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TECHNICAL NOTE

Effects of Rearing Density on Total Length and Survival of Lake Sturgeon Free Embryos

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Abstract

Experiments were conducted to identify aquaculture conditions that improve the growth and survival of free-embryo Lake Sturgeon *Acipenser fulvescens*. We quantified the effects of rearing density, family, and dissolved oxygen concentration on free-embryo TL and survival to the time of emergence. Experiments were conducted using free embryos from two full-sibling families that were reared in four densities in 3.0-L aquaria. A significant density and family effect on free-embryo TL at emergence was documented. Total length (22.9 ± 0.16 mm [mean ± SE]) at emergence for a rearing density of 9,688 individuals/m^2 was significantly greater than the mean TL for rearing densities of 19,375 and 32,292 individuals/m^2 (22.4 ± 0.14 mm and 21.8 ± 0.17 mm, respectively). Mean TL at emergence differed significantly between families (22.8 ± 0.13 mm versus 22.1 ± 0.11 mm). Mean dissolved oxygen concentration (mg/L) decreased significantly as a function of increasing fish density. Mean dissolved oxygen concentration (7.89 ± 0.004 mg/L) for densities with 3,229 individuals/m^2 was significantly greater than the mean dissolved oxygen concentration for densities with 19,375 and 32,292 individuals/m^2 (7.77 ± 0.018 mg/L and 7.71 ± 0.035 mg/L, respectively). Mean proportional survival to emergence decreased as rearing density increased (0.976 ± 0.008 versus 0.928 ± 0.023; $P = 0.97$) from the 9,688-individuals/m^2 treatment to the 32,292-individuals/m^2 treatment. There was no significant difference in survival between families. Results reveal that the rearing density of free embryos should be considered because of its effects on TL and, to a lesser extent, survival at emergence. These results are useful for the development of standard operating procedures in traditional and conservation aquaculture facilities where free embryos are raised.

Survival and growth during early ontogeny and through subsequent ontogenetic life periods is important to commercial as well as conservation aquaculture production programs. Rearing conditions experienced by free embryos, the phase after hatch when endogenous feeding occurs (Balon 2001), affect TL of larvae and are a significant predictor of the likelihood of first feeding and of mortality linked to starvation (Cushing 1972; Heming and Buddington 1988; Hardy and Litvak 2004). However, there are few studies that identify aquaculture conditions that improve free-embryo TL at the initiation of exogenous feeding, and results vary among taxa (Heming and Buddington 1988; Kamler 2008).

During the free-embryo phase, fish rely entirely on endogenous yolk sac reserves for energy and growth development (Heming and Buddington 1988; Kamler 2008). The rate or

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efficiency at which yolk reserves are utilized for growth is dependent upon abiotic (i.e., dissolved oxygen, light, or temperature) and biotic factors (i.e., maternal provisioning; Heming and Buddington 1988; Kamler 2008). These factors dictate how yolk reserves are allocated with respect to either body tissue development or competing anaerobic processes, such as those experienced during respiration in response to stress (Kamler 2008). If free embryos are reared under stressful conditions, there can be profound direct (mortality) and indirect (lower growth) effects (Bates et al. 2014; Boucher et al. 2014). For example, stressful rearing conditions, such as those experienced by negatively phototactic free embryos in the absence of refugia, can increase the rate at which yolk reserves are devoted to respiration (for locomotion) versus somatic development (Hansen and Moller 1985; Finn et al. 1995; Bates et al. 2014; Boucher et al. 2014). Additionally, biotic factors such as maternal provisioning (i.e., egg size) are documented to have significant direct and indirect effects on free embryos (Gisbert et al. 2000; Kamler 2008; Regnier et al. 2012). In general, environmental conditions experienced during early life stages can also affect traits during later ontogenetic stages (ontogenetic contingency; Crossman et al. 2011) and thus performance in aquaculture settings. Understanding the direct and indirect effects of environmental conditions on yolk reserve allocation, and thus TL, helps define aquaculture practices that reduce stressful conditions and improve growth (e.g., as estimated by TL at the time of emergence).

Sturgeons, given their high market value and worldwide conservation status, are an important species group (Bronzi et al. 1999; IUCN 2010; Rosenthal et al. 2010) in aquaculture. Recent research conducted with White Sturgeon *Acipenser transmontanus* and Lake Sturgeon *A. fulvescens* has improved our understanding of species-specific behaviors (Hastings et al. 2013; Boucher et al. 2014), which, in turn, has informed research needs for best aquaculture practices. For example, Boucher et al. (2014) showed that providing cover to free-embryo rearing tanks improved TL and survival at emergence as well as survival to subsequent life periods. However, other attributes of the rearing environment, such as rearing density and dissolved oxygen concentration, can be important when rearing free-embryo sturgeons (Ceskleba et al. 1985), yet data are lacking.

Our primary objective was to evaluate the effects of rearing conditions on free-embryo TL and survival of Lake Sturgeon at emergence. Specifically, we quantified the effects of rearing density and family as well as dissolved oxygen concentration on free-embryo Lake Sturgeon TL and survival at emergence. Our hypothesis was that Lake Sturgeon TL and survival would decrease as a function of increasing rearing density from the time of hatch to emergence.

METHODS

*Study site.—*Use of conservation streamside rearing facilities, such as the Black River Streamside Rearing Facility (BR-SRF), have been widely advocated in the Great Lakes basin as the preferred method for culturing Lake Sturgeon in situations where restoration goals to repatriate or enhance populations can be met by stocking (Holtgren et al. 2007). The BR-SRF is a flow-through system, supplied with ambient river water (~680 L/min) from the Kleber Reservoir, located near primary spawning areas for Lake Sturgeon in the upper Black River in Cheboygan County, Michigan. This study was conducted in June 2013, and the BR-SRF water temperature ranged from 17.7°C to 20.5°C (mean, 19.06°C).

*Fertilization and incubation.—*Gametes were collected from male and female (two families) Lake Sturgeon spawning in the upper Black River following procedures described by Crossman et al. (2011). Gametes were transported to the BR-SRF for fertilization, which took place within 4 h of collection. Prior to fertilization, we subsampled 20 eggs from each female and preserved them separately in a 90% solution of ethanol to measure egg size at a later date. Approximately 200 mL of eggs per female were placed into separate dry bowls. Milt samples from a separate male per female were activated using a 1:200 dilution of ambient river water and immediately poured over the eggs, allowing 90 s for fertilization. Excess milt was then removed, and eggs were rinsed once with ambient river water. Egg de-adhesion procedures began by applying a fuller's earth solution (Sigma Aldrich) and gently mixing for 50 min. After 50 min, fuller's earth was rinsed from the eggs and a 15-min, 50-mg/L iodophor disinfection treatment was administered. Following a 10-min rinse to remove residual iodophor using ambient river water, eggs were transferred to Aquatic Eco-Systems (Pentair) J32 Mini Egg-Hatching jars for incubation. Beginning 2 d post-fertilization, eggs were treated daily using a 500-mg/L, 15-min bath treatment of hydrogen peroxide until 24 h prior to hatch. Preserved eggs ($n = 20$/female) were photographed with a ruler for scale, and egg diameter was measured at the greatest linear distance (mm ± SE) using Image J version 1.43u.

*Experimental treatments.—*Density experiments were conducted using free embryos in four different rearing densities ($n = 50$, 150, 300, or 500 offspring/3.0-L tank; 3,229; 9,688; 19,375; or 32,292 individuals/m^2, respectively). Densities are representative of currently utilized protocols in sturgeon production facilities (DiLauro et al. 1998; Deng et al. 2003; Boucher et al. 2014; Wisconsin Department of Natural Resources, unpublished data). Rearing density was based on total rearing area of the tank bottom. We kept families separate for the duration of this experiment to account for differences in body size or survival associated with family. At hatch, free embryos were randomly placed into tanks by family until four replicates of each tank density level were filled (32 tanks in total). During the free-embryo phase, Lake Sturgeon seek refuge in substrate (Hastings et al. 2013). Therefore, we utilized 3.0-L polycarbonate tanks (Aquatic Habitats) filled with a single layer of 2.54-cm^3 sinking Bio-Balls ($n = 24$; CBB1-S; Pentair) covering the tank bottom. Bio-Balls were not removed from tanks for the duration of this study. Water flow was set at a rate of 57 L/h (~20 complete tank turnovers/h) and checked daily. We used a 12 h light : 12 h dark environment for the 9-d duration of this study. Mortalities were removed and enumerated at the start of each rearing day to quantify survival to emergence. Dissolved oxygen (mg/L) was recorded (YSI ProODO Optical DO–Temp meter) multiple times daily in each tank and was reported as mean dissolved oxygen concentration. Percent oxygen saturation was calculated from dissolved oxygen concentrations and temperatures according to Lasee (1995). As endogenous resources are absorbed, Lake Sturgeon emerge from the substrate (or Bio-Balls, in this case) and begin a "swim-up" behavior. At this point, we used a digital camera to photograph and measure a random subsample ($n = 30$) of fish from each treatment, each family, and each replicate for total length (TL, mm) using Image J version 1.43u.

Statistical analysis.—We tested for differences in mean (± SE) egg size, mean TL at emergence, mean dissolved oxygen concentration, and mean proportional survival as a function of fixed effects, including rearing density and family, using SAS version 9.3 (SAS Institute, Cary, North Carolina). Tank was the experimental unit for all response variables used in the analysis. Normality assumptions were assessed using the Shapiro–Wilk test. Homogeneity of variance assumptions were assessed using the Levene's test. A P-value < 0.05 was considered statistically significant for all analyses.

We compared mean egg size between families using a two-sample t-test. We used a general linear model using ANOVA to analyze TL at emergence and mean dissolved oxygen concentration, and a generalized linear model using a logit link function and ANOVA to analyze survival data. Survival-to-emergence data were modeled as a binomially distributed variable and reported as the mean proportion of survival from hatch to emergence. We used Tukey–Kramer multiple pairwise comparison tests for all response variables.

RESULTS

Duration of Free-Embryo Phase and Size at Emergence

The duration of the free-embryo phase was 9 d across treatments, and fish began exhibiting swim-up behavior in synchrony among density treatments and between families. Total length at emergence decreased as a function of increasing rearing density. We documented significant density ($F_{3,25} = 31.61$, $P < 0.0001$) and family ($F_{1,25} = 82.36$, $P < 0.0001$) effects on free-embryo TL at emergence. The mean TL at emergence for a rearing density of 50 fish/tank was significantly greater than the mean TL for rearing densities of 300 and 500 fish/tank ($t_{25} = -3.10$, $P = 0.0228$; $t_{25} = 7.77$, $P < 0.0001$, respectively; Table 1). The mean TL at emergence for a rearing density of 150 fish/tank was significantly greater than the mean TL for rearing densities of 300 and 500 fish/tank ($t_{25} = 4.16$, $P = 0.0017$; $t_{25} = 8.96$, $P <$ 0.0001, respectively; Table 1). Also, the mean TL at emergence for a rearing density of 300 fish/tank was significantly greater than that for a rearing density of 500 fish/tank ($t_{25} = 4.95$, $P = 0.0002$; Table 1). The mean TL at emergence for family 2 was significantly greater than that for family 1 ($t_{25} = -9.08$, $P < 0.0001$), and the mean egg diameter measured from female 2 (3.060 ± 0.025 mm) was significantly larger than the mean egg diameter from female 1 (2.780 ± 0.028 mm; $t_{19} = -7.57$, $P < 0.0001$).

TABLE 1. Lake Sturgeon mean (± SE) TL (mm) differs at emergence as a function of rearing density (individuals/tank) and between families. Fixed effects in the overall column and row with identical lowercase letters are not significantly different (Tukey–Kramer: $P < 0.05$).

Rearing density	Family 1	Family 2	Overall
50	22.3 ± 0.07	23.3 ± 0.04	22.7 ± 0.21 v
150	22.5 ± 0.14	23.2 ± 0.05	22.9 ± 0.16 v
300	22.0 ± 0.11	22.7 ± 0.06	22.4 ± 0.14 w
500	21.5 ± 0.21	22.1 ± 0.16	21.8 ± 0.17 x
Overall	22.1 ± 0.11 y	22.8 ± 0.13 z	22.4 ± 0.11

Dissolved Oxygen Concentration

We documented a significant density effect on dissolved oxygen concentration ($F_{3,10.34} = 22.62$, $P < 0.0001$), which decreased as a function of increasing fish density. However, the effects of family were not significant ($F_{1,7.67} = 2.03$, $P = 0.1934$). The mean dissolved oxygen concentrations from the 50 fish/tank treatment levels were statistically greater than those from the 300 and 500 fish/treatment levels ($t_{7.32} = -6.91$, $P = 0.0002$; $t_{6.09} = 5.20$, $P = 0.0019$, respectively; Table 2). Additional mean comparison tests revealed that the mean dissolved oxygen concentrations from the 150 fish/tank treatment levels were statistically greater than those from the 300 and 500 fish/tank treatment levels ($t_{11.2} = 4.55$, $P = 0.0045$; $t_{6.96} = 4.20$, $P = 0.0078$, respectively; Table 2). Oxygen saturation values for the range of water temperatures free embryos experienced in this study (17.7°C to 20.5°C; mean, 19.06°C) were from ~79.8–87.8%, and oxygen saturation averaged ~84.3%.

Survival to Emergence

Mean proportional survival from hatch to emergence appeared to decrease as rearing density increased from 150 to 500

TABLE 2. Mean (± SE) dissolved oxygen concentration (mg/L) as a function of rearing density (individuals/tank) and family. Fixed effects (density and family) in the overall column and row with identical lowercase letters are not significantly different (Tukey–Kramer: $P < 0.05$).

Rearing density	Family 1	Family 2	Overall
50	7.89 ± 0.005	7.88 ± 0.007	7.89 ± 0.004 x
150	7.85 ± 0.003	7.87 ± 0.018	7.86 ± 0.009 x
300	7.81 ± 0.011	7.73 ± 0.018	7.77 ± 0.018 y
500	7.79 ± 0.031	7.65 ± 0.034	7.71 ± 0.035 y
Overall	7.84 ± 0.012 z	7.78 ± 0.027 z	7.81 ± 0.016

TABLE 3. Mean (± SE) proportional survival to emergence as a function of rearing density (individuals/tank) and family. Fixed effects in the overall column and row with identical lowercase letters are not significantly different (Tukey–Kramer: $P < 0.05$).

Rearing density	Family 1	Family 2	Overall
50	0.995 ± 0.005	0.940 ± 0.020	0.971 ± 0.014 y
150	0.972 ± 0.010	0.980 ± 0.013	0.976 ± 0.008 y
300	0.946 ± 0.025	0.961 ± 0.003	0.954 ± 0.012 y
500	0.929 ± 0.055	0.928 ± 0.019	0.928 ± 0.023 y
Overall	0.963 ± 0.013 z	0.953 ± 0.009 z	0.958 ± 0.008

fish/tank; however, differences were not statistically significant ($F_{3,25} = 0.08$, $P = 0.9721$; Table 3). There was also no significant difference in survival between families ($F_{1,25} = 0.01$, $P = 0.9274$; Table 3).

DISCUSSION

Investigations focused on Lake Sturgeon during the ontogenetic period immediately after hatch advance our understanding of a life period for which there is little information pertaining to TL and survival. We documented significant effects of rearing density as well as family on the TL of free embryos at emergence, and demonstrated a corresponding, potentially related effect associated with dissolved oxygen concentration. We have also extended the results of previous literature, specifically Gisbert et al. (2000) and Regnier et al. (2012) who suggested maternal provisioning (i.e., egg size) has indirect effects on TL.

Impact of Density on Total Length, Dissolved Oxygen Concentration, and Survival

Total length.—Mean TL decreased as a function of increasing rearing density such that fish raised in densities of 150 fish/tank (or 9,688 individuals/m^2) or less were larger (+ 0.5–1.1 mm) at emergence, indicating that density should be considered when rearing free embryos. Some facilities raise free-embryo sturgeons at densities exceeding 21,000/m^2, which, based on our data, would result in a significant decrease in TL at emergence. Similar studies for demersal larval species such as Walking Catfish *Clarias batrachus* and African Sharptooth Catfish *C. gariepinus* have also shown that increasing density results in a decrease in TL (Hossain et al. 1998; Sahoo et al. 2004). These studies attributed decreased growth to increased competition for or reduced availability of food (Irwin et al. 1999; Sahoo et al. 2004). However, during the free-embryo phase fish are not feeding exogenously, which directs attention to other potential stress mechanisms such as increased swimming activity. Although we did not measure free-embryo activity (i.e., swimming) during this experiment, we suspect that stress related to swimming activity may have increased with density, as free embryos competed for the limited refuge space available. Increased swimming activity would explain the difference in TL at emergence because free embryos would have been diverting energy away from somatic tissue growth in favor of swimming activity. Although dissolved oxygen concentrations declined as treatment density increased, it is unlikely that dissolved oxygen differences among treatments were responsible for the observed size difference at emergence because dissolved oxygen levels were relatively high and close to saturation.

Survival.—Mean proportional survival from hatch to emergence appeared to decrease as rearing density increased from 150 to 500 fish/tank. However, treatment differences were not significant. Survival to emergence of free embryos was high in all tanks (mean, 0.96 [or 96%]) and was comparable to that in other sturgeon studies (Gisbert et al. 2000; Boucher et al. 2014). Our results suggest that although indirect effects on TL were evident, no direct effects on survival to emergence were associated with rearing free embryos in high densities. Similar results have been reported for other fish species (Hossain et al. 1998). However, reduced TL at emergence as well as other associated stresses resulting from high-density conditions may lead to lower survival during subsequent ontogenetic life periods (Bates et al. 2014; Boucher et al. 2014). We did not continue the study beyond the onset of exogenous feeding to validate this.

Impact of Family on Total Length and Survival

Mean egg size and TL of free embryos from family 2 were significantly larger than those from family 1. The difference in TL is likely due to initial egg size, which would suggest a maternal effect that has been documented previously for sturgeons (Gisbert et al. 2000) as well as for other fish species (Regnier et al. 2012). Our analysis was limited by the numbers of families represented ($n = 2$); however, our results suggest that additional studies with a greater number of families are needed to assess the potential impacts of family on free-embryo TL. Aquaculture facilities that mix offspring from several families during early rearing may provide free embryos born from females with larger eggs an advantage as larvae at emergence or initiation of exogenous feeding. Given that these larvae are larger, they may outcompete others in the hatchery environment during early life periods, which could result in unequal family sizes at the end of the production cycle or stockout.

The rearing density of free embryos in traditional as well as in conservation aquaculture facilities should be considered due to its indirect effects on TL. Larger TL at emergence or at initiation of exogenous feeding is documented to increase the likelihood of first feeding at this critical period, reducing mortality linked to starvation (Cushing 1972; Yufera and Darias 2007). Based on our study utilizing Lake Sturgeon free embryos and a total rearing area of 0.015 m^2, if free-embryo rearing density exceeds 9,688 individuals/m^2, TL at emergence will likely be significantly negatively affected. These results are useful for the development of standard operating procedures in traditional and conservation aquaculture facilities where free embryos are raised.

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