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Effects of stocking density on growth and survival of young Gulf killifish in recirculating aquaculture systems

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ABSTRACT

Gulf killifish, *Fundulus grandis*, is a hardy marine baitfish with established rearing techniques in ponds and static pools, but there is little information about the use of recirculating aquaculture systems (RAS) for growing killifish. The current trial investigated the effects of stocking density on growth and survival of young killifish in RAS. Young fish (28-day posthatch) of 51.6 ± 0.9 mg (mean $\pm$ SE) were stocked at 2, 5, 8, and 11 fish/L in 31 L tanks in RAS with triplicate groups for 16 weeks. Cannibalism was a major problem in the study, which increased with increasing initial stocking density, affecting survival negatively. Survival decreased ($P < 0.03$) with increasing initial stocking density and culture period. At the end of the trial, the 2, 5, 8 and 11 fish/L initial stocking density reduced to 1.66, 1.42, 0.86, and 0.74 fish/L respectively. A significant linear relationship existed between the initial stocking density and weight ($y = 0.077x + 2.3$; $R^2 = 0.8$; $P = 0.003$), whereas an inverse relationship occurred between initial stocking density and survival parameters ($y = -9.43x + 97.4$; $R^2 = 0.89$; $P < 0.001$). From the trial, the optimum density for culturing young of Gulf killifish in RAS appears to be around two fish/L.

KEYWORDS

Baitfish; cannibalism; optimum density; pond studies; recirculating technology; techniques

Introduction

The culture of marine fish for live bait is a new segment of the high-value aquaculture industry (Green, Gothreaux, and Lutz 2010) that provides anglers with baitfish for recreational fishing (Oesterling, Adams, and Lazur 2004; Oesterling, Sennett, and Kilduff 2005). Baitfish are usually sold on a per-fish, rather than weight, basis (Adams and Lazur 2001; Adams, Lazur, and Zajicek 1997) and can be grown to market size in a shorter period of time than most food fish. Fish used as live bait have a ready market and high demand, mostly met through capture fisheries (Oesterling, Adams, and Lazur 2004).

Gulf killifish, *Fundulus grandis*, is a hardy baitfish species with tolerance to a wide range of environmental conditions (Umminger 1971; Perschbacher,

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Aldrich, and Strawn 1990). Although Gulf killifish have a ready market and demand throughout the year, supply has not kept up with demand. This is because the majority of killifish sales rely upon harvests from the wild that show seasonal variations in abundance and irregular harvest sizes (Hanson, Wallace, and Latch 2004; Oesterling, Adams, and Lazur 2004). Due to these challenges, supply is inconsistent.

The economic viability of production in intensive aquaculture depends on the density at which the species are stocked (Huang, Chun, and Chiu 2002). Stocking density is a major factor affecting production parameters such as growth, survival, and yield (Ellis et al. 2002; Jones and Ruscoe 2000; Rahman et al. 2006; Tremblay-Bourgeois et al. 2010; Zhu et al. 2011). Therefore researchers and aquaculturists have been attracted to investigating the different densities at which fish growth can be optimized. Optimal stocking density is highly species specific (Ashley 2007; Boujard, Labbé, and Aupérin 2002) and varies with age and life stage (Huang and Chiu 1997; Jörgensen, Christiansen, and Jobling 1993). Finding this optimum density is very crucial for every cultured species and should cover a wide range of sizes (Tremblay-Bourgeois et al. 2010).

Previous studies have shown that a market size Gulf killifish (adults 6.3 cm) can be obtained in less than 115 days (Perschbachner and Strawn 1983, 1991; Phelps et al. 2010; Tatum et al. 1982; Waas and Strawn 1982). However, most of these studies focused on starting sizes between 0.3 and 0.5 g in ponds and static pools. This is because such sizes have high survival in grow-out ponds and result in the shortening of growth period. Therefore reports on the period needed to obtain market-size individuals and subsequent survivals have not addressed culture with initial weights lower than 0.1 g and their growth characteristics.

Recently, environmental concerns coupled with increasing cost of coastal property make coastal ponds and static pools less attractive for the production of baitfish. Current technology such as recirculating aquaculture systems (RAS) can be employed in baitfish production. The use of indoor RAS can enable a year-round production of killifish to meet market demands. In addition, it offers the flexibility of siting farms away from the coast and/or close to marketing centers. In comparison with traditional ponds, the use of RAS usually has an added advantage of allowing aquaculturists to grow fish at very high densities. This can have a positive effect on the number of fish that can be raised in captivity and may lead to an increase in number of fish that can be marketed per unit volume of water. Ofori-Mensah, Green, and Nunoo (2013) did a preliminary study in which juvenile Gulf killifish were assigned to treatments at a density of two or five fish/L in RAS for 82 days. The least stocking density of two fish/L (2/L) was 25 times the initial stocking density in Trimble, Tatum, and Styron's (1981) study and 1,000 times the density in Waas and Strawn's (1983) study. Compared to Tatum et al. (1982),

this 2/L density was approximately 143, 71, and 36 times the 0.014, 0.028, or 0.056 fingerlings per liter stocking densities. At initial weight of approximately 0.45 g, Ofori-Mensah, Green, and Nunoo (2013) obtained a good survival rate ($\geq 83\%$) in the preliminary study. Additionally, similar growth results (≥ 1.43 g) were obtained between the two and five fish/L. Therefore, the authors recommended higher densities to determine the relationship between stocking density and growth of Gulf killifish in RAS. Based on this recommendation and the need to contribute to filling the knowledge gap in the larval rearing of killifish, the present study evaluated the effects of stocking density on growth and survival in killifish with an initial weight below 0.1 g.

Materials and methods

Culture system

The trial was conducted at the Aquaculture Research Station (ARS; Louisiana State University Agricultural Centre, Baton Rouge, LA, USA) in an indoor RAS. The Institutional Animal Care and Use Committee of the Louisiana State AgCenter approved in advance all procedures used in this trial under protocol AE2010-14. The RAS had 12 plastic cylindrical tanks holding 31 L of water each. Water used in the system originated from a dechlorinated municipal source and was maintained at a salinity of 10 g/L using Crystal Sea Marinemix (Marine Enterprises International Inc., Baltimore, MD, USA) and was aerated continuously with a regenerative blower that had individual airstones (≈ 15 cm³) in each tank. The photoperiod was set at 12 h light and 12 h darkness. Sodium bicarbonate was added to the water in the system to maintain an alkalinity of > 150 mg/L. The temperature of the system was maintained at 25°C by means of a metal heater fitted to a digital temperature controller (NEMA 4X Outdoor Enclosure, Aqua Logic Inc., San Diego, CA, USA). Water was added throughout the study as needed to account for losses through evaporation. The recirculating system was provided with a bubble-bead filter (≈ 100 L) and a 40 W ultraviolet sterilizer. Prior to stocking, the system was allowed to run for approximately one month to ensure the absence of leakages and permit the accumulation of microbes (bacteria) in the bead filter to aid the nitrification process.

Stocking and feeding

Young fish (28 days posthatch) of an initial body weight of 51.6 ± 0.9 mg that had been adapted to microencapsulated diets and granule or grounded artificial diets were randomly assigned to the RAS at densities of 2, 5, 8, and 11 fish/L. Each density treatment had three replicates. Fish were fed

commercially available extruded feed (40% crude protein, 9% crude fat, 4% crude fiber; Burris Mill and Feed, Franklinton, LA, USA) that was ground and sieved through a mesh size of 500 μm . Fish were fed daily at 10% body weight divided into three feeding regimes: 0900, 1200, and 1500 h for 16 weeks (October to February) with particle size increasing gradually to 600–850 μm for the final 2 weeks of trial. Prior to feeding, the water flow to each tank was turned off to allow fish to feed for approximately 30 minutes, after which it was turned on.

Sampling protocol

About 20 fish were randomly sampled biweekly from each culture tank (60 individuals per density treatment) with their weight and standard length (SL) recorded. Survival was assessed every 2 weeks by counting the fish in tanks. At the end of trial, all fish were individually weighed and their SL recorded for evaluating final growth and survival rate parameters.

Growth and survival parameters

Growth parameters recorded included weight gain (W), growth rate (GR), specific growth rate (SGR), condition factor (CF), coefficient of variation (CV), and gross yield (GY). GR calculated as $\text{GR} = 100 \times [(W_f - W_i)/W_i]$, SGR as $\text{SGR} = 100 \times [(\log_e W_f - \log_e W_i)/t]$, coefficient of variation as $\text{CV} = [(SD/W_n)] \times 100$, and condition factor as $\text{CF} = 100 \times (W_n/L_s^3)$ with $\log_e$ being natural log; W_f final weight (g); W_i initial weight; t time (days); W_n mean fish weight (g); L_s standard length (cm); and SD being standard deviation of the killifish weight. Gross yield (Y) was determined by multiplying the mean final weight by survival.

Tanks were inspected thrice daily to remove dead fish. Fish with intact body parts were separated from those with missing parts. Mortality was assessed by counting dead fish that had intact bodies, whereas fish with missing body parts were recorded separately (C). Survival rate (SR) was calculated as $\text{SR} (\%) = [100 \times (N_f/N_i)]$ and cannibalism (C_t) calculated as $C_t = 100 \times \{C + [N_i - (M + N_f)]\}$ where M, N_f and N_i are mortality, final, and initial number of fish respectively.

Water quality

Water quality parameters such as temperature, salinity, dissolved oxygen (DO), pH, total alkalinity, total hardness, total ammonia-nitrogen (TAN), and nitrite were recorded weekly. Total alkalinity and hardness were determined with standard titration techniques, while TAN (salicylate method) and nitrite (diazotization method) were determined with a Hach DR 4000

spectrophotometer (Hach Co., Loveland, CO, USA). DO was measured with YSI Model 55 DO meter (YSI, Inc., Yellow Springs, OH, USA). Salinity and temperature were measured with YSI Model 30 salinity–conductivity–temperature meter. pH was determined with Orion Model 330 pH meter (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Data analysis

Data were reported as means $\pm$ standard error (SE). Percentage data were arcsine transformed prior to statistical analysis. After an initial exploration for normality using the Shapiro-Wilk and Anderson-Darling tests, data were subjected to one-way analysis of variance (ANOVA) to test if there were significant differences in growth and survival parameters among the density treatments. A Ryan-Einot-Gabriel-Welsch multiple-range (REGWQ) test was performed when significant differences were detected ($P \leq 0.05$). Linear regression was used to determine the relationship between stocking density and growth and survival parameters. Data were analyzed using XLSTAT 2012 computer software.

Results

Weight of killifish is presented in Table 1. Initial weight did not differ among treatment groups. Fish kept in 5 and 11 fish/L density treatments had attained higher ($P \leq 0.002$) weight 2 weeks after stocking. After week 4, weight of fish stocked at two and eight fish/L were higher ($P \leq 0.01$) than the other groups. After 6 weeks of stocking, fish weight increased ($P = 0.04$) with increasing stocking density from 2 to 8 fish/L, although fish weight did not differ ($P = 0.237$) between the 8 and 11 fish/L density treatments. From week 10 until the end of the trial, killifish at the 11 fish/L density treatment had the

Table 1. Weight (mean $\pm$ S.E in mg) of killifish during culture period at initial stocking densities of 2, 5, 8, and 11 fish/L for 112 days.

Stocking density				
Culture period	2 fish/L	5 fish/L	8 fish/L	11 fish/L
Week 0	50.9 $\pm$ 0.9	51.2 $\pm$ 0.7	51.0 $\pm$ 0.9	51.4 $\pm$ 0.6
Week 2	52.2 $\pm$ 0.2 ^a	59.4 $\pm$ 0.7 ^b	51.2 $\pm$ 0.7 ^a	58.5 $\pm$ 0.5 ^b
Week 4	87.1 $\pm$ 0.1 ^b	789 $\pm$ 0.7 ^a	90.3 $\pm$ 0.8 ^b	80.8 $\pm$ 1.6 ^a
Week 6	119.1 $\pm$ 2.1 ^a	124.1 $\pm$ 4.0 ^{ab}	137.1 $\pm$ 1.1 ^c	132.6 $\pm$ 1.8 ^{bc}
Week 8	153.7 $\pm$ 8.5 ^a	197.3 $\pm$ 37.2 ^a	221.2 $\pm$ 24.9 ^a	195.9 $\pm$ 13.8 ^a
Week 10	175.8 $\pm$ 13.2 ^a	236.4 $\pm$ 17.7 ^{ab}	295.4 $\pm$ 66.0 ^{ab}	447.4 $\pm$ 65.8 ^b
Week 12	223.3 $\pm$ 11.8 ^a	321.2 $\pm$ 8.0 ^a	460.9 $\pm$ 80.1 ^b	499.5 $\pm$ 76.0 ^b
Week 14	287.0 $\pm$ 16.7 ^a	403.4 $\pm$ 10.5 ^b	659.3 $\pm$ 90.9 ^c	738.2 $\pm$ 87.8 ^d
Week 16	333.2 $\pm$ 14.8 ^a	511.8 $\pm$ 16.9 ^b	1161.6 $\pm$ 116.5 ^c	1438.1 $\pm$ 106.2 ^c

Values with different superscript letters within a row denote significant differences between density treatments during a given culture period (Ryan-Einot-Gabriel-Welsch multiple-range test; $P \leq 0.05$).

highest weight. At the end of the trial, there was a positive linear relationship between initial stocking density and final weight ($y = 0.077x + 2.3$; $R^2 = 0.8$; $P = 0.003$).

Survival during the trial is shown in Figure 1. Fish stocked at 11 fish/L had the lowest survival (about 6%), whereas the highest survival of 83% was recorded at the lowest density treatment. There was a rapid decline in survival rate until week 10. Survival decreased with increasing stocking density and with culture period ($P < 0.001$). An inverse relationship ($y = -9.43x + 97.4$; $R^2 = 0.89$; $P < 0.001$) was observed between survival and stocking density.

Figure 2 shows the CV during the trial. At the beginning of the trial, fish of homogeneous sizes were stocked ($CV < 1\%$). CV increased rapidly (above 10%) between weeks 6 and 12. After week 12, CV gradually declined until the end of the trial. At the end of the study, CV was below 10% at the two and five fish/L density treatments. The high stocking densities had significantly higher ($P < 0.001$) final CV.

Cannibalism during the trial is reported in Table 2. There were no significant differences ($P > 0.05$) within density treatments, so data are presented as the average (mean $\pm$ SE) values. Cannibalism was observed in the culture tanks (density treatments) after 4 weeks of culture and increased rapidly till week 8. After 8 weeks poststocking, approximately half of fish in the 8 and 11 fish/L treatment tanks had been lost through cannibalism. The rate of cannibalism then decreased after week 12 until the end of the trial. Cannibalism reduced the initial stocking numbers to a common level

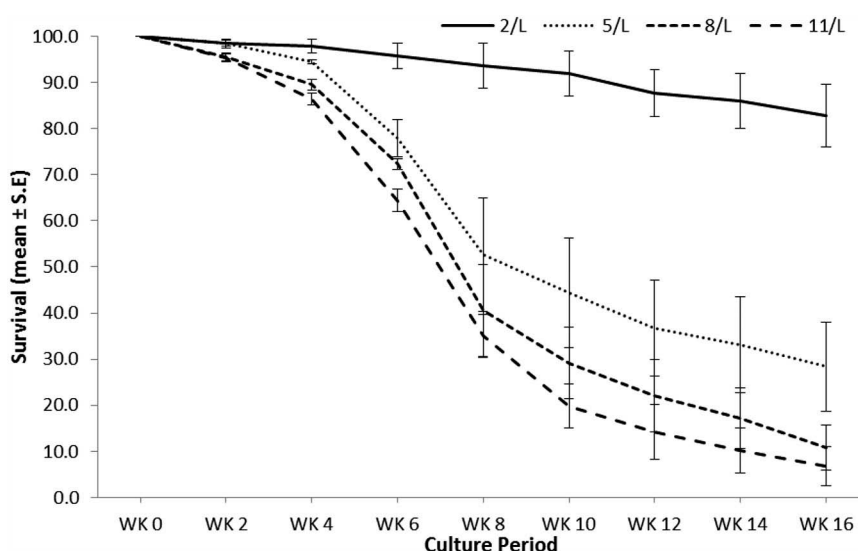


Figure 1. Survival rate of fry of Gulf killifish in culture unit at stocking densities of 2, 5, 8, and 11 fish L^{-1} for 112 days.

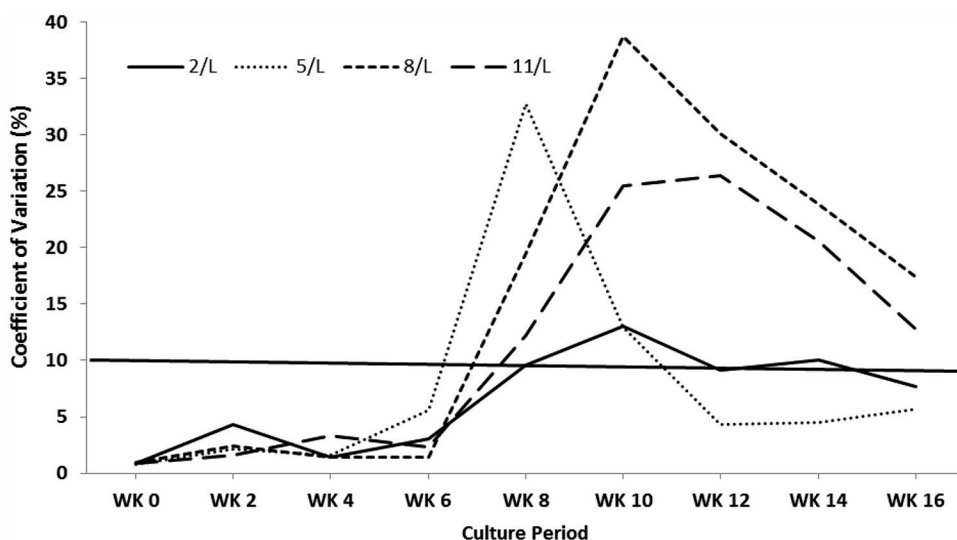


Figure 2. Coefficient of variation of young Gulf killfish in culture unit at stocking densities of 2, 5, 8, and 11 fish L⁻¹ for 112 days.

Table 2. Percentage (mean $\pm$ SE) of killfish cannibalized during the culture unit at initial stocking densities of 2, 5, 8, and 11 fish/L for 112 days.

Culture period	Stocking density			
	2 fish/L	5 fish/L	8 fish/L	11 fish/L
Week 0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
Week 2	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
Week 4	0.0 $\pm$ 0.0 ^a	2.2 $\pm$ 0.5 ^b	3.8 $\pm$ 0.2 ^c	3.6 $\pm$ 0.4 ^c
Week 6	1.6 $\pm$ 0.3 ^a	12.3 $\pm$ 2.5 ^b	15.7 $\pm$ 0.6 ^{bc}	20.5 $\pm$ 1.4 ^d
Week 8	2.2 $\pm$ 0.8 ^a	24.5 $\pm$ 6.9 ^b	31.2 $\pm$ 5.9 ^b	28.5 $\pm$ 1.9 ^b
Week 10	0.0 $\pm$ 0.0 ^a	6.5 $\pm$ 0.1 ^b	10.9 $\pm$ 1.3 ^c	13.4 $\pm$ 0.2 ^c
Week 12	4.3 $\pm$ 0.4 ^a	7.1 $\pm$ 1.6 ^c	7.1 $\pm$ 0.1 ^c	5.8 $\pm$ 0.2 ^b
Week 14	1.6 $\pm$ 0.1 ^a	3.2 $\pm$ 0.1 ^b	4.8 $\pm$ 0.3 ^c	3.7 $\pm$ 0.1 ^b
Week 16	3.2 $\pm$ 0.1 ^a	4.7 $\pm$ 0.8 ^b	6.5 $\pm$ 0.9 ^c	3.5 $\pm$ 0.1 ^a

Values with different superscript letters within a row denote significant differences between density treatments during a given culture period (Ryan-Einot-Gabriel-Welsch multiple-range test; $P \leq 0.05$).

between weeks 10 and 12 of poststocking. Cannibalism was highest in the high-density tanks (Table 2). After 16 weeks of culture, cannibalism had modified initial stocking densities (Table 3). A linear relationship existed between stocking density and cannibalism ($y = 22.3x + 1.64$; $R^2 = 0.703$; $P = 0.001$).

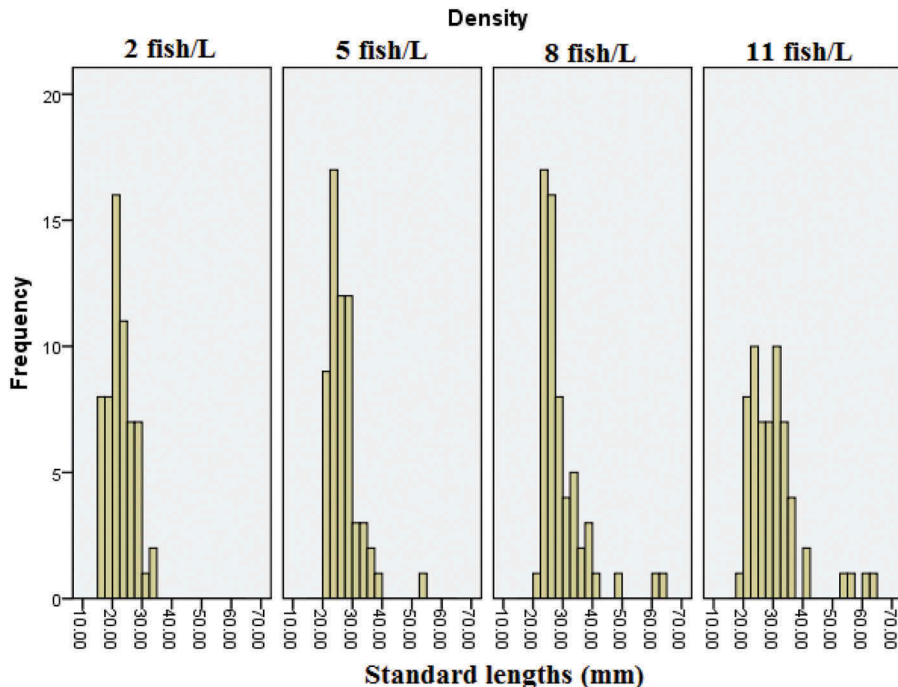
Final growth and survival parameters of Gulf killfish are shown in Table 3. Final weight was highest at 11 fish/L, although initial weights did not differ ($P > 0.3$) among density treatments. Weight increased with increasing initial stocking density and culture period ($P < 0.04$). GR, SGR, mortality, and proportion of killfish lost through cannibalism increased ($P < 0.05$) with increasing initial stocking density.

Table 3. Harvest results of Gulf killifish at the stocking densities after 112 days in 31 L RAS.

	2 fish/L	5 fish/L	8 fish/L	11 fish/L
Growth Parameters				
Initial weight (mg)	50.9 ± 0.9	51.2 ± 0.7	51.0 ± 0.9	51.4 ± 0.6
Final weight (g)	0.34 ± 0.02 ^a	0.51 ± 0.02 ^b	1.16 ± 0.12 ^c	1.44 ± 0.11 ^c
Total weight (g)	16.77 ^a	22.45 ^b	31.06 ^c	1.44 ^c
Standard length (cm)	2.28 ^a	2.68 ^{ab}	2.96 ^b	3.05 ^c
Yield (g/L)	0.541 ^a	0.724 ^b	1.001 ^c	1.061 ^c
Final CV (%)	4.69 ^a	5.70 ^b	17.37 ^c	17.79 ^c
GR (%)	552.9 ± 1.7 ^a	903.9 ± 3.4 ^b	2178.4 ± 20.6 ^c	2719.6 ± 34.2 ^d
SGR (%)	1.67 ± 0.1 ^a	2.03 ± 0.2 ^{bc}	2.63 ± 0.9 ^c	2.81 ± 1.3 ^c
CF (g/cm ³)	2.05 ^a	2.66 ^a	4.47 ^b	5.09 ^b
Final numbers of fish	51.3 ± 3.2 ^a	44.0 ± 10.4 ^{ab}	26.7 ± 8.7 ^b	23.0 ± 11.2 ^b
Final density (fish/L)	1.66 ^a	1.42 ^a	0.86 ^b	0.74 ^b
Survival Parameters				
Mortality rate (%)	6.5 ± 1.2 ^a	10.1 ± 0.2 ^b	9.4 ± 1.0 ^b	14.6 ± 1.2 ^c
Cannibalism (%)	12.9 ± 4.6 ^a	62.1 ± 10.0 ^b	80.0 ± 4.1 ^c	78.8 ± 3.8 ^c
Survival rate (%)	82.8 ± 3.7 ^a	28.4 ± 6.7 ^b	10.8 ± 1.9 ^c	6.7 ± 1.3 ^c

Values with different superscript letters within a row denote significant differences between density treatments during a given culture period (Ryan-Einot-Gabriel-Welsch multiple-range test; $P \leq 0.05$).

Figure 3 shows the size distribution of young Gulf killifish after 112 days of culture. At the end of the trial, sizes ranged from 1.58 to 3.37, 2.03 to 5.32, 2.14 to 6.4, and 1.97 to 6.44 cm at 2, 5, 8, and 11 fish/L density treatments respectively. It can be seen that size variation increased with increasing initial stocking density.

**Figure 3.** Size distribution (standard lengths) of young Gulf killifish at the end of 112 days of culture.

The mean temperature for the system was $24 \pm 0.4^{\circ}\text{C}$ with a recorded low of 18°C and high of 25.2°C for the duration of the trial. The mean DO of the RAS (7.76 ± 0.01) was above 5 mg/L. Salinity of the system was maintained at approximately 10 g/L with a minimum of 9.8 and a maximum of 10.2 g/L. pH of the system fluctuated between 8.54 and 8.02. The mean ammonia and nitrite were 0.012 ± 0.001 ppm and 0.025 ± 0.002 mg/L respectively. Alkalinity and hardness were above 120 and 1500 mg/L respectively.

Discussion

In intensive aquaculture, stocking densities tend to be high in order to maximize profits. High stocking densities can have negative effects, such as abnormal swimming behavior, increased aggression and competitive behaviors (including feed competition, territoriality, and dominance/subordination hierarchies), and cannibalism (Baras and Jobling 2002; Ellis et al. 2002; Greaves and Tuene 2001) and subsequently, low survival (Li et al. 2003; Mohanty 2004). Cannibalism was a major problem in the current trial, as it contributed to $\geq 70\%$ loss of killifish at the high-density treatments. The loss of individuals through cannibalism was noticed after 4 weeks poststocking. Cannibalism has been reported in killifish species (Able, Hagan, and Brown 2006; Able et al. 2007; Rozas and LaSalle 1990; Sagarese et al. 2016). In pond studies, Perschbacher and Strawn (1986) reported that piscivory in Gulf killifish increases with increasing fish size and age. Cannibalism is very common in cultured fish (Baras and Jobling 2002) and is governed by gape-size limitations (Baras, Kestemont, and M  lard 2003). With 10% feeding by biomass per day, the 11 fish/L density treatment received over five times the quantity fed the 2 fish/L, which led to a higher feed density at the high-density treatments. In enclosed environments, high feed density fosters prey encounters (Turesson and Br  nmark 2007) and drops search time, both beneficial for growth (Hart and Connellan 1984). Fish that adapted relatively early to the artificial diet in the present trial eventually became bigger and were antagonistic toward the smaller individuals. This resulted in the creation of dominance hierarchies and the emergence of cannibals that bit the tails of the relatively smaller fish. Fish prey has a higher content of digestible nutrients (Kubitza and Lovshin 1999), hence fish that feed totally or partly on conspecifics generally grow faster (Baras and Jobling 2002; Brabrand 1995). Although cannibalism is seen as an adaptive response to starvation and underfeeding, Hecht and Pienaar (1993) and Baras and Jobling (2002) reported that cannibalism can still occur even in the abundance of food supply. The 10% daily feeding by biomass has been recommended for young Gulf killifish (Wallace and Waters 2004) and to be gradually reduced as fish grew. Hence, the quantity of feed dispensed (10% body weight) in the present trial did not lead to low survival through underfeeding. Gulf killifish

are kept at densities below two fish/L in RAS in our lab. These densities are associated with very high survival rates (>95%, unpublished data). However, survival was low at densities above two fish/L treatment in the current trial. Redoing the high-density treatments with similar initial weight of fish presented similar results (unpublished data) and was indicative of the low survival rate in young Gulf killifish at densities higher than two fish/L. One of the ways of ensuring size homogeneity is to regularly grade fish, which can lead to improved growth and size homogeneity at harvest (Saoud et al. 2005). Manipulation of turbidity is another way to ensure uniform size distribution and decrease the size advantage of the largest fish (McEntire et al. 2015). However, the objectives of the current trial made it impossible to implement such practices.

A relatively slow growth was recorded during the first month of the culture period. Test fish might have taken a longer period to fully adapt to the diet provided in the trial and could have contributed to fish practically not feeding or the inability to digest and/or utilize the nutrients in the diets at the early stage of the trial. Further, the absence of natural production in the tanks could be one of the reasons for the slower GR and SGR of killifish. Studies have shown that natural feeds are very important in the diet of killifish (Ogle and Solang 1982; Tatum and Helton 1977; Trimble, Tatum, and Styron 1981). Perschbacher and Strawn (1983, 1991) and Phelps et al. (2010) showed a positive relationship between killifish growth and natural production. Wallace and Waters (2004) reported difficulties in obtaining good growth when Gulf killifish was fed solely on artificial diets.

Although the 11 fish/L had the highest final weight, size variation was highest in this group. Growing killifish at such a density may lead to production of irregular sizes and may not be financially prudent. This is because the marketing and distribution of baitfish are highly specialized due to the specific size requirements (Pounds, Engle, and Dorman 1992). The value of the fish diminishes significantly once it outgrows a specific size range, with peak demand dictating the number and sizes of fish that are maintained by baitfish farmers (Engle, Stone, and Park 2000). To reap the highest economic benefits, size variation of fish produced should be kept at a minimum. Therefore, a consistent supply of fish produced within a desired size range through culture has economic benefits for the producers. Further, it can help in alleviating pressure on wild stocks by providing growers with an alternative environmentally sustainable crop. The high-density treatments had very low survivals. One of the aims of fish farmers, including baitfish producers, is to raise and market more fish per unit volume of water. Hence, farmers do not stand to benefit economically by keeping fish at such densities, as they are likely to harvest very few fish.

Optimal stocking density in any culture facility is defined by the carrying capacity of the environment. It is also defined by the amount

of individual space needed by the fish, which is very species specific. In this trial, growth parameters (final weight, growth rate, specific growth rate, condition factor, and CV) increased with increasing stocking density. According to Greaves and Tuene (2001), Baras and Jobling (2002), Ellis et al. (2002), and Ashley (2007), increasing stocking density leads to increased aggression and cannibalism. A similar trend was observed in this current trial and impacted survival negatively. Although the lowest density (two fish/L) had the lowest growth, it had the highest survival (above 82%). At the end of study, the 2, 5, 8, and 11 fish/L had been reduced to 1.66, 1.42, 0.86, and 0.74 fish/L respectively. This implies that getting to the end of the study, two fish/L density treatment became the highest density at which fish were stocked. This may have contributed to the low growth, as seen here. Densities of 5–11 fish/L resulted in survivals below 28%. Therefore, the optimum density for young Gulf killifish appears not to be higher than two fish/L.

Water quality parameters recorded in the duration of the present trial show that conditions were conducive for the growth of young killifish. Similar values were obtained by Ofori-Mensah, Green, and Nunoo (2013), Patterson, Bodinier, and Green (2012) and Phelps et al. (2010) for optimal growth of Gulf killifish in static pools and RAS. It therefore suggests that young killifish can be cultured in RAS to densities as high as 11 fish/L without adversely affecting body weights but with low survival.

From the present trial, growth rate was low at the early stages of trial, although cultured fish were fed a high-protein diet thrice a day. Survival decreased with increasing stocking density and was below 10% at the 11 fish/L density, altering the initial stocking densities. At the end of the study, weight was highest at 8 and 11 fish/L density treatments but with low survival rate. Cannibalism increased with increasing stocking density and culture period. By the end of the study, it had contributed to over 70% loss of young Gulf killifish in the high-density tanks. With cannibalism defining survival in the trial, further studies are needed to evaluate the relationship between stocking density and cannibalism in killifish in RAS. It is also important to include natural foods (plankton) in the feeding protocol of killifish in RAS. From this trial, densities around two fish/L appear to be ideal for growing young Gulf killifish utilizing recirculating technology.

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