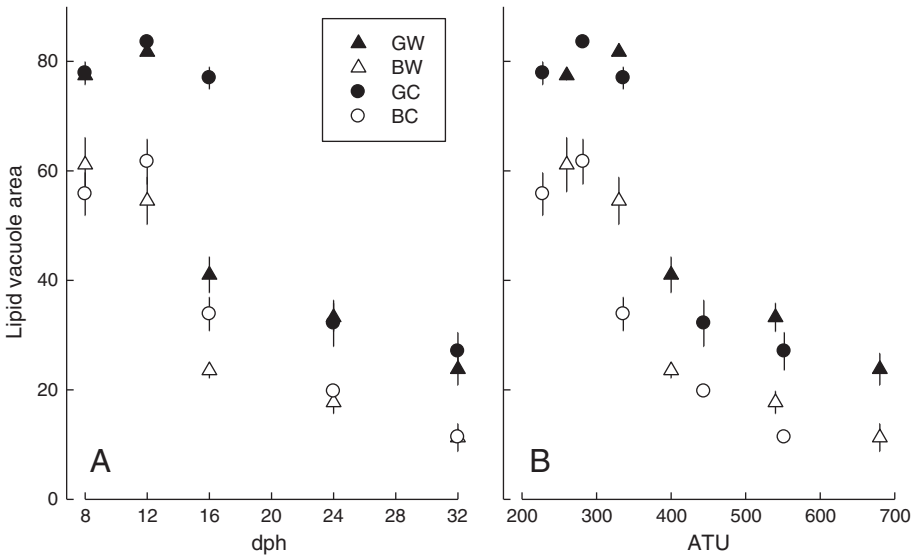

Fig. 7. Percent area of lipid in the liver by (A) days post hatch (dph) and by (B) accumulated thermal units (ATU) from longitudinal histological sections of white sturgeon. Symbols described in Fig. 2. Error bars represent ± 1 standard error.

been observed for Atlantic salmon (Hansen and Møller, 1985; Peterson and Martin-Robichaud, 1995). Both the greater *K* and higher liver lipid content for fish reared in gravel substrate suggest an energetic advantage over fish reared in bare tanks.

The positive relationship between temperature and yolk absorption rate is consistent with reports for other species of sturgeon (Gershanovich and Taufik, 1992; Hardy and Litvak, 2004; Wang et al., 1987) and several marine teleosts (Pepin, 1991). When plotted against ATU, yolk area was still significantly affected by temperature, likely due to higher metabolic rate at the warmer temperature. In contrast, yolk absorption efficiency was not affected by temperature, which agrees with Wang et al. (1987). The significant interaction between temperature and substrate for YAE, however, suggests that the effect of temperature may be altered by the presence or absence of substrate during YSL development. This difference in conversion efficiency became apparent at the end of the endogenous phase, as larvae reared in gravel grew much larger than those reared without gravel, despite absorbing yolk at the same rate for a given temperature. Similar to Atlantic salmon (Peterson and Martin-Robichaud, 1995), such effects could result from reduced yolk conversion efficiencies as a result of increased swimming and movement. The rates of absorption and absorption efficiency of yolk are important determinants of development, growth, and survival in larval fish (Heming and Buddington, 1988). The lower YAE and smaller size at both temperatures in bare reared larvae could be attributed to greater metabolic costs associated with maintenance and/or activity.

As YAR did not differ between substrate treatments, it suggests that white sturgeon larvae grow at a maximal rate, and that growth is determined by allocation of energy reserves. Yolk protein serves two main functions, amino acids for tissue growth and energy for catabolic processes (Heming and Buddington, 1988). Difference in absorption efficiency between larvae reared with and without gravel, therefore, suggests there was greater catabolism of yolk reserves in larvae reared without gravel, likely related to increased energy required for locomotory activity (Brett and Groves, 1979). The greater efficiency with which yolk was transformed to body tissue as a result of gravel rearing

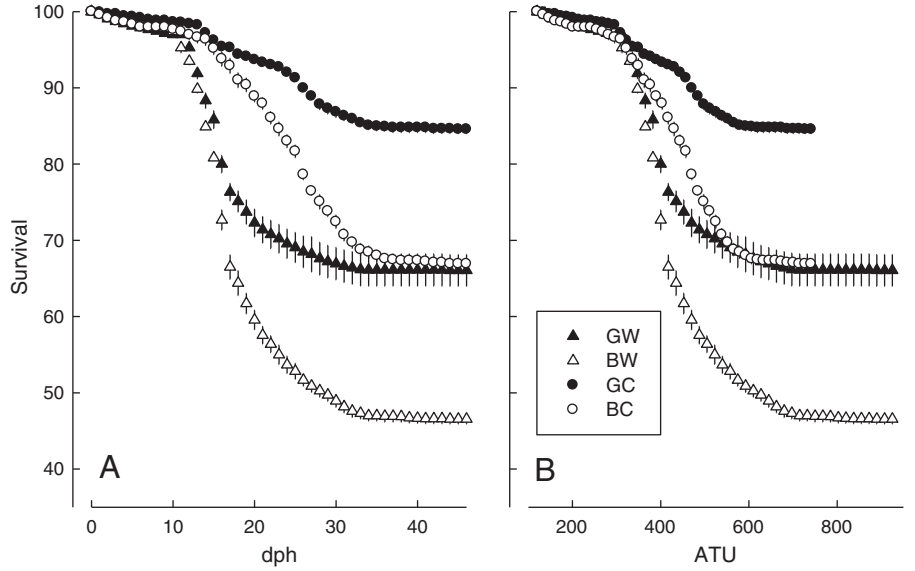

Fig. 8. Mean percent survival of white sturgeon plotted against (A) days post hatch (dph) and (B) accumulated thermal units (ATU), reared with and without gravel during the first ~300 ATU, at two different temperature regimes: 13.5 °C (cool) and 17.5 °C (warm). Symbols described in Fig. 2. Error bars represent ± 1 standard error.

is ecologically significant in that larger larvae may be expected to be stronger, better swimmers and less susceptible to damage or predation (Heming and Buddington, 1988). The decrease in lipid storage in the liver of YSL reared in the bare tanks may also reflect a difference in yolk catabolism between substrate treatments. Lipid catabolism is a significant source of metabolic energy for many yolk-feeding fish, especially for those from eggs with high lipid content, such as sturgeon (Gershanovich, 1989; Kamler, 2008). A reduction in liver lipid quantity, therefore, suggests a difference in the overall physiology and nutritional status of the juvenile sturgeon and likely contributes to reduced growth potential of larvae reared in bare conditions.

The effects of substrate and temperature on the survival of larvae were dramatic, greatest for larvae reared in gravel at 13.5 °C and poorest for larvae reared in bare conditions at 17.5 °C. While temperature has been shown to have a strong relationship with cumulative mortality in salmonids (Murray, 1980) and marine teleosts (Pepin, 1991), the relationship between mortality and rearing substrate is poorly understood. Gessner et al. (2009) found that the use of gravel rearing substrate significantly increased the survival of Atlantic sturgeon. The period of highest mortality, however, was delayed in the cooler treatments (even when plotted against ATU). Survival was high among all treatments, until the onset of exogenous feeding; a finding consistent with other work on white sturgeon (Conte et al., 1988) and other sturgeon, including lake sturgeon (Nilo et al., 1997), Siberian sturgeon (Gisbert and Williot, 1997), green sturgeon (Gisbert et al., 2001), Adriatic sturgeon (Boglione et al., 1999) and Atlantic sturgeon (Gessner et al., 2009). While the onset of exogenous feeding may play a role in mortality, the large difference in mortality (and cumulative survival) between temperature and substrate treatments suggest that additional factors, apart from the onset of exogenous feeding, may play a significant role in determining mortality.

Decreased survival at a warmer temperature could be due to the higher metabolic cost associated with elevated temperature. This would be particularly problematic if metabolic scope was limited. Metabolic scope is defined as the difference between resting and maximal metabolic rate (Fry, 1947) and represents the potential for energy allocation to functions other than metabolism such as locomotion and growth (among other traits) (Killen et al., 2007). Bailey and Houde (1989) reported that limited metabolic scope is likely the basis for the high mortality rates in larval marine fish in response to environmental fluctuations. The increase in metabolic costs due to rapid growth (with an increase in temperature) could be greater than the scope allows and result in increased mortality (Killen et al., 2007). Increased energy needs for locomotion (substrate effects) and basal metabolism (temperature effects) therefore limit energy availability and ultimately affect survival.

The period of transition from endogenous to exogenous nutrition was a time of significant change, not only in nutrition. It coincided with emergence from gravel, a decrease in rate of growth, and large variations in daily mortality. Significant changes in behavior were also observed at this time in fish reared in both bare and gravel tanks, with larvae emerging from gravel and larvae reared without gravel moving from a “clumping” behavior, as described by Conte et al. (1988), to free swimming and actively feeding. Significant mortality of larval fish, including sturgeon, occurs at this time influencing year-class strength (Cushing, 1972; Gessner et al., 2009). Although our laboratory experiments cannot fully represent all factors that control growth and survival of larval sturgeon in natural systems, they provide a general understanding of the importance gravel rearing may play in the wild. The provision of cover by substrate was previously shown to decrease predation (Gadomski and Parsley, 2005; McAdam, 2011); however, we provide evidence of survival effects even in the absence of predation. Larger larvae, produced by gravel rearing, would likely be stronger swimmers, more resistant to starvation and less susceptible to predation (Blaxter and Hempel, 1963; Ware, 1975). Gravel rearing may also put larvae in close proximity with an abundance of small benthic invertebrates at the onset of exogenous feeding.

Our results also provide valuable insights regarding the dual goals of improving larval quality and survival under culture conditions. Low larval survival is regularly reported in sturgeon culture (Boglione et al., 1999; Conte et al., 1988; Gessner et al., 2009; Gisbert and Williot, 1997; Gisbert et al., 2001; Nilo et al., 1997), and our findings suggest that typical hatchery culture conditions are not ideal. Rearing yolk sac larvae in conjunction with even a small addition of substrate may offer a significant benefit. We have also shown that optimal rearing temperatures based on survival may be lower than previously reported optima based on growth.

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