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Evaluation of Stocking Density during Second-Year Growth of Largemouth Bass, *Micropterus salmoides*, Raised Indoors in a Recirculating Aquaculture System

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Abstract

Largemouth bass (LMB), *Micropterus salmoides*, are a highly desirable food fish especially among Asian populations in large cities throughout North America. The primary production method for food-size LMB (>500 g) has been outdoor ponds that require two growing seasons (18 mo). Indoor, controlled-environment production using recirculating aquaculture system (RAS) technologies could potentially reduce the growout period by maintaining ideal temperatures year-round. Researchers conducted a 26-wk study to evaluate optimal stocking densities for growout of second-year LMB to food-fish size in an indoor RAS. LMB fingerlings (112.0 ± 38.0 g) were randomly stocked into nine 900-L tanks to achieve densities of 30, 60, or 120 fish/m^3 with three replicate tanks per density. The RAS consisted of a 3000-L sump, 1/4 hp pump, bead filter for solids removal, mixed-moving-bed biofilter for nitrification, and a 400-watt ultraviolet light for sterilization. Fish were fed a commercially available floating diet (45% protein and 16% lipid) once daily to apparent satiation. At harvest, all fish were counted, individually weighed, and measured. Total biomass densities significantly increased ($P \leq 0.05$) with stocking rate achieving 6.2, 13.2, and 22.9 kg/m^3 for fish stocked at 20, 60, and 120 fish/m^3, respectively. The stocking densities evaluated had no significant impact ($P > 0.05$) on survival, average harvest weight, or feed conversion ratio which averaged 92.9 ± 5.8%, 294.5 ± 21.1 g, and 1.8 ± 0.3, respectively. After approximately 6 mo of culture, LMB did not attain target weights of >500 g. Observed competition among fish likely resulted in large size variability and overall poor growth compared to second-year growth in ponds. Additional research is needed to better assess the suitability of LMB for culture in RAS.

Largemouth bass (LMB) have been cultured in the United States since the 1890s, primarily for sport-fish stock enhancement programs. More recently, production of larger sized LMB have increased (Brandt 1991) based on their increased use for corrective stocking in sport fish ponds (JSA 1983), fee fishing (Dupree and Huner 1984), managed trophy fisheries (JSA 1983), and especially for live sales as food fish in Asian markets (Tidwell et al. 1996).

LMB are a highly desirable food fish among ethnic Asian populations in large cities throughout North America. It is estimated that 1,500,000 kg of 500–700 g LMB are produced in the United States for live food fish markets at a relatively high selling price of >$10.00 US/kg, as compared to channel catfish, *Ictalurus punctatus*, of approximately $2 US/kg. The relatively high selling price of LMB has generated increased commercial interest in development of improved production technologies. The JSA listed determination of efficient growout procedures under intensive conditions as one of the research priorities for development of LMB aquaculture (JSA 1983). If this information can be generated, there appears to be a favorable financial potential for increased commercial production of this species in some states.

Historically, the primary production method for LMB has been in outdoor ponds, requiring two growing seasons (18–24 mo) to reach minimum food-fish size of 0.5 kg average weight. LMB are typically spawned in ponds by stocking brood fish and allowing reproduction to occur

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naturally (Tidwell et al. 2000). After spawning has occurred, LMB fry are raised in nursery ponds where they feed on zooplankton until they are large enough (>4 cm) to be feed trained in tanks (Heidinger 2000). Once feed trained, the fingerlings are then stocked back into ponds for growout. These procedures were described by Snow in the 1960s (Snow 1965, 1968; Snow and Maxwell 1970) and have changed little since.

Pond culture of finfish, although generally considered the most economical approach for most fish production, requires significant land, labor, and water resources; has a greater range of potential environmental impacts; and is highly vulnerable to weather (Tucker and Hargreaves 2012). Considering the relative high value of LMB, it might be possible and economically advantageous to intensify and shorten the growth period culturing the fish indoors in temperature-controlled tanks; however, very little research has been conducted on rearing LMB indoors. Indoor, controlled-environment production using recirculating aquaculture system (RAS) technologies could potentially reduce the growout period by maintaining ideal temperatures year-round.

Widespread commercial usage of RAS technology has not yet been widely adopted. In the 2013 USDA census of aquaculture, farmers reported that 35.8% of the total value of US aquaculture products was raised in ponds compared to only 8.7% for RAS systems (USDA 2014). However, RAS systems allow for year-round production with consistent volumes of product throughout the year as compared to seasonal availability and slow winter growth, which are a characteristic of pond production. RASs can also allow for higher production per unit area, production per unit water volume, and per unit worker than ponds (Ebeling and Timmons 2012).

Conversely, higher production rates require higher stocking densities per unit of volume. For example, in pond culture of LMB, maximum harvest density is equivalent to approximately 0.5 kg/m^3 (5000 kg/ha) whereas reported maximum densities for species well suited for high density culture in RAS, such as tilapia, *Oreochromis* sp., are 50–100 kg/m^3 (Ebeling and Timmons 2012). High population densities are known to affect fish health, food intake, and growth (Petit et al. 2001). Fish living in crowded conditions can become stressed from altered social interactions, restrictions of their ability to move freely or to otherwise behave normally (Wedemeyer 1996). Baker and Ayles (1990) reported that the level of antagonistic interaction between cohorts of arctic charr, *Salvelinus alpines*, and rainbow trout, *Oncorhynchus mykiss*, changed with changes in stocking density.

The determination of appropriate stocking densities is the logical first step in determining the species suitability to RAS production. The objective of this research was to compare the growth, feed conversion, and survival of 1-yr-old LMB stocked at three different densities in an RAS.

Materials and Methods

Researchers conducted a 26-wk study (approximately 6 mo and equivalent to the second summer growing season) with second-year (1-yr-old) LMB in an indoor recirculating system to evaluate optimal stocking densities for growout to food-fish size. LMB were raised initially in 12, 0.04-ha ponds at Kentucky State University's Aquaculture Research Center (Frankfort, KY, USA). Pond-reared LMB fingerlings were visually graded to a similar size. Average stocking weight (112.0 ± 38.0 g) was determined by individually weighing 100 individuals from the graded population. The fingerlings were randomly stocked into nine, 900-L tanks to achieve stocking densities of 30, 60, or 120 fish/m^3 (three replicates of each) and initial biomass densities of 2.6, 5.1, and 10.2 kg/m^3, respectively. Experimental stocking densities were chosen based on the assumed growth of the fish to an average size of approximately 500 g with associated target final biomass densities of 15–60 kg/m^3.

Culture tanks were 1-m^3 polyethylene cone bottom tanks (Polytank Inc, Litchfield, MN, USA). A shared recirculating system consisted of a 3000-L sump, a $^1\!/_4$ hp pump, a 1.5-m^3 automatic-backflush bead filter for solids removal (PolyGeyser, Aquaculture Systems Technologies LLC, New Orleans, LA, USA), a 2.0-m^3 mixed-moving-bed biofilter (Low Space Bioreactor, Pentair, Apopka, FL, USA) for nitrification, and 400-watt ultraviolet light for sterilization. Aeration in each tank was provided by a regenerative blower through a single 6.25 × 6.25 × 30 cm^3 medium pore silica diffuser (Pentair Inc., Apopka, FL, USA) providing approximately 1 ft^3/min of air. The average water flow rate supplying each tank was approximately 9 L/min providing approximately 14 water exchanges per tank per day.

The fish were fed a commercially available floating diet containing 45% protein and 16% lipid (Steelhead formulation, Skretting USA, Tooele, UT, USA) once daily to apparent satiation. Approximately 30 min after feeding, uneaten feed was removed from the tank surface with a small dip net. Tanks were covered with plastic mesh to prevent fish from jumping out. At harvest, all fish were counted, individually weighed to the nearest gram, and measured (total length; cm). Uniformity Index was measured as UI_{10} (%) = (n_{10}/N) × 100, where n_{10} is fish number (frequency) between "mean x 0.9" and "mean x 1.1" and N is total fish number measured (Bell 2002).

The room was kept on a 12:12 light cycle throughout the experiment. Temperature, dissolved oxygen, and pH in each tank were monitored once daily (0800 and 1600 h) using a YSI 556 multiprobe meter (Yellow Springs Instruments, Yellow Springs, OH, USA). Total ammonia-nitrogen and nitrite-nitrogen were measured from the common sump twice weekly using an HACH Odyssey digital spectrophotometer (HACH Company, Loveland, CO, USA). Total hardness and alkalinity were also measured two times per week from the sump using an HACH digital titrator. Alkalinity was adjusted twice weekly to 100 mg/L by addition of sodium bicarbonate.

The bead filter was set to automatically backwash approximately 10 times per day and waste solids were discharged three times weekly. Dechlorinated municipal water was added to make up water lost through waste discharge and that lost to evaporation, approximately 600-L per week. Water temperature was maintained at approximately 26–27 C by ambient room temperature (Tidwell et al. 2003).

Data to be compared statistically were first evaluated by Levene's test for homogeneity of variance and yielded P values greater than 0.05, indicating no differences between variances in the population. This meets the requirements for ANOVA ($P \leq 0.05$), which was used to statistically compare average dissolved oxygen concentrations and harvest data using Statistix version 10.0 (Statistix Analytical Software, Tallahassee, FL, USA). If significant differences were found among treatments, treatment means were separated using Fisher's least significant difference test (Steele and Torrie 1980). All percentage and ratio data were arc sin transformed prior to analysis (Zar 1984). However, data are presented untransformed to facilitate comparisons.

Results and Discussion

Water quality variables averaged (±SD): temperature, 27.1 ± 0.1 C; pH, 7.3 ± 0.5; total ammonia-N, 0.42 ± 0.24 mg/L; unionized ammonia-N, 0.01 ± 0.01 mg/L; and nitrite-N, 0.24 ± 0.10 mg/L. Overall mean dissolved oxygen concentrations were significantly higher ($P \leq 0.05$) in the low density treatment (6.7 ± 0.1 mg/L) compared to the high density treatment (6.1 ± 0.2 mg/L), which was not significantly different ($P > 0.05$) from the intermediate density (6.4 ± 0.2 mg/L). The experimental system maintained suitable water quality conditions throughout the experiment for good growth and survival of LMB. As such, water quality parameters are a statement of condition and are not biologically significant.

At harvest, all fish were counted, individually weighed, and measured (total length). Total biomass densities significantly increased ($P \leq 0.05$) with stocking rate achieving 6.2 kg/m^3 at 30 $fish/m^3$, 13.2 kg/m^3 at 60 $fish/m^3$, and 22.9 kg/m^3 at 120 $fish/m^3$ (Table 1). The stocking densities evaluated in this trial had no significant impact ($P > 0.05$) on survival, average harvest weight, percent weight gain, uniformity index (UI_{10}), or feed conversion ratio (FCR), which averaged 93%, 263%, 26.2, and

TABLE 1. *Means (±SD) of average harvest weight (AHW) (g), minimum/maximum of individual harvest weight (g), total production (kg/m^3), survival (%), feed conversion ratio (FCR), percent weight gain (% Wt Gn), and uniformity index$_{10}$ (%) of largemouth bass raised in tanks at three densities and fed a commercially available extruded pellet diet*[a]

	30/m^3	60/m^3	120/m^3
AHW (g)	301.3 ± 31.9^a	298.7 ± 20.1^a	283.5 ± 17.5^a
Min/max (g)	151/492	124/556	108/598
Tot prod (kg/m^3)	7.6 ± 1.0^c	16.4 ± 1.1^b	28.2 ± 3.9^a
Surv (%)	90.7 ± 4.6^a	98.7 ± 2.3^a	89.3 ± 7.1^a
FCR[b]	2.0 ± 0.3^a	1.6 ± 0.1^a	1.7 ± 0.3^a
% Wt Gn[c]	269.0 ± 28.5^a	266.7 ± 17.9^a	253.1 ± 15.6^a
UI$_{10}$ (%)[d]	25.1 ± 5.9	27.1 ± 2.7	26.5 ± 8.8

[a] Values within columns within trials followed by different superscripts are significantly different ($P \leq 0.05$).

[b] FCR = g dry feed fed/g wet weight gain.

[c] Percent weight gain (% Wt Gn) = (average harvest weight (g)/average stock weight (g))×100.

[d] UI$_{10}$ (%) = (n_{10}/N) × 100, where n_{10} is fish number (frequency) between "mean x 0.9" and "mean x 1.1" and N is total fish number measured.

1.8, respectively (Table 1). Feeding the fish once daily does not seem to have effected overall growth in LMB. Similar feeding methods are reported for LMB (Petit et al. 2001) and for feeding other predacious fish such as sea bass, *Dicentrarchus labrax* (Sammouth et al. 2009; d'Orbcastel et al. 2010).

Although survival and feed conversion were acceptable, after 6 mo of culture the LMB did not achieve target sizes of 500 g, which is considered market size in the food-fish marketplace. Also, size variation increased as densities increased. The UI$_{10}$ for body weight was 25 at 30/m^3, 27 at 60/m^3, and 27 at 120/m^3. Observations indicate that each tank had one or two fish that did achieve market size, but the remainder of the population did not. The size ranges are illustrated by minimum and maximum sizes (min/max) (Table 1). Again, these ranges became larger as densities increased (though average weights did not differ significantly). Smaller fish were observed to show signs of tail biting and bullying from cohorts, probably indicating intraspecific competition and aggression.

These data are in agreement with Park et al. (2015a) who evaluated juvenile LMB at stocking rates of 15, 20, 25, 30, 35, and 40 kg/m^3 in semienclosed RAS. The reported survival rates were above 95% in all treatments, and there were no significant differences ($P < 0.05$) in FCR or specific growth rate. Gross yield was significantly different, ranging from 51 kg/m^3 at the lowest density to 126 kg/m^3 at the highest density. They also reported no significant differences ($P < 0.05$) in final individual weight (g), condition factor, and coefficient of variation (for total body length, body weight, condition factor). However, they did find differences among treatments among all Uniformity Indexes (for total body length, body weight, condition factor), indicating that with increased density, there was decreased uniformity. This agrees with findings in the current study.

When Park et al. (2015b) evaluated stocker-sized LMB in semiclosed indoor systems, they evaluated stocking densities at 4.5, 9, 18, 36, 54, and 72 kg/m^3. Results indicated that FCR and specific growth rate increased until they reached the two highest densities, where they began to diminish. Survival rates were 92% in all treatments except at the lowest density, which was 82%. As is to be expected, gross yield increased with increasing stocking densities. In size variability analysis, there were no significant differences ($P < 0.05$) for condition factor or among coefficient of variation (for total body length, body weight, condition factor).

Tidwell et al. (1998) evaluated the stocking density of LMB in ponds and determined no difference in weight gain for fish stocked at either 6175/ha or 12,350/ha. Petit et al. (2001) compared juvenile LMB growth and size variation in aquaria stocked at different densities. The authors evaluated initial biomass densities of 1, 2, and 3 kg/m^3 compared to the initial biomass densities of 2.6, 5.1, and 10.2 kg/m^3 evaluated in this trial. They found that growth decreased with increased stocking density, which is not in agreement with what was found previously. This could be due to the size difference at stocking and rapid growth in juveniles as compared to adult fish.

Fish diets must be manufactured into pellets that the fish will readily accept and utilize most efficiently (Jobling et al. 2001). Extruded floating feeds are typically used in commercial production of LMB in ponds as they allow the farmer to see how much, and how actively, the fish eat. However, it may be beneficial to feed a slow sinking pellet to LMB in RAS to reduce the ability of a few aggressive fish to prevent others from efficiently feeding at the water's surface. Petit et al (2001) observed antagonism while feeding and reported that there were larger fish who ate first and at the water surface while the majority of the population ate food later in the water column. They further speculate that when LMB are stocked at lower densities, this feeding behavior may not be detrimental but could become problematic when stocked at higher densities. Multiple daily feedings might also allow more aggressive individuals to become satiated reducing antagonism during subsequent feedings. Development of genetic strains of LMB better suited for high-density tank culture would likely also be beneficial. Additional research is needed to better assess or improve the suitability of LMB for culture in RAS.

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