Effects of Family, Feeding Frequency, and Alternate Food Type on Body Size and Survival of Hatchery-Produced and Wild-Caught Lake Sturgeon Larvae

John M. Bauman* and Brittany M. Woodward
Department of Fisheries and Wildlife, Michigan State University, 13 Natural Resources Building, East Lansing, Michigan 48824, USA

Edward A. Baker
Michigan Department of Natural Resources, Fisheries Division, 484 Cherry Creek Road, Marquette, Michigan 49855, USA

Terence L. Marsh
Department of Microbiology and Molecular Genetics, Michigan State University, 6169 Biomedical Physical Sciences, East Lansing, Michigan 48824, USA

Kim T. Scribner
Department of Fisheries and Wildlife, Michigan State University, 13 Natural Resources Building, East Lansing, Michigan 48824, USA; and Department of Integrative Biology, Michigan State University, 288 Farm Lane, East Lansing, Michigan 48824, USA

Abstract

Studies that evaluate the effects of different feeding strategies (e.g., feeding frequency and food type) on larval fish growth and survival are important for aquaculture productivity. We quantified the effects of common feeding strategies on the growth and survival of larval Lake Sturgeon Acipenser fulvescens. At 30 d after initiation of exogenous feeding, the TL and body weight of larvae reared under a treatment regime of three feedings per day (TL [mean ± SE] = 46.83 ± 0.43 mm; body weight = 0.41 ± 0.01 g) were significantly greater than those of fish reared under a regime of 12 feedings per day (TL = 45.73 ± 0.54 mm; body weight = 0.39 ± 0.02 g). Alternate food type and family were documented to have significant effects on the body weight of hatchery-produced larvae at 14 d postexogenous feeding: body weight (mean ± SE) was significantly greater for larvae that were fed brine shrimp Artemia spp. (family 1: 0.31 ± 0.004 g; family 2: 0.35 ± 0.006 g) than for larvae that received formulated food (family 1: 0.06 ± 0.006 g; family 2: 0.08 ± 0.014 g). Furthermore, alternate food type had a significant effect on the survival of hatchery-produced larvae at 14 d postexogenous feeding, as survival was greater for larvae that were fed Artemia (97.0 ± 3.0%) than for those that were given formulated food (34.0 ± 7.0%). In addition, alternate food type had significant effects on the body weight and survival of wild-caught Lake Sturgeon larvae, with both variables being greater for fish that received Artemia (body weight [mean ± SE] = 0.41 ± 0.02 g; survival = 94.0 ± 2.0%) than for fish that were given formulated food (body weight = 0.03 ± 0.01 g; survival = 17.0 ± 6.0%). Our results provide guidance to direct feeding strategies that can be used to develop standard operating procedures for the culture of Lake Sturgeon, a species of conservation concern.

Larval fish commonly experience periods of reduced growth and high mortality in aquaculture settings. Ontogenetic periods during which growth and survival can be negatively affected include the period of transition from endogenous to exogenous feeding as well as several weeks after the initiation of exogenous feeding (Kamler 1992). During these critical periods, nutrient deprivation or starvation can contribute to low growth and high mortality (Heming et al. 1982; Li and Mathias 1982; Theilacker 1986; Alves et al. 1999; Kamali et al. 2006). In aquaculture settings, if proper feeding strategies are not employed at the onset of exogenous feeding, larvae reach a “point of no return” whereby they either fail to feed exogenously or discontinue...
feeding, which leads to significant mortality (Blaxter and Hempel 1963; Kamler 1992). Therefore, studies that evaluate the effects of different feeding strategies (e.g., feeding frequency and food type) on the growth and survival of larval fish are important for aquaculture productivity.

Feeding frequency (the number of feedings administered each day) has been found to significantly affect the growth and survival of larval fish (Mollah and Tan 1982; Wang et al. 1998; Cho et al. 2003). For example, Li et al. (2014) measured physiological stress factors in Blunt Snout Bream Megalobrama amblycephala and found that individuals feeding at higher frequency exhibited greater immunity to disease and greater growth and survival. Tung and Shiau (1991) observed that greater feeding frequency resulted in greater growth and survival due to more efficient carbohydrate metabolism. Investigations of alternate food types in aquaculture settings have also found that food type significantly affects larval growth and survival and that administering an appropriate food type can reduce annual production costs (Hamre et al. 2013). For example, Atlantic Halibut Hippoglossus hippoglossus that received live brine shrimp Artemia spp. as food during early development had greater growth and survival than fish that were fed live wild zooplankton (Naess et al. 1995); improved growth and survival were attributed to greater assimilation efficiency or greater palatability. More notably, formulated commercial diets have been studied extensively to identify low-cost, nutrient-rich alternatives to live food (Hamre et al. 2013). However, despite extensive research on alternate feeding strategies, the results are highly variable and warrant evaluation of species-specific feeding regimes, particularly for species of economic and conservation concern (e.g., sturgeons; Auer 1996; Rosenthal et al. 2010).

Sturgeons are highly marketable species that are sought for their roe and flesh worldwide; however, 85% of the world’s sturgeons are in danger of extinction (IUCN 2010). Several anthropogenic factors have contributed to the low levels of sturgeon abundance, including overfishing, poaching, habitat degradation, and interruption of migration routes that are utilized for reproduction (Auer 1996; Rosenthal et al. 2010). As a result, aquaculture programs are being implemented to rebuild natural populations, and recovery programs are used to repatriate or improve recruitment of remnant populations (Memis et al. 2009; Rosenthal et al. 2010). However, low growth and high mortality during the larval period, which are attributed to inadequate feeding strategies (Buddington and Christofferson 1985), serve as bottlenecks to the successful aquaculture production of sturgeons. Research on sturgeons in aquaculture settings has demonstrated that species-specific preferences for live food, the palatability or attractiveness of certain food types, and the use of live food in combination with artificial or formulated food all offer benefits in the form of improved growth and survival during early life periods (Kappenman et al. 2011). Data on optimal feeding strategies (Giberson and Litvak 2003), such as feeding frequency or beneficial alternate food types, are lacking for larval Lake Sturgeon Acipenser fulvescens.

Although Lake Sturgeon have been cultured for over 100 years (Post 1890; Leach 1920), documentation of the optimal feeding frequency and food type in relation to growth and survival of larvae has been limited. Alternate food types for larval Lake Sturgeon have been investigated in some capacity, and live food has outperformed formulated food types (Anderson 1984; Ceskleba et al. 1985; DiLauro et al. 1998). However, these prior studies were confounded because larvae were fed Artemia prior to the onset of experiments that introduced alternate food. Research has been conducted to improve juvenile growth and survival in aquaculture (Moreau and Dabrowski 1996), but mortality is especially high during the larval period (Harkness and Dymond 1961; Ceskleba et al. 1985), and therefore this ontogenetic period is deserving of further attention.

High mortality of larval Lake Sturgeon reduces the cost effectiveness of current aquaculture restoration programs, such as streamside rearing facilities (SRFs), and may impact the overall success of restoration efforts. In the Great Lakes basin, SRFs have been widely advocated as the preferred method for culturing Lake Sturgeon in situations where restoration goals (repopulation or enhancement of populations) can be met by stocking (Holtgren et al. 2007). However, recent experience has shown that targeted stocking goals (~900 fish/year from each facility) are difficult to achieve when SRFs are used; this is largely attributable to the high mortality rates that occur during the larval period.

Experiments have not been conducted to quantify and compare the effects of feeding frequency and alternate food types on the growth and survival of larval Lake Sturgeon. In addition, feeding frequency and food type effects have not been investigated by using fish from different sources. Most of the SRFs currently in place in the Great Lakes region are used to incubate and hatch Lake Sturgeon eggs that are collected directly from adults. However, several SRFs are raising wild-caught larvae that have been collected as they disperse from spawning sites in their natal rivers (Crossman et al. 2011). These wild-caught larvae may have begun feeding on natural foods and may respond differently to foods provided in the aquaculture setting. Therefore, the objectives of this study were to (1) quantify and compare the effects of feeding frequency on the TL, body weight, and survival of hatchery-produced larval Lake Sturgeon; and (2) quantify and compare the effects of alternate food types on the body weight and survival of hatchery-produced and wild-captured larval Lake Sturgeon. Results from this study can be utilized in the development of standard operating procedures to be used for Lake Sturgeon larvae, which currently experience high mortality in aquaculture settings.
METHODS

Study Site

This study was conducted in spring 2013 and 2014 at the Black River SRF (BR-SRF) on the upper Black River, located in Cheboygan County, Michigan. The BR-SRF is a flow-through system that is supplied with ambient Black River water (681 L/min) from Kleber Reservoir. The BR-SRF provides conditions that are suitable for the evaluation of culture techniques utilized for sturgeon at multiple life stages due to the facility’s large size (316 m²) and accessibility to the gametes and larvae of Lake Sturgeon.

Collection, Fertilization, and Incubation of Hatchery-Produced Larvae

Using the procedures described by Crossman et al. (2011), gametes were collected from four male and four female Lake Sturgeon spawning in the upper Black River. Procedures for fertilization were summarized by Bauman et al. (2015). Each day from 2 d postfertilization until the 24 h prior to hatch, eggs were given a 15-min bath treatment of hydrogen peroxide (500 mg/L). After hatch, Lake Sturgeon free embryos seek refuge in available substrate (Hastings et al. 2013). Therefore, free embryos were raised in 10-L, polycarbonate flow-through tanks (Aquatic Habitats) in which the bottom was covered with a single layer of 2.54-cm³ sinking Bio-Balls (Pentair CBB1-S). Lake Sturgeon were raised until the endogenous resources were absorbed and the fish began to exhibit “swim-up” behavior (∼7–10 d posthatch) signaling the onset of exogenous feeding. During feeding experiments, the water flow rate was set at 20 L/h (∼7 complete tank turnovers per hour) and was checked daily. A photoperiod of 12 h light : 12 h dark was used for the duration of the study.

Collection of Wild Larvae

We used D-frame drift nets to capture wild Lake Sturgeon larvae as they drifted immediately downstream of spawning areas, and we transported the larvae to the BR-SRF for rearing. The methodology and timing of drift capture are detailed further by Auer and Baker (2002) and Smith and King (2005).

Feeding Rate

To ensure that larval Lake Sturgeon were fed consistently in all experiments, we used previously established dry weight feeding rates for sturgeon (similar to Deng et al. 2003): larvae in all tanks were fed 26% of body weight (BW) per day during the first and second weeks postexogenous feeding, 13% of BW/d during the third week, and 11% of BW/d during the fourth week. For all feeding treatments that included Artemia, the fish were offered live Artemia nauplii (premium grade, 90% hatch rate, Great Salt Lake strain; Brine Shrimp Direct). The Artemia were incubated overnight and were harvested in the morning prior to the first feeding of the day. In addition, reservoir detritus that was retained by serial filtration (hereafter, “filtrate”) through 100-μm and then 50-μm Bag Filter Vessels (Pentair Aquatic Eco-Systems) was collected prior to the first feeding each day and was used in three of the feeding treatments to represent a “natural” food type for experiments with wild-caught larvae. Similar to the methods of Agh et al. (2012), we created and used a wet weight : dry weight conversion to calculate the Artemia and filtrate feeding rates for the Lake Sturgeon larvae. In all experiments, food was administered manually by using a laboratory pipette to ensure that the tanks received equal rations.

Experimental Treatments

Feeding frequency experiment with hatchery-produced larvae.—Feeding frequency treatments with hatchery-produced larvae were evaluated from June 3 to July 2, 2013, when the water temperature at BR-SRF ranged from 15.3°C to 25.8°C (mean = 19.7°C). At the onset of exogenous feeding, 50 larvae were placed into each of 32 (2 feeding frequencies × 2 families × 8 replicates) 3.0-L, polycarbonate flow-through tanks (Aquatic Habitats). Each tank was randomly assigned to one of the two feeding frequency treatments, with eight replicates per treatment. Feeding frequency treatment groups included (1) fish that were fed 12 times per day and (2) fish that were fed three times per day. Fish were fed over a period of 10 h (from 0900 to 1900 hours), and the amount of food administered per day was equivalent for the two treatments. In the low feeding frequency treatment, larvae were given equal amounts of food three times per day (0900, 1400, and 1900 hours). In the high feeding frequency treatment, larvae were given equal amounts 12 times (every 54 min) per day from 0900 to 1900 hours. By the end of each day, each feeding frequency treatment group had been given equal quantities of food. Feeding frequency treatments were designed to mimic current SRF feeding protocols: (1) frequent daily feedings with relatively small quantities of food versus (2) infrequent daily feedings with large quantities of food. Fish were acclimated in tanks for 24 h prior to the start of the experiment. Mortalities were removed and enumerated at the start of each rearing day to quantify survival through 30 d postexogenous feeding. At 30 d postexogenous feeding, body size (TL, mm; body weight, g) was determined for all surviving fish.

Alternate food type experiment with hatchery-produced larvae.—Alternate food types for use with hatchery-produced larvae were evaluated from June 21 to July 4, 2013, when the water temperature at BR-SRF ranged from 19.2°C to 25.8°C (mean = 21.9°C). At the onset of exogenous feeding, 25 larvae were placed into each of 24 (3 alternate food types × 2 families × 4 replicates) 3.0-L, polycarbonate flow-through tanks (Aquatic Habitats). Fish were acclimated to the tanks for 24 h prior to the start of the experiment. Each tank was then randomly assigned to one of three treatments representing alternate food types that are currently utilized in SRFs (each treatment expressed in percentages of the recommended ration.
TABLE 1. Nutritional composition (%) of alternate food types (Otohime B2 Larval Diet and brine shrimp Artemia spp.) that were fed to Lake Sturgeon larvae (NA = value not available).

<table>
<thead>
<tr>
<th>Component</th>
<th>Otohime B2</th>
<th>Artemiaa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>51.0</td>
<td>52.2</td>
</tr>
<tr>
<td>Lipid</td>
<td>NA</td>
<td>18.9</td>
</tr>
<tr>
<td>Fat</td>
<td>11.0</td>
<td>NA</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>3.0</td>
<td>NA</td>
</tr>
<tr>
<td>Ash</td>
<td>15.0</td>
<td>9.7</td>
</tr>
<tr>
<td>Calcium</td>
<td>2.3</td>
<td>NA</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>1.5</td>
<td>NA</td>
</tr>
<tr>
<td>Moisture</td>
<td>6.5</td>
<td>NA</td>
</tr>
</tbody>
</table>

a Nutrient composition information derived from Leger et al. (1987).

[BW/d]: (1) 100% Artemia; (2) 50% Artemia and 50% Otohime B2 Larval Diet (hereafter, “Otohime B2”; particle size = 0.36–0.60 mm; Marubeni Nisshin Feed, Tokyo, Japan); and (3) 100% Otohime B2. The nutrient composition of Otohime B2 was derived from product packaging, and the nutrient composition of Artemia was obtained from the peer-reviewed literature (Table 1). Fish were fed equal amounts three times daily at 0900, 1400, and 1900 hours. Mortalities were removed and enumerated at the start of each rearing day to quantify survival. Significant mortality occurred in some treatments; therefore, this experiment was halted at 14 d postexogenous feeding.

Alternate food type experiment with wild-caught Larvae.—An experiment in which wild-caught larvae were given alternate food types was conducted from June 4 to June 17, 2014, when the BR-SRF water temperature ranged from 18.0°C to 23.1°C (mean = 20.3°C). At 24 h postcapture, 20 wild larvae were placed into each of 48 (6 alternate food types × 8 replicates) 3.0-L, polycarbonate flow-through tanks (Aquatic Habitats). Each tank was randomly assigned to one of six food types designed to mimic those currently utilized in SRFs, including filtrate gathered from a nearby reservoir (used to represent a natural forage type in the river; see Feeding Rate section). Alternate food types for this experiment (expressed in percentages of the recommended ration [BW/d]) included (1) 100% Artemia; (2) 50% Artemia and 50% Otohime B2; (3) 100% Otohime B2; (4) 90% Otohime B2 and 10% filtrate; (5) 90% Artemia and 10% filtrate; and (6) 45% Artemia, 45% Otohime B2, and 10% filtrate. Nutrient composition for Otohime B2 and Artemia was the same as described for hatchery-produced larvae. Nutrient composition for the filtrate was not determined in this study. Fish were fed equal amounts three times daily (at 0900, 1400, and 1900 hours). Fish were acclimated to the tanks for 24–48 h prior to the start of the experiment. Mortalities were removed and enumerated at the start of each rearing day to quantify survival. Significant mortality occurred in some treatments, so the experiment was ended at 14 d postexogenous feeding.

Statistical Analysis

All statistical analyses to quantify response variables were performed in SAS version 9.3 (SAS Institute, Cary, North Carolina). Summary statistics (mean ± SE) for all response variables were calculated, with tank as the experimental unit. Body size measurements (mean TL and body weight) were analyzed by using a general linear model and ANOVA. We also used a generalized linear model and ANOVA for mean proportional survival data, which were modeled using a beta distribution. For hatchery-produced larvae, families were kept separate to quantify the main effect of family and the potential treatment × family interaction effect. Least-squares means or Tukey–Kramer pairwise comparison tests (based on the number of comparisons within an experiment) were used to detect significant differences between treatments. For all results, P-values less than 0.05 represented statistical significance.

RESULTS

Feeding Frequency Experiment with Hatchery-Produced Larvae

We detected a significant effect of feeding frequency treatment on the mean TL of hatchery-produced Lake Sturgeon larvae at 30 d postexogenous feeding (P = 0.04). However, there was no significant effect of family on larval TL (P = 0.06). Mean TL was significantly greater in groups that were fed three times per day than in fish that were fed 12 times per day (P = 0.04; Table 2). The TL at 30 d postexogenous feeding was 2.4% greater for larvae that were fed three times per day than for larvae that were fed 12 times per day.

The mean body weight of larvae at 30 d postexogenous feeding was significantly affected by feeding frequency treatment (P = 0.03). However, there was no significant effect of family on mean body weight (P = 0.09). Larvae that were fed three times per day had significantly greater body weight than fish

<table>
<thead>
<tr>
<th>Feeding frequency</th>
<th>Family 1</th>
<th>Family 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>TL (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 times/d</td>
<td>45.16 ± 0.61 y</td>
<td>46.30 ± 0.46 y</td>
</tr>
<tr>
<td>3 times/d</td>
<td>46.43 ± 0.26 z</td>
<td>47.22 ± 0.60 z</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 times/d</td>
<td>0.37 ± 0.02 y</td>
<td>0.40 ± 0.01 y</td>
</tr>
<tr>
<td>3 times/d</td>
<td>0.40 ± 0.01 z</td>
<td>0.42 ± 0.01 z</td>
</tr>
<tr>
<td>Percent survival</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 times/d</td>
<td>93.0 ± 6.0 z</td>
<td>98.0 ± 1.0 z</td>
</tr>
<tr>
<td>3 times/d</td>
<td>97.0 ± 1.0 z</td>
<td>98.0 ± 1.0 z</td>
</tr>
</tbody>
</table>

Notes: Tukey–Kramer pairwise comparison tests (based on the number of comparisons within an experiment) were used to detect significant differences between treatments. For all results, P-values less than 0.05 represented statistical significance.
that were fed 12 times per day ($P = 0.03$; Table 2). Mean body weight at 30 d postexogenous feeding was 6.5% greater in the fish that received three feedings per day than in fish that were given 12 feedings per day.

Feeding frequency and family did not have significant effects on mean percent survival at 30 d postexogenous feeding ($P = 0.63$ and 0.79, respectively; Table 2). Mean percent survival was high for both treatments and both families, ranging from 93.0% to 98.0%. The feeding frequency × family interaction term was not significant for mean TL ($P = 0.7371$), mean body weight ($P = 0.9829$), or mean percent survival ($P = 0.8278$) and was therefore removed from each model.

**Alternate Food Type Experiment with Hatchery-Produced Larvae**

Mean body weight of hatchery-produced Lake Sturgeon larvae at 14 d postexogenous feeding was significantly affected by alternate food type ($P < 0.0001$) and family ($P = 0.0009$). Mean body weight was significantly greater in the 100% *Artemia* treatment group than in the 50% *Artemia*–50% Otohime B2 group ($P < 0.0001$) or the 100% Otohime B2 group ($P < 0.0001$; Table 3). Additionally, mean body weight of fish that received 50% *Artemia*–50% Otohime B2 was significantly greater than that of fish fed 100% Otohime B2 ($P < 0.0001$; Table 3). Mean body weight was significantly greater for fish in family 2 than for fish in family 1 ($P < 0.0009$; Table 3).

The effect of alternate food type on mean percent survival at 14 d postexogenous feeding was significant ($P = 0.0008$); however, survival was not different between the two families ($P = 0.10$). Mean percent survival for larvae that were given 100% *Artemia* was significantly greater than the survival of fish that were fed 100% Otohime B2 ($P = 0.002$; Table 3). Additionally, mean percent survival for fish that were fed 50% *Artemia*–50% Otohime B2 was significantly greater than the survival of fish receiving 100% Otohime B2 ($P = 0.004$; Table 3). The alternate food type × family interaction term was not significant for mean weight ($P = 0.62$) or mean survival ($P = 0.59$) and was thus removed from each model.

**Alternate Food Type Experiment with Wild-Caught Larvae**

Mean body weight of wild Lake Sturgeon larvae at 14 d postexogenous feeding was significantly affected by alternate food type ($P < 0.0001$). Mean body weight in the 100% *Artemia*, 90% *Artemia*–10% filtrate, 50% *Artemia*–50% Otohime B2, and 45% *Artemia*–45% Otohime B2–10% filtrate treatment groups was significantly greater than mean weight in the 100% Otohime B2 group (all $P < 0.0001$) or the 90% Otohime B2–10% filtrate group (all $P < 0.0001$; Table 4). Additionally, the mean body weight of fish that received 100% *Artemia* was significantly greater than the mean weight of larvae that were fed 45% *Artemia*–45% Otohime B2–10% filtrate ($P = 0.0370$; Table 4).

Survival at 14 d postexogenous feeding varied significantly among alternate food types ($P < 0.0001$). Mean percent survival of larvae that received 100% *Artemia*, 50% *Artemia*–50% Otohime B2, 90% *Artemia*–10% filtrate, and 45% *Artemia*–45% Otohime B2–10% filtrate was significantly greater than the survival of fish that were given 100% Otohime B2 or 90% Otohime B2–10% filtrate (all $P < 0.0001$; Table 4). Mean percent survival was higher from alternate food type treatments that included *Artemia* ($94.0 ± 2.0\%$) than from treatments that did not include *Artemia* ($12.0 ± 4.0\%$; Table 4).

**DISCUSSION**

During the transition from endogenous to exogenous feeding and for several weeks after the onset of exogenous feeding, larval fish in aquaculture typically exhibit periods of low growth and high mortality. We quantified the effects of commonly utilized feeding strategies on the body size and survival of Lake Sturgeon larvae during this critical early life period. Significant

Table 3. Effects of alternate food types (brine shrimp *Artemia* spp. and Otohime B2 Larval Diet) on body weight and percent survival (mean ± SE) at 14 d postexogenous feeding for two families of hatchery-produced Lake Sturgeon larvae. For a given variable and a given family, values with a lowercase letter in common are not significantly different ($P > 0.05$).

<table>
<thead>
<tr>
<th>Alternate food type</th>
<th>Body weight (g)</th>
<th>Percent survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% <em>Artemia</em></td>
<td>0.31 ± 0.004 x</td>
<td>98.0 ± 2.0 y</td>
</tr>
<tr>
<td>50% <em>Artemia</em> and 50% Otohime B2</td>
<td>0.22 ± 0.011 y</td>
<td>95.0 ± 4.0 y</td>
</tr>
<tr>
<td>100% Otohime B2</td>
<td>0.06 ± 0.006 z</td>
<td>99.0 ± 1.0 y</td>
</tr>
</tbody>
</table>

Table 4. Effects of alternate food types (brine shrimp *Artemia* spp., Otohime B2 Larval Diet, and filtrate) on body weight and percent survival (mean ± SE) at 14 d postexogenous feeding for wild-caught Lake Sturgeon larvae. For a given variable, values with a lowercase letter in common are not significantly different ($P < 0.05$).

<table>
<thead>
<tr>
<th>Alternate food type</th>
<th>Body weight (g)</th>
<th>Percent survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% <em>Artemia</em></td>
<td>0.41 ± 0.024 z</td>
<td>94.0 ± 1.8 y</td>
</tr>
<tr>
<td>50% <em>Artemia</em> and 50% Otohime B2</td>
<td>0.29 ± 0.020 zy</td>
<td>90.0 ± 2.3 y</td>
</tr>
<tr>
<td>100% Otohime B2</td>
<td>0.03 ± 0.006 x</td>
<td>96.0 ± 2.0 y</td>
</tr>
<tr>
<td>50% <em>Artemia</em> and 10% Otohime B2 and 10% filtrate</td>
<td>0.02 ± 0.002 x</td>
<td>12.0 ± 3.2 y</td>
</tr>
<tr>
<td>90% <em>Artemia</em> and 10% filtrate</td>
<td>0.37 ± 0.014 zy</td>
<td>96.0 ± 2.0 y</td>
</tr>
<tr>
<td>45% <em>Artemia</em>, 45% Otohime B2, and 10% filtrate</td>
<td>0.26 ± 0.010 y</td>
<td>94.0 ± 3.1 z</td>
</tr>
</tbody>
</table>

**TABLE 3.** Effects of alternate food types (brine shrimp *Artemia* spp., Otohime B2 Larval Diet) on body weight and percent survival (mean ± SE) at 14 d postexogenous feeding for two families of hatchery-produced Lake Sturgeon larvae. For a given variable and a given family, values with a lowercase letter in common are not significantly different ($P > 0.05$).
differences in mean TL and mean body weight at 30 d postexoge-
nous feeding were identified as a function of feeding frequency in
hatchery-produced larvae. In addition, we identified signifi-
cant differences in mean body weight and survival as a function
of alternate food types fed to hatchery-produced and wild-caught
larvae. When hatchery-produced larvae were utilized, families
were kept separate (not typically done in aquaculture) to quan-
tify family effects in response to hatchery feeding strategies, as
such information is lacking in the literature. Our results provide
information on feeding strategies that can be used to develop
standard operating procedures for culture of the Lake Sturgeon,
which is a species of conservation concern.

Feeding Frequency Experiment with Hatchery-Produced
Larvae
Increasing the feeding frequency has been documented to
improve larval growth and survival for many fish species (Tung
and Shiau 1991; Cho et al. 2003; Li et al. 2014; among others).
However, in this study, mean body size of Lake Sturgeon larvae
that were fed three times per day was significantly greater than
that of fish fed 12 times per day. Our results are similar to those
reported for demersal species, wherein fewer feedings either
improved growth or did not reduce growth (Petkam and Moodie
2001; Giberson and Litvak 2003).

The observed differences in body size during this experiment
could have been due to differences in Artemia size or energy con-
tent as a function of feeding time (Sorgeloos et al. 2001). For
example, fish that were fed three times daily received a third
of the total daily ration soon after Artemia had been harvested,
whereas fish that were fed 12 times per day did not (cumula-
tively) receive a third of the daily ration until 4 h later. Accord-
ing to Sorgeloos et al. (2001), Artemia nauplii that are kept alive
may after several hours develop into a second larval stage that
contains lower amounts of amino acids and is less digestible
than recently hatched Artemia (Leger et al. 1987). In addition,
more developed Artemia nauplii, despite their reduced energy
content, may be larger and exhibit greater swimming and predatu-
ror evasion abilities (Sorgeloos et al. 2001), which may have
made feeding more difficult for the Lake Sturgeon larvae that
were fed 12 times per day. Neither Artemia energy content nor
Artemia size was measured in this study. Because differences in
larval body size due to feeding frequency may have been a re-
sult of nested effects related to Artemia harvest time, we suggest
that facilities utilizing a protocol of one Artemia harvest per day
for feeding larval fish should acknowledge the potential effects
on fish body size. Additional studies incorporating food types
that do not lose energy content or change in size as a function
of time are needed to further support our results that a feeding
frequency of three times per day can improve larval body size
relative to more frequent feedings (e.g., 12 times per day).

Survival of Lake Sturgeon larvae to 30 d postexogenous feed-
ing was not significantly different as a function of feeding fre-
quency; however, it was higher than expected and higher than
previously reported in studies of sturgeon. At 30 d postexoge-
nous feeding, differences were not detected in larval body size or
survival between the families used in this experiment. Although
our sample size was small, these results are surprising given the
amount of research that has associated larval body size during
early and later ontogenetic life periods with genotypic origin
(Nunez et al. 2011), including recent work on Lake Sturgeon
(Dammerman et al. 2015). It could be, however, that genetic
effects diminish as a function of age, similar to the early life
periods in Chinook Salmon Oncorhynchus tshawytscha (Heath
et al. 1999). Similar studies with larger sample sizes of different
families are needed to quantify the effects of family on body
size.

Alternate Food Type Experiment with Hatchery-Produced
Larvae
At 14 d postexogenous feeding, significant differences were
detected in the mean body weight of hatchery-produced larvae
as a function of three alternate food types. Larvae that received
live Artemia alone or in combination with formulated food types
exhibited greater body sizes than fish whose feeding regime did
not include Artemia. These results are similar to those of other
studies (Petkam and Moodie 2001; Agh et al. 2012; Piotrowska
et al. 2013). Previous research has suggested that sturgeons im-
print to the food that is provided at the onset of exogenous
feeding (Buddington and Christofferson 1985; DiLauro et al.
1998); therefore, additional studies using alternate food types
for hatchery-produced larvae that have not been pre-exposed
to other food types (namely Artemia) are warranted. Our re-
sults supplement previous research on food types for hatchery-
produced larvae, although those studies may have also been
confounded by offering Artemia to fish prior to initiating the
experiments (Anderson 1984; DiLauro et al. 1998). Similar to
the study by DiLauro et al. (1998), hatchery-produced larvae
in our study were not observed to feed on the formulated diet
(Otohime B2), which suggests that larval Lake Sturgeon either
do not imprint on or do not prefer formulated feed up to 14 d
postexogenous feeding. These findings are further supported by
the lower mean body weight of hatchery-produced larvae that
were given Otohime B2. Conversely, Kappenman et al. (2011)
reported promising results for Otohime B2 during a study of
hatchery-produced larval Pallid Sturgeon Scaphirhynchus al-
lus; those authors found that larvae receiving Otohime B2 ex-
hibited mean growth rates of 7.9% per day and survival rates of
54–72%. This could be due to the fact that Kappenman et al.
(2011) mixed Otohime B2 in equal proportions with the Oto-
hime B1 Larval Diet, which had a smaller particle size that
was more easily ingested; thus, the Pallid Sturgeon larvae
were offered a range of food particle sizes from 0.20 to 0.62 mm.
Artemia nauplii typically range in size from 0.43 to 0.52 mm
(Leger et al. 1987), so a gape limitation due to the particle size
of Otohime B2 (0.36–0.62 mm) is unlikely to have been a prob-
lem in our study. Mean percent survival of hatchery-produced
lactes were higher when the feeding treatment included *Artemia* than when Otohime B2 was administered in the absence of *Artemia*. Survival of hatchery-produced larvae that were subject to feeding regimes containing *Artemia* was higher than expected and was higher than previously reported survival rates for sturgeon.

Mean body weight of hatchery-produced larvae at 14 d postexogenous feeding significantly differed between the two families reared separately during this experiment. Differences in body size between Lake Sturgeon families could be due to differences in maternal provisioning (i.e., egg size and free embryo size), as was observed in Siberian Sturgeon *Acipenser baeri* (Gisbert et al. 2000). However, results of our feeding frequency experiment showed that family effects on body size were not significant at 30 d postexogenous feeding. The difference in results of the feeding frequency and alternate food type experiments suggests that family effects associated with maternal provisioning may diminish as a function of age after 14 d postexogenous feeding. Additional studies with a greater number of evaluated families would be needed to confirm this.

**Alternate Food Type Experiment with Wild-Caught Larvae**

At 14 d postexogenous feeding, significant differences in mean body weight of wild-caught Lake Sturgeon larvae were detected as a function of six alternate food types. Larvae that were fed live *Artemia* alone or in combination with formulated food and filtrate exhibited greater body weight, comparable to the results of similar studies (Petkam and Moodie 2001; Agh et al. 2012; Piotrowska et al. 2013). However, we used wild-caught larvae in this experiment, thereby expanding upon previous research by providing results from a progeny source for which no alternate food type information had existed previously. Early rearing of wild-caught larvae in aquaculture has been difficult due to the inability of larvae to transition to *Artemia* near the onset of exogenous feeding (Holtgren et al. 2007; Crossman et al. 2014). It has been suggested that wild-collected larvae have already begun to feed in the river and thus are imprinted on natural food types (DiLauro et al. 1998; Crossman et al. 2014). However, comparisons of body size suggested—and visual observations confirmed—that wild-produced larvae in this study only fed on *Artemia*. These results are similar to those for the hatchery-produced larvae and to those reported by DiLauro et al. (1998), given that wild-produced larvae were not observed feeding on the formulated food (Otohime B2). In addition, wild-produced larvae were not observed to consume food that was derived from filtrate. Our results indicate that wild Lake Sturgeon only fed on *Artemia*, which is further supported by the significantly lower body weight as a function of decreasing *Artemia* proportion in the feeding regime.

Mean percent survival was significantly higher when the wild-produced larvae were given food types that contained *Artemia* than when the larvae received only Otohime B2 or a mixture of Otohime B2 and filtrate. Survival of wild-produced larvae that were fed *Artemia* was higher than expected and exceeded survival values reported in previous studies.

**Survival of Larvae in the Feeding Strategy Experiments**

Larvae in all experiments exhibited mean survival rates near 96.0% when fed *Artemia* at 26% of BW/d during the first and second weeks after the onset of exogenous feeding, 13% of BW/d during the third week, and 11% of BW/d during the fourth week. This result suggests that the amount of food provided (rather than the frequency of feedings) may be causing the high mortality rates of Lake Sturgeon during the larval stage. Additionally, imprinting to specific food types among wild-caught larvae may not be a factor affecting survival, as larvae in this study consumed live *Artemia* and showed faster growth and higher survival than larvae that were fed alternate food types. Our review of Holtgren et al. (2007) revealed that SRFs as currently configured (two 2.6-L *Artemia* hatchers to produce up to 900 fingerling Lake Sturgeon) are only able to provide 1–5% of BW/d during the first through fourth weeks postexogenous feeding and thus may be limiting larval survival. Based on the *Artemia* wet weight : dry weight conversion (dry weight = 0.1767·[sieved wet weight] – 0.0541) determined here and based on feeding rates predetermined for White Sturgeon *Acipenser transmontanus* (Deng et al. 2003), we calculated that approximately two 17-L *Artemia* hatchers (CA-BSH1; Florida Aqua Farms, Inc.) would be needed to effectively feed up to 900 Lake Sturgeon larvae. Recommended feeding rates for Lake Sturgeon are not readily available in the literature, thus warranting investigation of optimal feeding rates for this species during the larval period. Lake Sturgeon feeding rates are especially needed for facilities (e.g., SRFs) that utilize ambient-temperature river water with the potential to exceed 26°C, given that underfed larvae have a reduced level of heat-shock proteins, which allow larval fish to respond to elevated levels of heat-related stress (Deng et al. 2009).

As larvae begin exogenous feeding, several factors may also affect their survival in hatchery environments, including tank complexity (e.g., water volume and water flow) as well as aquaculture management practices (e.g., disease treatment and tank cleaning; Conte 1988; Crossman et al. 2014). In this study, 3.0-L, polycarbonate flow-through aquaria were used, and the tanks were populated with different numbers of fish for each of the three experiments due to the availability of wild-caught or hatchery-produced fish at the onset of the experiment. Given the similarity in results (high survival) across experiments, we do not suspect that different sample sizes for the treatment groups added significant variability to the results. Except for temperature, water quality variables were not measured during this study. However, water flow in each tank was monitored routinely and set at a rate that would achieve approximately seven complete water changes per hour. Based on visual observation, that flow rate allowed for sufficient evacuation of excess food and waste approximately 30 min after the food was added to a tank. Therefore, it is unlikely that water quality differed among tanks as a
function of experimental treatment. The feeding rates and concomitant growth rates reported here are based on the ranges of temperature experienced by fish during each experiment, and that temperature variation was experienced uniformly across all treatments. Fish were not given any preventative chemotherapeutic prophylactic during the experiments. Furthermore, tanks were cleaned once weekly, which may have reduced handling stress relative to facilities with daily tank cleaning.

Based on the present results, we recommend feeding Artemia to Lake Sturgeon larvae three times per day at 26% of BW/d during weeks 1 and 2 postexogenous feeding, 13% of BW/d during week 3, and 11% of BW/d during week 4 to improve body size and survival. Continued investigations seeking more economical food types that either maintain or improve body size without compromising survival are still needed. Furthermore, survival in traditional and SRF aquaculture settings is likely to improve if optimal feeding rates are established for larval sturgeon.

ACKNOWLEDGMENTS

 Funding for this study was provided by the Great Lakes Fishery Trust, the Michigan Department of Natural Resources (MDNR), and the Federal Aid in Sport Fish Restoration Program. We thank Jim Holser (MDNR); Nathan Barton, Kari Dammerman, Adam Umstead, Troy Smith, Lindsey Adams, and especially Sarah Walton (Department of Fisheries and Wildlife, Michigan State University [MSU]); David Reyes-Gastelum (Center for Statistical Training and Support, MSU) for statistical consultation; Jim Tucker (Tower-Kleber Limited Partnership); and Gerald R. Gray (Sturgeon for Tomorrow, Cheboygan, Michigan).

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