

**Optimization of intensive rearing strategies for largemouth  
bass (*Micropterus nigricans*) during the early stages**

by

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## ABSTRACT

The transition from endogenous to exogenous feeding of largemouth bass (*Micropterus nigricans*) limits production under intensive recirculating aquaculture systems (RAS). This thesis had two main objectives aimed at improving larviculture in indoor RAS. Objective 1 determined the optimal larval stocking density (LD; 25, 50, 100 larvae/L) and prey density (PD; 2, 4, 8 *Artemia*/mL) combination. Objective 2 evaluated larval performance at 100 larvae/L under static (PD<sub>12</sub>, PD<sub>16</sub>, PD<sub>20</sub>) and progressive PD regimens (PD<sub>8-12</sub>, PD<sub>8-12-16</sub>, PD<sub>8-12-16-20</sub>), relative to a control (LD<sub>25</sub>×PD<sub>8</sub>). Morphometrics, survival, cannibalism, biomass, individual weights, condition index, and specific growth rate (SGR) were measured throughout early ontogeny. In Objective 1, larvae were largest at LD<sub>25</sub>×PD<sub>8</sub> (23 to 128 mm<sup>2</sup>) and smallest at LD<sub>100</sub>×PD<sub>2</sub> (13 to 64 mm<sup>2</sup>). Survival (90%) and cannibalism (<1%) were most favorable at LD<sub>25</sub>×PD<sub>8</sub>, whereas biomass peaked at LD<sub>100</sub>×PD<sub>8</sub> (200 g/tank). In Objective 2, the largest fish were observed in the control and PD<sub>20</sub> treatments, followed by the PD<sub>8-12</sub> regimen. Survival was highest in control (87%) and lowest in PD<sub>20</sub> (51%). Highest biomass was achieved in PD<sub>20</sub> (572 g) followed by PD<sub>16</sub> (560 g) and PD<sub>8-12-16-20</sub> (546 g). Cannibalism was <10% in control but increased to 40% in the PD<sub>20</sub> treatment. Overall, progressive PD regimens supported growth comparable to the control while facilitating intensive larviculture.

## **Artificial Intelligence (AI) Use Disclosure Statement**

In the preparation of this thesis, the following Artificial Intelligence (AI) tools were used: Microsoft co-pilot and Grammarly. These tools were used primarily to improve the sentence composition and structures. The author acknowledges full responsibility for the intellectual content of this work and has ensured that all AI-assisted sections have been reviewed and revised for accuracy and appropriate academic style. All AI-generated content was reviewed and validated for relevance, appropriateness, and accuracy before incorporation into the final document to maintain scholarly integrity of this research.

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## Chapter 1

### **Predator and prey density impacts largemouth bass (*Micropterus nigricans*) performance during early life history when cultured in an indoor recirculation aquaculture system**

#### **Abstract**

Largemouth bass, *Micropterus nigricans*, is a valuable freshwater aquaculture species that holds a rapidly growing market in North America. Proper transition of larvae from endogenous to exogenous feeding is critical, yet early feeding strategies remain poorly defined. Our study evaluated how larval stocking density (LD), prey density (PD), and their interactions shape early larvae performance in an indoor recirculating aquaculture system. At hatch, fry (~27°C) were stocked into 15 L tanks at LD<sub>25</sub>, LD<sub>50</sub>, or LD<sub>100</sub> (larvae/L). Starting 2 days post-hatch (DPH), fry were factorially fed *Artemia* at PD<sub>2</sub>, PD<sub>4</sub>, or PD<sub>8</sub> (*Artemia*/mL) every 2-3 h. All treatments received rotifers (2-8 DPH) and a starter diet from 120 °days until 27 DPH. Larvae were reared under a 16:8 light cycle (~500 lux). Mortalities were removed daily. Weekly samples were analyzed for morphometric traits (notochord length, total length, eye diameter, myotome height, jaw length, body area, and fin-fold area). Endpoints metrics included survival, specific growth rate (SGR), biomass, and cannibalism. Both LD and PD, and their interaction, significantly influenced morphometric traits (except jaw length and myotome height) and endpoint outcomes (except biomass). Larvae length and body area were the largest at LD<sub>25</sub>×PD<sub>8</sub> (22.6±3.4 mm; 128±12 mm<sup>2</sup>) and the smallest at LD<sub>100</sub>×PD<sub>2</sub> (12.5±1.8 mm; 64±8 mm<sup>2</sup>), with other traits exhibiting similar trajectories. Survival (90.3±4.1%) and SGR (6.86±0.07) were highest at LD<sub>25</sub>×PD<sub>8</sub>, whereas LD<sub>100</sub>×PD<sub>8</sub> yielded the greatest biomass (199.5±5.6 g/tank). Cannibalism was lowest at LD<sub>25</sub>×PD<sub>8</sub>

(<1%) and highest at LD<sub>100</sub>×PD<sub>2</sub> (13.24±5.81). Overall, LD<sub>25</sub>×PD<sub>8</sub> is recommended for maximizing size and SGR, whereas LD<sub>100</sub>×PD<sub>8</sub> favors biomass yield.

## 1.0. Introduction

Fisheries and aquaculture are among the primary sectors that contribute to global food security, provide employment opportunities, preserve cultures, and impact world economics (Baluyut, 1989; Beveridge and Little, 2002; Partelow et al., 2025; Troell et al., 2023). In 2024, global production from fisheries and aquaculture reached a record 223.2 Mt, valued at \$472 billion. Aquaculture accounted for 130.9 Mt and \$312.8 billion which represent 59% of total yield and surpassed fisheries for the first time (FAO, 2024). The United Nations has estimated that, between now and 2030, the global demand for fish products will increase by at least 40 Mt per year (FAO, 2020).

Commercial cultured finfish species in the United States include catfish (*Ictalurus* spp.), hybrid striped bass (*Morone chrysops* × *Morone saxatilis*), tilapia (*Oreochromis* spp.), rainbow trout (*Oncorhynchus mykiss*), and largemouth bass (LMB; *Micropterus* spp.) (USDA, 2024). LMB is a member of the Centrarchidae family, a native freshwater finfish to North America, and is one of the most desirable recreational fish in the United States. Historically, the propagation of LMB began about a century ago in the early 1890s (Tidwell et al., 2000; Kim et al., 2022). This was limited to stock enhancement programs for natural water bodies and was primarily done by hatcheries (Worth, 1895). LMB is cultured for conservation, fee fishing, and live sales as food fish (Tidwell et al., 2000; Long et al., 2015; Caporelli, 2019). The LMB industry generated \$35.23

million in revenue in 2023, ranking fourth in farm-gate sales among all freshwater species in U.S. The market value for LMB was \$7.53 per pound, which is higher than other commercial food fish species like catfish, tilapia, and rainbow trout, which sell for \$1.25 to \$4.09 per pound (USDA, 2023). LMB has also been introduced outside its native range into South America, Europe, Africa, and Asia. China remains the leading global producer of LMB and reached a production level of ~728,000 Mt in 2022 (Liu et al., 2024). To put these figures into perspective, the United States catfish industry, the largest aquaculture sector, produced about 87,028 Mt of food-size fish in 2024 (National Agricultural Statistics Service, 2025). Overall, LMB has the potential to be a globally important freshwater food fish due to its desirable aquaculture traits, including adaptability, strong disease resistance, rapid growth rate, faster sexual maturity, and higher economic return (Wang et al., 2020; Pěnka et al., 2021; Zhu et al., 2022).

Historically, LMB has been primarily propagated in outdoor earthen ponds over two grow-out seasons (18-24 months) to attain minimum commercial size, where farmers mainly depended on natural reproduction from broodstock (Tidwell et al., 1996, 1998, 2000). Using this production strategy, natural production of LMB relied heavily on live food, beginning with zooplankton and transitioning to forage feed when fish reached a desirable stocking size. This practice led to high production costs, as many ponds were used for LMB, plus additional costs associated with forage fish. For instance, 2.27 kg of forage fish were used to raise a 0.45 kg LMB (Nelson et al., 1974). This practice was prevalent until artificial diets were used in manipulated environments (Snow 1963, 1972). By the early 20<sup>th</sup> century, many spawning and rearing methods developed for LMB were well-established and are still used today. Recently, indoor spawning and the use of hatcheries have become more prominent. For this approach, broodstock are transferred indoors and allowed

to spawn on mats in raceways, fiberglass troughs, or concrete ponds. Following fertilization, spawning mats are moved to nutrient-fertilized ponds where hatched fry are left to feed on zooplankton. When fingerlings are large enough (>4 cm), they are harvested and brought indoors to be feed-habituated for 3 to 4 weeks (Heidinger, 2000). After fingerlings actively forage on commercial diets, they are transferred back to ponds for the grow-out phase until harvest (Quintero et al., 2019).

Although pond culture of finfish is generally considered the most economical for fish production, it requires significant land, labor, and water resources and has a wider range of environmental impacts (Tucker and Hargreaves, 2012). Farmers have reported high mortality rates during multiple stages of LMB production, especially during stages associated with earthen ponds. This mortality is a result of poor survival due to predation/cannibalism (Radwan, 2022), disease (Hasan et al., 2014), harmful algae blooms (Seymour, 1980), frequent transfer between facilities (Portz et al., 2006), or poor water quality (Park et al., 2015). Considering the relatively high value of LMB, indoor intensive culture from hatch to fingerling stages could be economically advantageous and a possible alternative to the highly variable nursery pond phase of production (Skudlarek et al., 2013; Watts et al., 2016). Indoor production of LMB using RAS would also allow for better environmental control (Ende et al., 2024), making year-round production feasible and circumventing the high mortality associated with initial pond stages (Maher et al., 2023). Overall, developing RAS techniques for food fish, such as LMB, could increase per-unit area production efficiency while reducing water requirements (Ebeling and Timmons, 2012).

Establishing LMB production in RAS requires that optimal conditions are met during “critical” early life stages (embryo to fingerlings). Many rearing conditions, such as stocking density (Stejskal et al., 2021; Seo and Park, 2023), temperature (Aguilar et al., 2023), and food availability (Sifa and Mathias, 1987; Duffy et al., 1996), can be manipulated while fish are reared indoors using RAS. Stocking density is a crucial factor in determining fish growth, particularly during the early stages of development (Webb et al., 2007). Given the increasing spatial constraints in modern aquaculture, the primary goal of RAS has been to optimize stocking density for maximizing output within limited land and water resources (Saraiva et al., 2022; Heimann and Delzeit, 2024). High stocking densities can cause fish stress, and ultimately affect growth, survival, behavior, reproduction, and water quality (Gomes et al., 2000; Calderon Herrera, 2014). Other negative aspects, such as cannibalism and aggressive behavior, can lead to mortality (Smith and Reay, 1991) and disrupt production (Rowland et al., 2006). Cannibalism in the early stage does not require a size differentiation and is proportional to the probability of encountering siblings (Slembrouck et al., 2009). Conversely, a low stocking density could reduce competition, leading to a decrease in feed intake (Turnbull et al., 2008; Fatima et al., 2020; Saraiva et al., 2022). A lower stocking density at the larval stage may also result in better larval quality and size. However, this generally necessitates a substantial volume of infrastructure, which raises costs and decreases production efficiency (Geng et al., 2019).

Studies on stocking density across various species have shown variable results. In rainbow trout, growth and survival were unaffected across densities of 10, 40, and 80 kg/m<sup>3</sup> (North et al., 2006). However, higher densities led to fin erosion, while the lowest density resulted in size variation and reduced body condition. Tambaqui (*Colossoma macropomum*) larvae reared in RAS

performed similarly at 60, 120, and 180 larvae/L, although the lowest density resulted in negative net revenue (Santos et al., 2022). Arifin et al. (2019) found that growth in giant gourami (*Osphronemus goramy*) declined at ~20 larvae/L vs. ~1 to 10 larvae/L, despite stable survival and size uniformity. Similarly, Fazekas et al. (2022) reported higher growth in sterlet (*Acipenser ruthenus*) larvae when stocked at 5 and 10 larvae/L vs. 20 larvae/L. Meanwhile, in Siberian sturgeon (*A. baerii*) larvae reared at 30 larvae/L showed improved growth and muscle development compared to those reared at 120 larvae/L (Aidos et al., 2020). These findings highlight that optimal stocking density relies on species, rearing conditions, developmental stage, producer goals, and priorities (Saoud et al., 2008; Niazi et al., 2022). The literature on LMB larviculture also reflects diverse approaches to stocking density, with representative values compiled in Table 1. 1.

It is widely recognized that balanced nutrition and diet are essential for maintaining optimal larval welfare and performance. Larval diets should be easily digested and provide nutritional elements to sustain growth and metabolic demand (Rønnestad et al., 2013; Madkour et al., 2023). First-feeding is considered a major bottleneck for larviculture due to high mortality events (Agh and Sorgeloos, 2005; Siriwardena, 2007; Malzahn et al., 2022), and any nutrient deficiencies during the larval stage are often manifested later in ontogeny (Rathore et al., 2016; Øie et al., 2017). LMB (Molinari et al., 2021) and many fish species (Yúfera and Darias, 2007; Pan et al., 2022) have a rudimentary digestive tract with no functional stomach or gastric glands during early ontogeny. This makes it difficult for them to wean onto dry feed (Zambonino-Infante & Cahu, 2010) and often requires live prey organisms, such as rotifers (*Branchionus* spp.) and/or *Artemia*. Since LMB larvae are believed to be 'visual feeders', adapted to attack moving prey, the movement of these live prey items is likely to stimulate feeding responses (Pedersen et al., 1987; Conceição

et al., 2010). Moreover, evidence suggests that LMB has poor artificial diet utilization capacity during the first 21 days of exogenous feeding and requires a co-feeding period of both live prey and an artificial diet for optimal growth during the early larval stage (Skudlarek et al., 2013; Kwasek et al., 2021).

Live prey feeding strategies vary across species and management practices. For example, Lukić et al. (2024) used between 100 and 500 *Artemia* /larvae at a stocking density of ~20 larvae/L in a LMB weaning experiment. Bryant and Matty (1980) optimized *Artemia* feeding rates based on larval biomass (100–250% of body weight) in common carp (*Cyprinus carpi*). Chen and Zeng (2021) used a 1.5 *Artemia*/mL density to transition orchid dotyback (*Pseudochromis fridmani*) larvae from rotifers to *Artemia*. Together, these studies demonstrated the flexibility and effectiveness of *Artemia* as suitable prey for larvae. Nevertheless, optimizing prey density is still a challenge. For instance, in Atlantic cod (*Gadus morhua*) larvae, growth and survival increased with elevated levels of prey density but plateaued beyond 4 prey/mL, which indicated diminishing returns at higher levels. Conversely, larvae reared under lower prey concentration (<1000 prey/L) exhibited complete mortality by 3 weeks post-hatch, underscoring a critical threshold for survival (Puvanendran and Brown, 1999). Another study with freshwater prawns (*Macrobrachium rosenbergii*) showcased that ingestion was highest at moderate prey densities (4 and 8 *Artemia*/mL), and excessively high densities (>12 *Artemia*/mL) led to diminishing or negative returns (Barros and Valenti, 2003). Such a phenomenon might be related to the "confusion effect," where larvae exhibit reduced predatory efficiency when overwhelmed by high prey concentration (Landeau and Terborgh, 1986; Jeschke and Tollrian, 2007). The diminishing return might also be due to elevated total biomass in the rearing environment, which compromises water quality by

increasing organic load. On the other hand, underfeeding during early larval stages can severely compromise survival and development, as larvae rely on a very short window to transition to exogenous feeding before utilizing endogenous resources (Ribeiro et al., 2022).

Despite much research on LMB larvae, no published data on the effects of different prey concentrations and stocking density on larval growth and survival are available. Our study aimed to determine the impact of initial yolk-sac fry stocking density (predator density), prey density (*Artemia*), and their interaction on the growth and survival of LMB larvae (*Micropterus nigricans*). Recognizing that morphology responds to environmental pressures, we followed the following traits: notochord length ( $N_L$ ), total length ( $T_L$ ), eye diameter ( $E_D$ ), myotome height ( $M_H$ ), jaw length ( $J_L$ ), body area ( $B_A$ ), fin-fold area ( $FF_A$ ) through early ontogeny to assess interactive effects of larval density (LD; 25, 50, 100 larvae/L) and prey density (PD; 2, 4, 8 *Artemia*/ mL) on growth and survival. These traits are related to locomotion and maneuvering (Osse et al., 1997; Voesenek et al., 2018; Bamba, 2021), prey capture capacity (Ferry, 2015), visual acuity (do Nascimento et al., 2015), and body condition (Lloret et al., 2014; Kamimura et al., 2021). Collectively, they indicate an individual's capacity to acquire and allocate energy (Brosset et al., 2023). We hypothesize that both stocking density and prey density, as well as their interaction, significantly influence these morphometric traits, along with other metrics such as survival, biomass, cannibalism and growth in general. This multi-faceted approach will provide greater knowledge and technological innovation to enhance LMB hatchery production, industry sustainability, and global food security. Moreover, these findings will have broader application across other high-value fish species, supporting sector-wide growth and economic impact.

## 1.1. Materials and methods

### 1.1.1. *Animal care*

Protocols for fish experimentation complied with the Institutional Animal Care and Use Committee (IACUC# 2023-5268).

### 1.1.2. *Broodstock management*

LMB broodstock were raised at Red Hills Fishery in Boston, Georgia, USA (30.8478°N, -83.7606°W) and were satiation-fed a combination of goldfish and a broodstock diet (Richloam Bass Fry #14; 64% fish meal, 13% corn gluten meal, 2% fish oils, 1% vitamin supplements, UT, USA) at ~2% body weight/ day. All fish were fed to satiation. Brooders were stocked in a 27 m × 3 m × 1 m concrete raceway with a flow rate of 340 L/min. Freshwater sourced from well was used for raceways. To initiate spawning, brooders were reared on a 10 h photoperiod for the first four weeks, then an 8 h photoperiod for 3 to 4 weeks, then back up to a 10 h photoperiod for two weeks, and finally 14 h for the last two weeks. Spawning mats (Spawntex, Pentair Aquatic Eco-Systems, FL, USA) were evenly distributed along the length of the raceway. During the spawning season, the water temperature and dissolved oxygen (DO) levels in the raceways were ~21°C and ~7 mg/L, respectively. Mean total ammonia nitrogen (TAN) was 0.02 mg/L at embryo collection. An alkalinity of 120-160 mg/L was maintained throughout the rearing period with the addition of sodium bicarbonate.

### *1.1.3. Embryos collection and incubation*

Eggs/embryos spawned on 27 Feb 2024 were transported from Red Hills Fishery to the Auburn University E.W. Shell Fisheries Center (32.6526°N, -85.4859°W) on 28 Feb 2024. Transport was done in 114 L aerated coolers (Coleman, Chicago, IL, USA) containing 40 L of raceway water, with each cooler holding three spawning mats (0.30 m<sup>2</sup>). Upon arrival, eggs/embryos were subjected to drip acclimatization for 2 h. Spawning mats were then suspended 15 cm below the water surface in 6 × 75 L black aquaria for incubation (~21 °C). Each aquarium represented a distinct spawning mat.

Aquaria were integrated into RAS containing a UV filter (Emperor smart DC2305, Pentair Aquatic Eco-Systems, FL, USA), bead filtration system (Bubble Bead Filter XS10000, Aquaculture Systems Technology, LA, USA), bag filter (Pall x-100, Pall Corporation, NY, USA), 0.5 hp pump (PerformancePro Cascade, Cascade Pump Company, CA, USA), 27 × 15L tanks (S-19508BLU, straight line container, Uline, WI, USA), three 795 L circular blue tanks (PT-5430, Polytank Inc., MN, USA), two 190 L sump tanks (Pentair, FL, USA), and a heat-pump (AquaLogic Delta Star DSHP-9, Aqua Logic Inc, CA, USA). Individual tanks were aerated with diffused air (DO > 6.0 mg/L), and a flow rate of ~ 1 L/min was maintained.

Temperature and DO were monitored daily (~08:00; YSI model 58 with 550A probe; YSI, Yellow Springs, OH, USA). Other water quality parameters were tested twice weekly using a spectrophotometer (D/R 2000 Direct Reading, Hach, CO, USA) and a pH meter (Orion™ Star A211 pH, Thermo Scientific, MA, USA). Nitrite and nitrate levels were maintained between 0 and 0.02 mg/L, ammonia 0 to 0.05 mg/L, pH 7.2 to 7.7, alkalinity 90 to 125 mg/L CaCO<sub>3</sub>, and hardness 80 to 90 mg/L CaCO<sub>3</sub>. Facility air temperature was kept at 23 ± 0.5°C, and embryo incubation

occurred under a 16 h light/8 h dark photo period at ~250 lux. The source of water for the experiment was municipal water which went through a series of dichlorination processes (trickling followed by sodium thiosulfate treatment and intensive aeration) before being introduced into the RAS.

#### 1.1.4. Larval rearing and experimental design

Eggs/embryos were monitored every 3 h, starting at ~48 h post-fertilization. Once >50% of larvae hatched, a representative proportion of larvae from all six aquaria were collected in a 22.9 × 30.5 × 5.1 cm white porcelain tray. This ensured representation from all spawning mats, minimizing family-related effects. Larvae were gently transferred into 38.1 × 30.5 × 24.1 cm rectangular blue culture tanks (n=27), each filled with 15 L of water.

The experimental setup consisted of nine treatments, which combined three larval densities (LD: 25, 50, and 100 larvae/L) and three prey densities (PD: 2, 4, and 8 *Artemia*/mL), and each factorial combination consisted of three replicate culture tanks. Each tank received 375 (25 larvae/L), 750 (50 larvae/L), or 1,500 (100 larvae/L) yolk-sac larvae. Altogether, 24,648 larvae were stocked, including 648 larvae added post-stocking; 12 larvae/tank to compensate for potential handling-related mortality + 12 larvae/ tank to replace individuals removed for initial sampling × 27 culture tanks = 648 larvae.

Once tanks were stocked with yolk-sac larvae, water temperature was slowly raised from 21 to 27 °C over two days (~1 °C/8h), and flow rate was set to 0.5 L/min. At 2 days post-hatch (DPH), larvae were fed 2, 4, or 8 *Artemia*/mL. Premium Grade A *Artemia* (INVE SEP-ART D-FENSE ARTEMIA, INVE Aquaculture, Belgium) were offered every 2-3 h, which resulted in 8 feedings per day, spanning from 07:00 to 23:00 h. All larvae were also fed S-type rotifers (*Brachionus*

*plicatilis*), at 5 rotifers/mL, from 2 to 9 DPH. At 5 DPH, larvae were gradually weaned from rotifers over four days, with a reduction of 1 rotifer/mL daily.

To complement live feed, Otohime A1 micro-diet (75-250  $\mu\text{m}$ ) (Marubeni Nisshin Feed, Tokyo, Japan) was supplied to each tank ( $\sim 0.5$  g/tank). The selection of appropriate micro-diet and pellet size was based on fish size at each sampling point and their receptivity to the diet. Larvae were progressively transitioned from smaller to larger pellets and this transition strategy involved co-feeding 25% of the larger pellets with 75% of the smaller pellets on the first day, then the proportion of larger pellets was gradually increased until they constituted 100% of the diet. Larvae were fed Otohime B1 from 13 DPH, Otohime B2 from 17 DPH, and Otohime C1 from 24 DPH (Figure. 1. I).

Dead larvae were removed daily (7:00-9:00) to assess mortality rates. Tanks were siphoned twice daily using a thin air hose to remove excess feed, fecal matter, and organic waste. Water exchanges were routinely performed at 25%, with larger exchanges made, when necessary, based on water conditions. Final survival was assessed at 27 DPH and adjusted for the 6.9% larvae removed for morphometric and molecular analyses.

#### *1.1.5. Data collection*

##### *Morphological traits*

Fish were sampled at 2, 9, 16, 23, and 27 DPH. At each sampling point, 12 fish were randomly selected from each tank. For this study, 0 DPH was defined as the time when  $>50\%$  of eggs hatched. Selected fish were euthanized with 100 ppm MS-222 (tricaine methane sulphonate; Argent Laboratories Inc., Redmond, WA, USA) buffered in rearing water, and digital images were

captured using a Zeiss stereomicroscope (SterREO Discovery V12) equipped with 0.5 to 1.0× objectives and ZEN 2.5 imaging software (blue edition). The following morphometric traits were obtained from each individual using ImageJ software (Ver. 1.54 G); including  $N_L$  (distance from tip of snout to end of myotome, just before the tail begins),  $T_L$  (distance from tip of snout to tip of tail),  $E_D$ ,  $M_H$  (body height measured posterior to anus),  $J_L$  (length of lower jaw),  $B_A$  (body area excluding fin-fold area and yolk sac), and  $FF_A$  (body area including fin area and yolk sac).

### *Specific growth rate*

Specific growth rate (SGR) was calculated based on total length ( $T_L$ ) measurements at 2 DPH and 27 DPH using the formula:  $SGR = [(lnL_t - lnL_0) / t] \times 100$ , where  $L_0$  represents the initial total length,  $L_t$  is the final total length, and  $t$  is the duration in days.

### *Survival, biomass, and percent cannibal*

Survival was assessed at 27 DPH. To ensure that fish were accurately counted, fish were first collected and sedated in a buffered MS-222 solution (20 mg/L) until 20 to 30% showed loss of equilibrium or reduced movement. Sedated fish were transferred to a flat  $60.96 \times 38.10 \times 7.62$  cm white tray with minimal water to prevent overlapping. Digital images were analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, USA) with the Cell Counter plugin (version 3.0.0) to quantify surviving fish from each tank. Survival was determined by subtracting the fish left in each tank from the original stocking number. After digital images were taken, 50 fish were randomly selected from each tank to determine individual wet weights ( $\pm 0.001$  g), which were then used to calculate total tank biomass. Final tank biomass was determined by multiplying the average wet weight of the individual fish by the total number of survivors. Cannibalism was

estimated by: (initial larval count - final larval count) - cumulative daily recorded mortality in each tank.

### *Statistical analysis*

Data were analyzed using SAS statistical software (version 9.1, SAS Institute Inc., NC, USA). Residuals were tested for normality using the Shapiro-Wilk test and plotted against predicted values to test for homogeneity of variance. Survival and percent cannibalism were arcsine square-root transformed before statistical analysis. Tukey's HSD was used for all pairwise comparisons.

### *Morphological traits*

Larval morphological traits such as  $N_L$ ,  $T_L$ ,  $J_L$ ,  $M_H$ ,  $E_D$ ,  $B_A$ , and  $FF_A$  were log-transformed and analyzed using repeated measures factorial mixed-model ANOVAs (PROC MIXED; SAS Institute, 2024; Model 1):

$$Y_{abci} = \mu + L_a + P_b + LP_{ab} + T_c + LT_{ac} + PT_{bc} + LPT_{abc} + R_i + \varepsilon_{n(abci)} \quad (\text{Model 1})$$

where  $\mu$  is the true mean;  $L_a$  is the LD main effect (where  $a = 25, 50, 100$  larvae/L);  $P_b$  is the PD main effect (where  $b = 2, 4, 8$  *Artemia*/mL);  $LP_{ab}$  is the LD  $\times$  PD interaction;  $T_c$  is the time main effect (where  $c = 5$  sampling days; 2, 9, 16, 22, 27 DPH);  $LT_{ac}$  is the LD  $\times$  Time interaction;  $PT_{bc}$  is the PD  $\times$  Time interaction;  $LPT_{abc}$  is the LD  $\times$  PD  $\times$  Time interaction;  $R_i$  is the replicate tank effect (where  $i =$  tank ID); and  $\varepsilon_{n(abci)}$  is the residual error. Fish LD, PD, sampling days, and their interactions were considered fixed effects. An autoregressive covariance  $[AR(1)]$  structure was applied to account for temporal correlation in repeated measurements. The Satterthwaite procedure

(Satterthwaite, 1946) was used to approximate the denominator degrees of freedom for all F-tests (Spilke et al., 2005).

When a significant second-order interaction ( $LD \times PD \times Time$ ) was detected, Model 1 was decomposed into factorial ANOVAs at 16 and 27 DPH (Model 2).

$$Y_{abi} = \mu + L_a + P_b + LP_{ab} + R_i + \varepsilon_{n(abi)} \quad (\text{Model 2})$$

The sampling times 16 DPH and 27 DPH were selected as they captured two critical phases in larval development. Specifically, before 9 DPH, larvae were transitioning from endogenous to exogenous feeding. At this point, larvae had not fully exploited the resources provided. However, between 9 and 16 DPH, larvae achieved a juvenile stage with a morphology like that of an adult (streamlined body). Moreover, noticeable size variation and traces of cannibalism were also observed at this time. Day 16 represented a stage when morphological differentiation, resource utilization, and competitive interactions strongly influenced culture conditions. In contrast, 27 DPH marked the experimental endpoint, and gave us cumulative growth outcomes to treatment conditions.

The main effects of LD and PD were interpreted for traits where no significant  $LD \times PD$  interaction was detected. On the contrary, if a significant  $LD \times PD$  interaction was detected, the model was decomposed using a series of one-way ANOVAs to examine the effects of LD at each PD and PD at each LD.

#### *Specific growth rate, survival, biomass, and percent cannibalism*

Specific growth rate, survival, biomass, and percent cannibalism were analyzed using Model 2. Main effects and interactions were interpreted, as above (*Section 2.6.1*).

### *Correlation matrix*

To examine relationships among morphological traits, we performed a correlation analysis using OriginPro (Ver. 2025, OriginLab Corporation, Northampton, MA, USA) at 0, 16, and 27 DPH. A Pearson correlation matrix was used to quantify linear associations between traits. The Pearson correlation coefficient ( $r$ ) ranged from -1 to +1, where values close to +1 indicate strong positive correlations, values near -1 indicate strong negative correlations, and values around 0 denote weak or no linear association. Normality of variables was checked before analysis, and significance was set at  $\alpha = 0.05$ .

### *Artemia cost analysis*

We estimated profit per treatment (in terms of only one variable: *Artemia* supplied) in three steps (Table 2):

(1) First, we calculated how much *Artemia* was used per treatment and the associated cost. Total live *Artemia* offered per treatment (AF) was calculated as PD (*Artemia*/mL; 2, 4, or 8 *Artemia*/mL)  $\times$  feeding frequency ( $F = 8$  times/day; see *Section 2.4*)  $\times$  culture tank water volume ( $V = 15,000$  mL)  $\times$  number of culture days (DPH) in relation to standardized length.

$$(AF) = (PD \times F \times V \times D)$$

Then, to calculate *Artemia* cost per treatment, we took the total amount of live *Artemia* offered per treatment (AF) and divided by the number of hatched *Artemia* /g of cysts ( $H = \sim 100,000$  *Artemia*/g) to get the total amount of *Artemia* cysts (G) used.

$$(G) = (AF) / H$$

Next, we multiplied the total amount of *Artemia* cysts (G) by their price (C; USD/g) to determine *Artemia* cost (AC) per treatment ( $AC = G \times C$ ).

(2) Second, we multiplied the standardized length (SL) of fish across all treatment (in mm) by the selling price of fish per inch (P), i.e. \$0.51, to get the average selling price of fish per treatment (SP).

$$(SP) = SL \times P$$

Then, we multiplied the average selling price of fish per treatment (SP) by the number of survivors (N) in each treatment to get the total gross return from each treatment (TGR)

$$(TGR) = N \times SP$$

(3) Finally, profit per treatment was calculated by subtracting *Artemia* cost per treatment (AC) from the total gross return per treatment (TGR)

$$\text{Profit} = TGR - AC$$

Moreover, as an extension of our cost analysis, we conducted predictions using simple linear regression models (Table 1. 2) to estimate days taken for larvae in each of our nine treatments to reach a standardized size of 17.98 mm. This threshold was based on the lowest growth output observed in the LD<sub>100</sub>×PD<sub>4</sub> treatment. Incorporating this model into our analysis allowed us to compare growth trajectories across treatments and speculate how quickly fish could attain market-relevant sizes relative to *Artemia* input.

## 1.2. Results

### 1.2.1. Morphological traits

From 2 to 27 DPH,  $N_L$  increased from 5.20 to 22.60 mm (Figure. 1. 2A) and  $FF_A$  increased from 4.96 to 127.61 mm<sup>2</sup> (Figure. 1. 3D). Morphometric traits, such as  $T_L$ ,  $J_L$ ,  $M_H$ ,  $E_D$ , and  $B_A$  exhibited a similar pattern (Figure. 1. 2, Figure. 1. 3). Repeated measures factorial mixed-model ANOVA showed a significant second-order interaction ( $LD \times PD \times Time$ ) for  $N_L$ ,  $T_L$ ,  $E_D$ ,  $B_A$ , and  $FF_A$  ( $F_{16, 64} = 1.80$ ,  $P = 0.051$ ; Table 1. 3; Figure. 1. 2, Figure. 1. 3). The saturated models were decomposed into a series of mixed-model factorial ANOVAs at 16 and 27 DPH (Table 1. 4, Growth trajectories of largemouth bass (*Micropterus nigricans*) from 2-27 days post-hatch (DPH) and post hoc comparisons under three larval densities (25, 50, 100 larvae/L) and three prey densities (2, 4, 8 Artemia/mL). Growth parameter panels (A, D, G, J, M) show trajectories for all nine treatment combinations ( $LD \times PD$ ), with fish silhouettes illustrating the measured traits. Post hoc comparisons are shown for notochord length (A: growth; B: 16 DPH; C: 27 DPH), total length (D; E; F), jaw length (G; H; I), myotome height (J; K; L), and eye diameter (M; N; O). In post hoc plots, bars show prey-density (PD) effects within each larval density (LD), while color-coded connection lines above the bars show LD effects within each PD; connected lines indicate no significant difference ( $P > 0.05$ ), and unconnected lines indicate significant differences. Different letters above bars denote significant differences ( $P < 0.05$ ). The legend shows the nine  $LD \times PD$  treatments. (Figure. 1. 3).

At 16 DPH, the  $LD \times PD$  first-order interaction was significant for  $N_L$ ,  $T_L$ ,  $E_D$ ,  $B_A$ , and  $FF_A$  ( $F_{4,18} = 5.83$ ,  $P = 0.003$ ; Table 1. 4). In order to evaluate the independent main effect of LD and PD, one-way ANOVAs were performed, which revealed that both factors significantly affected most morphometric traits (Table 1. 5). As larvae morphometrics traits were significantly influenced

by the interactions, we decomposed the model into a series of mixed model ANOVAs to determine the effect of PD at each LD and LD at each PD (Figure. 1. 2, Figure. 1. 3). At LD<sub>25</sub>, increasing PD impacted morphometrics, such that fish reared at PD<sub>4</sub> and PD<sub>8</sub> were larger than those reared at PD<sub>2</sub> for N<sub>L</sub>, T<sub>L</sub>, E<sub>D</sub>, B<sub>A</sub> and FF<sub>A</sub>. A similar trend was observed at LD<sub>50</sub>, where PD<sub>8</sub> produced the largest fish regarding N<sub>L</sub>, T<sub>L</sub>, E<sub>D</sub>, B<sub>A</sub> and FF<sub>A</sub>. At LD<sub>100</sub>, fish fed PD<sub>8</sub> continued to produce fish with larger morphometric traits (Figure. 1. 2, Figure. 1. 3). When fish were fed 2 *Artemia*/mL (PD<sub>2</sub>), those stocked at LD<sub>50</sub> had significantly bigger N<sub>L</sub>, T<sub>L</sub>, E<sub>D</sub>, B<sub>A</sub>, and FF<sub>A</sub> (Figure. 1. 2, Figure. 1. 3) compared to LD<sub>25</sub> and LD<sub>100</sub>. Meanwhile, at PD<sub>4</sub>, larvae stocked at LD<sub>25</sub> were significantly larger for all morphometric traits except for E<sub>D</sub> (Figure. 1. 2, Figure. 1. 3) When the fish were given 8 *Artemia*/mL (PD<sub>8</sub>), the lowest larval stocking density (LD<sub>25</sub>) yielded significantly larger fish for N<sub>L</sub>, T<sub>L</sub>, E<sub>D</sub>, B<sub>A</sub>, and FF<sub>A</sub> compared to LD<sub>100</sub>.

At 27 DPH, the LD × PD first-order interaction was significant for N<sub>L</sub>, T<sub>L</sub>, M<sub>H</sub>, B<sub>A</sub>, and FF<sub>A</sub> ( $F_{4,18} = 2.46, P = 0.007$ ; Table 1. 4). To evaluate the independent effects of each factor, one-way ANOVAs were also performed at this stage. Our analyses revealed that PD significantly influenced most morphometric traits, whereas the main effect of larval density was no longer significant across treatments (Table 1. 6). Tukey post-hoc analysis revealed distinct trends when examining the effect of LD on the different PD treatments (Figure. 1. 2, Figure. 1. 3). For instance, at PD<sub>2</sub>, the fish stocked at LD<sub>25</sub> had the largest T<sub>L</sub>, B<sub>A</sub> and FF<sub>A</sub> ( $P < 0.05$ , Figure. 1. 2F, Figure. 1. 3C, F). Meanwhile, N<sub>L</sub> was significantly larger when fish were stocked at LD<sub>25</sub> and LD<sub>50</sub> compared to LD<sub>100</sub> (Figure. 1. 2C). E<sub>D</sub> was markedly larger when fish were stocked at LD<sub>25</sub> than at LD<sub>100</sub>. No significant differences in E<sub>D</sub> were observed between LD<sub>25</sub> and LD<sub>50</sub> or between LD<sub>50</sub> and LD<sub>100</sub> (Figure. 1. 2O). At PD<sub>4</sub>, we observed a consistent trend, where fish stocked at LD<sub>25</sub> and LD<sub>50</sub> were

significantly larger than those stocked at LD<sub>100</sub> (Figure. 1. 2, Figure. 1. 3). At PD<sub>8</sub>, a similar trend was observed, where fish stocked at LD<sub>25</sub> had a longer length and a larger area than those stocked at LD<sub>100</sub>. E<sub>D</sub>, on the other hand, had no significant difference among LD stocking levels at PD<sub>8</sub> (Figure. 1. 2).

The second-order interaction (LD × PD × Time) was not significant for J<sub>L</sub> and M<sub>H</sub>. However, significant LD × Time ( $F_{6,51.9} = 3.00, P = 0.004$ ) and PD × Time ( $F_{8,64.4} = 4.22, P = 0.001$ ) interactions were detected (Table 1. 3, Figure. 1. 2). For J<sub>L</sub>, differences in LD emerged at 16 DPH, where larvae stocked at LD<sub>25</sub> and LD<sub>50</sub> had significantly longer jaws than those at LD<sub>100</sub> ( $F_{2,6} = 16.61, P = 0.002$ ). At 27 DPH, LD effects remained significant ( $F_{2,6} = 5.46, P = 0.006$ ), with LD<sub>25</sub> treatment consistently producing the longest J<sub>L</sub>. A similar trend was observed across PD levels: at 16 DPH, tanks fed PD<sub>8</sub> had fish with significantly longer jaws than PD<sub>4</sub> and PD<sub>2</sub> ( $F_{2,6} = 18.51, P = 0.004$ ). At 27 DPH, the trend continued, where fish fed PD<sub>8</sub> consistently had the longest J<sub>L</sub> ( $F_{2,6} = 37.83, P = 0.012$ ).

For M<sub>H</sub>, differences across LD levels were first observed at 16 DPH, where larvae stocked at LD<sub>25</sub> and LD<sub>50</sub> had wider M<sub>H</sub> compared to those at LD<sub>100</sub> ( $F_{2,6} = 21.53, P \leq 0.001$ ). At 27 DPH, fish M<sub>H</sub> were significantly wider at lower densities, following the trend LD<sub>25</sub> > LD<sub>50</sub> > LD<sub>100</sub> ( $F_{2,6} = 15.30, P \leq 0.001$ ). In contrast, PD differences in M<sub>H</sub> were only detected at 16 DPH, where larvae fed PD<sub>8</sub> had wider M<sub>H</sub> than those at PD<sub>2</sub> ( $F_{2,6} = 4.61, P = 0.003$ ), while no significant difference was observed at 27 DPH ( $F_{2,6} = 2.60, P = 0.700$ ; Figure. 1. 2L).

### 1.2.2. Morphological traits correlation matrix

Pairwise correlations among morphometric traits ( $N_L$ ,  $T_L$ ,  $E_D$ ,  $M_H$ ,  $J_L$ ,  $B_A$ , and  $FF_A$ ) were assessed at 0, 16, and 27 DPH under the best-performing treatment ( $LD_{25} \times PD_8$ ; Figure. 1. 5). At 0 DPH, significant correlations were observed among morphological traits, with the strongest correlation between  $N_L$  and  $B_A$  ( $r = 0.91$ ,  $P \leq 0.05$ ), while  $Y_{S_A}$  showed no significant associations. By 16 DPH, correlations became more pronounced, with  $T_L$  and  $B_A$  showing the strongest relationship ( $r = 0.96$ ,  $P \leq 0.001$ ), whereas the weakest significant correlation was between  $E_D$  and  $J_L$  ( $r = 0.47$ ,  $P \leq 0.001$ ). At 27 DPH,  $N_L$  and  $B_A$ ,  $T_L$  and  $FF_A$  exhibited near-perfect correlations ( $r = 0.97$ ,  $P \leq 0.001$ ). In contrast,  $J_L$  had the weakest correlations with other traits with coefficients ranging from 0.34 to 0.45 ( $P \leq 0.05$ ).

### 1.2.3. Cumulative survival and survival rate

Highest cumulative survival was observed in treatments with  $LD_{25} \times PD_8$  combinations, followed by  $LD_{25} \times PD_4$  and  $LD_{100} \times PD_8$  treatments at ~94, 92, and 91 % respectively. Treatments with the  $LD_{25}$  and  $PD_2$  combination resulted in the lowest cumulative survival at 83% (Figure. 1. 4A).

Two-way ANOVA models showed a significant  $LD \times PD$  interaction for LMB survival ( $F_{4,18} = 2.84$ ,  $P = 0.0551$ ; Table 1. 7). Therefore, the models were decomposed into a one-way ANOVA model at each LD and PD level (Figure. 1. 4B). At  $LD_{25}$ , a higher PD concentration of 8 Artemia/mL resulted in  $\sim 90\% \pm 4.1$  survival rate, significantly higher than  $PD_4$  and  $PD_2$  treatments. No significant difference was observed between prey density at  $LD_{50}$ . However, at  $LD_{100}$ ,  $PD_8$  yielded significantly higher survival than  $PD_2$ . When evaluating the influence of LD across PD treatments, LD had no significant effect on  $PD_2$  and  $PD_4$ . At  $PD_8$ , larvae stocked at 25/L showed

the highest survival, which significantly exceeded LD<sub>100</sub>, while LD<sub>50</sub> yielded intermediate results (Figure. 1. 3B).

#### 1.2.4. Specific Growth Rate (SGR)

The main effect of LD was significant for larval SGR ( $F_{2,18} = 56.06, P < 0.001$ ) whereas PD had no significant effect ( $F_{2,18} = 0.1, P = 0.902$ ; Table 1. 7). A significant first-order interaction between LD  $\times$  PD was detected ( $F_{4,18} = 2.63, P = 0.069$ ; Table 1. 7). Therefore, one-way ANOVAs were run on PD at each LD, and on LD at each PD at 27 DPH (Figure. 1. 4C). The effect of LD levels on PD was not significant. When considering the effect of PD on different LD levels, at PD<sub>2</sub>, larvae reared at LD<sub>25</sub> and LD<sub>50</sub> yielded significantly higher SGR than those reared at LD<sub>100</sub>. A similar trend was observed at PD<sub>4</sub>, where LD<sub>25</sub> and LD<sub>50</sub> had a significantly higher SGR than LD<sub>100</sub>. At PD<sub>8</sub>, larvae stocked at LD<sub>25</sub> had significantly higher SGR than at LD<sub>100</sub>. Overall, the highest mean SGR was observed at LD<sub>25</sub> and PD<sub>8</sub>, whereas the lowest SGR was observed at LD<sub>100</sub> and PD<sub>4</sub>.

#### 1.2.5. Biomass

Biomass was significantly influenced by both LD ( $F_{2,24} = 19.64, P < 0.0001$ ; Figure. 1. 3) and PD ( $F_{2,24} = 4.374, P \leq 0.001$ ; Table 1. 4). No significant LD  $\times$  PD interaction was detected ( $F_{2,18} = 1.53, P = 0.2345$ , Table 1. 4). Therefore, two factors were interpreted independently (Figure. 1. 7). Post hoc comparisons showed that biomass increased progressively with higher LD and PD levels. Biomass at LD<sub>100</sub> ( $159.43 \pm 5.88$  g) was significantly greater than at LD<sub>25</sub> and LD<sub>50</sub>. For PD, biomass at 8 *Artemia*/mL was significantly higher than at 2 *Artemia*/mL, although the difference between 4 and 8 *Artemia*/mL was not statistically significant. The highest biomass was

achieved at 100 larvae/L and 8 *Artemia*/mL treatments ( $199.535 \pm 4.31$  g). Final yield from the combination of all LD and PD levels is illustrated in Figure. 1. 6.

#### 1.2.6. Cannibalism

Two-way ANOVA revealed a significant first-order LD  $\times$  PD interaction ( $F_{4,18} = 4.15$ ,  $P = 0.017$ ; Table 1. 4). Among the interacting factors, only the main effect of LD was statistically significant ( $F_{2,18} = 6.20$ ,  $P < 0.001$ ; Table 1. 4). Overall, the percentage of cannibalism was highest when larvae were fed 2 *Artemia*/mL and stocked at 100 larvae/L ( $13.24 \pm 2.20\%$ ). The lowest percentage of cannibalism was noticed when larvae were fed 8 *Artemia*/mL and stocked at 25 larvae/L ( $0.267 \pm 1.51\%$ ).

Post hoc analysis revealed no significant difference in percent cannibalism across PD levels within each LD group. (Figure. 1. 4E). However, LD effects were examined within individual PD levels, at least one LD group differed significantly in each case. At PD<sub>2</sub>, cannibalism was significantly lower at LD<sub>25</sub> compared to LD<sub>50</sub> and LD<sub>100</sub>. At PD<sub>4</sub>, larvae stocked at different levels of LD did not have a significant difference in cannibalism. At PD<sub>8</sub>, larvae reared at LD<sub>25</sub> exhibited the lowest percentage of cannibalism

#### 1.2.7. *Artemia* cost analysis

Highest profits occurred at LD<sub>100</sub> $\times$ PD<sub>8</sub> (\$413.7), followed by LD<sub>100</sub> $\times$ PD<sub>4</sub> (\$396.0) and LD<sub>100</sub> $\times$ PD<sub>2</sub> (\$367.1). The lowest profit was at LD<sub>25</sub> $\times$ PD<sub>8</sub> (\$93.1). At the intermediate density (LD<sub>50</sub>), profit was minimal at PD<sub>8</sub>, generating \$164.4 (Table 1. 2).

### 1.3. Discussion

During the endogenous feeding stage, larvae growth is primarily shaped by abiotic factors such as temperature, oxygen, and light (Villamizar et al., 2011; Dubey et al., 2023). Once larvae shift to exogenous feeding, larval density (LD) and prey density (PD) become the primary drivers that influence energy acquisition, metabolic costs, behavior, and growth (Jenkins et al., 1991; Fessehaye et al., 2006; Giacomini, 2022). Here, we measured seven morphometric traits:  $N_L$ ,  $T_L$ ,  $E_D$ ,  $M_H$ ,  $J_L$ ,  $B_A$ , and  $FF_A$  to capture the effects of LD and PD on morphology, function, and condition of larvae throughout their early life.

There was a significant LD  $\times$  PD  $\times$  Time interactions for both measures of larval length ( $N_L$  and  $T_L$ ). For example, at 16 DPH, larvae stocked at LD<sub>25</sub> were the largest at PD<sub>4</sub>, while LD<sub>50</sub> and LD<sub>100</sub> grew longest at PD<sub>8</sub>. However, LD<sub>100</sub> $\times$ PD<sub>8</sub> larvae were shorter than LD<sub>25</sub> $\times$ PD<sub>8</sub>, suggesting that 8 *Artemia*/mL may be insufficient at higher LD. Geng et al. (2019) found a similar result in Japanese flounder, (*Paralichthys olivaceus*) where quadrupling PD yielded parallel growth across lower and higher stocking levels. This shows that to a certain extent, the effect of stocking density can be masked if sufficient prey is provided. At LD<sub>25</sub>, increasing PD beyond 4 *Artemia*/mL yielded no significant return; similar results were seen in pollock (*Theragra chalcogramma*) and prawn (*Macrobrachium rude*) studies where overfeeding larvae stocked in lower larvae densities yielded no additional growth benefits (Porter et al., 2005; Hinzano et al., 2023). Interestingly, at PD<sub>2</sub>, larvae stocked at 50 larvae/L grew longest, indicating moderate density (LD<sub>50</sub>) optimizes length in LMB larvae, consistent with findings in northern pike (*Esox lucius*) where in a stocking-density experiment (10, 20, 40, 80 individual/L), larvae stocked at 20 and 40 individual/L had significantly higher body weight and SGR than the other groups (Imentai et al., 2024).

During the experiment, we observed that larvae stocked at LD<sub>25</sub> were more skittish than those stocked at LD<sub>50</sub> and LD<sub>100</sub>. Although we did not scientifically observe the behavior and movement of larvae in each treatment, such a response may be associated with an underlying social facilitation or sensory process. A study on zebrafish (*Danio rerio*) showed that the presence of conspecifics suppressed aversive responses (Wee et al., 2022) and impacted their behavior. Puvanendran and Brown (1998) demonstrated that larval motor action patterns, such as swimming, orientation, or attack, shift in response to environmental stimuli (light intensity in their study), and impact feeding efficiency. Studies conducted on stocking densities by Pitcher and Parrish (1993) and Hoare et al. (2000) support the notion that when larvae are stocked in higher densities, they show improved foraging activity. Moreover, from an evolutionary perspective, LMB larvae form swarms or schools around their parents during early life stages, where a male parent guards them (Heidinger, 2000). Stocking larvae in larger groups or higher LD might trigger an increased sense of security in larvae, as this mimics their natural grow-out period during the early stage, where they forage in groups. Although our interpretations remain speculative, future studies could be done by adopting a modal action pattern (MAP) framework to systematically test whether stocking density influences movement and feeding behavior in LMB.

By 27 DPH, PD had minimal effect, and this was likely due to the transition to pellet feed. In our experiment, we observed that larvae were quickly transitioning to formulated pellet faster than we were anticipating and this underscores the need to optimize timing of weaning for future context. Some studies show that at later larval stage, nauplii become too small relative to the larval gape (Huebert & Peck, 2014), and the overall nutritional contribution of *Artemia* might not be adequate to sustain larval growth. Considering this, a better alternative at a later stage would be providing larger prey such as cladocerans (Graeb et al., 2004) or using enriched *Artemia* (Akbar)

et al., 2011). Across PD levels, larvae stocked at 25/L and 50/L were larger than larvae stocked at 100/L, with the LD<sub>25</sub>×PD<sub>8</sub> combination yielding the largest larvae overall. This result aligns with studies in African catfish (*Clarias gariepinus*), blunt snout bream (*Megalobrama amblycephala*), and rainbow trout (*Oncorhynchus mykiss*), where stocking fish at higher density elevated stress and suppressed growth despite adequate feeding (North et al., 2006; van de Nieuwegiessen et al., 2008; Liu et al., 2024).

At hatch, larval eye diameter ( $E_D$ ) was relatively large to their body size. As larvae progressed in their ontogeny,  $E_D$  exhibited an allometric growth trajectory, mirroring development patterns described in other larval structures (Osse and van den Boogaart, 2004). Larvae reared at moderate density (LD<sub>50</sub>) had larger eyes under PD<sub>2</sub>. Whereas at PD<sub>8</sub>, larvae stocked at low density (LD<sub>25</sub>) had the largest eyes. Adequate prey availability during early ontogeny likely provided sufficient energy for both somatic and organ specific development, which thereby helped in feed accumulation and prey capture. Our observation aligns with a conceptual framework proposed by J.W.M. Osse and J.G.M. van den Boogaart (2004) where they suggested that when energy reserve is limited, larvae in general direct their available resource towards primary needs which yields them greatest return in terms of enhancing the larvae's ability to find, capture and assimilate additional food and to avoid being prey to other conspecifics or other predators. Nevertheless, having larger  $E_D$  at early stages may be especially advantageous in pond culture, where green water and turbid backgrounds reduce contrast and challenge prey detection (McLean, 2021). By 27 DPH, the difference in ED between treatments disappeared, suggesting that the early stages are a critical window for eye development (Gao et al., 2023).

Jaw length ( $J_L$ ) is another key determinant of early feeding success. It is well established that mouth gape sets the upper limit of prey size that a larvae can consume (Singh et al., 2015). In our experiment, significant differences across treatments were observed from 16 DPH. Similar patterns have been reported where craniofacial elements linked to feeding showed phase-specific growth and clear inflection points during larval development (Peña and Dumas, 2009; Kupren et al., 2014). At 16 DPH, larvae stocked at LD<sub>25</sub> and LD<sub>50</sub> followed by treatments fed 8 *Artemia*/mL had wider jaws. Interestingly, at 27 DPH, larvae fed the lowest PD (2 *Artemia*/mL) had longer  $J_L$  than those fed PD<sub>4</sub>. We speculate that when prey is limited, larvae tend to exhibit cannibalism where they adapt to consume conspecifics. This adaptation might have driven larvae at lower LD to attain faster jaw development. Such findings align with the broader concept that fish larvae can plastically adjust organ growth in response to environmental challenges (Muschick et al., 2012). We did not observe a significant second-order interaction for fish  $M_H$ . At 16 and 27 DPH, larvae stocked at 25/L consistently had greater  $M_H$  than those at higher densities. This highlights the negative impact of stocking fish at high density on fish myotome (Aidos et al., 2019). Moreover, the effect of PD was limited, as it was only significant at 16 DPH. At this sampling point, larvae fed PD<sub>8</sub> exhibited deeper myotomes.

Across species, a strong correlation between  $B_A$  and length exists and the trend is evident in teleost larvae also (Kamimura et al., 2021). However,  $B_A$  might reveal differences not captured by length alone. In our study, it was observed that fish NL at LD<sub>25</sub>, was not significantly different when fed either PD<sub>4</sub> or PD<sub>8</sub>. However, their  $B_A$  was significantly larger when fed 8 *Artemia*/mL compared to the PD<sub>4</sub> treatment. Moreover, when examining LD effects under PD<sub>8</sub>, larval  $N_L$  followed the LD<sub>25</sub>  $\geq$  LD<sub>50</sub>  $\geq$  LD<sub>100</sub> trend, where LD<sub>25</sub> significantly outperformed LD<sub>100</sub>. In contrast,  $B_A$  showed a clearer separation (LD<sub>25</sub>  $>$  LD<sub>50</sub>  $>$  LD<sub>100</sub>), which demonstrates that  $B_A$  integrates both

elongation and fullness, and exposes density-related constraints more than length alone (Pepin, 1995; Konovalov et al., 2019). Across sampling points,  $B_A$  decreased as LD increased. This pattern is consistent with other fish species, where increased feeding levels or frequency promoted greater  $B_A$  (Rahman et al., 2021; Benini et al., 2023). By 27 DPH,  $B_A$  differences were primarily driven by LD and the effect of PD diminished, just like prior observation with other morphometrics traits above.

In our study, larvae exhibited faster finnage development until 16 DPH. This timing matches findings from other studies. Many perciforms and *Cyprinus* spp. larvae begin to resorb their fin-fold before reaching 8 mm in  $T_L$ , and their swimming patterns shift from anguilliform to sub-carangiform where they exhibited faster finnage development (Fukuhara, 1985; Osse & van den Boogaart, 2000). Higher PD facilitated increased  $FF_A$ , but we also observed that  $PD_4$  often matched  $PD_8$  across different LD. At low prey ( $PD_2$ ),  $LD_{50}$  fish had the largest  $FF_A$ , again showing the advantage of moderate density in low prey availability conditions. By 27 DPH, PD effects faded, where  $LD_{25}$  and  $LD_{50}$  fish had comparable  $FF_A$ . The overall pattern suggests that there are diminishing returns at higher PD, similar to the plateau effects of prey density reported by Puvanendran & Brown (1999). Aside from the morphometric measures, a higher stocking density has been shown to negatively affect fin health and condition (Stejskal et al., 2020). During the experiment, we did not observe any cases of fin tears or missing fins in high-density treatments, yet larvae reared at higher LD had smaller fin areas. Overall, fish with larger  $FF_A$  at the early stage are expected to swim more efficiently, avoid predation, and allocate more energy toward growth instead of expending it on basic locomotion (Webb and Weihs, 1986; van den Boogaart et al., 2012) therefore, selecting proper LD and PD conditions is intrinsic for early larviculture success.

It is well established that a single live-feed type protocol often yields lower survival rates (Harzevili et al., 2003; Busch et al., 2011; Lahnsteiner et al., 2023). Co-feeding larvae with customized prey size and type, according to their developmental stage such as rotifers, *Artemia*, and pellets, is likely to improve growth outcomes. A review study conducted on use of live feeds on early stage of fish showed a similar outcome where matching prey size/type/ration to larval stage improved performance (Conceição et al., 2010). Cumulative survival was highest in LD<sub>25</sub>×PD<sub>8</sub> (~94%), followed by LD<sub>25</sub>×PD<sub>4</sub> and LD<sub>100</sub>×PD<sub>8</sub> (~92–91%), while LD<sub>25</sub>×PD<sub>2</sub> was lowest (~83%). Despite being stocked at highest density (LD<sub>100</sub>), larvae fed at PD<sub>8</sub> achieved survival comparable to those reared at low stocking density of LD<sub>25</sub>. Interestingly, survival at LD<sub>25</sub>×PD<sub>2</sub> was lower than at LD<sub>100</sub>×PD<sub>2</sub>. This outcome could simply be the result of limitations of prey availability (Slembrouck et al., 2009) or this may be partly due to proportional effects, as the same number of mortalities represents a greater percentage loss in low density treatments compared to higher LD treatments. It could also be due to potential confusion effect. Larvae stocked at lower LD encounters more nauplii per individual and potentially, gets confused to detect ideal prey for consumption. This perceptual constraint causes larvae to spend greater energy reserves during prey capture attempts. Such foraging inefficiency, especially at early developmental stage, have pronounced effect and can potentially culminate into starvation mortality (Jeschke and Tollrian, 2007).

Regardless of these outcomes, further feeding studies on LMB should be conducted to examine the effect of PD higher than 8 *Artemia*/mL. For instance, study conducted on tambaqui post-larvae (*Colossoma macropomum*), where they were fed  $\geq 1000$  *Artemia* nauplii/L in the second week of rearing, had very high survival and growth rates (Melo et al., 2024). Another study on spotted scat (*Scatophagus argus*), where they were offered a range of *Artemia* densities (4, 8,

12, 16, and 20 *Artemia*/mL), showed increased survival as PD increased, reaching ~100% (Noornissabegum et al., 2022). That said, we must be cautious about the impact of increasing PD, as the effect of higher PD may lead to more complicated husbandry practices (Slembrouck et al., 2009) as well as diminishing returns in terms of costs and an increased likelihood of the confusion effect which reduces predator efficiency.

Larvae SGR was also significantly influenced by the LD × PD interaction. At LD<sub>25</sub>, growth increased with prey supply and was highest at PD<sub>8</sub>. At LD<sub>50</sub>, the highest SGR was observed at PD<sub>4</sub>, while at LD<sub>100</sub>, PD<sub>4</sub> unexpectedly produced the lowest SGR. In contrast to our findings, studies on *Pangasius hypophthalmus* (Vu & Huynh, 2020) and *Hoplias lacerdae* (Luz & Portella, 2015) reported consistent gains in SGR as PD increased, highlighting a discrepancy that may reflect species-specific and density related responses. When examining the effect of LD within each PD, larvae at LD<sub>25</sub> and LD<sub>50</sub> consistently outperformed LD<sub>100</sub> at PD<sub>2</sub> and PD<sub>4</sub>. At PD<sub>8</sub>, larvae stocked at 25/L had the highest SGR. Similar outcomes were observed in sunshine bass (*Morone chrysops* × *Morone saxatilis*) and crucian carp (*Carassius carassius*), where individual growth declined at high LD despite higher biomass yield (Ludwig & Lochmann, 2011; Żarski et al., 2011). Nevertheless, growth across treatments remained strong, where even the most crowded groups exceeded 5% SGR per day. This indicates that while stocking density strongly influenced growth, LMB larvae can still achieve commercially relevant growth under intensive conditions when prey rations, feeding strategy, and transitions are carefully designed and managed.

Total biomass increased with increasing LD and PD. The highest yield was observed at LD<sub>100</sub>×PD<sub>8</sub>, showing that even though individual growth was slower at high LD, cumulative production per tank (in terms of biomass) was maximized when enough prey was provided. A

similar pattern was reported for sunshine bass, where Ludwig & Lochmann (2011) found that individual size decreased with density, but total tank yield showed a curvilinear increase, with maximum biomass at ~87 larvae/L. Źarski et al. (2011) also documented that crucian carp larvae reared between 50-600/L grew largest at low density, yet final biomass was positively correlated with LD, with the highest observed at 600/L. Their result demonstrates that intensification could increase production if survival is maintained. Biomass was significantly higher at PD<sub>8</sub> than at PD<sub>2</sub>, although the gain beyond PD<sub>4</sub> was marginal. This agrees with Lee et al. (2017), who showed that larval sablefish (*Anoplopoma fimbria*) biomass rose as rotifer density increased (2.5-17.5/mL), though improvements plateaued at the highest levels. Together, these findings emphasize that while higher LD and PD maximize biomass, producers must consider their existing farm infrastructure and weigh these gains against trade-offs in growth, animal welfare, and efficiency.

Cannibalism was highest at LD<sub>100</sub>×PD<sub>2</sub> (~13%) and lowest at LD<sub>25</sub>×PD<sub>8</sub> (<1%). This shows that stocking fish at higher density combined with lower prey supply increases cannibalism significantly, while low LD with high PD minimizes it (Pereira et al., 2017). Trends across studies suggest that sibling cannibalism is the main cause of early mortality at high LD (Hatzithanasiou et al., 2002.; Wenzel et al., 2022). However, when fish were fed a higher PD of 8 *Artemia*/mL, the percentage of cannibalism was usually low, even at higher LD treatment. This may be due to the suppression of dominance hierarchies at higher LD, where many larvae compete but no individual can consistently assert dominance. Furthermore, when prey availability is sufficient, even smaller individuals retain enough energy to resist subordination, which further weakens size-based asymmetries and prevents formation of stable hierarchies (Baras and Jobling, 2002; Wipf et al., 2011; Arechavala-López et al., 2020; Sarma et al., 2023). Overall, our results showed that cannibalism in LMB is most severe under high LD × low PD. Therefore, stocking larvae with

adequate prey and executing size control measures such as grading and culling should help minimize losses.

Fish quality and quantity play an important role in determining the selling price of fish. In this study, economic profit (in relation to *Artemia* cost) varied across treatments. At LD<sub>100</sub>×PD<sub>8</sub>, returns were nearly three times greater than those at LD<sub>25</sub>. While SGR peaked at LD<sub>25</sub>×PD<sub>8</sub>, the highest economic gain was observed at LD<sub>100</sub>×PD<sub>8</sub> treatments. Further optimization of prey rations and weaning strategies could help achieve growth at LD<sub>100</sub>×PD<sub>8</sub> comparable to LD<sub>25</sub>×PD<sub>8</sub> and thereby increase profit. It is important to note that our cost analysis did not include additional operational expenses such as labor, electricity, water, equipment, or depreciation costs. The selling price of individual fish used in this analysis was based on an average mid-range value derived from a small survey of producers and retailers. Despite these limitations, this analysis provides a useful framework for estimating production costs relative to live feed inputs, which constitute a major share of expenses during early rearing.

Overall, this study demonstrated that LMB could be successfully cultured at high larval density (100 larvae/L) using RAS technology without compromising survival rates or biomass yield. This provides a strong foundation for scaling production efficiency in commercial hatcheries. Furthermore, our findings can be used to tailor the rearing strategy based on a producer's goal; producers seeking maximum yield from given resources could stock fish at high density (LD<sub>100</sub>) and feed 8 *Artemia*/mL, whereas those targeting larger, faster growing fingerlings for the recreational angling market in shorter time spans could opt for culture conditions that involves lower stocking density (LD<sub>25</sub> or LD<sub>50</sub>) paired with moderate PD<sub>4</sub> or high PD<sub>8</sub>. Looking to the future, several research directions are crucial for advancing intensive LMB production using

RAS technology. A study on evaluating higher PD concentrations to achieve the threshold of diminishing returns should be conducted. Moreover, studies beyond feed management, such as early monosexing techniques, could be carried out to improve yield consistency. Ultimately, integrated approaches will enable producers to develop science-based hatchery protocols that drive the expansion of the LMB industry. By combining our findings with future research directions, we anticipate that biological and production-related challenges can be alleviated, thereby positioning LMB as a globally competitive species.

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Table 1. 1. Stocking densities (Larvae/L or fish/L) reported in previous Centrarchidae larviculture studies, organized by species, life stage (days post-hatch, DPH), culture system, culture duration, prey/diet type, and rearing temperature.

| Species        | Lifestage | Culture system | Culture time | Stocking density          | Diet type                 | Temperature | Authors                   |
|----------------|-----------|----------------|--------------|---------------------------|---------------------------|-------------|---------------------------|
| LMB            | 2 DPH     | RAS            | 27 days      | 110 larvae/L              | <i>Art.</i>               | 24/27°C     | Aguilar et al., 2023      |
| LMB            | 4 DPH     | RAS            | 21 days      | ~10 larvae/L              | FD                        | 25±1°C      | Molinari et al., 2023     |
| LMB            | 5 DPH     | RAS            | 18 days      | ~21 larvae/L              | Ro, <i>Art.</i> , FD      | 25±1°C      | Lukić et al., 2024        |
| LMB            | 5 DPH     | RAS            | 80 days      | ~10 larvae/L              | FD                        | 25±1°C      | Kwasek et al., 2021       |
| LMB            | 5 DPH     | RAS            | 10 days      | 10-50 larvae/L            | Ro, <i>Art.</i> , FD      | 24±1°C      | Skudlarek et al., 2013    |
| LMB            | 7 DPH     | CE             | 4 days       | 25 larvae/L               | Zoo                       | 19±1°C      | Laurence 1972             |
| LMB            | 13 DPH    | RAS            | 27 days      | ~1 larvae/L               | FD                        | 26±1°C      | Chen et al., 2024         |
| LMB            | 19 DPH    | RAS            | 32 days      | 1 larvae/L                | FD                        | 26±1°C      | Zhang et al., 2025        |
| SMB            | 5 DPH     | Pond           | 35 days      | 56,790 larvae/ha          | CO, Zoo                   | 25-31°C     | Robel and Fisher, 1999    |
| SMB            | 10 DPH    | RW             | 21 days      | 5 larvae/L                | Ro, <i>Art.</i> , FD      | 25±1°C      | Ehrlich et al., 1989      |
| Bluegill       | 7 DPH     | RAS            | 14 days      | 133 larvae/L              | <i>Art.</i> , FD          | 20-26°C     | Mischke et al., 1998      |
| Bluegill       | 3 DPH     | FT             | 30 days      | .                         | <i>Art.</i> , Dap, FD     | 23-26°C     | Bryan et al., 1994        |
| Bluegill       | 10 DPH    | Pond           | 360 days     | ~80 larvae/m <sup>3</sup> | Rot, <i>Art.</i> , CO, CL | 23-27°C     | Partridge et al., 1999    |
| Bluegill       | 15 DPH    | CE             | 21 days      | .                         | Zooplanktons              | 23-26°C     | Oplinger and Wahl, 2014   |
| Black Crappie  | 15 DPH    | RAS            | 120 days     | 10-15 fish/L              | Krill, <i>Art.</i>        | 26±2°C      | Dudenhoeffer et al., 2014 |
| Black Crappie  | 6 DPH     | RAS            | 35 days      | 3 fish/L                  | <i>Art.</i> , FD          | 26±1°C      | Dudenhoeffer et al., 2012 |
| Redear Sunfish | 6 DPH     | RAS            | 35 days      | 3 fish/L                  | <i>Art.</i> , FD          | 26±1°C      | Dudenhoeffer et al., 2012 |

Abbreviations: LMB = largemouth bass (*Micropterus nigricans*); SMB = smallmouth bass (*Micropterus dolomieu*); FD = formulated diet; RAS = recirculating aquaculture system; CO = copepods; CL = cladocerans; ZP = zooplankton, CE= controlled environment, FT = flow through, RW = raceways, Ro = Rotifers, Art. = Artemia, Zoo = Zooplanktons, FD = formulated diets

Table 1. 2. Stepwise estimation of profit per treatment in largemouth bass (*Micropterus nigricans*) reared under different larval densities (LD: 25, 50, 100 larvae/L) and prey densities (PD: 2, 4, 8 Artemia/mL).

| LD  | PD | G (g) | AC (\$) | N    | L (mm) | SL | DPH  | DS  | R <sup>2</sup> | SP (\$) | TGR (\$) | Profit (\$) | Equation ( $y=mx+b$ )  |
|-----|----|-------|---------|------|--------|----|------|-----|----------------|---------|----------|-------------|------------------------|
| 25  | 2  | 43.2  | 8.1     | 285  | 25.1   | 18 | 18.0 | 7.0 | 0.92           | 0.36    | 102.6    | 94.5        | SL = 1.77 + 0.90 × DPH |
| 25  | 4  | 79.2  | 14.9    | 307  | 25.5   | 18 | 16.5 | 8.5 | 0.96           | 0.36    | 110.5    | 95.6        | SL = 2.46 + 0.94 × DPH |
| 25  | 8  | 154   | 28.9    | 339  | 25.9   | 18 | 16.0 | 9.0 | 0.95           | 0.36    | 122.0    | 93.1        | SL = 2.68 + 0.96 × DPH |
| 50  | 2  | 46.6  | 8.8     | 539  | 22.5   | 18 | 19.4 | 5.6 | 0.95           | 0.36    | 194.0    | 185.3       | SL = 2.71 + 0.79 × DPH |
| 50  | 4  | 87.4  | 16.4    | 576  | 24.7   | 18 | 18.2 | 6.8 | 0.91           | 0.36    | 207.4    | 190.9       | SL = 2.49 + 0.85 × DPH |
| 50  | 8  | 175   | 32.9    | 548  | 22.3   | 18 | 18.2 | 6.8 | 0.98           | 0.36    | 197.3    | 164.4       | SL = 3.40 + 0.80 × DPH |
| 100 | 2  | 52.8  | 9.9     | 1047 | 19.5   | 18 | 22.0 | 3.0 | 0.92           | 0.36    | 376.9    | 367.0       | SL = 3.24 + 0.67 × DPH |
| 100 | 4  | 115   | 21.7    | 1160 | 18.0   | 18 | 24.0 | 1.0 | 0.97           | 0.36    | 417.6    | 395.9       | SL = 3.69 + 0.60 × DPH |
| 100 | 8  | 191   | 36.0    | 1249 | 20.0   | 18 | 19.9 | 5.1 | 0.97           | 0.36    | 449.6    | 413.7       | SL = 3.95 + 0.70 × DPH |

Note: Profit was calculated in three steps: (1) *Artemia* cost per treatment (AC) = PD × feeding frequency (F = 8/day) × tank volume (V = 15,000 mL) × culture days (DPH), divided by hatch rate ( $\approx 100,000$  *Artemia*/g) and multiplied by cyst price ( $\approx 0.2$  USD/g); (2) Total gross return (TGR) = standardized larval length (SL, mm; converted to inches) × selling price per inch (SP = 0.51 USD/inch) × number of survivors (N); (3) Profit (USD) = TGR - AC. DPH values represent the predicted number of days for larvae in each treatment to reach a minimum target length of 18 mm (SL), based on treatment-specific linear growth equations ( $y = mx + b$ ). SL (mm) was standardized length across treatments to enable direct economic comparisons. DS indicates the number of days saved relative to the slowest-growing treatment ( $LD_{100} \times PD_4$ ).  $R^2$  values correspond to the goodness of fit of the growth regressions.

Table 1. 3. Results of three-way repeated measures ANOVA that evaluated morphometric traits of largemouth bass (*Micropterus nigricans*) larvae.

| Main effect | DFN | Notochord length |           | Total length |           | Eye-diameter |           | Myotome height |          | Jaw length |           | Body area |           | Fin-fold area |          |
|-------------|-----|------------------|-----------|--------------|-----------|--------------|-----------|----------------|----------|------------|-----------|-----------|-----------|---------------|----------|
|             |     | DFD              | F value   | DFD          | F value   | DFD          | F value   | DFD            | F value  | DFD        | F value   | DFD       | F value   | DFD           | F value  |
| LD          | 2   | 32.3             | 63.2****  | 33.7         | 62.3****  | 36           | 47.1****  | 47.2           | 33.8**** | 28         | 29.4****  | 34.2      | 77.1****  | 40.5          | 68.8**** |
| PD          | 2   | 32.3             | 22.5****  | 33.7         | 21.4****  | 36           | 26.03**** | 47.2           | 8.93**** | 28         | 29.62**** | 34.2      | 23.92**** | 40.5          | 17.2**** |
| LD×PD       | 4   | 32.3             | 4.25***   | 33.7         | 4.9***    | 36           | 5.47***   | 27.2           | 4.17***  | 28         | 3.84**    | 34.2      | 4.31***   | 40.5          | 2.74**   |
| Day         | 4   | 63.8             | 2493****  | 64           | 2832****  | 59.7         | 4601****  | 64.4           | 2151**** | 51.9       | 1461****  | 63.9      | 3212****  | 64.4          | 2448**** |
| Day×LD      | 8   | 63.8             | 9.92****  | 64           | 9.98****  | 59.7         | 6.46****  | 64.4           | 5.85**** | 51.9       | 3.62***   | 63.9      | 10.45**** | 64.4          | 7.95**** |
| Day×PD      | 8   | 63.8             | 12.89**** | 64           | 11.85**** | 59.7         | 6.38****  | 64.4           | 4.22**** | 51.9       | 8.23****  | 63.9      | 13.98**** | 64.4          | 9.79**** |
| Day×LD×PD   | 16  | 63.8             | 1.83**    | 64           | 1.8*      | 59.7         | 1.91*     | 64.4           | 0.65     | 51.9       | 1.52      | 63.9      | 2.13**    | 64.4          | 1.93**   |

Note: Fixed effects included larval density (LD), prey density (PD), day (DPH), and their interactions (LD × PD, Day × LD, Day × PD, Day × LD × PD). For each trait, F values are reported with numerator (DFN) and denominator (DFD) degrees of freedom. Traits analyzed were notochord length, total length, eye diameter, myotome height, jaw length, body area, and fin-fold area. Significance levels: \*  $P \leq 0.10$ ; \*\*  $P \leq 0.05$ ; \*\*\*  $P \leq 0.01$ ; \*\*\*\*  $P \leq 0.001$

Table 1. 4. Results of two-way ANOVAs that evaluate morphometric traits of largemouth bass (*Micropterus nigricans*) larvae showed significant interactions between larval density (LD) and prey density (PD) at 16 and 27 days post-hatch (DPH).

| Main effects |     |     | NL        | TL        | ED        | BA        | FFA       |
|--------------|-----|-----|-----------|-----------|-----------|-----------|-----------|
| Day 16       | DFN | DFD | F value   | F value   | F value   | F value   | F value   |
| LD           | 2   | 18  | 26.36**** | 26.88**** | 9.27***   | 35.42**** | 39.11**** |
| PD           | 2   | 18  | 92.92**** | 72.89**** | 40.59**** | 88.55**** | 96.64**** |
| LD×PD        | 4   | 18  | 6.31***   | 5.83***   | 5.91***   | 5.92***   | 6.44***   |
| Day 27       | DFN | DFD | F value   | F value   | F value   | F value   | F value   |
| LD           | 2   | 18  | 53.73**** | 50.37**** | 23.07**** | 54.46**** | 59.85**** |
| PD           | 2   | 18  | 0.12      | 0.11      | 0.79      | 0.06      | 0.14      |
| LD×PD        | 4   | 18  | 2.46*     | 2.58*     | 0.77      | 2.65*     | 2.75*     |

Note: Traits analyzed were notochord length (NL), total length (TL), eye diameter (ED), body area (BA), and fin-fold area (FFA). For each trait, F values are reported with numerator (DFN) and denominator (DFD) degrees of freedom. Significance levels: interactions (\*

$P \leq 0.10$ ; \*\*  $P \leq 0.05$ ; \*\*\*  $P \leq 0.01$ ; \*\*\*\*  $P \leq 0.001$ ); fixed effects of LD, PD, and day (\*  $P \leq 0.05$ ; \*\*  $P \leq 0.01$ ; \*\*\*  $P \leq 0.001$ ; \*\*\*\*  $P \leq 0.0001$ ).

Table 1. 5. Results of one-way ANOVAs (Type III test of fixed effects) evaluating the independent influence of prey density (PD) and larval density (LD) on morphometric traits of largemouth bass (*Micropterus nigricans*) larvae at 16 days post-hatch (DPH).

| Main effect on Day 16 |     |     | NL         | BL        | ED        | MH        | JL        | BA         | FFA        |
|-----------------------|-----|-----|------------|-----------|-----------|-----------|-----------|------------|------------|
| PD                    | DFN | DFD | F value    | F value   | F value   | F value   | F value   | F value    | F value    |
| 2                     | 2   | 6   | 25.07**    | 36.42**   | 42.42**   | 18.51*    | 16.61*    | 23.17*     | 33.89**    |
| 4                     | 2   | 6   | 7.21*      | 7.70*     | 2.1       | 9.57*     | 4.61      | 8.66*      | 9.22*      |
| 8                     | 2   | 6   | 63.40***   | 62.63***  | 24.97*    | 0.38      | 10.41*    | 91.28***   | 80.43***   |
| LD                    | DFN | DFD | F value    | F value   | F value   | F value   | F value   | F value    | F value    |
| 25                    | 2   | 6   | 26.80****  | 25.4****  | 15.42**** | 1.47      | 21.53**** | 26.39****  | 27.79****  |
| 50                    | 2   | 6   | 45.48****  | 46.69**** | 9.76**    | 41.95**** | 13.4****  | 37.80****  | 54.13****  |
| 100                   | 2   | 6   | 156.21**** | 29.75**** | 51.31**** | 76.74**** | 30.19**** | 102.73**** | 126.16**** |

Note: Traits analyzed were notochord length (NL), total length (TL), eye diameter (ED), body area (BA), and fin-fold area (FFA). For each trait, F values are reported with numerator (DFN) and denominator (DFD) degrees of freedom. Significance levels for LD and

PD: \*  $P \leq 0.05$ ; \*\*  $P \leq 0.01$ ; \*\*\*  $P \leq 0.001$ ; \*\*\*\*  $P \leq 0.0001$

Table 1. 6. Results of one-way ANOVAs (Type III test of fixed effects) evaluating the independent influence of prey density (PD) and larval density (LD) on morphometric traits of largemouth bass (*Micropterus nigricans*) larvae at 27 days post-hatch (DPH).

| Main Effect at 27 DPH |     |     | NL        | BL        | ED      | MH        | JL      | BA        | FFA       |
|-----------------------|-----|-----|-----------|-----------|---------|-----------|---------|-----------|-----------|
| PD                    | DFN | DFD | F value   | F value   | F value | F value   | F value | F value   | F value   |
| 2                     | 2   | 6   | 25.63**   | 28.63**** | 12.50** | 37.83**** | 5.46*   | 22.87**   | 28.77**** |
| 4                     | 2   | 6   | 43.14**** | 36.69**** | 10.92** | 41.30**** | 15.30** | 40.49**** | 40.22**** |
| 8                     | 2   | 6   | 8.36*     | 7.77*     | 4.15    | 11.11**   | 2.6     | 9.08*     | 9.94*     |
| LD                    | DFN | DFD | F value   | F value   | F Value | F value   | F value | F value   | F value   |
| 25                    | 2   | 6   | 0.91      | 0.98      | 1.14    | 0.83      | 4.53    | 0.61      | 0.58      |
| 50                    | 2   | 6   | 3.1       | 3.18      | 0.84    | 3.45      | 1.19    | 3.14      | 3.58      |
| 100                   | 2   | 6   | 1.18      | 1.22      | 0.7     | 1.37      | 1.42    | 1.32      | 1.27      |

Note: Traits analyzed were notochord length (NL), total length (TL), eye diameter (ED), body area (BA), and fin-fold area (FFA). For each trait, F values are reported with numerator (DFN) and denominator (DFD) degrees of freedom. Significance levels for LD and PD: \*  $P \leq 0.05$ ; \*\*  $P \leq 0.01$ ; \*\*\*  $P \leq 0.001$ ; \*\*\*\*  $P \leq 0.0001$

Table 1. 7. Results of two-way ANOVAs evaluating larval biomass, survival, cannibalism, and specific growth rate (SGR) of largemouth bass (*Micropterus nigricans*) at 27 days post-hatch (DPH).

| Day 27 | Main effect |     | Biomass    | Survival   | Cannibalism | SGR        |
|--------|-------------|-----|------------|------------|-------------|------------|
|        | DFN         | DFD | F value    | F value    | F value     | F value    |
| LD     | 2           | 18  | 85.9*****  | 9.62***    | 31.01*****  | 52.06***** |
| PD     | 2           | 18  | 35.04***** | 11.12***** | 0.65        | 0.1        |
| LD×PD  | 4           | 18  | 1.53       | 2.84*      | 4.16**      | 2.63*      |

Notes: Analyses included main effects of larval density (LD), prey density (PD), and their interaction (LD × PD). For each response variable, F values are reported with numerator (DFN) and denominator (DFD) degrees of freedom. Significance levels: \*  $P \leq 0.05$ ; \*\*  $P \leq 0.01$ ; \*\*\*  $P \leq 0.001$ ; \*\*\*\*\*  $P \leq 0.0001$ .

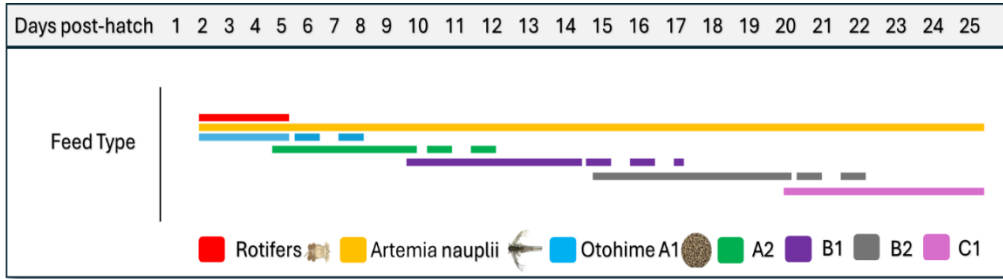


Figure. 1. 1. Different types of feed used during the rearing of largemouth bass (*Micropterus nigricans*) in our experiment. The upper grey bar represents the timeline in days post-hatch (DPH), from Day 2 to Day 25. Colored bars indicate the different feeds administered during larval rearing. Dashed sections represent weaning periods, which begin when a larger pellet is gradually introduced alongside the current feed. Otohime pellets were used during the experiment, with the pellet size progressively increased: A1 (light blue), A2 (green), B1 (purple), B2 (grey), and lastly C1 (pink) which was fed until 27 DPH.

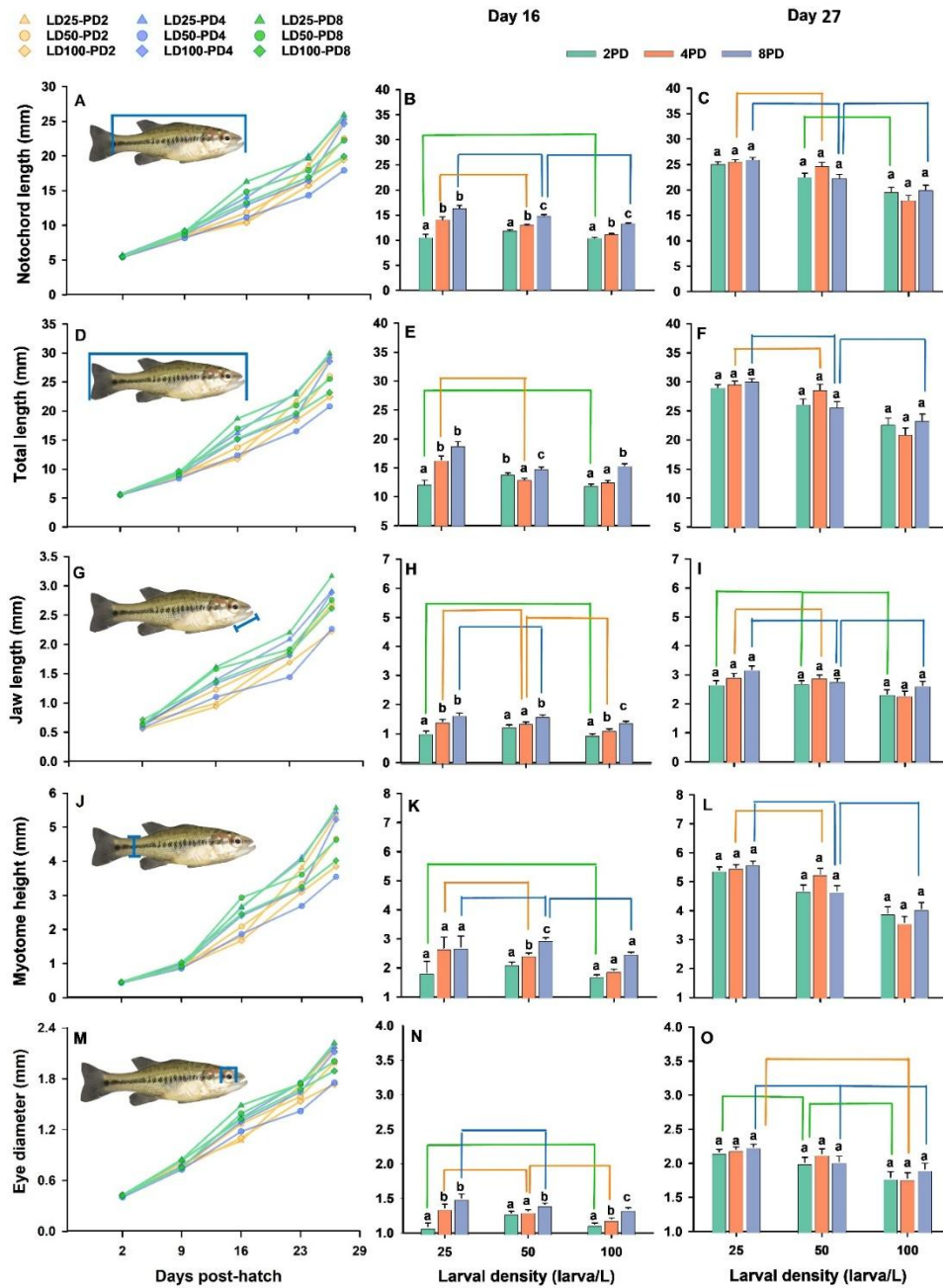


Figure. 1. 2. Growth trajectories of largemouth bass (*Micropterus nigricans*) from 2-27 days post-hatch (DPH) and post hoc comparisons under three larval densities (25, 50, 100 larvae/L) and three prey densities (2, 4, 8 *Artemia*/mL). Growth parameter panels (A, D, G, J, M) show trajectories

for all nine treatment combinations ( $LD \times PD$ ), with fish silhouettes illustrating the measured traits. Post hoc comparisons are shown for notochord length (A: growth; B: 16 DPH; C: 27 DPH), total length (D; E; F), jaw length (G; H; I), myotome height (J; K; L), and eye diameter (M; N; O). In post hoc plots, bars show prey-density (PD) effects within each larval density (LD), while color-coded connection lines above the bars show LD effects within each PD; connected lines indicate no significant difference ( $P > 0.05$ ), and unconnected lines indicate significant differences. Different letters above bars denote significant differences ( $P < 0.05$ ). The legend shows the nine  $LD \times PD$  treatments.

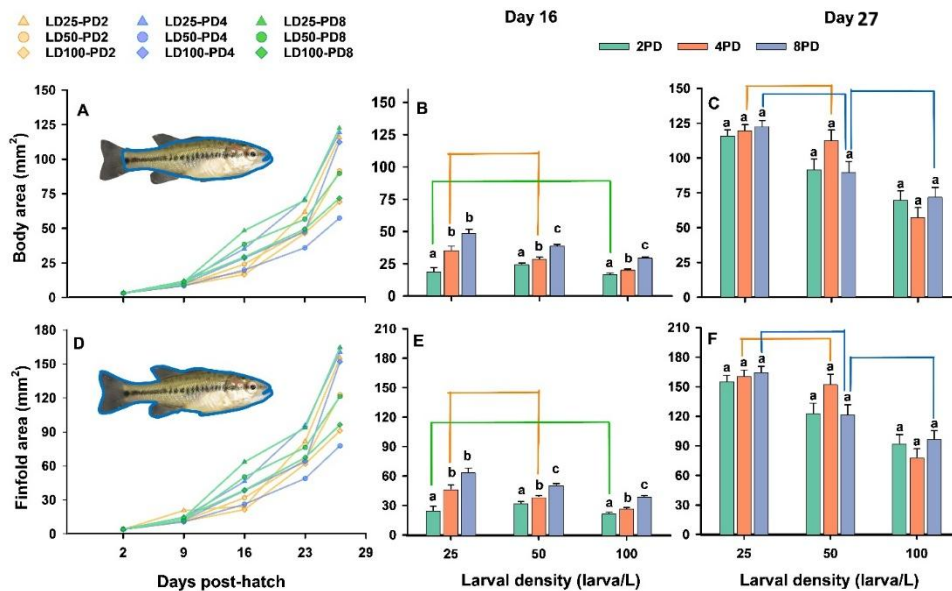


Figure. 1.3. Growth trajectories (2-27 days post-hatch, DPH) and post hoc comparisons of largemouth bass (*Micropterus nigricans*) body area (A: growth; B: post hoc at 16 DPH; C: post hoc at 27 DPH) and fin-fold area (D; E; F) under three larval densities (25, 50, 100 larvae/L) and three prey densities (2, 4, 8 *Artemia*/mL). Growth panels (A, D) show trajectories for all nine LD × PD treatment combinations, with fish silhouettes illustrating the measured traits. In post hoc plots, bars represent PD effects within each LD, while color-coded connection lines above the bars represent LD effects within each PD; connected lines indicate no significant difference ( $P > 0.05$ ), while unconnected lines indicate significant differences. Different letters above bars denote significant differences ( $P < 0.05$ ). The legend shows the nine LD × PD treatments.

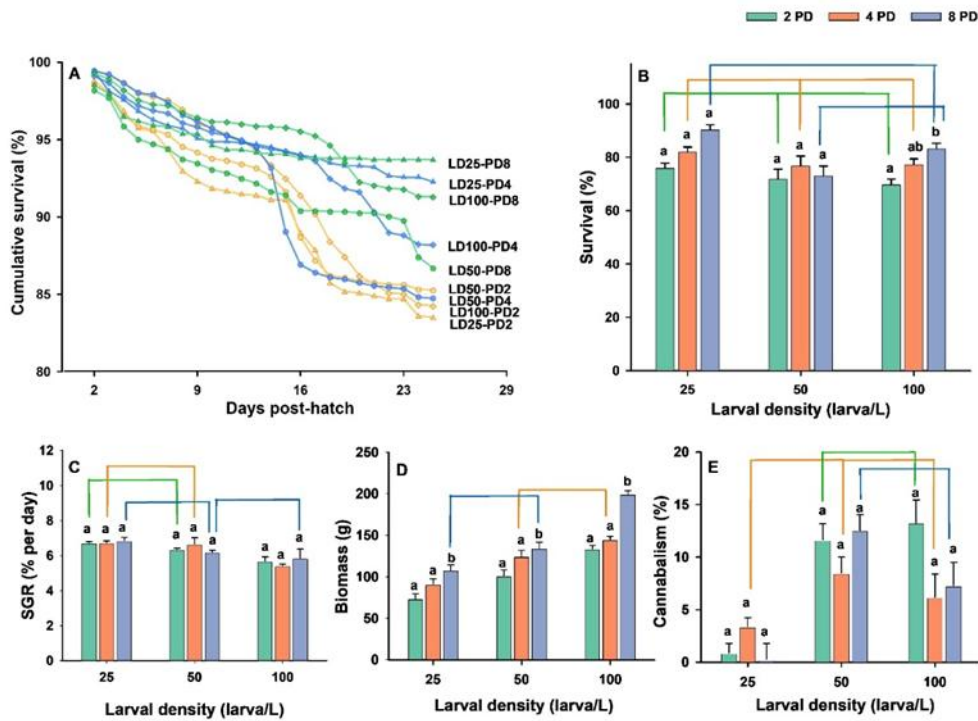


Figure. 1. 4. Effect of larval density (LD: 25, 50, 100 larvae/L) and prey density (PD: 2, 4, 8 *Artemia*/ mL) on cumulative survival (A) and endpoint post hoc comparisons at 27 DPH for survival (B), specific growth rate (C), biomass (D), and cannibalism (E) in largemouth bass (*Micropterus nigricans*) larvae. Cumulative survival for each tank was recorded to 25 DPH. In bar plots, bars represent PD effects within each LD, and color-coded lines above the bars represent LD effects within each PD; connected lines indicate no significant difference ( $P > 0.05$ ), while unconnected lines show significant differences. Different letters above bars denote significant differences ( $P < 0.05$ ).

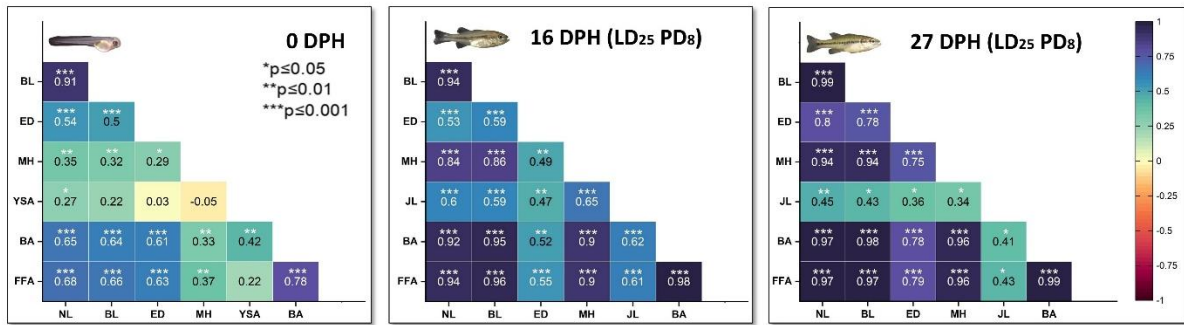


Figure. 1. 5. Correlation matrices of morphometric traits in largemouth bass (*Micropterus nigricans*) at 0, 16, and 27 days post-hatch (DPH) under a stocking density of 25 larvae/L (LD<sub>25</sub>) and a prey density of 8 *Artemia*/mL (PD<sub>8</sub>). Traits include notochord length (NL), body length (BL), eye diameter (ED), myotome height (MH), jaw length (JL), body area (BA), and fin-fold area (FFA). Numbers represent Pearson correlation coefficients (r), with color intensity indicating the strength and direction of association (scale bar: -1 to +1). Significance levels are denoted by asterisks (\* P ≤ 0.05, \*\* P ≤ 0.01, \*\*\* P ≤ 0.001).



**LD25 × PD2**

**Biomass:** 79 g  
**Mean Condition Index:** 0.106  
**Avg. Length:** 25.06



**LD25 × PD4**

**Biomass:** 100 g  
**Mean Condition Index:** 0.114  
**Avg. Length:** 25.51



**LD25 × PD 8**

**Biomass:** 124 g  
**Mean Condition Index:** 0.122  
**Avg. Length:** 25.94



**LD50 × PD 2**

**Biomass:** 118 g  
**Mean Condition Index:** 0.120  
**Avg. Length:** 22.51



**LD50 × PD 4**

**Biomass:** 132 g  
**Mean Condition Index:** 0.117  
**Avg. Length:** 24.67



**LD50 × PD 8**

**Biomass:** 144 g  
**Mean Condition Index:** 0.124  
**Avg. Length:** 22.28



**LD100 × PD 2**

**Biomass:** 138 g  
**Mean Condition Index:** 0.119  
**Avg. Length:** 19.53



**LD100 × PD 4**

**Biomass:** 155 g  
**Mean Condition Index:** 0.127  
**Avg. Length:** 17.977



**LD100 × PD 8**

**Biomass:** 204 g  
**Mean Condition Index:** 0.131  
**Avg. Length:** 19.95

Figure. 1. 6. Visual comparison of final yield of largemouth bass (*Micropterus nigricans*) across nine treatments from a 3 × 3 factorial design, combining three larval densities (LD; 25, 50, 100 larvae/L) and three prey densities (PD; 2, 4, 8 *Artemia*/mL). Each image represents the final larval

yield from a tank randomly selected for each unique combination of LD and PD. Average lengths are in mm. Condition Index is the ratio of myotome height to total length.

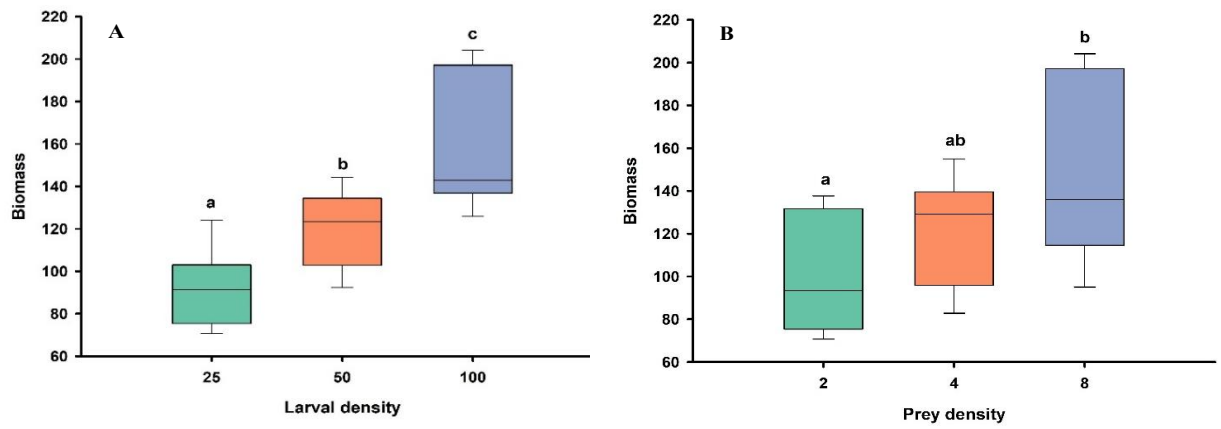


Figure. 1. 7. Final biomass of largemouth bass (*Micropterus nigricans*) at harvest by (A) larval density (LD: 25, 50, 100 larvae/L) and (B) prey density (PD: 2, 4, 8 *Artemia*/mL). One-way ANOVAs were conducted to test main effects of larval density and prey density. Differences among levels were assessed with Tukey post hoc comparisons. Values are mean  $\pm$  SD (n = 3 tanks). The y-axis shows final biomass (g/tank). Different letters denote significant differences ( $\alpha = 0.05$ )

## Chapter 2

### **Effects of static and progressively increasing prey density regimes on larval performance of largemouth bass (*Micropterus nigricans*) reared under intensive recirculating aquaculture system**

#### **Abstract**

This study evaluates how static and progressively increasing prey density (PD; *Artemia*/mL) regimens influence growth, survival, and performance of largemouth bass (*Micropterus nigricans*) larvae reared in an indoor recirculating aquaculture system. Beginning at 2 days post-hatch (dph), larvae were fed either four static rations (PD<sub>8</sub>, PD<sub>12</sub>, PD<sub>16</sub>, PD<sub>20</sub>) or three progressive regimens (PD<sub>8-12</sub>, PD<sub>8-12-16</sub>, PD<sub>8-12-16-20</sub>) where, PD was increased stepwise at 6, 10, and 14 dph. All treatments were stocked at 100 larvae/L except for the Control (25 larvae/L; static PD<sub>8</sub>). Morphometric traits (notochord length, myotome height, and body area), total weight, and condition index were assessed weekly at 2, 9, 13, 23, and 29 dph, while survival, biomass, percent cannibal, average weight, and specific growth rate (SGR) were evaluated at 29 dph. Notochord length, body area, and condition index showed no treatment effects. Total weight differed across PD regimens and increased with age, while myotome height exhibited a significant Treatment × Day interaction. The Control and PD<sub>20</sub> treatments yielded the largest fish, followed by a progressive incremental PD<sub>8-12</sub> regimen. PD significantly influenced all endpoint metrics except SGR. Survival was highest in the Control (86.57±10.31%) and lowest in PD<sub>20</sub> treatment (51.34±4.15%). On the contrary, highest biomass was achieved in PD<sub>20</sub> (572.34±46.76 g) followed by PD<sub>16</sub> (560 ± 31.7 g) and PD<sub>8-12-16-20</sub> (545.68 ± 16.76 g). Percent cannibal was <10% in control, but increased significantly under high static PD, reaching 40 ± 0.7% in PD<sub>20</sub>. Progressively

increasing PD regimens, particularly PD<sub>8-12</sub>, supported growth outcomes comparable to the control as well as the high static PD regimens.

## 2.0. Introduction

Global aquaculture has expanded rapidly over the past decades, increasing its share from 15 % in 1986 to 51% in 2022, and hence surpassing capture fisheries for the first time (FAO, 2024). Despite this growth, the successful rearing of early life stages of fish remains a major bottleneck across many culture species largely due to the demanding nutritional, physical, and microbial requirements during larval development (Research Council of Norway, 2009; Molinari et al., 2023). During the early developmental stages, larvae undergo drastic morphological and physiological changes, including differentiation of craniofacial structures (Peña and Dumas, 2009), shifts in swimming ability and pattern (Voesenek et al., 2018), and maturation of digestive structures (Rønnestad et al., 2013). The transition from endogenous to exogenous feeding is particularly critical, as their digestive tract remains rudimentary with little to no-functional stomach (Yúfera and Darias, 2007). While species such as rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*) have undergone series of selective breeding programs that allow larvae to transition directly to formulated feeds, most carnivorous species still require live prey during early development to sustain growth and survival. This transition period is frequently associated with high mortality caused by starvation (Zhang et al., 2015), cannibalism (Pereira et al., 2017) and growth heterogeneity (Kestemont et al., 2003), which is primarily a result of mismatches between larval prey requirements and feeding regimes (Hecht and Appelbaum, 1988; Baras and Jobling, 2002).

These constraints are also evident in largemouth bass (LMB: *Micropterus nigricans*), a high value aquaculture species with a growing aquaculture importance worldwide. Although the grow-out phases for market-size fish have flourished due to advancements in nutrition, technology and husbandry practices, LMB larval rearing and weaning stages still remain the most sensitive phase, which is often characterized by high mortality and inconsistent production (Li et al., 2020). Total annual production of LMB in U.S. is around 7,000 – 8,000 tonnes, whereas China remains the dominant global producer, accounting for > 90% of total world supply with production reaching around 728,000 tonnes in 2022 (Yu et al., 2024). In the U.S., production still relies on outdoor earthen ponds, where fish are grown over the span of two seasons, typically takes 18–24 months. Broodstocks are naturally spawned, and larvae are introduced in freshly fertilized ponds where they sustain on zooplanktons blooms (Tidwell et al., 1996, 1998, 2000). This approach requires a lot of space, labor and forage fish, which leads to high production costs. Moreover, pond-based culture is prone to fluctuations in critical biotic and abiotic factors such as plankton abundance (Mao et al., 2025), water and soil chemistry (Boyd et al., 2002; Rocha et al., 2022), weather conditions, disease and parasite introduction from external sources (Assefa and Abunna, 2018; Mrambra et al., 2023) and so on. As a result, producers are now opting to shift to an indoor recirculating aquaculture system (RAS) condition where they have better control over the environment and feeding strategies, which in turn facilitates year-round production of consistent quality juveniles.

With that said, successful culture of early stage LMB in RAS depends on optimization of some key rearing parameters such as stocking density, temperature (Aguilar et al., 2023), prey availability (Aguilar et al., 2025), water quality and various other interacting factors (Webb et al., 2007; Stejskal et al., 2021; Seo and Park, 2023). These parameters interact strongly to influence

larval performance (Parajuli et al., *unpublished data*), among which food availability and feeding strategies are influential factors. Moreover, evidence suggests that LMB has poor artificial diet utilization capacity during the first 21 days of exogenous feeding, and they require a co-feeding period with artificial diets and live prey, such as rotifers (*Branchionus* spp.) and *Artemia* (*Artemia nauplii*), to achieve optimal growth (Skudlarek et al., 2013; Kwasek et al., 2021; Aguilar et al., 2025). These live feeds are predominantly used due to their ideal size in relation to larval gape, ease of availability, simple culture protocols, and their motility patterns which stimulate the feeding pattern of visually oriented predators like LMB (Sorgeloos et al., 2001; Conceição et al., 2010).

Previous work (Parajuli et al., *unpublished data*) evaluated the combining effect of larval stocking density (LD; 25, 50 or 100 larvae/L) and prey density (PD; 2, 4 or 8 *Artemia*/mL) on growth and performance of LMB larvae reared in indoor RAS. Results showed that a combination of LD<sub>100</sub>×PD<sub>8</sub> yields the greatest biomass, whereas specific growth rate (SGR) was highest at low LD and low PD treatments (LD<sub>25</sub>×PD<sub>8</sub>). However, this experiment maintained constant PD throughout ontogeny and did not account for the developmental changes that can affect feeding capacity and prey capture efficiency.

Most larval fish undergo marked ontogenetic shifts in morphology and behavior that directly influence their feeding behaviors (Pepin et al., 2023). At hatch, larvae typically have low prey capture success due to relatively small gape size and weak swimming ability, which makes introduction of very high PD in the water column inefficient and wasteful. As, larvae move further into ontogeny, their mouth gape widens, visual acuity improves (do Nascimento et al., 2015), and their swimming form changes (Voesenek et al., 2018), which consequently improves their capture efficiency (Kestemont et al., 2007; Gisbert et al., 2004). Nevertheless, most protocols developed

for larviculture use a fixed PD from the beginning to the end of the live feeding period, often at high levels which are intended to avoid prey limitations. While this ensures high survival and growth, it also leads to unnecessary feed usage during early stages, which inflates production costs and potentially degrades water quality (Barros and Valenti, 2003; Bryant et al., 1980). On the other hand, underfeeding during critical periods can exacerbate size heterogeneity, trigger cannibalistic behavior, and reduce survival, leading to significant losses for producers (Baras and Jobling, 2002; Hatzithanasiou et al., 2002; Pereira et al., 2017)

At present, few studies have been conducted to systematically evaluate PD regimens, where the prey supply is adjusted according to developmental stage. Thus, the objective of this study was to examine the effects of different PD regimens, both static and progressive increasing, on the performance, growth, and survival of LMB larvae reared in intensive RAS. We hypothesized that a progressive increase in PD in combination with larval ontogeny would sustain growth and survival comparable to high static PD treatments, while reducing overall live feed usage and associated costs. Results are expected to provide guidelines for optimizing live feed management during the critical early life stages of LMB and contribute to the development of more cost-effective and sustainable hatchery practices.

## **2.1. Materials and Methods**

### *2.1.1. Animal care*

Protocols for fish experimentation complied with the Institutional Animal Care and Use Committee (IACUC# 2023-5268).

### *2.1.2. Broodstock management*

LMB broodstock were raised at Malone Lake Fish Hatchery in Lonoke, AR, U.S. (34.786815°N, -91.901101°W) and were fed to satiation with Rangen 4818 LMB diet (Rangen, ID, U.S.). Broodstock were kept at 30.5 m diameter 3800 L round plastic tanks with constant flow of filtered well water at ~19°C. To initiate spawning, broodstock were reared on a 12 h light: 12 h dark photoperiod that simulated natural spring day length. Individual spawning mats (Spawntex, Pentair Aquatic Eco-Systems, FL, U.S.) were assigned to each pair of broodstock. During the spawning season, dissolved oxygen (DO) levels were maintained at ~9 mg/L. Mean total ammonia nitrogen (TAN) was 0.04 mg/L at embryo collection. An alkalinity of 80–140 mg/L was maintained throughout the rearing period with the addition of sodium bicarbonate.

### *2.1.3. Embryo collection and incubation*

Eggs/embryos spawned on 19 April 2025 were transported from Malone Lake fish hatchery to the Auburn University E.W. Shell Fisheries Center (32.6526°N, -85.4859°W) on 21 April 2024. Embryos were placed in three insulated coolers (43 × 25 × 27 cm; Uline, WI, U.S.). Each cooler housed two double-layered plastic bags, and each bag represented a distinct family of 2 days post-hatch (dph) embryos. The bags were filled with 10 L of well water where DO and water temperature were maintained at ~10 mg/L and ~21 °C. Upon arrival, larvae were subjected to drip acclimatization for 2 h. Embryos were incubated in 6 different aquaria at ~21 °C where each aquarium had a distinct family.

Aquaria were integrated into RAS containing a UV filter (Emperor smart DC2305, Pentair Aquatic Eco-Systems, FL, U.S.), bead filtration system (Bubble Bead Filter XS10000, Aquaculture Systems Technology, LA, U.S.), bag filter (Pall x-100, Pall Corporation, NY, U.S.), 0.5 hp pump (PerformancePro Cascade, Cascade Pump Company, CA, USA), 21 × 20L tanks (H-3687BL, Uline, WI, U.S.), three 795 L circular blue tanks (PT-5430, Polytank Inc., MN, U.S.), two 190 L sump tanks (Pentair, FL, U.S.), and a heat-pump (AquaLogic Delta Star DSHP-9, Aqua Logic Inc, CA, U.S.). Individual tanks were aerated with diffused air (DO > 6.0 mg/L), and a flow rate of ~ 1 L/min was maintained.

Temperature and DO were monitored daily (~08:00; YSI model 58 with 550A probe; YSI, Yellow Springs, OH, U.S.). Other water quality parameters were tested twice weekly using a spectrophotometer (D/R 2000 Direct Reading, Hach, CO, U.S.) and a pH meter (Orion™ Star A211 pH, Thermo Scientific, MA, U.S.). Nitrite and nitrate levels were maintained between 0–0.03 mg/L, ammonia 0.0–0.2 mg/L, pH 7.2–7.7, alkalinity 90–140 mg/L CaCO<sub>3</sub>, and hardness 80–120 mg/L CaCO<sub>3</sub>. Facility air temperature was kept at 23 ± 0.5°C, and embryo incubation occurred under a 16 h light/8 h dark photo period at ~250 lux. The source of water for the experiment was municipal water which went through a series of dichlorination processes (trickling followed by sodium thiosulfate treatment and intensive aeration) before being introduced into the RAS.

#### *2.1.4. Larval rearing and experimental design*

Following incubation period of 12 hours, a representative portion of larvae from all six aquaria were collected in a 22.9 × 30.5 × 5.1 cm white porcelain tray which ensured representation

from all six family. Larvae were then transferred to 69 × 56 cm circular black tanks (n=21), each filled with 20 L of water.

The experimental setup consisted of seven PD regimens applied from 2–18 dph. The treatments were designed to reflect both static and progressive increases in PD in relation to ontogenetic development. In the static treatments, *Artemia* were fed at a constant density of PD<sub>8</sub>, PD<sub>12</sub>, PD<sub>16</sub>, or PD<sub>20</sub> (*Artemia*/mL). In the progressive treatments, PD was increased stepwise at specific developmental intervals. All larvae initially received 8 *Artemia*/mL from 2–6 days post-hatch (dph), after which the PD was raised to 12 *Artemia*/mL during days 6–10 dph. From this point, three progressive regimens were applied: (i) PD<sub>8-12</sub>, where PD was maintained at PD<sub>12</sub> for the remainder of the trial (ii) PD<sub>8-12-16</sub> where PD increased to PD<sub>16</sub> from 10 dph onward and (iii) PD<sub>8-12-16-20</sub>, where PD increased to PD<sub>16</sub> between 10–14 dph and further to PD<sub>20</sub> from 14–18 dph (Figure. 2. 1). Each treatment was replicated three times, with 2,000 larvae/tank, corresponding to the fixed larval density of 100 larvae/L in 20 L rearing units. Altogether 38,004 larvae were stocked, including 504 larvae post-stocking; 12 larvae/tank to compensate for potential handling-related mortality + 12 larvae/tank to replace individuals removed for initial sampling × 21 culture tanks = 504 larvae.

Once tanks were stocked with yolk-sac larvae, water temperature was slowly raised from 21 to 27°C over two days (~1°C/8h), and flow rate was set to 0.5 L/min. At 2 dph, larvae were fed 8,12,16 or 20 *Artemia*/mL. Premium Grade A *Artemia* (INVE SEP-ART D-FENSE ARTEMIA, INVE Aquaculture, Belgium) were offered every 2/3 h, which resulted in 8 feedings per day, spanning from 07:00 to 23:00 h. All larvae were also fed S-type rotifers (*Brachionus plicatilis*), at 5 rotifers/mL, from 2–11 dph. At 7 dph, larvae were gradually weaned from rotifers over four days, with a reduction of 1 rotifer/mL daily. To complement live feed, Otohime A1 micro-diet (75–250

µm) (Marubeni Nisshin Feed, Tokyo, Japan) was supplied to each tank (~0.5 g/tank). The selection of appropriate micro-diet and pellet size was based on fish size at each sampling point and their receptivity to the diet. Larvae were progressively transitioned from smaller to larger pellets and this transition strategy involved co-feeding 25% of the larger pellets with 75% of the smaller pellets on the first day, then the proportion of larger pellets gradually increased until they constituted 100% of the diet.

Larvae were first fed Otohime A2 from 2–6 dph. Weaning started at 4 dph when Otohime B1 was introduced. B1 was given from 4–7 dph. At 7 dph, Otohime B2 was introduced while B1 was gradually reduced (25% per day). By 11 dph, the larvae were fully transitioned to B2, which was fed until 16 dph. Otohime C1 was introduced at 16 dph, and its amount progressively increased until it became the only feed by 21 dph. Juvenile fish were then fed C1 until 26 dph, after which Otohime C2 was introduced. The amount of C2 was increased until it became the sole feed at 28 dph, and it was fed until 30 dph (Figure. 2. 2).

Dead fish were removed daily (7:00–9:00) to assess mortality rates. Tanks were siphoned twice daily using a thin air hose to remove excess feed, fecal matter, and organic waste. Water exchanges were routinely performed at 25%, with larger exchanges made, when necessary, based on water conditions. Final survival was assessed at 29 dph and adjusted for the 2.65% larvae removed for morphometric and molecular analyses.

#### *2.1.5. Data collection*

##### *Morphological traits*

Fish were sampled at 2, 9, 16, 23, and 29 dph, where 12 fish were randomly selected from each tank. For this study, 0 dph was defined as the time when >50% of eggs hatched. Selected fish were euthanized with 100 ppm MS-222 (tricaine methane sulphonate; Argent Laboratories Inc., WA, U.S.) buffered in rearing water. Digital images were captured using a Zeiss stereomicroscope (SterREO Discovery V12) equipped with 0.5 to 1.0× objectives and ZEN 2.5 imaging software (blue edition). Larval morphometric traits included notochord length ( $N_L$ : distance from tip of snout to the end of myotome, just before the tail begins), Myotome height ( $M_H$ : body height measured posterior to anus) and body area ( $B_A$ : body area excluding fin-fold area).

#### *Relative Condition Index*

Relative condition index ( $K_n$ ) was used to assess individual larval body condition independently of size effects. The index was calculated following Le Cren (1951) as:

$$K_n = \frac{W}{aL^b}$$

where,

W = the observed individual wet weight (g)

L = length (mm)

a = scaling constant/ intercept at unit length of fish (represents the condition of body)

b = slope/ allometric exponent (indicates growth types; isometric vs allometric),

These parameters are derived from the length-weight relationship using allometric growth formula used by Ricker (1973) in the form of  $W = aL^b$ , where ‘a’ is the constant and ‘b’ is the exponent.

This equation was transformed into a logarithmic as suggested by Le Cren (1951) and expressed

as:  $\{\ln W = \ln a + (b \times \ln L)\}$ , where ‘a’ is the constant being initial growth and ‘b’ is the growth coefficient.

The regression was fitted using all individuals’ length and weight pooled across treatments and time points to provide a common reference curve. Predicted weights ( $\widehat{W} = aL^b$ ) were then used to calculate  $K_n$  for each treatment, where values  $\geq 1$  indicate fish heavier than expected for their length, and values  $\leq 1$  indicate lighter individuals. The index was computed for each sampling day (9, 16, 23 and 29 dph) to evaluate temporal changes in larval condition among treatments. Since the intercept and slope in Le Cren’s condition index describe baseline length-weight relationship for a given developmental stage, data were pooled by sampling day (dph) rather than by PD treatments. Similar approaches were used in previous studies for determining the length-weight relation of fish in aquaculture settings or in their natural habitat (Datta et al., 2013; Kachari et al., 2017; Gubiani et al., 2020). Similarly, growth coefficient (b) depicts different growth patterns, where when  $b > 3$  means positive allometry,  $b = 3$  means isometry and  $b < 3$ , means negative allometry growth.

#### *Survival, biomass, percent cannibal, and average weight*

Survival was assessed at 29 dph. To ensure that fish were accurately counted, fish were first collected and sedated in a buffered MS-222 solution (20 mg/L) until 20–30% showed loss of equilibrium or reduced movement. Sedated fish were transferred to a flat  $61 \times 38 \times 8$  cm white tray with minimal water (~200 mL) to prevent overlapping. Digital images were analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, U.S.) with the Cell Counter plugin (v. 3.0.0) to quantify surviving fish from each tank. Survival was determined by subtracting the fish left in each tank from the original stocking number, following the formula:

Survival (%) =  $(N_F / N_I) \times 100\%$ , where  $N_F$  is the number of surviving fish at 29 dph and  $N_I$  is the initial number of larvae stocked at 2 dph (adjusted for fish used for sampling).

After digital images were taken, 50 fish were randomly selected from each tank to determine individual wet weights ( $\pm 0.001$  g), which were then used to calculate total tank biomass. Final tank biomass was determined by multiplying the average wet weight of the individual fish by the total number of survivors, following the formula:

Biomass (g) =  $N_F \times W_M$ , where  $N_F$  is the final number of fish that survived at 29 dph, and  $W_M$  is the mean weight of fish from each treatment at 29 dph.

Percent Cannibal was estimated by:  $[(I_C - F_C) - M_C] / I_C \times 100\%$ , where  $I_C$  is initial number of larvae stocked at 2 dph,  $F_C$  is number of surviving fish at 29 dph, and  $M_C$  is cumulative daily recorded mortality in each tank.

### *Specific growth rate*

Specific growth rate (SGR) was calculated both for length and weight. Notochord length was used as a benchmark for length based SGR whereas, average weight of fish was used to access weight based SGR. Length based specific growth rate ( $SGR_L$ ) was calculated by using 2 dph and 29 dph as initial and final time points, using the following formulae:

$SGR = [(\ln L_t - \ln L_0) / t] \times 100$ , where  $L_0$  represents the initial notochord length,  $L_t$  is the final notochord length, and  $t$  is the duration in days.

Similarly, Weight based specific growth rate ( $SGR_W$ ) was calculated as:

$SGR = [(\ln W_t - \ln W_0) / t] \times 100$ , where  $W_0$  represents the initial average weight,  $W_t$  is the final average weight, and  $t$  is the duration in days.

### 2.1.6. Statistical analysis

Data were analyzed using SAS statistical software (version 9.1, SAS Institute Inc., NC, USA). Residuals were tested for normality using the Shapiro-Wilk test and plotted against predicted values to test for homogeneity of variance. Survival and percent cannibal were arcsine square-root transformed before statistical analysis. Tukey's HSD was used for all pairwise comparisons.

#### *Morphological traits*

Morphological traits such as notochord length ( $N_L$ ), myotome height ( $M_H$ ), body area ( $B_A$ ) along with total weight ( $T_W$ ) were log-transformed and analyzed using repeated-measures mixed model ANOVAs (PROC MIXED; SAS Institute, 2025; Model 1):

$$Y_{abi} = \mu + T_a + D_b + DT_{ab} + R_i + \varepsilon_{n(abi)} \quad (\text{Model 1})$$

where  $\mu$  is the true mean;  $T_a$  is the fixed effect of treatment (where 'a' ranges from treatment 1–treatment 7);  $D_b$  is the fixed effect of sampling day (where  $c = 5$  sampling days; 2, 9, 16, 23, and 29 dph);  $DT_{ab}$  is the *Treatment*  $\times$  *Day* interaction;  $R_i$  is the random effect of tank replicates (where  $i = \text{tank ID}$ ); and  $\varepsilon_{n(abi)}$  is the residual error. An autoregressive covariance [*AR (1)*] structure was applied to account for temporal correlation in repeated measurements. The Satterthwaite procedure (Satterthwaite, 1946) was used to approximate the denominator degrees of freedom for all F-tests (Spilke et al., 2005).

A significant treatment  $\times$  day interaction was observed only for myotome height ( $M_H$ ). For this trait, Model 1 was decomposed into separate one-way ANOVAs at each sampling day using:

$$Y_{ai} = \mu + T_a + R_i + \varepsilon_{n(ai)} \quad (\text{Model 2})$$

Within each sampling day, Tukey's HSD test was used to perform pairwise comparisons between treatments. Although the effect of sampling day within each treatment was also tested, only among-treatment comparisons at each sampling point are reported.

For traits without significant interaction, Model 1 was used to evaluate main effects. To facilitate interpretation of final size differences, larval morphometric traits were additionally analyzed at 29 dph using one-way ANOVA (Model 2) with Tukey's HSD to compare treatment means.

#### *Specific growth rate, survival, biomass, and percent cannibal*

Specific growth rate in length (SGR<sub>L</sub>), specific growth rate in weight (SGR<sub>W</sub>), survival, biomass, and percent cannibal were measured at the end of the trial and therefore, analyzed using a single factor mixed model (Model 1; *Section 2.6.1*). Treatment was included as a fixed effect, and tank ID was included as a random effect. When a significant treatment effect was detected, Tukey's HSD post hoc test was used to compare endpoint means among the seven PD regimens.

#### *Artemia cost analysis*

We estimated profit per treatment (in terms of only one variable: *Artemia* supplied) in three steps (Table 2):

(1) First, we calculated how much *Artemia* was used per treatment and the associated cost. Total live *Artemia* offered per treatment (AF) was calculated as PD (Progressive and static PD regimen) × feeding frequency (F = 8 times/day; see *Section 2.4*) × culture tank water volume (V = 20,000 mL) × number of culture days (dph) in relation to standardized length.

$$(AF) = (PD \times F \times V \times D)$$

Then, to calculate *Artemia* cost per treatment, we took the total amount of live *Artemia* offered per treatment (AF) and divided by the number of hatched *Artemia* /g of cysts ( $H = \sim 100,000$  *Artemia*/g) to get the total amount of *Artemia* cysts (G) used.

$$(G) = (AF) / H$$

Next, we multiplied the total amount of *Artemia* cysts (G) by their price (C; USD/g) to determine *Artemia* cost (AC) per treatment ( $AC = G \times C$ ).

(2) Second, we multiplied the standardized length (SL) of fish across all treatments (in mm) by the selling price of fish per inch (P), i.e. \$0.51, to get the average selling price of fish per treatment (SP).

$$(SP) = L \times P$$

Then, we multiplied the average selling price of fish per treatment (SP) by the number of survivors (N) in each treatment to get the total gross return from each treatment (TGR)

$$(TGR) = N \times SP$$

(3) Finally, profit per treatment was calculated by subtracting *Artemia* cost per treatment (AC) from the total gross return per treatment (TGR)

$$\text{Profit} = \text{TGR} - \text{AC}$$

Moreover, as an extension of our cost analysis, we conducted predictions using simple linear regression models (Table 2.4) to estimate days taken for larvae in each of our seven PD regimens to reach a standardized size of 28.20 mm. This threshold was based on the lowest growth output observed in the progressively increasing PD regimen of PD<sub>8-12-16-20</sub>. Incorporating this model into

our analysis allowed us to compare growth trajectories across treatments and speculate how quickly fish could attain market-relevant sizes relative to *Artemia* input.

## 2.2. Results

### 2.2.1. Morphological traits and average weight across time

Notochord length ( $N_L$ ), myotome height ( $M_H$ ), body area ( $B_A$ ) and total individual weight ( $T_W$ ) of LMB larvae increased over time across all PD treatments (Figure 2. 3A, C, E, G). Fish  $N_L$  showed significant effects of sampling day (Day), but neither the main effect of treatment ( $P=0.28$ ) nor the *Treatment*  $\times$  *Day* interaction ( $P=0.36$ ; Table 2.1) was significant. Fish  $N_L$  increased from 6.5 mm at 2 dph to 30.6 mm by 29 dph. Across PD regimens,  $N_L$  ranged from 28.4 to 31.2 mm at 29 dph. The longest  $N_L$  was observed in Control ( $LD_{25} \times PD_8$ ) and  $PD_{20}$  treatment, whereas the shortest  $N_L$  value was observed in  $PD_{8-12-16-20}$  condition. Although overall effect of PD regimens was non-significant, Tukey's HSD assessed at 29 dph revealed that the Control treatment had significantly bigger  $N_L$  compared to  $PD_{8-12-16-20}$  ( $P<0.05$ ), while fish reared in remaining treatment had similar  $N_L$ .

Mixed-model factorial ANOVAs model revealed *Treatment*  $\times$  *Day* interaction for  $M_H$  ( $P<0.01$ ; Table 2.1). Therefore, the model was broken down, and effect of treatments were evaluated with each sampling day (Figure. 2. 4). Fish  $M_H$  differed among treatments at 9, 16, 23 and 29 dph ( $P<0.05$ ). At 9 dph,  $M_H$  was greater in Control and  $PD_{20}$  treatment compared to the rest of the treatments. At 16 dph, the Control treatment had bigger  $M_H$  than  $PD_{12}$  and  $PD_{8-12-16-20}$  and at 23

dph,  $M_H$  was bigger in the Control than PD<sub>20</sub>. By 29 dph,  $M_H$  of fish in the Control and PD<sub>20</sub> treatments were comparable and significantly greater than those in PD<sub>16</sub>. All other PD treatments showed intermediate  $M_H$  values that did not differ significantly from either high (Control, PD<sub>20</sub>) or PD<sub>16</sub> treatment (Figure 2. 3D, Figure. 2. 4) No significant differences across the treatments were observed for larval  $B_A$  (Figure 2. 3F). Although at 29 dph, fish reared in Control ( $198 \pm 19\text{mm}^2$ ) and at PD<sub>20</sub> ( $192 \pm 10\text{mm}^2$ ) treatment had overall bigger mean  $B_A$  whereas the smallest mean  $B_A$  was recorded in PD<sub>8-12-16-20</sub> treatment. Total weight of fish ( $T_W$ ) differed significantly among treatment ( $F_{6,70} = 7.43$ ,  $P < 0.001$ ) and showed a significant main effect of Day ( $F_{3,70} = 2143.24$ ,  $P < 0.001$ ). However, no *Treatment*  $\times$  *Day* interaction ( $P = 0.533$ ) was observed. For  $T_W$ , we noticed that Control (LD<sub>25</sub> $\times$ PD<sub>8</sub>) and PD<sub>20</sub> treatment performed significantly better than PD<sub>8-12-16-20</sub> at 29 dph (Figure 2. 3H).

### 2.2.2. End point metrics

Final survival differed significantly among PD regimen ( $F_{6,13} = 6.46$ ,  $P = 0.0025$ , Table. 2.3). The highest survival was recorded from the Control treatment ( $86.6 \pm 7.8\%$ ) whereas the lowest was observed when fish were fed PD<sub>20</sub> ( $51.3 \pm 7.8\%$ ). Survival in Control (LD<sub>25</sub> $\times$ PD<sub>8</sub>) was significantly higher than in all other treatments ( $P < 0.01$ ; Figure. 2. 5A). Final survival accounted for percent cannibal, removal of mortalities and sampling losses. Biomass production also varied significantly among PD regimens ( $F_{6,13} = 60.04$ ,  $P < 0.0001$ , Table. 2.3). Control treatment ( $224 \pm 31.72\text{g}$ ) produced significantly lower biomass compared to other PD regimens (Figure. 2. 5B). Fish fed 20 *Artemia*/mL (PD<sub>20</sub>) yielded the highest biomass per tank with an average of  $572 \pm 31.72\text{g}$ , followed PD<sub>16</sub> treatment ( $560 \pm 31.72\text{g}$ ). Among 100 larvae/L treatments, fish fed 12 *Artemia*/mL (PD<sub>12</sub>) resulted in lowest biomass ( $488 \pm 31.72\text{g}$ ).

Similar results were observed for percent cannibal where PD regimens had a significant effect on cannibalistic behavior of larvae ( $F_{6,13} = 14.50$ ,  $P < 0.0001$ , Table. 2.3). The lowest cannibal percentage was observed in the Control treatment ( $LD_{25} \times PD_8$ ) with percent cannibal being around 10% (Figure. 2. 5C), whereas highest percent cannibal activity was recorded when fish were reared in  $PD_{20}$  treatment ( $40 \pm 0.7\%$ ). Average weight differed significantly among PD regimens ( $F_{6,13} = 3.64$ ,  $P = 0.0242$ , Table. 2.3). Fish average weight at the end was significantly higher in the Control treatment ( $LD_{25} \times PD_8$ ;  $0.58 \pm 0.02g$ ) and  $PD_{20}$  treatment ( $0.57 \pm 0.02g$ ) in contrast to  $PD_{8-12-16-20}$  which was around at  $0.43 \pm 0.02g$  (Figure. 2. 5D). No significant treatment effects were detected for either length-based specific growth rates ( $SGR_L$ ;  $F_{6,13} = 2.21$ ,  $P = 0.1088$ ) or weight-based specific growth rates ( $SGR_L$ :  $F_{6,13} = 1.20$ ,  $P = 0.3670$ ) at 29 dph (Figure. 2. 5E, F; Table 2.3). Nevertheless,  $SGR_L$  exceeded 5% per day and  $SGR_W$  was 15% per day across all treatments.

### 2.2.3. Cumulative survival (%) and Condition index ( $K_n$ )

Cumulative survival (%) was recorded every morning and evening. We observed the highest cumulative survival when fish were fed 20 *Artemia*/mL ( $PD_{20}$ ; ~90%), followed by  $PD_{8-12-16-20}$  (~89%), and  $PD_{8-12}$  (88%) treatments respectively. Cumulative survival for Control ( $LD_{25} \times PD_8$ ) was ~80% at 28 dph. Condition index ( $K_n$ ) was around 1.0 during 9 dph and steadily increased over time reaching almost 1.5 at 29 dph. Mixed model indicated no overall treatment effect, but pairwise comparison showed differences at 16 dph where the Control treatment had significantly higher condition index ( $K_n$ ) than  $PD_{8-12-16-20}$  and  $PD_{12}$  treatments.

### 2.2.4. Length-weight regression

Condition Index ( $K_n$ ) between different PD regimen was statistically similar at all sampling points except for 16 dph, where  $K_n$  of larvae stocked in the Control was significantly higher than

PD<sub>8-12-16-20</sub> and PD<sub>12</sub> treatment. To further examine the allometric relationship between body weight and length, a  $\log(\text{length})$  vs  $\log(\text{weight})$  regression analysis was performed. Data was pooled across all sampling points to establish the single length-weight relationship for each PD regimen. A strong positive correlation between body length and weight was observed across treatments ( $P < 0.001$ ). The strength of this relationship varied slightly among treatments, with the highest coefficient of determination ( $R^2$ ) recorded in PD<sub>12</sub> ( $R^2 = 0.920$ ), followed by PD<sub>16</sub> ( $R^2 = 0.915$ ). The lowest  $R^2$  values were found in PD<sub>8-12</sub> ( $R^2 = 0.822$ ) and PD<sub>8-12-16</sub> ( $R^2 = 0.846$ ) treatments. To characterize ontogenetic shifts in growth pattern irrespective of PD treatment, data were then pooled across all PD regimens at each sampling day. Resulting regression slope/ growth coefficient (b) at 9, 16, 23 and 29 dph were 4.36, 3.01, 2.88 and 2.36 respectively.

#### 2.2.4. *Artemia* cost analysis

Highest profit occurred when fish were fed progressively increasing PD<sub>8-12-16-20</sub> regimen (\$653). This was followed by progressively increasing PD<sub>8-12-16</sub> (\$632.3) and then by PD<sub>8-12</sub> (\$601.4) regimen. Lowest profit was observed in Control (\$213.2). Second lowest profit was observed in high static PD<sub>20</sub> regimen with a total net profit of \$528 (Table 2.4). One way ANOVAs showed that PD had significant impact on economic returns where total net profit generated by Control treatment was significantly lower than the rest of the PD regimen (Figure. 2. 9).

### 2.3. Discussion

This study examined how static vs. progressive prey density (PD) regimens influence growth, survival, and behavior of LMB larvae reared intensively in RAS. Throughout early life stages, larvae undergo drastic changes in morphology, where development of head, respiratory function,

tail and escape reaction occurs prior to full development of the stomach. (Osse and van den Boogaart, 1995). This rapid shift in growth trajectory is an evolutionary strategy where larvae prioritize growth and development of organs that are of absolute necessity for food accumulation and survival (Gao et al., 2023). During this time, larvae go through different stages of metamorphosis and the energy required to undergo these changes varies. Therefore, to optimize productivity, feeding regimens should correlate to these energy requirements of larvae (Pepin, 2014; Ribeiro et al., 2022). Despite this, hatchery and producers often prefer a static high PD regimen during the early phase of production. These regimens and/or protocols are often developed to minimize starvation mortality risk during the transition from endogenous to exogenous feeding (Sorgeloos et al., 2001; Yúfera and Darias, 2007; Conceição et al., 2010). A static high PD regimen might ensure higher survival, but the associated trade-offs such as deterioration of water quality (Kong et al., 2020), mortality of larvae that undergoes slower morphological change, confusion effects (Neill and Cullen, 1974; Krause and Ruxton, 2002), waste of live feed and so on could offset its overall benefit.

During our previous experiment, we observed a significant interaction between LD and PD, which influenced overall performance (Parajuli et al., *manuscript submitted for publication*). Results showed that combining high stocking density with abundant prey supply maximizes total biomass yield ( $LD_{100} \times PD_8$ ). However, this combination also resulted in reduced survival, increased percent cannibal, and lower SGR compared to the low LD and high PD treatment ( $LD_{25} \times PD_8$ ; Control treatment in this current experiment). Therefore, based on this observation, we hypothesized that adjusting PD in relation to different growth stages would sustain growth comparable to static high-PD protocols, while maintaining similar survival and performance observed under low larval density  $\times$  high PD treatment ( $LD_{25} \times PD_8$ ).

All morphometric traits ( $N_L$ ,  $M_H$ ,  $B_A$ ) as well as total weight of the juvenile fish increased significantly over time. LMB larvae showed positive allometry at 9 dph ( $b= 4.36$ ), transitioning to isometric growth by 16 dph ( $b= 3.01$ ). During the later stages (23 and 29 dph), a negative allometric growth pattern occurred ( $b= 2.88$  and  $b= 2.36$  respectively). Many studies have shown similar stage dependent growth patterns during early life history stages of fish. Osse (1995) reported that growth coefficient/slope with respect to weight increased with length, prior to juvenile stage of *Cyprinus carpio* was around 4.48, and was reduced to 3.12. Kupren et al. (2023) also observed similar growth priorities during early development in multiple cyprinid species. A high allometric growth of head and tail sections was favored during the beginning of the larval period, followed by a tendency towards isometric growth at the end of larval phase. Based on these findings, it is plausible that 16 dph ends the larval period in LMB, represented by a shift in isometric growth. However, further observation on a daily or even shorter temporal scales would help clarify the precise timing of the transition.

Morphometric comparisons across PD regimens showed that the Control ( $LD_{25} \times PD_8$ ) and  $PD_{20}$  treatments produced the largest larvae by 29 dph, with  $PD_{8-12}$  also resulting in sizes comparable to these groups. In contrast,  $PD_{8-12-16-20}$  consistently produced the smallest fish. Progressive increases from 8 to 12 *Artemia*/mL ( $PD_{8-12}$ ) may have provided a more balanced match between early prey requirements and later energy demands, whereas static  $PD_{20}$  likely provided surplus prey throughout. Moreover, the introduction of very high prey density such as 20 *Artemia*/mL or even 16 *Artemia*/mL can disturb water parameters before the system stabilizes (Rojas-Tirado et al., 2017), which might also have contributed to the subtle performance difference among treatments. Such transient effects have been documented in RAS, where total ammonia nitrogen (TAN) and ammonia levels spike within hours following feeding (Lee et al., 2012; Godoy-Olmos et al., 2022).

Evidence from other taxa also supports the idea that increasing PD does not always translate to improved growth. Studies conducted on different varieties of prawns (*M. carcinus* and *M. rosenbergii*) demonstrated that as the density of nauplii increased in the culture environment, the ingestion of nauplii also increased in almost all developmental stages, except for stage IV. However, it was also noted that in a closed culture environment, the highest PD's do not always yield the best growth because the excess intake can reduce food transit time in the digestive tract and consequently, the assimilation of nutrients becomes inefficient (Barros and Valenti, 2003; Coelho-Filho et al., 2018).

Another study conducted on Macquarie perch (*Macquaria australasica*) at several stocking densities found that an increase in *Artemia* concentration from 1500 to 3000 *Artemia*/L at higher stocking density spectrum (8, 16 and 32 larvae/L) did not result in improved length and weight gain (Sheikh-Eldin et al, 1997). On the other hand, a study conducted on spotted scat (*Scatophagus argus*) larvae compared 4, 8, 12, 16 and 20 *nauplii* per ml per day and showed that a higher prey density of 20 *Artemia*/mL yielded significantly higher growth in comparison to other PD (Noornissabegum et al., 2022). The static regimen used in the spotted scat experiment was similar to the static PD regimen that we used, however, our results demonstrated that PD<sub>20</sub> did not outperform PD<sub>8-12</sub>.

While  $N_L$  did not differ between treatments (except for Control and PD<sub>8-12-16-20</sub>), fish  $M_H$  and  $T_W$  were higher in PD<sub>20</sub>. These differences were not captured by Le Cren's condition index ( $K_n$ ), which showed no significant PD effect. Le Cren's index is generally considered robust as it incorporates actual allometric parameters of the population and therefore avoids bias associated with non-isometric growth. However, studies have noted that Le Cren's condition index may lack sensitivity when differences among treatments are driven primarily by muscular growth or when

fish undergo rapid morphological change (Blackwell et al., 2000; Datta et al., 2013). This likely explains why elevated  $M_H$  and  $T_W$  in high static PD<sub>20</sub> treatment were not captured by  $K_n$ .

End point metrics such as survival, biomass, percent cannibal and average weight were significantly impacted by PD regimens, whereas specific growth rates ( $SGR_L$  and  $SGR_W$ ) did not differ between treatments. Highest survival was recorded in control treatments (LD<sub>25</sub>×PD<sub>8</sub>) regardless of the lowest cumulative survival percentage. The lower cumulative survival percent in control treatment (LD<sub>25</sub>×PD<sub>8</sub>) was due to a proportional effect where a small number of mortality resulted in greater proportional decline in survival rate compared to high density treatment (LD<sub>100</sub>). The second highest survival rate was recorded in progressively incremental PD<sub>8-12-16-20</sub> treatment, which consisted of an increase of PD from 2, 6, 10, 14 dph, respectively.

The progressive PD<sub>8-12-16-20</sub> regimen appears to address the major constraints that typically limit survival during early stages. Newly hatched larvae exhibit limited visual acuity (Khan et al., 2023), reduced feeding performance (China and Holzman, 2014), poor depth perception and locomotion (Voesenek et al., 2018) making excessively high PD counterproductive due to sensory overload and confusion effects. A confusion effect is when the rapid erratic movement of numerous preys make it difficult for larvae to focus and capture (Turesson and Brönmark, 2004). Hence, beginning exogenous feeding of LMB larvae at PD of 8 *Artemia*/mL likely extended the effective feeding window, as lower biological load in water columns typically extends the persistence of *Artemia* (Sorgeloos et al., 2001; Grajeda et al., 2008). This makes live feed more accessible to slower and smaller larvae. As larvae matured, improvement in sensory systems, swimming capacity, and improved behavioral adjustments in response to their physiological needs (Herbing, 2001, Corradi and Filosa, 2021), facilitated efficient transition to 12 and 16 *Artemia*/mL. Furthermore, larvae are known to learn and adjust accordingly to changing feeding regimens and

prey distribution (Rosenlund et al., 1997). Therefore, by the time PD was increased to 20 *Artemia*/mL at 14 dph, larvae could exploit short feeding windows, behaviorally adapting to changing PD's which met their increased metabolic and energy demand.

This pattern is also consistent with the idea that older larvae require substantially more prey. Ribeiro et al. (2022) reported that feeding intensity in older larvae were several folds higher than younger stage, as reflected by higher numbers of prey in their guts. Comparable observations have been made in different studies. For example, in gilthead seabream (*Sparus aurata*) larvae, increasing *Artemia* and rotifers density to 250/mL and 25/mL after 20 dph resulted in significantly higher survival rate. On the other hand, this progressively increasing PD<sub>8-12-16-20</sub> regimen had the lowest average weight, significantly lower than Control and high static PD<sub>20</sub> treatment. A negative relationship between survival and individual weight has been reported in other species such as great sturgeon (*Huso huso*) larvae (Vaghei et al., 2023). Results from this experiment support the notion that high food availability improves growth but not necessarily survival (Kestemont et al., 2003) as reflected by static PD<sub>20</sub> treatment, where fish had higher average weight and length yet lowest survival among treatments.

Regardless of the rankings, survival rates in treatments where larvae were stocked at 100 larvae/L were significantly lower than Control (LD<sub>25</sub>×PD<sub>8</sub>). Inadequate prey availability and early size disparities can intensify competitive interactions (Kastemont et al., 2003). These survival outcomes might also be closely related to the cannibalism dynamics observed when fish are fed different PD regimens. Cannibalism in general is the act of eating all or part of an individual of the same species irrespective of its developmental stage (Smith and Reay, 1991). This behavior is mostly predominant during the larval stage where mortality can reach as high as 50%. Intra-cohort cannibalism during larval stage can be influenced by various factors including sex, stocking

density, rearing tanks, size variation, genetic factors and so on (Hetenyi et al., 2025). Sexual growth dimorphism during early developmental stage has been reported in various fish species (Naumowicz et al., 2017). This physiological difference between sexes also leads to size heterogeneity in larval fish. Stocking density is another factor that impacts homogeneity of larval size (Millot et al., 2024), which in turn promotes dominance hierarchies and cannibalistic behavior (Manley et al., 2014; Duk et al., 2017; Mushtaq et al., 2024). The consequences of size variation at the larval stage are generally more dramatic than the juvenile or adult stages, as larvae have a large mouth relative to body size, and they tend to exert cannibalism on prey that are just slightly smaller (Baras et al., 2003).

During the present experiment, we observed high size variations within treatments, which may have resulted in higher incidences of cannibalism. In the context of LMB, females tend to grow faster and attain larger size than males later in their adult stage (Wang et al., 2024). An accepted believe in the LMB industry is that male larvae typically grow faster during larval stage and later on female fish surpass them during juvenile or broodstock stages. However, no clear scientific records are established which shows males larvae tend to outgrow female fish during early stages. Regardless of the underlying mechanism behind size heterogeneity in our experiment, it was clear that the fish that grows faster within the cohort population, establishes dominance (Hecht & Pienaar, 1993). During feedings, the larger fish are known to ambush for feed rapidly, leaving smaller conspecifics with limited food. This behavior worsens survival chances of smaller fish within treatments. Therefore, to mitigate unequal feeding opportunities in our experiment, each ration was divided into four or five small sprinkle feedings. But despite this effort, pronounced size variation and elevated cannibalism persisted.

Considering *Artemia* as an only input cost, the economic analysis between different PD regimens showed a clear trade-off among growth, survival, biomass yield, and net profit across PD regimens. All three progressively increasing PD regimens generated higher net profits than the static regimens. The progressive PD<sub>8-12-16-20</sub> regimen produced the highest survival and biomass along with greatest profit per tank, even though larvae in this treatment were smaller than the other regimens. In contrast, the static PD<sub>20</sub> regimen produced good growth, on par with Control but had highest associated cost of production. This resulted in lower net profit compared to any other progressive PD regimens. At the other extreme, static PD<sub>8</sub> (Control) yielded biggest fish, but produced lowest total biomass, resulting in the poorest economic return despite minimal *Artemia* input cost. The intermediate progressive PD<sub>8-12</sub> regimen emerged as an efficient compromise. This progressively increasing PD regimen achieved growth comparable to both the Control and static PD<sub>20</sub>, generated high survival and net profit while using roughly half the *Artemia* used in PD<sub>20</sub>. Thus, when the goal is maximizing economic return per tank, PD<sub>8-12-15-20</sub> is preferred regimen because the slower growth is outweighed by its higher survival rate and profit. Similarly, when *Artemia* supply or feed budget is limited, PD<sub>8-12</sub> is likely the most attractive option. This PD regimen maintains similar survival and profit to PD<sub>8-12-16-20</sub>, while sustaining comparable growth to Control and high PD<sub>20</sub> and also uses substantially lower input *Artemia*. Overall, progressive increase in PD consistently improved economic returns relative to static PDs by better aligning *Artemia* inputs with changing ontogenetic demand of larvae in terms of live feed.

In conclusion, this study demonstrated that largemouth bass reared under intensive RAS conditions exhibited distinct growth and survival responses to static and progressively increasing PD regimes. Rearing larvae at a lower density (25 larvae/L) and feeding 8 *Artemia*/mL (Control

treatment) yielded high survival and rapid growth but often resulted in superfluous feeding during early stages. Increasing LD to 100 larvae/L optimized live-feed utilization and overall tank biomass, although final individual weights were lower. To refine production efficiency at 100 larvae/L stocking density, three levels of static and progressive prey density treatments were tested. The static high-prey treatment (20 *Artemia*/mL) produced growth comparable to the low-density Control but with reduced survival. In contrast, the progressive PD regimen, where fish were fed 8 *Artemia*/mL initially and increased to 12 *Artemia*/mL after 6 dph (PD<sub>8-12</sub>), achieved similar growth and survival outcomes while using approximately half the amount of live feed. Further research direction would be to evaluate what factors are interacting to impact size heterogeneity during early larval stage of LMB so that survival rates can be optimized. Overall, our study provides a practical framework for enhancing hatchery efficiency by enabling producers to strategically balance cost-effectiveness, intensification level, and productivity.

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Table 2. 1. Two-way mixed-model ANOVA results for larval morphometric traits of largemouth bass (*Micropterus nigricans*) reared under seven feeding treatments from 2 to 29 days post-hatch (dph). Fixed effects included sampling day (Day), treatment (Treat), and their interaction (Day × Treat)

| Metrics        | Effect     | Num DF | Den DF | F value | P value |
|----------------|------------|--------|--------|---------|---------|
| N <sub>L</sub> | Day        | 4      | 88     | 4294.36 | <.0001  |
|                | Treatment  | 6      | 1      | 6.96    | 0.2823  |
|                | Day×Treat. | 24     | 65     | 1.1     | 0.3678  |
| M <sub>H</sub> | Day        | 4      | 89     | 357.29  | <.0001  |
|                | Treatment  | 6      | 89     | 2.3     | 0.0414  |
|                | Day×Treat. | 24     | 65     | 2.58    | 0.0013  |
| B <sub>A</sub> | Day        | 4      | 89     | 747.76  | <.0001  |
|                | Treatment  | 6      | 89     | 1.84    | 0.1005  |
|                | Day×Treat. | 24     | 65     | 1.18    | 0.2962  |

Note: Traits analyzed were notochord length (N<sub>L</sub>), myotome height (M<sub>H</sub>), and body area (B<sub>A</sub>). For each trait, F-values are reported with numerator (Num DF) and denominator (Den DF) degrees of freedom. Statistical significance for main effects was assessed at  $\alpha = 0.05$ , whereas interaction effects were considered significant at  $\alpha = 0.10$

Table 2. 2. Two-way mixed-model ANOVA results for total body weight and condition index (K) of largemouth bass (*Micropterus nigricans*) larvae reared under seven feeding treatments from 2 to 29 days post-hatch (dph). Fixed effects included sampling day (Day), treatment (Treat), and their interaction (Day × Treat).

| Metrics         | Effect     | Num DF | Den DF | F value | P value |
|-----------------|------------|--------|--------|---------|---------|
| Weight          | Day        | 3      | 70     | 2143.24 | <.0001  |
|                 | Treatment  | 6      | 70     | 7.43    | <.0001  |
|                 | Day×Treat. | 18     | 52     | 0.94    | 0.5334  |
| Condition index | Day        | 3      | 69     | 0.02    | 0.9969  |
|                 | Treatment  | 6      | 10.01  | 1.07    | 0.441   |
|                 | Day×Treat. | 18     | 51     | 1.08    | 0.3989  |

Notes: Traits analyzed were total body weight and condition index (K). For each trait, F-values are reported with numerator (Num DF) and denominator (Den DF) degrees of freedom. Statistical significance for main effects was assessed at  $\alpha = 0.05$ , whereas interaction effects were considered significant at  $\alpha = 0.10$ .

Table 2. 3. One-way ANOVA results for endpoint (29 days post-hatch, dph) performance traits of largemouth bass (*Micropterus nigricans*) larvae reared under seven feeding treatments (Treat).

| Metrics     | Effect | Num DF | Den DF | F value | P value |
|-------------|--------|--------|--------|---------|---------|
| Survival    | Treat  | 6      | 13     | 6.46    | 0.0025  |
| Avg. Weight | Treat  | 6      | 13     | 3.64    | 0.0242  |
| Biomass     | Treat  | 6      | 13     | 60.04   | <.0001  |
| SGRL        | Treat  | 6      | 13     | 2.21    | 0.1088  |
| SGRW        | Treat  | 6      | 13     | 1.2     | 0.367   |
| Cannibal %  | Treat  | 6      | 13     | 14.5    | <.0001  |

Notes: Traits analyzed included survival (%), average individual weight (Avg. Wt., g), biomass (g), specific growth rate in length (SGRL, % per day), specific growth rate in weight (SGRW, % per day), and percent cannibalism (%). For each trait, F-values are reported with numerator (Num DF) and denominator (Den DF) degrees of freedom. Statistical significance for treatment effects was assessed at  $\alpha = 0.05$ .

Table 2. 4. Stepwise estimation of profit per treatment in largemouth bass (*Micropterus nigricans*) reared under different and prey densities (PD) regimens

| Treat. | G (g) | AC (\$) | N    | L (mm) | SL   | DPH  | DS  | R <sup>2</sup> | SP (\$) | TGR (\$) | Profit (\$) | Equation ( $y=mx+b$ )   |
|--------|-------|---------|------|--------|------|------|-----|----------------|---------|----------|-------------|-------------------------|
| 1      | 204.8 | 38.6    | 391  | 32.17  | 28.2 | 26.2 | 2.8 | 0.88           | 0.51    | 251.8    | 213.2       | SL = -0.86 + 0.96 × dph |
| 2      | 281.6 | 53.0    | 1043 | 31.38  | 28.2 | 27.2 | 1.8 | 0.87           | 0.51    | 654.4    | 601.4       | SL = -0.44 + 0.98 × dph |
| 3      | 332.8 | 62.6    | 1151 | 30.18  | 28.2 | 28.2 | 0.8 | 0.87           | 0.51    | 694.9    | 632.3       | SL = -1.08 + 1.04 × dph |
| 4      | 358.4 | 67.5    | 1277 | 28.20  | 28.2 | 28.8 | 0.2 | 0.86           | 0.51    | 720.5    | 653.0       | SL = -0.99 + 1.06 × dph |
| 5      | 307.2 | 57.8    | 1026 | 30.22  | 28.2 | 28.3 | 0.7 | 0.86           | 0.51    | 620.3    | 562.5       | SL = -0.50 + 1.02 × dph |
| 6      | 409.6 | 77.1    | 1124 | 30.02  | 28.2 | 28.3 | 0.7 | 0.87           | 0.51    | 674.8    | 597.7       | SL = -1.00 + 1.04 × dph |
| 7      | 512   | 96.4    | 1002 | 31.14  | 28.2 | 27.2 | 1.8 | 0.84           | 0.51    | 624.3    | 527.9       | SL = -0.40 + 0.98 × dph |

Note: Net profit/ profit was calculated in three steps: (1) *Artemia* cost per treatment (AC) = Prey density (All levels of static and progressively increasing prey density regimens: *Artemia*/mL) × feeding frequency (F = 8/day) × tank volume (V = 20,000 mL) × culture days (dph), divided by hatch rate ( $\approx 100,000$  *Artemia*/g) and multiplied by cyst price ( $\approx 0.2$  USD/g); (2) Total gross return (TGR) = standardized larval length (SL, mm; converted to inches) × selling price per inch (SP = 0.51 USD/inch) × number of survivors (N); (3) Profit (USD) = TGR - AC. The dph values represent predicted number of days for larvae in each treatment to reach a minimum target length of 28.20 mm (SL), based on treatment-specific linear growth equations ( $y = mx + b$ ). SL (mm) was standardized length across treatments to enable direct economic comparisons. DS indicates the number of days saved relative to the slowest-growing treatment (PD<sub>8-12-16-20</sub>; Treatment 4). R<sup>2</sup> values correspond to the goodness of fit of the growth regressions. Treatments 1, 5, 6, and 7 correspond to static PD regimens of 8, 12, 16, and 20 *Artemia*/mL, respectively, maintained

throughout the experimental period. Treatments 2 (PD<sub>8-12</sub>), 3 (PD<sub>8-12-16</sub>), and 4 (PD<sub>8-12-16-20</sub>) represent progressively increasing PD regimens applied according to larval developmental stage.

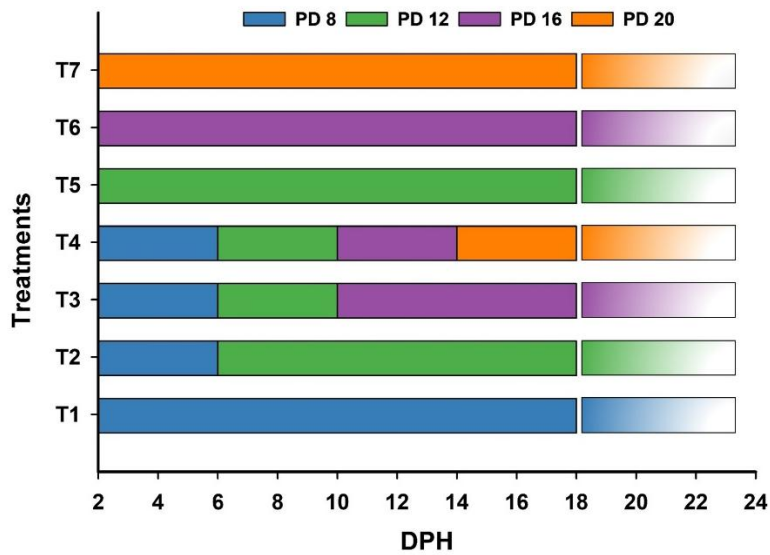


Figure. 2. 1. Different prey densities (PD; *Artemia*/mL) used across treatments during the experiment during the larval rearing of largemouth bass (*Micropterus nigricans*).

Note: The Y-axis represents the seven experimental treatments (Treatment 1 – Treatment 7), and the X-axis represents days post-hatch (dph) which ranges from 2 – 24 dph. The experimental setup consisted of seven PD regimens applied from 2–18 dph. The treatments were designed to reflect both static and progressive increases in PD in relation to ontogenetic development. In the static treatments, *Artemia* were fed on a constant density of PD<sub>8</sub>, PD<sub>12</sub>, PD<sub>16</sub>, or PD<sub>20</sub> (*Artemia*/mL) as represented by treatment 1, 5, 6 and 7, respectively. In progressive treatments, PD was increased stepwise at specific developmental intervals. All larvae initially received 8 *Artemia*/mL from 2–6 days post-hatch (dph), after which the PD was raised to 12 *Artemia*/mL during days 6–10 dph. From this point, three progressive regimens were applied: (i) PD<sub>8-12</sub>, where PD was maintained at PD<sub>12</sub> for the remainder of the trial (ii) PD<sub>8-12-16</sub> where PD increased to PD<sub>16</sub> from 10 dph onward and (iii) PD<sub>8-12-16-20</sub>, where PD increased to PD<sub>16</sub> between 10–14 dph and further to PD<sub>20</sub> from 14–

18 dph as represented by treatment 2, 3 and 4 respectively. Each treatment was replicated three times, with 2,000 larvae/tank, corresponding to the fixed larval density of 100 larvae/L in 20 L rearing units. Gradient line following the bars represents that the treatments were weaned over a period of 4 days.

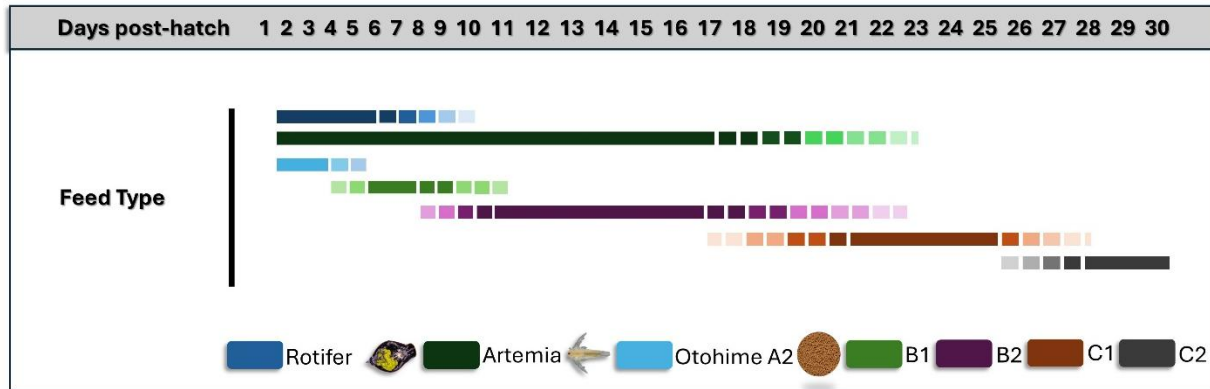


Figure. 2. 2. Feeding and weaning schedule for largemouth bass (*Micropterus nigricans*) larvae. The diagram illustrates the timing and overlaps of different feed types (Otohime A2, B1, B2, C1, and C2) from 2 to 30 days post-hatch (dph).

Note: Colored bars represent the feeding periods for each diet, with dotted sections indicating gradual weaning transitions. The red line represents smaller strain rotifers (*Brachionus plicatilis*), which were fed until 9 dph, with gradual weaning starting at 5 dph by reducing 1 rotifer per day. Yellow line represents *Artemia* nauplii that were fed from 2 to 30 dph according to experimental treatments. Larvae were progressively transitioned from smaller to larger pellets through co-feeding, starting with 25% larger and 75% smaller pellets on the first day of transition, then gradually increasing the proportion of larger pellets until they reached 100% of the diet. Otohime A2 represented by sky-blue bar was fed from 2 to 6 dph, with weaning initiated at 4 dph as B1 was introduced. B1 represented by light green lines was provided from 4 to 7 dph and gradually replaced by B2 between 7 and 11 dph. B2 represented by the pink line was then fed exclusively until 16 dph, after which larvae were transitioned to C1 as represented by purple line, reaching 100% by 21 dph and continuing until 26 dph. C2 represented by grey line was introduced thereafter, reaching 100% by 28 dph and maintained until 30 dph.

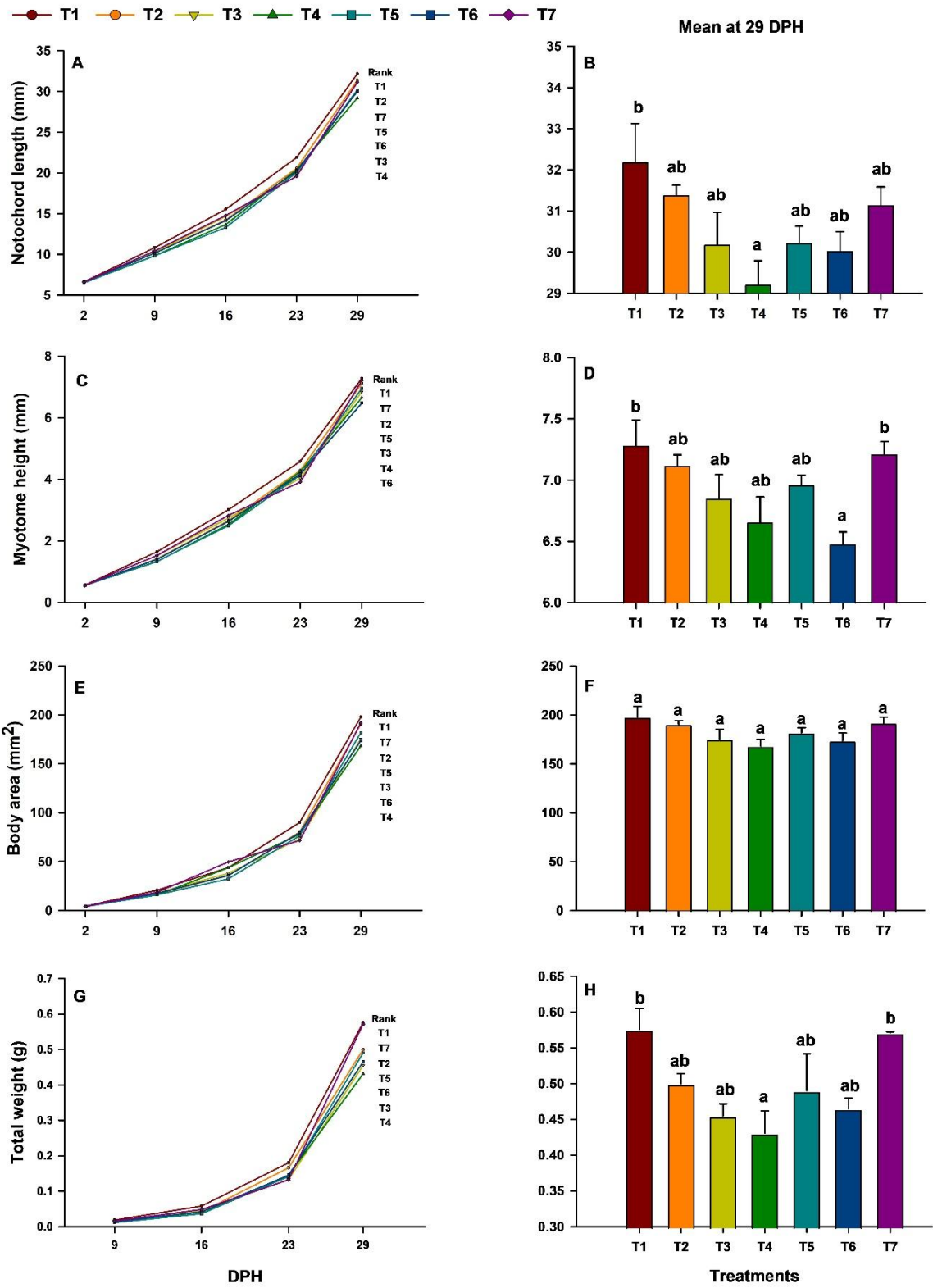


Figure 2. 3. Growth and morphological development of largemouth bass (*Micropterus nigricans*) larvae reared under seven feeding treatments (T1–T7) from 2 to 29 days post-hatch (dph). Treatments T1, T5, T6, and T7 correspond to static prey density (PD) regimens of 8, 12, 16, and 20 *Artemia*/mL, respectively, maintained throughout the experimental period. Treatments T2 (PD<sub>8-12</sub>), T3 (PD<sub>8-12-16</sub>), and T4 (PD<sub>8-12-16-20</sub>) represent progressively increasing PD regimens applied according to larval developmental stage. All larvae received an initial density of 8 *Artemia*/mL from 2–6 dph, after which PD was raised to 12 *Artemia*/mL during 6–10 dph. In the progressive regimens, PD was either maintained at 12 *Artemia*/mL (T2), increased to 16 *Artemia*/mL from 10 dph onward (T3), or further increased to 20 *Artemia*/mL from 14 dph (T4).

*Note:* Panels (A, D, G, J) show temporal changes in notochord length (N<sub>L</sub>), myotome height (M<sub>H</sub>), body area (B<sub>A</sub>), and total weight (T<sub>w</sub>), respectively. Panels (B, E, H, K) present pooled least-square means across sampling days, illustrating the main effect of Treatment, while panels (C, F, I, L) display treatment effects at the experimental endpoint (29 dph). Different lowercase letters above bars denote significant differences among treatments (Tukey's HSD,  $P < 0.05$ ). The  $y$ -axes represent morphometric traits (N<sub>L</sub>, M<sub>H</sub>, and B<sub>A</sub>) and average individual total weight (T<sub>w</sub>), and the  $x$ -axes denote treatments (T1–T7). Legends for the temporal growth plots are shown in top left corner above panel A, with each treatment represented by a distinct color and symbol combination. Units of the measured traits are indicated in parentheses on the respective  $y$ -axes. Ranks for each treatment are listed on the right side of the trendlines at 29 dph.

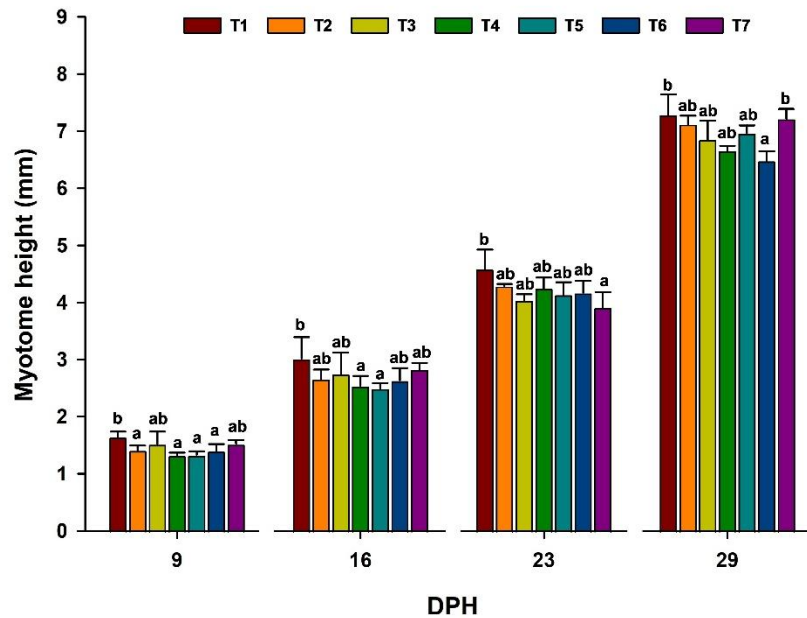


Figure. 2. 4. Myotome height of largemouth bass larvae (*Micropterus nigricans*) reared under seven feeding treatments (T1–T7) from 9 – 29 days post-hatch (dph). Treatments T1, T5, T6, and T7 correspond to static prey density (PD) regimens of 8, 12, 16, and 20 *Artemia*/mL, respectively, maintained throughout the experimental period. Treatments T2 (PD<sub>8-12</sub>), T3 (PD<sub>8-12-16</sub>), and T4 (PD<sub>8-12-16-20</sub>) represent progressively increasing PD regimens applied according to larval developmental stage. All larvae received an initial density of 8 *Artemia*/mL from 2–6 dph, after which PD was raised to 12 *Artemia*/mL during 6–10 dph. In the progressive regimens, PD was either maintained at 12 *Artemia*/mL (T2), increased to 16 *Artemia*/mL from 10 dph onward (T3), or further increased to 20 *Artemia*/mL from 14 dph (T4).

Different lowercase letters above bars denote significant differences among treatments (Tukey’s HSD,  $P < 0.05$ ). The y-axes represent myotome height ( $M_H$ ), and the x-axes denote Days-post hatch (9 – 29). Legends are shown above the panel, with each treatment represented by a distinct color bar. Units of the myotome height ( $M_H$ ) are indicated in parentheses on the y-axes.

T1 T2 T3 T4 T5 T6 T7

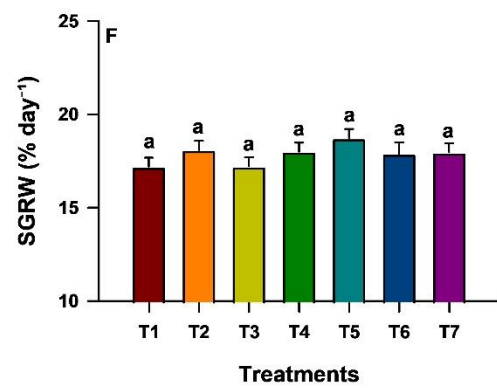
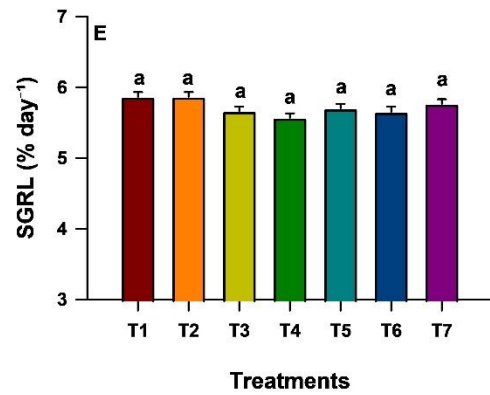
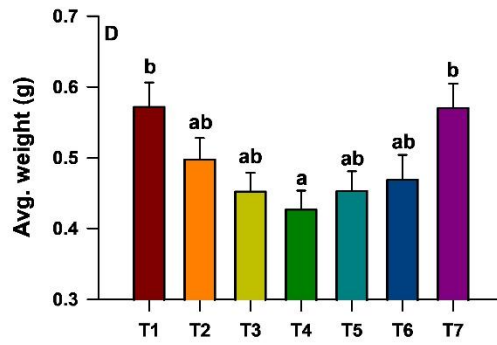
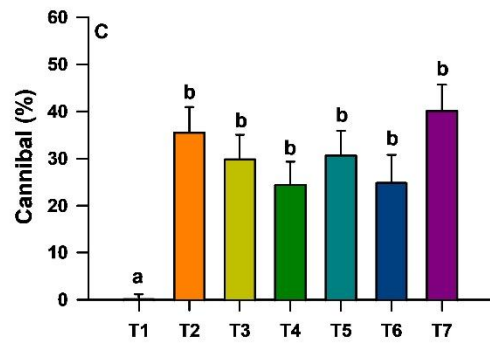
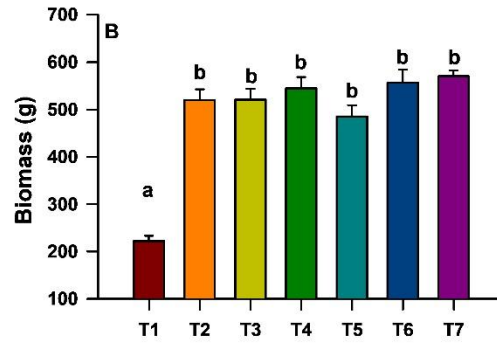
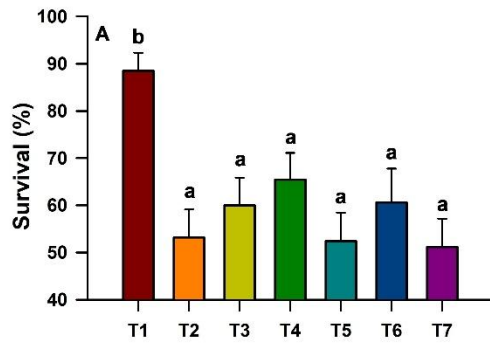


Figure. 2. 5. Endpoint performance of largemouth bass (*Micropterus nigricans*) larvae reared under seven feeding treatments (T1–T7) at 29 days post-hatch (dph). Treatments T1, T5, T6, and T7 correspond to static prey density (PD) regimens of 8, 12, 16, and 20 *Artemia*/mL, respectively, maintained throughout the experimental period. Treatments T2 (PD<sub>8-12</sub>), T3 (PD<sub>8-12-16</sub>), and T4 (PD<sub>8-12-16-20</sub>) represent progressively increasing PD regimens applied according to larval developmental stage. All larvae received an initial density of 8 *Artemia*/mL from 2–6 dph, after which PD was raised to 12 *Artemia*/mL during 6–10 dph. In the progressive regimens, PD was either maintained at 12 *Artemia*/mL (T2), increased to 16 *Artemia*/mL from 10 dph onward (T3), or further increased to 20 *Artemia*/mL from 14 dph (T4).

Note: Panels A, B, C, D, E and F show survival (%), total biomass (g), percent cannibal (%), individual endpoint weight (g), specific growth rate in length (SGRL, % per day), and specific growth rate in weight (SGRW, % per day), respectively. Different lowercase letters above bars indicate significant differences among treatments (Tukey's HSD,  $P < 0.05$ ). The  $y$ -axes represent the respective end point metrics, and the  $x$ -axis denotes treatments (T1–T7). Units of the measured variables are indicated in parentheses on each  $y$ -axis.

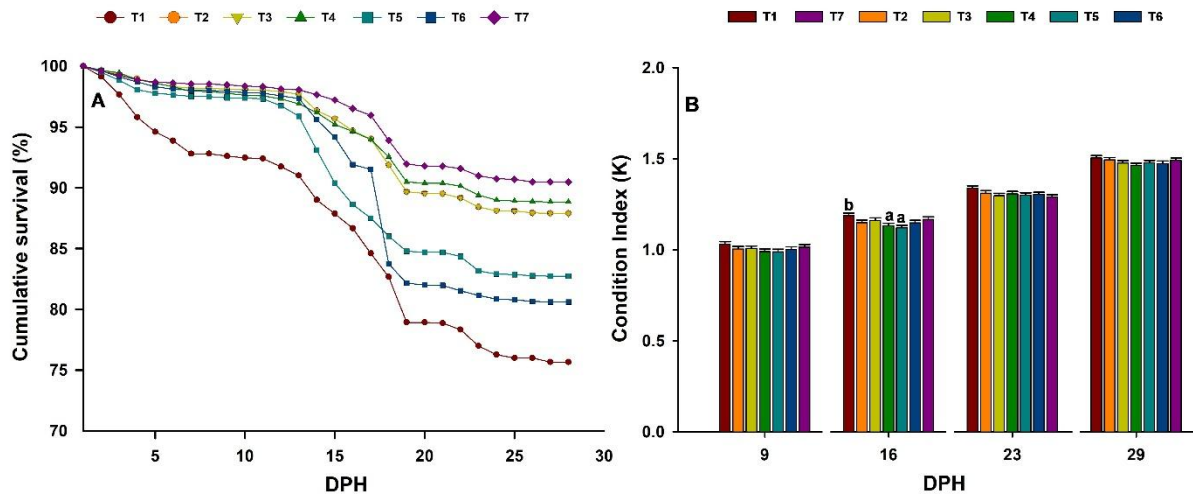


Figure. 2. 6. Cumulative survival and condition index (K) of largemouth bass (*Micropterus nigricans*) larvae reared under seven feeding treatments (T1–T7) from 2 to 29 days post-hatch (dph). Treatments T1, T5, T6, and T7 correspond to static prey density (PD) regimens of 8, 12, 16, and 20 *Artemia*/mL, respectively, maintained throughout the experimental period. Treatments T2 (PD<sub>8-12</sub>), T3 (PD<sub>8-12-16</sub>), and T4 (PD<sub>8-12-16-20</sub>) represent progressively increasing PD regimens applied according to larval developmental stage. All larvae received an initial density of 8 *Artemia*/mL from 2–6 dph, after which PD was raised to 12 *Artemia*/mL during 6–10 dph. In the progressive regimens, PD was either maintained at 12 *Artemia*/mL (T2), increased to 16 *Artemia*/mL from 10 dph onward (T3), or further increased to 20 *Artemia*/mL from 14 dph (T4).

Note: Panel A represents cumulative survival over time. All treatments began with 2000 larvae, except the control treatment (T1; 500 larvae, LD<sub>25</sub> × PD<sub>8</sub>), which is represented by the lower segment of the broken y-axis. The x-axis represents larval age (dph), and the y-axis shows the cumulative number of surviving larvae. Panel B represents Condition index (K) of larvae across sampling points, indicating relative body condition among treatments over time. The x-axis

represents sampling days (dph), and the *y*-axis represents the calculated condition index (K). Legends identifying treatments (T1–T7) are shown at the top center of the figure. Different lowercase letters above bars indicate significant differences among treatments (Tukey's HSD,  $P < 0.05$ ).

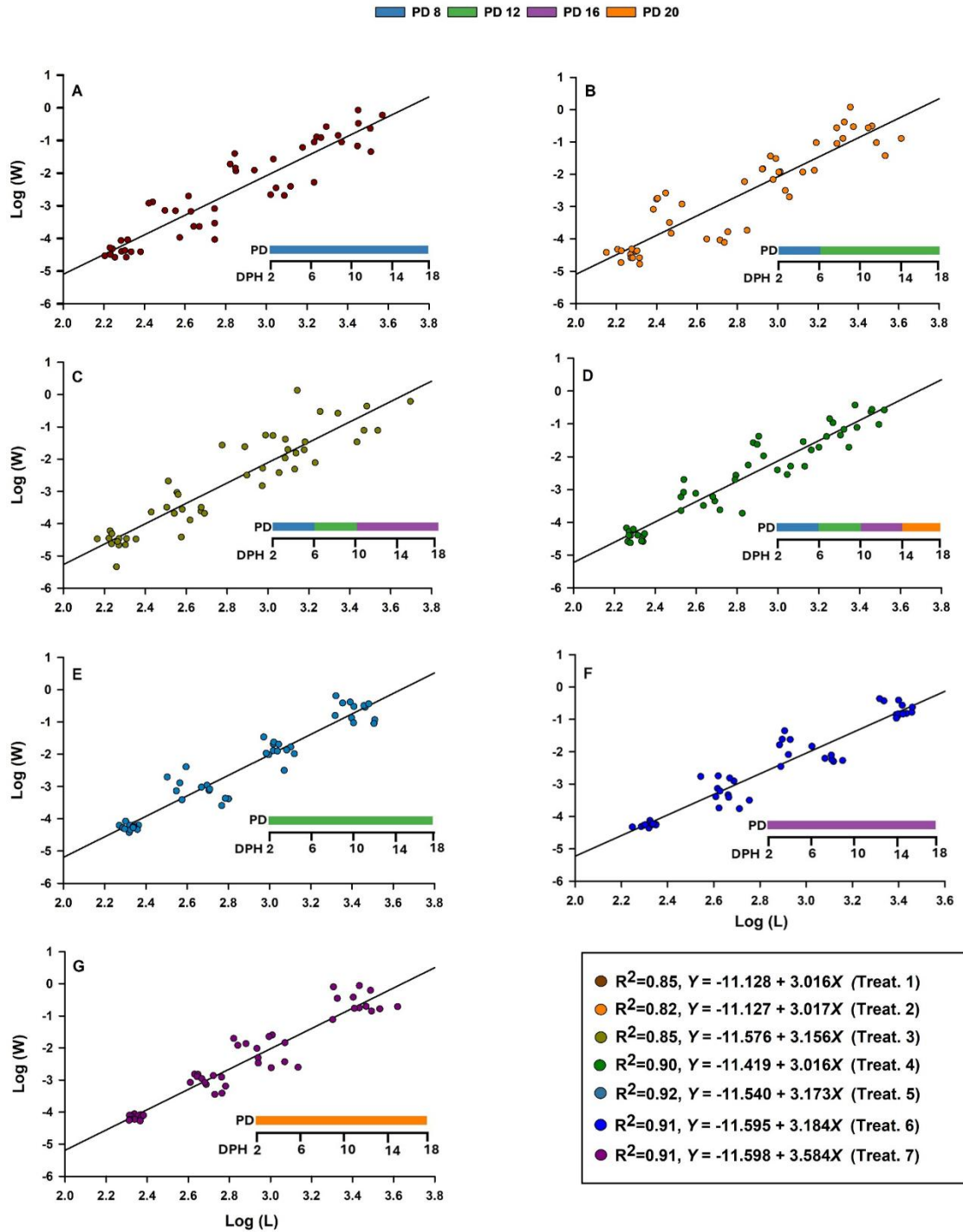


Figure. 2. 7. Length-weight relationships (log-log scale) of largemouth bass (*Micropterus nigricans*) larvae reared under seven treatment combinations (A-G correspond to Treatments 1–7, respectively). Treatments T1, T5, T6, and T7 correspond to static prey density (PD) regimens of

8, 12, 16, and 20 *Artemia*/mL, respectively, maintained throughout the experimental period. Treatments T2 (PD<sub>8-12</sub>), T3 (PD<sub>8-12-16</sub>), and T4 (PD<sub>8-12-16-20</sub>) represent progressively increasing PD regimens applied according to larval developmental stage. All larvae received an initial density of 8 *Artemia*/mL from 2–6 dph, after which PD was raised to 12 *Artemia*/mL during 6–10 dph. In the progressive regimens, PD was either maintained at 12 *Artemia*/mL (T2), increased to 16 *Artemia*/mL from 10 dph onward (T3), or further increased to 20 *Artemia*/mL from 14 dph (T4).

Note: Each panel represents a separate treatment, with individual data points pooled across all sampling days (dph). The regression line describes the relationship between log-transformed total length (Log L) and log-transformed body weight (Log W). Coefficient of determination ( $R^2$ ), significance level ( $P$ ), and fitted regression equation are shown within each panel. Color codes for treatments are indicated in the legend on the top panel.



Tank ID: Tank 5



Tank ID: Tank 12

**A**

**Control**  
 LD<sub>25</sub>×PD<sub>8</sub>  
 Survival: 86.57%  
 Biomass: 224 g  
 SGRL: 5.8% d<sup>-1</sup>  
 NL: 32.1 mm  
 TW: 0.58 g



Tank ID: Tank 7 Rep 1



Tank ID: Tank 7 Rep 1

**B**

**Treatment 7**  
 LD<sub>100</sub>×PD<sub>20</sub>  
 Survival: 55.20 %  
 Biomass: 572 g  
 SGRL: 5.6% d<sup>-1</sup>  
 NL: 31.1 mm  
 TW: 0.57g



Tank ID: Tank 18 Rep 1



Tank ID: Tank 18 Rep 1

**C**

**Treatment 2**  
 LD<sub>100</sub>×PD<sub>8-12</sub>  
 Survival: 54.1%  
 Biomass: 521 g  
 SGRL: 5.8% d<sup>-1</sup>  
 NL: 31.4 mm  
 TW: 0.50 g



Tank ID: Tank 3 Rep 1



Tank ID: Tank 3 Rep 1

**D**

**Treatment 4**  
 LD<sub>100</sub>×PD<sub>8-12-16-20</sub>  
 Survival: 65%  
 Biomass: 546 g  
 SGRL: 5.5% d<sup>-1</sup>  
 NL: 29.2 mm  
 TW: 0.43 g

Figure. 2. 8. Representative tank images of selected treatments showing final biomass and growth performance of largemouth bass (*Micropterus nigricans*) larvae at 29 dph. Treatments T1, T5, T6, and T7 correspond to static prey density (PD) regimens of 8, 12, 16, and 20 *Artemia*/mL, respectively, maintained throughout the experimental period. Treatments T2 (PD<sub>8-12</sub>), T3 (PD<sub>8-12-16</sub>), and T4 (PD<sub>8-12-16-20</sub>) represent progressively increasing PD regimens applied according to larval developmental stage. All larvae received an initial density of 8 *Artemia*/mL from 2–6 dph, after which PD was raised to 12 *Artemia*/mL during 6–10 dph. In the progressive regimens, PD was either maintained at 12 *Artemia*/mL (T2), increased to 16 *Artemia*/mL from 10 dph onward (T3), or further increased to 20 *Artemia*/mL from 14 dph (T4).

Note: Pictures represent total yield from four selected treatments (A: treatment 1, B: treatment 7, C: treatment 2, and D: treatment 4) at the 29 dph. LD denotes larval density (Larvae/L) and PD denotes prey density (*Artemia*/mL), with subscript indicating the PD levels provided to each treatment during the experiment. Two replicate tanks are shown for the control (Treatment 1) to illustrate variability in biomass yield. Treatments 7, 2, and 4 represent the highest-performing combinations based on overall survival, biomass production, and morphometric indices. Tank identifications (Tank ID) are indicated below each image. Each treatment label (left panel) lists the specific LD and PD applied. Growth metrics include survival (%), total biomass (g), length-specific growth rate (SGRL; % d<sup>-1</sup>), mean final notochord length (NL; mm), and mean total weight per individual (TW; g)

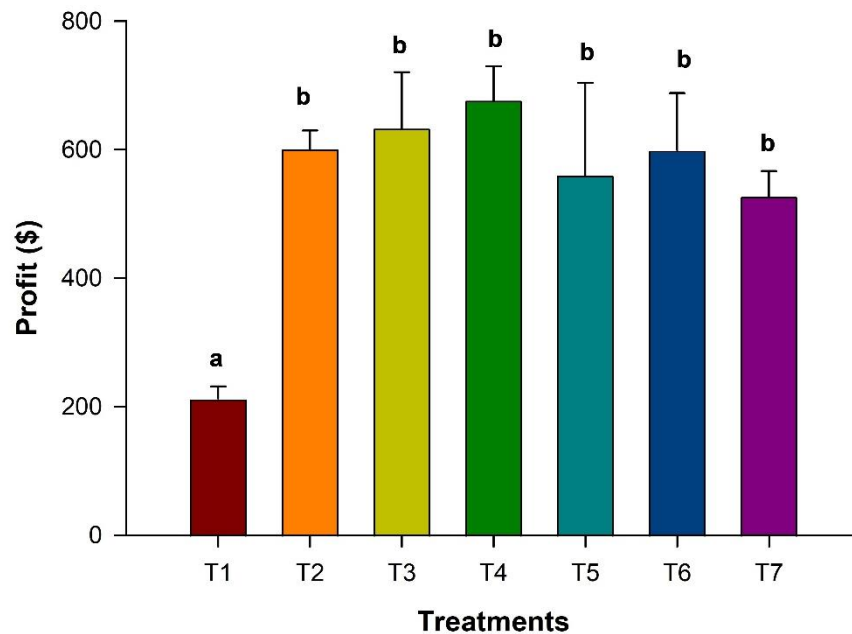


Figure. 2. 9. Net profit generated by different treatments used during larval rearing of largemouth bass (*Micropterus nigricans*) from 2–29 dph. Treatments T1, T5, T6, and T7 correspond to static prey density (PD) regimens of 8, 12, 16, and 20 *Artemia*/mL, respectively, maintained throughout the experimental period. Treatments T2 (PD<sub>8-12</sub>), T3 (PD<sub>8-12-16</sub>), and T4 (PD<sub>8-12-16-20</sub>) represent progressively increasing PD regimens applied according to larval developmental stage. All larvae received an initial density of 8 *Artemia*/mL from 2–6 dph, after which PD was raised to 12 *Artemia*/mL during 6–10 dph. In the progressive regimens, PD was either maintained at 12 *Artemia*/mL (T2), increased to 16 *Artemia*/mL from 10 dph onward (T3), or further increased to 20 *Artemia*/mL from 14 dph (T4).

