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Development of First-Feeding Protocols for Indoor Larviculture of Largemouth Bass (*Micropterus salmoides*)

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Largemouth bass (LMB) Micropterus salmoides *fry do not accept prepared diets at first feeding. Fry are initially reared in fertilized ponds on natural live foods until large enough to be feed trained. Unpredictable weather patterns and depletion of natural forages can affect nursery pond survival. A series of experiments was conducted to investigate the use of* Artemia *nauplii prepared diets and optimal feeding schedules to raise LMB fry from first feeding through habituation to a commercial dry diet. In Studies 1, 2, and 3, swim-up fry were transferred to a recirculating system and stocked into either 3-L (Studies 1 and 2) or 10-L (Study 3) acrylic aquaria. Study 1 screened candidate diets to evaluate whether LMB fry could be transitioned directly to prepared diets or if they required live foods. In Study 2 the optimum duration for feeding live* Artemia *(1, 2, or 3 weeks) and the appropriate size of commercial diets (<200 or 200–360 μm) were evaluated. Study 3 was designed to identify the best transitional feed. Results from Study 1 indicate that fry fed Otohime-A (<200 μm) and decapsulated* Artemia *cysts performed better than those fed other diets tested. However, survivals were low (6%–8%) indicating a need for live feed initially. In Trial 2, fry fed live* Artemia *nauplii for two weeks and then transitioned to a 200–360 μm diet (Otohime-B) performed better than other diet combinations tested. In Study 3, survival was significantly higher in treatments using decapsulated*

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Artemia *cysts or Otohime-B as transitional diets between initial live* Artemia *feeding and trout starter. These data indicate that LMB fry can be successfully raised from first feeding to fully habituated to a commercial trout starter by feeding live* Artemia *nauplii for two weeks, followed by a gradual transition to either decapsulated* Artemia *cysts or Otohime-B for one week, then gradually transitioning to trout starter. Surviving fish were easily transitioned to commercial floating feed (Study 4). This protocol yielded survival rates of approximately 70% and may improve the reliability of LMB fingerling production by eliminating the outdoor nursery pond phase.*

KEYWORDS Largemouth bass, larviculture

INTRODUCTION

Largemouth bass (LMB) *Micropterus salmoides* have been cultured in the United States since the 1890s, primarily for stock enhancement programs. More recently production of larger sized LMB has increased (Brandt 1991) based on their increased use for corrective stocking in sport-fish ponds (Joint Subcommittee on Aquaculture [JSA] 1983), fee fishing (Dupree and Huner 1984), managed trophy fisheries (JSA 1983), and for live sales as food fish in ethnic Asian markets (Tidwell et al. 1996). It is estimated that 1,000,000 kg of 400–700 g LMB are produced in the United States for live food-fish markets. The Joint Subcommittee on Aquaculture listed determination of efficient growout procedures under intensive conditions as one of the research priorities for development of LMB aquaculture (JSA 1983). If this information can be generated, there appears to be a favorable financial potential for increased commercial production of this species in some states.

LMB are primarily spawned in ponds by stocking brood fish and allowing reproduction to occur naturally (Tidwell et al. 2000). After spawning has occurred, LMB fry are raised in nursery ponds where they feed on zooplankton until they are large enough (>4 cm) to be feed trained in tanks (Heidinger 2000). Once feed trained, the fingerlings are then stocked back into ponds for growout. These procedures were described by Snow in the 1960s (Snow 1965, 1968, 1975) and have changed little since.

Both the spawning and nursery pond phases are highly vulnerable to weather perturbations, which cause abrupt changes in water temperature, resulting in variable success year to year. Even with favorable weather conditions, zooplankton populations in nursery ponds are notoriously difficult to manage and even a short period (1–2 days) of low abundance can negatively impact growth and survival of young fish and increase the incidence

of cannibalism. LMB can be spawned indoors in raceways using spawning mats under controlled conditions (Isaac et al. 1998). It might be possible to intensify and better control LMB fingerling production by spawning and rearing fry indoors in temperature-controlled tanks; however, very little research has been conducted on rearing LMB fry indoors.

One of the greatest obstacles to the intensification of LMB fry production is that swim-up fry do not directly accept prepared diets (Tidwell et al. 2000). In other aquaculture species such as channel catfish, *Ictalurus punctatus,* and rainbow trout, *Onchorhychus mykiss*, first-feeding fry will accept artificial feeds (Lovell 1989). This is a positive attribute for a commercial species as it reduces the uncertainty of relying on zooplankton populations in ponds in terms of their abundance, proper size, and even timing (Geiger 1983). In recent years progress has been made in the development of larval and fry diets and feeding, especially for marine fish species. In fish that do not accept dry diets at first feeding, live brine shrimp nauplii *Artemia* sp. represent a convenient and highly nutritious source of live food organisms (Webster and Lovell 1990). A proper combination of feeding live food (*Artemia*) during the initial period of exogenous feeding with a transition to artificial larval diets after a few days has proven feasible in the culture of other freshwater fish predators such as striped bass, *Morone saxatilis* (Webster and Lovell 1990; Chu and Ozkizilcik 1999), walleye, *Sander vitreus* (Nickum 1986; Dabrowski et al. 2000), Murray cod, *Maccullochella peelii peelii* (Ryan et al. 2007), and the alligator gar, *Atractosteus spatula* (Clay et al. 2011). These intensive methods of early rearing should be evaluated for LMB as a method to potentially eliminate the unpredictable nursery pond stage.

A series of studies were designed to evaluate different artificial diets with and without live feeds and to identify critical periods for the use of these diets. This research also compared the fatty acid profiles of fry fed different treatments as they can not only reflect the content of the different diets, but also selective retention of nutritionally important fatty acids (Tidwell et al. 1992) as there is some indication that largemouth bass may metabolize certain fatty acids differently that other freshwater species (Tidwell et al. 1996). The overall goal of the research is to develop protocols for the intensive rearing of LMB fry indoors from hatching through habituation to a commercial dry diet.

The objective of Study 1 was to screen artificial diets for acceptance by largemouth bass fry at the initiation of exogenous feeding. Study 2 was conducted to evaluate the best performing diets from Study 1, either alone or in combination with different length periods of initial live *Artemia* feeding. The objective of Study 3 was to evaluate different artificial diets as transitional feeds between *Artemia* feeding and final diets and compare gradual vs. immediate diet transitions. Study 4 followed the best treatments from Study 3 through the final stage of training to commercial floating pellets.

MATERIALS AND METHODS

For Studies 1, 2, and 3, LMB broodstock were paired into three 0.04-ha ponds at a rate of four pairs per pond. Spawning mats were placed at each of the four corners of the ponds at a depth of approximately 0.6 m. Ponds were observed daily for spawning behaviors and, when spawning or nest guarding behavior was observed, mats were visually checked for eggs. When presence of eggs was confirmed, mats were transferred to 380-L indoor tanks, incubated at 24°C using dechlorinated municipal water, and monitored for hatching. When visual observation indicated sufficient numbers of "swim-up" fry to indicate initiation of exogenous feeding, they were transferred to study tanks.

All studies were conducted in an AHAB recirculating aquarium system (Aquatic Habitats Inc., Apopka, FL, USA). First-feeding largemouth bass fry (0.008 g) were used for Studies 1, 2, and 3. Fry were individually counted into tanks until target densities were achieved. The stocking density for Study 1 was 33.3 fry per liter. Stocking density for Studies 2 and 3 were 50 fry per liter. Study 4 was stocked at a rate of 10 fish per liter and used fish from Study 3. Tanks were randomly assigned to treatments to eliminate any differences in tank location within the system. Aquaria were covered with transparent blue acrylic lids to prevent fish from jumping out and on the sides by black construction paper to maintain similar light intensities, allow for the fry to visually silhouette the food particles, and prevent visual interactions between fish in adjacent tanks. The room was kept on a 12:12 light cycle with light beginning at 0700 h and ending 1900 h.

Temperature, dissolved oxygen, and pH in the system were monitored twice daily (0700 h and 1500 h) using a YSI 556 multi probe meter (Yellow Springs Instruments, Yellow Springs, OH, USA). Total ammonia-nitrogen and nitrite-nitrogen were measured daily using a HACH Odyssey digital spectrophotometer (HACH Company, Loveland, CO, USA). Total hardness and alkalinity were measured three times per week (MWF) using a HACH digital titrator. Water temperature was maintained at approximately 24°C by controlling the ambient temperature of the room. Fry were fed to excess, based on visual response, three times per day at 0800 h, 1200 h, and 1600 h. Tanks were cleaned prior to the 0800 h feeding and after the 1200 h feeding by siphoning and wiping the sides. All of the above protocols were the same for Studies 1–3. The specifics of each trial are described below.

Study 1

Study 1 was designed to screen six larval feeds for acceptance by largemouth bass fry at either first feeding or after three days of feeding with live *Artemia* nauplii (Figure 1). The study was conducted in 36, 3-L tanks stocked with 100 swim-up fry per tank. There were 12 treatments with three replicate tanks

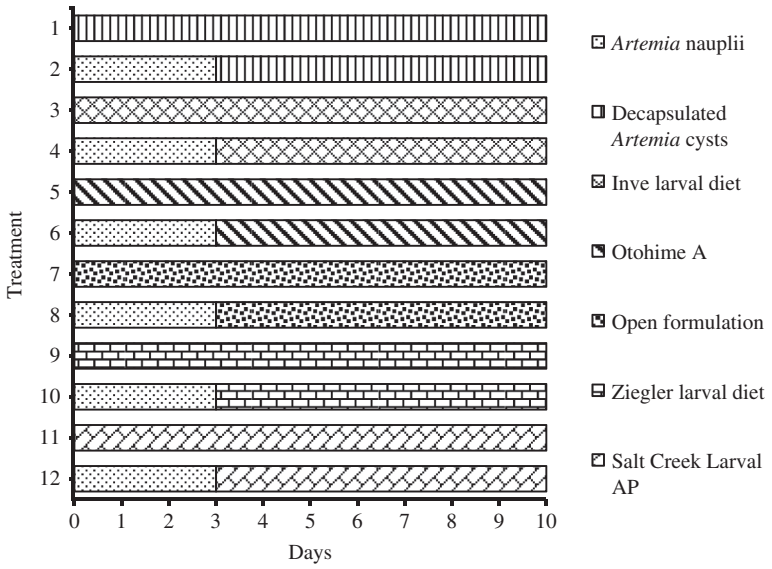

FIGURE 1 Feeding schedules for largemouth bass fry in Study 1. The changes between diets in all treatments were immediate.

each. The six diets were fed from either day 1 (no *Artemia*) or beginning on day 4 after three days of feeding live *Artemia* nauplii. The total duration of the study was 10 days. The six diets evaluated were decapsulated *Artemia* cysts, Inve larval diet, Otohime-A, an open formulation microencapsulated diet designed for freshwater fish fry (Fredrick Barrows, USFWS, Bozeman, Montana), Ziegler larval diet, and Salt Creek larval diet. These diets were fed to first-feeding LMB fry in treatments 1, 3, 5, 7, 9, and 11, respectively. For treatments 2, 4, 6, 8, 10, and 12, the same six diets were fed to LMB fry that had been communally fed live *Artemia* for three days prior to stocking. At the conclusion of the study all fish from each aquaria were bulk weighed and counted.

Study 2

Study 2 was designed to compare the best performing feeds from Study 1 as initial diets for first-feeding LMB or as transitional (intermediate) diets after either one or two weeks of feeding live *Artemia* (Figure 2). This study was conducted in 3-L tanks stocked with 50 fry/L. There were 12 treatments with three replicate tanks each. The duration of the study was five weeks. Treatments varied during weeks 1–3, with all treatments receiving trout starter during weeks 4 and 5. Treatment 1 (control) was unfed to determine how long fry would survive without feeding. In Treatment 2 fry were fed live *Artemia* for the first three weeks. Fish in Treatment 3 were

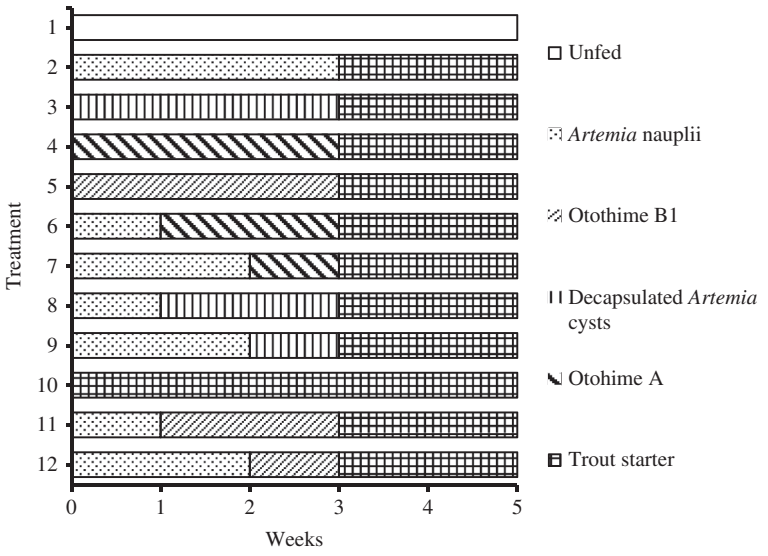

FIGURE 2 Feeding schedules for largemouth bass fry in Study 2. The changes between diets in all treatments were immediate.

fed decapsulated *Artemia* cysts for the first three weeks. Treatments 4 and 5 were fed Otohime-A (<200 μm) or Otohime-B (200–360 μm), respectively, for the first three weeks. Treatments 6 and 7 were fed live *Artemia* for one or two weeks, respectively, and then transitioned to Otohime-A for two or one week, respectively. Treatments 8 and 9 were fed live *Artemia* nauplii one or two weeks, respectively, then fed decapsulated *Artemia* cysts two or one week, respectively, as the transitional feed. Treatment 10 was fed the commercial trout starter for the entire five weeks. Treatments 11 and 12 used live *Artemia* nauplii for one or two weeks, respectively, then transitioned to the larger Otohime-B for two or one week, respectively. At the conclusion of the study all fish from each of the aquaria were counted and individually weighed.

Study 3

Building on results from Study 2, Study 3 was designed to determine the best transitional diet for LMB and the best protocol for the transition between diets (i.e., gradual or immediate). This study was scaled up to 10-L aquaria with a stocking density of 50 fry/L. Six treatments with three replicates each were evaluated. All treatments were fed live *Artemia* for two weeks, then a transitional diet for one week, and trout starter for the final two weeks (Figure 3). Fry in Treatment 1 were fed Otohime-B as the transitional diet and the transitions from live *Artemia* to Otohime-B and Otohime-B to trout starter were both immediate. Treatment 2 used the same diets and schedules

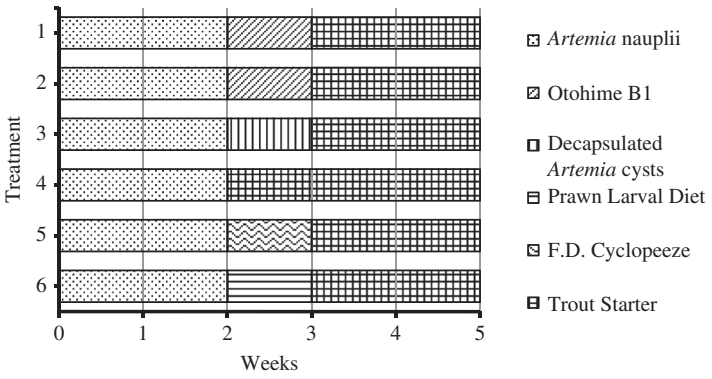

FIGURE 3 Feeding schedules for largemouth bass fry in Study 3. The transition between diets was immediate in Treatment 1 and gradual in all other treatments. Gradual transitions took place over a three-day period with day one feeding 75% original diet/25% new diet, day two 50% original diet/50% new diet, and day three 25% original diet/75% new diet.

as Treatment 1, but the transitions between diets were gradual. These transitions were each over three day periods with day one being 75% original diet and 25% new diet. Day 2 was 50% original and 50% new, day 3 was 25% original diet and 75% new diet, and day 4 was 100% new diet. Treatments 2, 3, 4, 5, and 6 were all gradual transitions and used the following diets as the transitional diet, respectively: Otohime-B, decapsulated *Artemia* cysts (Inve), trout starter (Rangen, Fast Start), or freeze-dried Cyclopeze. At the conclusion of Study 3 bulk weights and total number were recorded for each aquarium, and individual weights were recorded for 100 randomly selected individuals from each aquarium or all if less than 100 remained. Samples of fish from each tank were frozen with liquid nitrogen and subsequently analyzed for proximate composition, fatty acid profiles, and amino acid profiles (Woodson-Tenent Laboratories, Memphis, TN, USA). Samples of unfed swim-up fry at stocking, fry that had been fed *Artemia* for 2 weeks, and samples of all the diets (including *Artemia* nauplii) were also submitted for the same analyses.

Study 4

Study 4 was conducted to evaluate how well fish from the best performing treatments in Study 3 would transition to a floating pellet, the final stage in the largemouth bass feed training protocol. This study was conducted in 10-L aquaria with a stocking rate of 10 fish/L for a total of 100 fish/tank. Two treatments with four replicates each were evaluated. Fish from Treatments 2 and 3 (the best performing treatments) were harvested and immediately re-stocked to now represent Treatments 1 and 2, respective to Study 4. These fish were then gradually transitioned to a commercial 1.0 mm floating pellet

(Purina, AquaMax) over a three-day period then fed the floating diet for an additional six days. At the end of the study all fish from each tank were counted and individually weighed.

Statistical Analysis

Data from Studies 1–3 were statistically compared by ANOVA ($P \leq 0.05$) using Statistix version 7.0 (Statistix Analytical Software 2000). If significant differences were found among treatments, treatment means were separated using Fisher's least significant difference test. Study 4 treatments were compared with Students T-Test ($P \leq 0.05$) using Statistix version 7.0 (Statistix Analytical Software 2000). All percentage and ratio data were arc sin transformed prior to analysis (Zar 1984). All data are presented untransformed to facilitate comparisons.

RESULTS AND DISCUSSION

Study 1

During Study 1 water quality variables averaged (±SD): temperature, 23.2 ± 0.5°C; dissolved oxygen, 8.7 ± 0.12 mg/L; pH, 8.1 ± 0.06; total ammonia-N, 0.69 ± 0.35 mg/L; un-ionized ammonia-N, 0.11 ± 0.18 mg/L; and nitrite-N, 0.41 ± 0.27 mg/L.

At the end of the 10-day trial, fish in Treatments 1, 2, 5, and 6 had significantly higher ($P \leq 0.05$) survival (6.4%–8.4%) than fish in other treatments, which were not significantly different ($P > 0.05$) from each other (Table 1). Fry in Treatments 1 and 5 were fed only non-living diets from the time of swim-up, indicating that a small percentage of first-feeding largemouth bass fry can survive on dry diets without live foods. However, this study was only over a short time period (10 days).

Average weight was significantly higher ($P \leq 0.05$) in Treatment 2 than in any other treatment. In this treatment, first-feeding fry were fed live *Artemia* nauplii for three days and then decapsulated *Artemia* cysts for seven days. Average weights of fry in Treatments 1 and 6 were significantly larger ($P \leq 0.05$) than weights of fry in all treatments other than Treatment 2. Treatment 1 utilized decapsulated *Artemia* cysts from first feeding through the entire trial, while fry in Treatment 6 were fed live *Artemia* nauplii for three days and then Otohime-A for days four through ten.

Production (mg/L) combines both survival and average weight and can be a better measure of overall success than either individual measure. Production was significantly greater ($P \leq 0.05$) in Treatment 2 than in any other treatment. Production in Treatment 1 was greater ($P \leq 0.05$) than in Treatments 3–6. The third highest production was in Treatment 6 (fed live

TABLE 1 Study 1: Mean (±SE) for average final weight (g), survival (%), and production (mg/l) for largemouth bass fry fed either decapsulated *Artemia* cysts, Inve larval diet, Otohime-A, an open formulation microencapsulated diet, Ziegler larval diet, or Salt Creek larval diet. In odd number treatments (1–11) these respective diets were fed beginning at swim-up. In even number treatments (2–12) the respective diets were fed beginning at Day 4 after being communally fed live *Artemia* nauplii for three days.

Treatment	Average weight (mg)	Survival (%)	Production (mg/L)
1	45.2 ± 3.8y	7.8 ± 1.7z	178.7 ± 17.4y
2	89.3 ± 1.4z	7.1 ± 1.0z	317.8 ± 43.3z
3	0 ± 0v	0 ± 0y	0 ± 0v
4	0 ± 0v	0 ± 0y	0 ± 0v
5	19.4 ± 2.8x	8.4 ± 2.1z	76.6 ± 7.4w
6	35.1 ± 0.7y	6.4 ± 1.8z	114.0 ± 34.0x
7	8.9 ± 3.9x	2.2 ± 0.6y	20.6 ± 5.3wv
8	7.7 ± 7.7xwv	0.4 ± 0.4y	5.1 ± 5.1v
9	6.4 ± 6.4wv	0.9 ± 0.9y	8.6 ± 8.6v
10	0 ± 0v	0 ± 0y	0 ± 0v
11	17.5 ± 1.3xw	1.1 ± 0.2y	9.6 ± 1.7v
12	8.2 ± 8.2xwv	0.4 ± 0.4y	5.4 ± 54v

Significant differences ($P \leq 0.05$) are indicated by different letters within columns.

Artemia nauplii for three days then Otohime-A), which was not significantly different ($P > 0.05$) from Treatment 5 (fed Otohime-A throughout).

These data would indicate that an initial period of feeding live *Artemia* nauplii is advantageous. This agrees with Webster and Lovell (1990) and Nickum (1986), who found live *Artemia* nauplii and a transitional diet were successful for culturing striped bass *Morone saxatilis* and walleye *Sander vitreus* fry, respectively. Data from Study 1 also indicate that decapsulated *Artemia* cysts and Otohime-A are the most promising of the non-living diets evaluated.

Study 2

During Study 2, water quality variables averaged (±SD): temperature, 24.1 ± 0.8°C; dissolved oxygen, 7.9 ± 0.2 mg/L; pH, 7.9 ± 0.2; total ammonia-N, 0.29 ± 0.12 mg/L; un-ionized ammonia-N, 0.012 ± 0.004 mg/L; nitrite-N, 0.054 ± 0.031 mg/L; alkalinity, 71.6 ± 11.8 mg/L; and total hardness, 160.8 ± 20.5 mg/L.

In the unfed treatment (Treatment 1) there were no surviving fish by day five post swim-up at 24°C. At three days without food, fry appeared emaciated and beyond recovery. At the end of the study (5 weeks), survival was significantly greater ($P \leq 0.05$) in Treatment 12 (36%) than in Treatments 2, 3, 6, 7, 8, and 9, but not significantly greater ($P > 0.05$) than Treatment 11 (30%), which was not significantly different ($P > 0.05$) from Treatment 9 (24%; Table 2). Survivals in Treatments 3, 6, 7, and 8 were ≤21%. Average

TABLE 2 Study 2: Mean (±SE) of average final weight, survival (%), and production (g/L) of largemouth bass fry fed different combinations of live and prepared diets for three weeks and then trout starter for two weeks, with the exception of Treatment 1, which was unfed. Treatments 2, 3, 4, 5, and 10 were fed *Artemia* nauplii, decapsulated *Artemia* cysts, Otohime-A (<200 μm), Otohime-B (200–360 μm), and trout starter, respectively, for the first three weeks. Treatments 6, 8, and 11 were fed *Artemia* nauplii for one week then Otohime-A, decapsulated *Artemia* cysts, and Otohime-B, respectively, for two weeks. Treatments 7, 9, and 12 were fed *Artemia* nauplii for two weeks and then Otohime-A, decapsulated *Artemia* cysts, and Otohime-B, respectively, for one week. Treatments 1, 4, 5, and 10 had 0% survival and were omitted from results and statistical comparisons.

Treatment	Average Weight (g)	Survival (%)	Production (g/L)
2	0.17 ± 0.03x	20.9 ± 2.1x	1.77 ± 0.50x
3	0.02 ± 0.00w	7.3 ± 2.8w	0.05 ± 0.02w
6	0.04 ± 0.00w	6.4 ± 0.8w	0.09 ± 0.01w
7	0.18 ± 0.02x	6.4 ± 1.1w	0.53 ± 0.09w
8	0.35 ± 0.02z	19.8 ± 3.6x	3.27 ± 0.41y
9	0.30 ± 0.02zy	23.5 ± 0.8bx	3.34 ± 0.28y
11	0.12 ± 0.0x	29.5 ± 3.2zy	1.62 ± 0.19x
12	0.26 ± 0.02y	36.4 ± 3.5z	4.40 ± 0.13z

Significant differences ($P \leq 0.05$) are indicated by different letters within columns.

weights were significantly greater ($P \leq 0.05$) in Treatment 8 (0.35 g) than in treatments 2, 3, 6, 7, 11, and 12, but not significantly different ($P > 0.05$) from Treatment 9 (0.30 g), which was not significantly different ($P > 0.05$) from Treatment 12 (0.26 g). Production (g/L) was significantly greater ($P \leq 0.05$) in Treatment 12 (4.4 g/l) than in any other treatment. Production in Treatments 8 and 9 (at 3.3 and 3.4 g/l, respectively) were significantly greater ($P \leq 0.05$) than production in the remaining treatments.

The four treatments that had survivals >21% all had an initial period of live *Artemia* feeding. The best results were obtained in Treatment 12 with fry fed live *Artemia* for two weeks then fed Otohime-B (the larger particle size). The next most promising results were from Treatments 8 and 9 with fry fed live *Artemia* nauplii for one or two weeks, respectively, then transitioned to decapsulated *Artemia*.

The data from Study 2 indicate that first-feeding largemouth bass benefit from two weeks of feeding live *Artemia* nauplii with a transition to the larger Otohime-B. If feeding of live *Artemia* nauplii is limited to one week, decapsulated *Artemia* cysts are a better transitional feed.

Study 3

During Study 3, water quality variables averaged (±SD): temperature, 24.6 ± 0.6°C; dissolved oxygen, 7.7 ± 0.3 mg/L; and pH, 7.9 ± 0.14; total ammonia-N, 0.313 ± 0.100 mg/L; un-ionized ammonia-N, 0.018 ± 0.028 mg/L; nitrite-N, 0.066 ± 0.035 mg/L; alkalinity, 71.5 ± 16.9 mg/L; and total hardness, 169.4 ± 21.9 mg/L.

TABLE 3 Study 3: Mean (±SE) of average final weight, survival (%), and production (g/L) of largemouth bass fry fed live *Artemia* nauplii for two weeks, then a transitional diet for one week and then trout starter for two weeks. For Treatment 1, the change to the transitional diet was immediate and for all other treatments the change was gradual over a three-day period (75:25, 50:50, 25:75). The transitional diets were Otohime-B (Treatments 1 and 2), decapsulated *Artemia* cysts (Treatment 3), trout starter (Treatment 4), and freeze-dried Cyclopeeze (Treatment 5).

Treatment	Average Weight (g)	Survival (%)	Production (g/L)
1	0.25 ± 0.02y	51.53 ± 1.64y	4.54 ± 0.14x
2	0.26 ± 0.01y	71.0 ± 1.56z	6.35 ± 0.32y
3	0.33 ± 0.02z	76.3 ± 3.53z	8.79 ± 0.06z
4	0.33 ± 0.01z	8.47 ± 1.38w	0.98 ± 0.15w
5	0.12 ± 0.00x	27.8 ± 2.70x	1.64 ± 0.12w

Significant differences ($P \leq 0.05$) are indicated by different letters within columns.

After 35 days, survival was significantly higher ($P \leq 0.05$) in Treatment 3 (76%) and Treatment 2 (71%) than in the other treatments (Table 3). These treatments represented gradual transitions from live *Artemia* nauplii to decapsulated *Artemia* cysts and Otohime-B, respectively. Survival in Treatment 1 (52%; immediate transition to Otohime-B) was significantly greater ($P \leq 0.05$) than Treatments 5 (28%; freeze-dried Cyclopeeze) and 4 (8%; trout starter), which were significantly different ($P \leq 0.05$) from each other.

The average weight of fry in Treatments 3 and 4 were not significantly different ($P > 0.05$) from each other but both were significantly greater ($P \leq 0.05$) than the other treatments. However, the larger average weight of fish in Treatment 4 may have been largely due to low rearing density resulting from poor survival. Average weights of fish in the two Otohime-B-fed treatments (Treatment 1 immediate and Treatment 2 gradual) were not significantly different ($P > 0.05$) but were significantly greater ($P \leq 0.05$) than Treatment 5. Production levels were significantly different ($P \leq 0.05$) among all five treatments. The relationship was Treatment 3 (8.8 g/L) > Treatment 2 (6.4 g/L) > Treatment 1 (4.5 g/L) > Treatment 5 (1.6 g/L) > Treatment 4 (1.0 g/L).

A comparison of Treatments 1 and 2 allow for a direct comparison of immediate and gradual transitions between feed types. While there was no significant difference ($P > 0.05$) in final average weights between the two protocols, survival (%) and production (g/L) were both significantly greater ($P \leq 0.05$) with gradual transition. These data indicate that a process of providing first-feeding LMB with live *Artemia* nauplii for two weeks and then gradually transitioning them to decapsulated *Artemia* cysts or Otohime-B for one week is an effective method for habituating LMB to artificial diets, providing survival rates of >70%.

Tissue proximate analysis (Table 4) showed that fish in Treatment 3 had significantly less ($P \leq 0.05$) moisture than fish in Treatment 2. Fish in

TABLE 4 Mean (±SE) of whole body proximate composition of largemouth bass fry in Study 3 fed live *Artemia* nauplii for two weeks, a transitional diet for one week, and then trout starter for two weeks. For Treatment 1 the change to the transitional diet was immediate and for all other treatments the change was gradual over a three-day period (75:25, 50:50, 25:75). The transitional diets were Otohime-B (Treatments 1 and 2), decapsulated *Artemia* cysts (Treatment 3), trout starter (Treatment 4), freeze dried Cyclopeeze (Treatment 5).

Treatment	Moisture	Protein	Fat	Fiber	Ash
1	78.0 ± 0.3y	14.3 ± 0.1y	4.72 ± 0.1y	0.1 ± 0.1z	2.4 ± 0.0z
2	78.2 ± 0.2y	14.2 ± 0.2y	4.73 ± 0.0y	0.1 ± 0.1z	2.4 ± 0.0z
3	77.0 ± 0.3x	14.9 ± 0.3z	5.22 ± 0.2z	0.0 ± 0.0z	2.5 ± 0.1z
4	77.2 ± 0.1x	14.7 ± 0.2zy	4.56 ± 0.1y	0.0 ± 0.0z	2.5 ± 0.0z
5	79.6 ± 0.2z	13.5 ± 0.1x	4.09 ± 0.0x	0.0 ± 0.0z	2.4 ± 0.0z

Significant differences ($P \leq 0.05$) are indicated by different letters within columns.

Treatment 3 had significantly greater ($P \leq 0.05$) protein levels than fish in Treatments 1, 2, and 5, and greater fat percentage than those in Treatment 1, 2, 4, and 5. There were no significant differences ($P > 0.05$) in terms of fiber or ash. There were no significant differences ($P > 0.05$) in tissue fatty acid profiles of fry in Treatments 1, 2, 3, or 4 (Table 5) except for fry in Treatment 4, which had significantly lower concentrations of n-3 fatty acids than fry in other treatments. Fry from Treatment 5 fed Cyclopeez as a transitional diet had significantly greater ($P \leq 0.05$) percentages of all summary categories of fatty acids in the fish tissue (other than n−3/n−6 ratio) than fish in all other treatments, but performed poorly in the study.

TABLE 5 Mean (±SE) of whole body fatty acid composition (% relative) from largemouth bass fry in Study 3 fed live *Artemia* nauplii for two weeks, a transitional diet for one week, and then trout starter for two weeks. For Treatment 1 the change to the transitional diet was immediate and for all other treatments the change was gradual over a three-day period (75:25, 50:50, 25:75). The transitional diets were Otohime-B (Treatments 1 and 2), decapsulated *Artemia* cysts (Treatment 3), trout starter (Treatment 4), freeze-dried Cyclopeeze (Treatment 5).

	Treatment				
Fatty Acid	1	2	3	4	5
Saturates	28.8 ± 2.5y	27.7 ± 1.1y	25.9 ± 1.2y	26.1 ± 2.6y	34.1 ± 1.3z
Monenes	36.0 ± 3.7y	33.9 ± 1.0y	32.2 ± 0.9y	31.6 ± 3.2y	43.0 ± 2.1z
Dienes	18.3 ± 1.5y	17.5 ± 0.5y	16.6 ± 1.0y	16.7 ± 1.0y	22.3 ± 0.3z
HUFA	21.4 ± 2.0y	20.5 ± 1.6y	21.1 ± 1.5y	20.1 ± 2.1y	26.7 ± 0.2z
PUFA	26.4 ± 2.1y	24.9 ± 0.8yx	24.2 ± 1.0yx	22.8 ± 0.7y	32.3 ± 1.3z
n-3	9.6 ± 0.9y	8.8 ± 0.5yx	9.0 ± 0.5y	7.3 ± 0.1x	11.8 ± 1.0z
n-6	18.3 ± 1.5y	17.5 ± 0.5y	16.6 ± 1.0y	16.7 ± 1.0y	22.3 ± 0.3a
n-3/n-6	1.1 ± 0.1z	1.1 ± 0.0z	1.2 ± 0.0z	1.1 ± 0.1z	1.1 ± 0.0z

Significant differences ($P \leq 0.05$) are indicated by different letters within columns.

TABLE 6 Mean (±SE) of average final weight, survival (%), and production (g/L) of largemouth bass fry in Study 4 fed Purina Aquamax over a nine-day period. Treatment 1 used fish from Study 3 fed Otohim-B as a transitional diet, and Treatment 2 used fish from Study 3 fed decapsulated *Artemia* cysts as a transitional diet.

Treatment	Average Weight (g)	Survival (%)	Production (g/L)
1	0.59 ± 0.03y	87.5 ± 3.7z	17.2 ± 0.7y
2	0.70 ± 0.01z	95.8 ± 1.5z	22.5 ± 0.7z

Significant differences ($P \leq 0.05$) are indicated by different letters within columns.

Study 4

Study 4 followed fish from the best treatments in the previous study through final transition to floating pellets, which is considered the successful endpoint of largemouth feed training protocols (Heidinger 2000). Survival was not significantly different ($P > 0.05$) between the two treatments and averaged 92% overall (Table 6). Average weights and production were both significantly greater ($P \leq 0.05$) in fish gradually transitioned from live to artificial diets on decapsulated *Artemia* cysts (Treatment 2) than on Otohime-B (Treatment 1). However, Otohime-B would still be considered a successful transitional feed, achieving an average of 62% survival in Studies 3 and 4 compared to 73% survival for fish fed decapsulated *Artemia* cysts as the transitional diet.

These results suggest that feeding a diet of live *Artemia* for two weeks and transitioning gradually to decapsulated *Artemia* cysts for one week and then gradually transitioning to a commercial trout starter is the best method for rearing largemouth bass fry indoors. This protocol allows LMB fry to be habituated to a commercial floating pellet at sizes smaller (0.25 g) than are typically feed trained following the nursery pond phase. The traditional 30- to 45-day nursery pond phase normally produces LMB fingerlings of 3.8-5.0 cm (Heidinger 2000), which typically weigh 0.7–1.6 g when brought indoors for feed training (Piper et al. 1982).

CONCLUSION

To intensively culture largemouth bass completely indoors from hatch through feed training could be beneficial as a possible alternative to the highly variable nursery pond phase of production. The protocol of feeding live *Artemia* for a period of two weeks with a gradual transition to decapsulated *Artemia* cysts or Otohime-B for one week and a gradual transition to a commercial trout starter for two weeks worked well in these studies. This protocol produced high survivals ready to transition to a floating

pellet. The entire process took 45 days, which is comparable to the time it takes to produce feed-trained fingerlings using the traditional method of pond culture (Snow 1975; Heidinger 2000). The potential advantage of the intensive method developed in this study is that production could be more predictable and reliable. However, the resulting feed-trained fish from this study were smaller than fish produced by traditional nursery pond methods and should be evaluated for performance in the subsequent growout phase of production.

REFERENCES

Brandt, T. 1991. Temperate basses, *Morone* spp., and black basses, *Micropterus* spp. In *Handbook of nutrient requirements of finfish*, edited by R. P. Wilson, 161–168. Boca Raton, FL: CRC Press.

Chu, F. E., and S. Ozkizilcik. 1999. Acceptability of complex microencapsulated diets by striped bass (*Morone saxatilis*) larvae. *Journal of Experimental Marine Biology and Ecology* 237:1–9.

Clay, T., M. Suchy, A. Ferrara, Q. Fontenot, and W. Lorio. 2011. Early growth and survival of larval alligator gar, *Atractosteus spatula*, reared on artificial floating feed with or without a live *Artemia* spp. supplement. *Journal of the World Aquaculture Society* 42(3): 412–413.

Dabrowski, K., S. Czesny, S. Kolkovski, W. E. Lynch Jr., P. Bajer, and D. A. Culver. 2000. Intensive culture of walleye larvae produced out of season and during regular season spawning. *North American Journal of Aquaculture* 62: 219–224.

Dupree, H. K., and J. V. Huner. 1984. Propagation of black bass, sunfishes, tilapias, eels, and hobby fishes. In *Third report to the fish farmers*, edited by H. K. Dupree and J. V. Huner, 119–135. Washington, DC: U.S. Department of the Interior, Fish and Wildlife Service.

Geiger, J. G. 1983. A review of pond zooplankton production and fertilization for the culture of larval and fingerling striped bass. *Aquaculture* 35: 353–369.

Heidinger, R. 2000. Black bass/largemouth bass culture. In *Encyclopedia of aquaculture*, edited by R. R. Stickney, 108–117. New York: Wiley-Interscience.

Isaac, Jr., J., T. M. Kimmel, R. W. Bagley, V. H. Staats, and A. Barkoh. 1998. Spawning behavior of Florida largemouth bass in an indoor raceway. *The Progressive Fish-Culturist* 60:59–62.

Joint Subcommittee on Aquaculture (JSA). 1983. *Largemouth bass species plan. National Aquaculture development plan vol. II*. Washington, DC: Department of the Interior.

Lovell, T. 1989. *Nutrition and feeding of fish*. New York: Van Nostrand Reinhold.

Nickum, J. 1986. Walleye. In *Culture of non-salmonid freshwater fishes*, edited by R. R. Stickney, 115–126. Boca Raton, FL: CRC Press.

Piper, R. G., I. B. McElwain, L. E. Orme, J. P. McCraren, L. G. Fowler, and J. R. Leonard. 1982. *Fish hatchery management*. Washington, DC: United States Department of the Interior, Fish and Wildlife Service.

Ryan, S., B. Smith, R. Collins, and G. Turchini. 2007. Evaluation of weaning strategies for intensely reared Australian fish, Murray cod, *Maccullochella peeliii peelii*. *Journal of the World Aquaculture Society* 38(4): 527–535.

Snow, J. R. 1975. Hatchery propagation of the black basses. In *Black bass biology and management*, edited R. H. Stroud and H. Clepper, 344–356. Washington, DC: Sport Fishing Institute.

Snow, J. R. 1968. Production of six to eight-inch largemouth bass for special purposes. *The Progressive Fish-Culturist* 30:144–152.

Snow, J. R. 1965. Results of further experiments on rearing largemouth bass fingerlings under controlled conditions. *Proceedings of the Annual Conference Southeastern Association Game Fish Commission* 17:191–203.

Tidwell, J. H., S. D. Coyle, and T. A. Woods. 2000. *Species profile-largemouth bass. SRAC Publication No. 732*. Stoneville, MS: Southern Regional Aquaculture Center.

Tidwell, J., C. Webster, and J. Clark. 1992. Effects of feeding, starvation, and refeeding on the fatty acid composition of channel catfish, *Ictalurus punctatus*, tissues. *Comparative Biochemistry and Physiology* 103A(2): 365–368.

Tidwell, J. H., C. D. Webster, and S. D. Coyle. 1996. Effects of dietary protein level on second year growth and water quality for largemouth bass (*Micropterus salmoides*) raised in ponds. *Aquaculture* 145:213–223.

Webster, C. D., and T. Lovell. 1990. Comparison of live brine shrimp nauplii and nonliving diets as first food for striped bass larvae. *The Progressive Fish-Culturist* 52:171–175.

Zar, J. H. 1984. *Biostatistical analysis*. Englewood Cliffs, NJ: Prentice-Hall.