Growth and Production Characteristics of Palmetto Bass (*Morone saxatilis* female x *Morone chrysops* male) Reared at Three Densities in a Pilot-scale Recirculating Aquaculture System

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ABSTRACT

Production characteristics of palmetto bass (*Morone saxatilis* female x *Morone chrysops* male) reared at three stocking densities (36 fish/m³, 72 fish/m³, and 144 fish/m³) in a pilot-scale RAS were evaluated. A final mean ±SE fish weight of 412.1 ± 7.8 g at the high density was significantly lower than that of fish at the medium density weighing 542.1 ± 11.8 g (P < 0.05). Fish weight (676.1 ± 17.0 g) at the lowest density was significantly higher than at the high and medium densities (P < 0.05). The average daily weight gain at the low density (2.8 g/d) was 22% and 47% higher than fish reared at the medium and high densities, respectively. Total biomass gains of 733.8, 483.3, and 297.9 kg were obtained at the high, medium, and low densities. Feed conversion and survival rates were similar among densities averaging 1.4 and 97.1%, respectively. Higher mean daily and cumulative feed totals at the highest density contributed to significantly higher ammonia and nitrite concentrations and lower pH levels at harvest. All other measured water quality parameters were similar among densities and remained within known acceptable limits for fish growth. The results indicated that

palmetto bass reared in closed systems reached market size in 224 days at the low and medium densities. However, the relative biomass production may not justify such strategies when compared to the yield obtained at the highest rearing density.

INTRODUCTION

Wholesale distribution of hybrid striped bass in seafood markets helped to fill the void between consumer demand and market supply as striped bass availabilities from natural harvest declined during the 1980's. As a result, hybrid striped bass gained commercial importance from North Carolina to Massachusetts (Gempesaw et al. 1992).

Commercial production of striped bass hybrids was initially conducted in earthen ponds (Williams 1976; Kerby et al. 1983) as well as cages held in estuarine environments (Valenti et al. 1976; Williams et al. 1981; Woods et al 1983). However, environmental conditions promoting acceptable growth rates limited the growing season to between 6 and 8 months. This results in a production cycle, fry to market-size fish (454 to 525 g, Coale et al. 1990), ranging from 18 to 24 months in culture water subject to seasonal temperature variations (Gempesaw et al. 1992). This seasonal availability resembles commercial harvest of striped bass and results in fluctuating supply and tremendous variations in market value (Losordo et al. 1989). By eliminating temperature variations and maintaining near acceptable water quality conditions, a product of consistent quality and quantity can be produced, which may help to stabilize market pricing.

Near optimal environmental conditions can be maintained in the recirculating aquaculture system (RAS) through filtration techniques and routine additions of fresh water. However, growth limiting conditions often develop as the rate of fresh water replacement declines and the cumulative amount of feed delivered increases (Hirayama et al. 1988). These conditions are characterized by increased concentrations of sub-lethal nitrogenous compounds and suspended solids and periods of reduced dissolved oxygen.

Hybrid striped bass reared under sub-lethal conditions experience reduced growth rates, an increased occurrence of disease, and lower survival rates (Oppenborn and Goudie 1993). Production studies in open

culture systems (ponds, cages, and raceways) have demonstrated that hybrid striped bass can tolerate intensive culture environments characterized by elevated concentrations of metabolic wastes and dissolved organic compounds and periods of low dissolved oxygen (Smith and Jenkins 1985; Jenkins et al. 1989; Brown et al. 1993). As yet, little information exists which describes the impact of the RAS production environment on hybrid striped bass. Therefore, this study was conducted to evaluate the performance of hybrid striped bass reared in a recirculating aquaculture system. In as much as water quality is influenced by feeding rates and stocking density, three rearing densities were studied to determine their effect on growth rates, feed conversion, and survival.

MATERIALS AND METHODS

Palmetto bass (*Morone saxatilis* female x *Morone chrysops* male) obtained from Keo Fish Farms (Keo, AR, USA) were stocked into eight pilot-scale recirculating aquaculture systems at densities of 36 fish/m³ (450 fish total), 72 fish/m³ (900 fish total), or 144 fish/m³ (1800 fish total). Systems were housed at the Virginia Polytechnic Institute and State University (Virginia Tech, Blacksburg, VA, USA) aquaculture facility. The two lowest density treatments were conducted in triplicate while the highest density treatment was conducted in duplicate systems. Because of fingerling supply limitations, fish were stocked into the culture systems over a 30 day period, during which time fish were maintained on a maintenance diet ration (daily feed allotment = 1% of the estimated total biomass per system). This resulted in size differences between rearing densities at the start of the study, where the mean ±SE weights were 43.4 ± 2.4, 50.8 ± 1.9, and 34.9 ± 1.2 g for the low, medium, and high densities, respectively. The initial sampling began with a 2-week acclimation period before the start of the 224-d study.

System Design and Operation

Each recirculating system (Figure 1) consisted of a 8,330 L rectangular rearing tank from which water flowed into a multi-tube clarifier (1,970 L) containing corrugated polyvinyl chloride blocks (BIOdeck 12060, Munters Corp., Fort Myers, FL, USA) for solids removal. Two 0.19kW submerged pumps elevated clarified water 2.1 m to the first stage of a
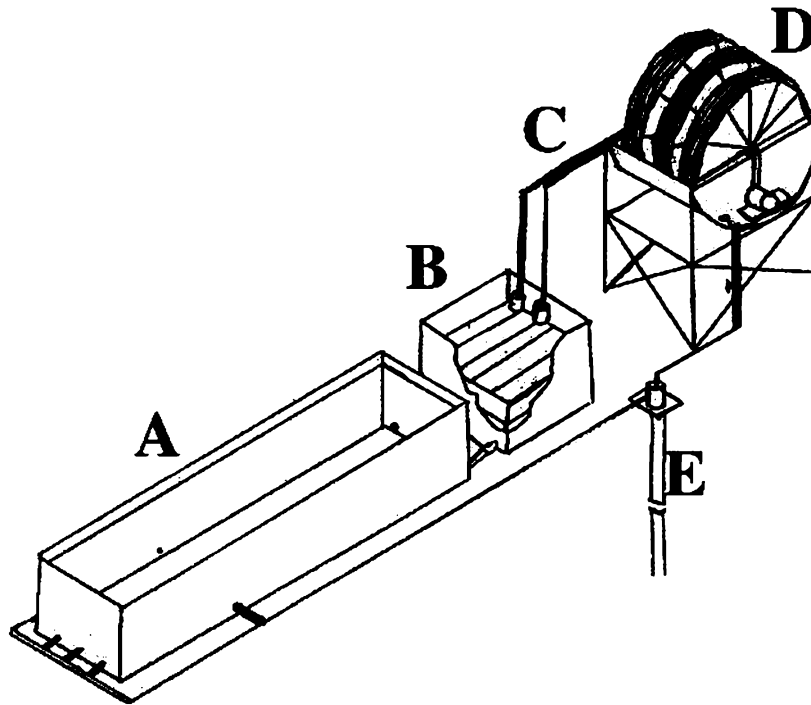
Figure 1. Schematic drawing of the pilot scale recirculating aquaculture system used to culture palmetto bass (Morone saxatilis female x Morone chrysops male). The system consisted of a rearing tank (A), a multi-tube clarifier (B), two submersible pumps (C), a rotating biological contactor (D), and a U-tube aeration device (E).

three-staged rotating biological contactor (RBC) vessel (1,990 L). Support media of the biofilter was constructed of BIOdeck material cut into disks (30 cm X 1.83 m diameter) and rotated at three revolutions/min by a 0.19 kW gear motor. Water gravity flowed through the RBC vessel at approximately 227 L/min and down a 12.2 m deep U-tube aeration device receiving pure oxygen injection. Oxygenated water entered the culture tank through five ports (one along each side and three at the front) positioned 2.5 cm from the bottom of the tank.

The same management protocol was followed for all rearing densities throughout the study. Water exchange and addition was conducted to make up for evaporative losses and wash down the clarifier to remove collected solids. Isolation and wash down of the clarifier was conducted after the delivery of 3 kg of feed to the system. No clarifier was washed

down more than once per day. The clarifier water volume accounted for approximately 15% of the total volume of the system, which resulted in one complete system volume exchange of each system per week. With every fresh water exchange, 1.5 kg of sodium bicarbonate ($NaHCO_3$) and 1 kg of calcium chloride ($CaCl$) were added to maintain alkalinity (for buffering capacity of pH) and hardness levels of 100-150 mg/L, respectively.

Fish Husbandry

Feed was hand-delivered twice daily at 08:30 and 17:00 h. Before the first feeding of each day, water quality measurements were taken to determine if conditions were within known acceptable limits for hybrid striped bass growth (Nicholson et al. 1990). Weight gain was estimated weekly based on an assumed feed conversion rate (FCR) of 1.5 and used to determine the daily feed ration, which was calculated as a percentage of the total biomass present. A commercial diet, "Bass Grower" (BioSponge Aquaculture Products Co., Sheridan, WY, USA) containing minimum crude protein, fat and crude fiber levels of 44, 8, and 3%, respectively, was provided to the fish.

Data Collection

Daily water quality measurements included temperature, total-ammonia-nitrogen (TAN), pH and dissolved oxygen (DO). Twice weekly, nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), alkalinity, and hardness levels were measured. A portable DO meter (Yellow Springs Instrument, Yellow Springs, OH, USA) was used to measure temperature and DO, and pH was measured with a hand-held portable pH pen (Hach Company, Loveland, CO, USA). The TAN, NO_2-N, and NO_3-N concentrations were measured with a spectrophotometer (DR/2000, Hach Company). Alkalinity and hardness levels were monitored following standard methods titration procedures (APAH 1989).

Fish were not fed for 24 h before sampling. A minimum sample of 5% (25, 50, and 100 fish per tank from the low, medium, and high stocking densities, respectively) of a tank's population was arbitrarily netted at 28-day intervals for weight and length measurements. Fish were placed in a 115 L holding tank containing 4000 mg/L NaCl and with 70 mg/L MS-222 (Sigma Chemical Co., St. Louis, MO, USA) during each

sampling procedure. Growth characteristics were calculated for each sampling period as follows:

1) Growth rate (g/d)

$$G = \frac{W_t - W_o}{T}$$

2) Specific growth (%)

$$SG = \frac{\log_e W_t - \log_e W_o}{T} \times 100$$

3) Condition factor

$$K = \frac{W_t}{L_t^3} \times 10^5$$

4) Feed conversion ratio

$$FCR = \frac{F_t - F_o}{W_t - W_o}$$

where:
W_t = mean weight (g), at time t,
W_o = mean weight (g), at time t-1,
L_t = length (mm), at time t,
F_t = total feed delivered (g), at time t,
F_o = total feed delivered (g), at time t-1, and
t = time (day).

The statistical analysis for growth and water quality measurements were performed using linear regression procedures, Proc Mixed and GLM, (SAS, SAS Institute, Inc., Cary, NC, USA). A split-plot complete randomized design was used to analyze treatment and time effects (tank nested within treatments and used as the error term). Mean weight differences at the start of the study were significant, therefore, initial weights were adjusted to a fixed intercept and analyzed. The slopes of the treatment growth regression models equaled the growth rate and were used to establish treatment effects. Multiple comparison tests were conducted with Duncan's multiple-range test. Statistical differences were determined at the $P < 0.05$ significance level.

RESULTS AND DISCUSSION

Water Quality

During the study period, temperatures ranged between 23 and 27°C and mean daily DO concentrations were similar among stocking densities, averaging 8.1 mg/L and ranging from 5.6 to 11.9 mg/L (Table 1). However, results of a diurnal study (Nunley 1992) revealed that DO levels decreased to 4.6 mg/L within 120 minutes after the day's last feeding at the highest stocking densities. Within 75 minutes, oxygen levels increased above desired minimum levels of 5 mg/L (Table 1).

Ammonia and nitrite concentrations and pH levels were significantly different between the high and low stocking densities, yet, they were not statistically different from those of the medium density. Nitrite levels at the medium and low stocking densities were significantly lower than levels detected at the high stocking density (Table 1). However, water quality conditions throughout the study were considered acceptable for hybrid striped bass growth at all rearing densities (Nicholson et al. 1990). Oppenborn and Goudie (1993) reported an un-ionized ammonia of 96 h LC_{50} for hybrid striped of 0.64 mg/L as NH_3-N. The range of un-ionized ammonia concentrations (0.001 to 0.155 mg/L) measured across all stocking densities remained below reported toxicity limits. Overall mean NH_3-N concentrations of 0.015, 0.017, 0.018 mg/L were calculated for the low, medium, and high treatments, respectively, and determined not to be significantly different. All other quality parameters were similar among stocking densities.

Observed differences in water quality were attributed to differences in cumulative feed totals. The rate of water quality decline in recirculating aquaculture systems was shown to be a function of the quantity of feed delivered and the fresh water replacement rate (Hirayama et al. 1988). During this study, this effect was identified by the fast increase in TAN concentration and pH decrease, particularly at the medium and high densities. Because the rate of fresh water replacement (maximum of one complete system volume per week) was the same for all treatments, the rate of accumulation of waste products and subsequent decline in environmental quality was attributed solely to the feed input. Average daily feed consumption and cumulative feed amounts are presented in Table 2. There was no difference in the percentage body weight of feed
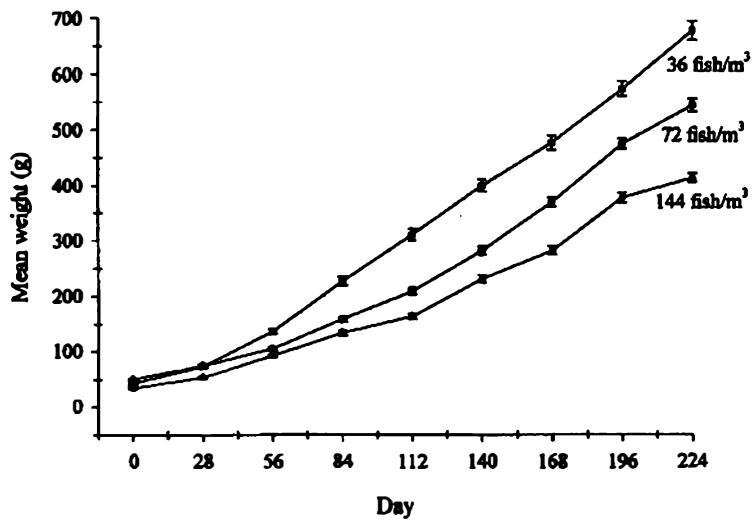
Figure 2. Mean +/- SE weights of palmetto bass (Morone saxatilis female x Morone chrysops male) cultured at different stocking densities in a pilot-scale recirculating aquaculture system for 224 days.

Figure 3. Mean +/- SE biomass standing crop total of palmetto bass (Morone saxatilis female x Morone chrysops male) cultured at different stocking densities in a pilot-scale recirculating aquaculture system for 224 days.
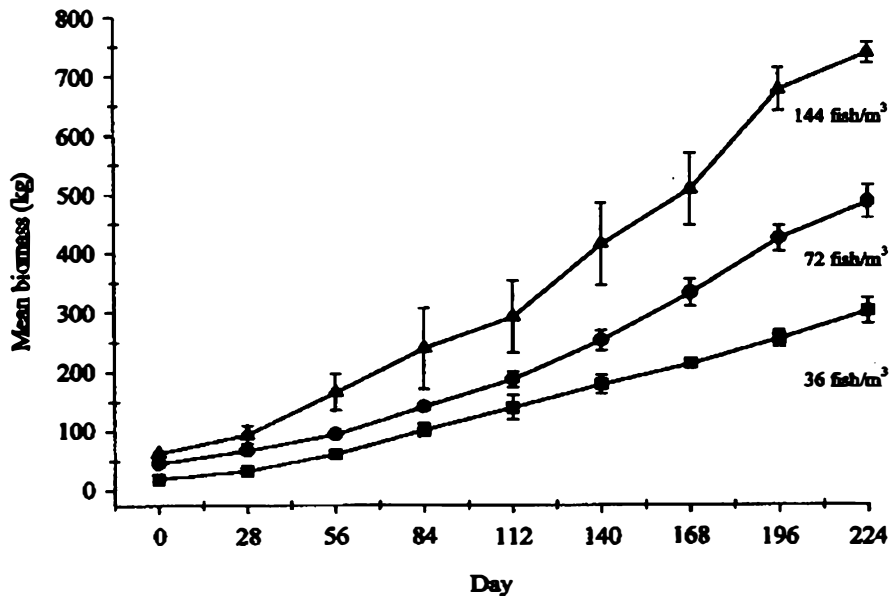
consumed (pooled mean of 1.6%) for similarly sized fish. However, significant growth differences were observed.

Biofiltration during this trial and routinely used in closed culture systems targets the removal and detoxification of ammonia to maintain growth promoting conditions. However, biofilters dominated by ammonia-oxidizing chemolithotrophs do not eliminate unidentified metabolic wastes excreted by the fish (Okabe et al. 1995) which can lead to the accumulation of growth inhibiting substances (Wedemeyer et al. 1979). Thus, the reduced growth observed at the higher rearing densities may have resulted from chronic exposure to sub-lethal concentrations of these accumulating growth inhibitory compounds.

Survival and Growth

Survival rates were higher than 95% across all stocking densities with no significant differences detected. However, significant differences in growth characteristics were observed in response to stocking density (Table 2). Growth rates of 2.8, 2.2, and 1.7 g/d were calculated for the low, medium, and high densities, respectively. Growth rate at the highest stocking density was significantly lower than those calculated at the medium and low densities. Growth at the lowest stocking density was significantly higher than at the medium stocking density. Mean ± SE fish weight at the low, medium, and high rearing densities were 676.7 ± 17.0, 542.1 ± 11.8, and 412.1 ± 7.8 g, respectively, at harvest. Mean specific growth rates were not significantly different among densities for similarly sized fish (Table 2). However, total biomass gains were significantly different. Final treatment biomass averaged 733.8, 483.3, and 297.9 kg at the low, medium, and high densities, respectively. No difference in length-weight regressions (calculated using the least squares methods with covariance analysis used to test for stocking density effects) among stocking densities was detected, thus fish measurements from all densities were pooled to compute a single predictive equation:

$$\log W = -13.9 + 3.497 \log TL, \ r^2 = 0.98$$

Final standing crop biomasses of 58.7, 38.7, and 27.3 kg/m^3 were produced at the high, medium, and low stocking densities, respectively.

Table 1. Mean +/- SE values, calculated from day 0 - 224, for monitored water quality parameters experienced by Morone saxatilis female X Morone chrysops male cultured at three stocking densities in a pilot-scale recirculating aquaculture system. Row values followed by different letters are statistically different (P = 0.05).

Parameter	Stocking density		
	Low 36 fish/m³	Medium 72 fish/m³	High 144 fish/m³
Temperature (°C)	24.6 +/- 0.4[a]	24.8 +/- 0.4[a]	24.7 +/- 0.5[a]
DO (mg/L)	8.2 +/-0.1[a]	8.1 +/- 0.1[a]	8.0 +/- 0.1[a]
pH	7.8 +/- 0.1[a]	7.6 +/- 0.1[a,b]	7.5 +/- 0.1[b]
TAN (mg/L)	0.51 +/- 0.1[a]	0.70 +/- 0.1[a,b]	1.01 +/- 0.2[b]
Alkalinity (mg/L)	139 +/- 12.7[a]	140 +/- 10.1[a]	139 +/- 14.0[a]
NO_2-N (mg/L)	0.16 +/- 0.03[a]	0.29 +/- 0.09[a]	0.88 +/- 0.25[b]
NO_3-N (mg/L)	53.0 +/- 18.0[a]	54.8 +/- 14.8[a]	64.5 +/- 18.7[a]
Hardness (mg/L)	282 +/- 22.2[a]	285 +/- 22.5[a]	311 +/- 31.3[a]

Table 2. Mean +/- SE values for production characteristics of palmetto bass (Morone saxatilis female X Morone chrysops male) cultured at three rearing densities for 224 days. Row values followed by different letters are statistically different (P = 0.05). Production characteristic lacking statistical designation were not analyzed due to inherent correlation to number of fish stock. Range shown in parentheses.

Production Chracteristic	Stocking density		
	Low 36 fish/m^3	Medium 72 fish/m^3	High 144 fish/m^3
Weight (g)	676.1 +/- 17.0[a]	542.1 +/- 11.8[b]	412.1 +/- 7.8[c]
Growth rate (g/d)	2.8 +/- 0.2[a]	2.2 +/- 0.1[b]	1.7 +/- 0.04[c]
Specific growth rate	1.2 +/- 0.04[a] (1.9 - 0.3)	1.1 +/- 0.02[a] (2.2 - 0.6)	1.1 +/- 0.01[a] (1.43 - 0.5)
Relative biomass production (% change)	93.5 +/- 0.6[a]	90.5 +/- 0.5[b]	91.4 +/- 0.3[b]
Absolute biomass harvested (kg)	297.9 +/- 22.5	483.3 +/- 26.8	733.8 +/- 17.7
Final biomass density (kg/m^3)	23.8 +/- 1.8[a]	38.7 +/- 2.1[b]	58.7 +/- 1.4[c]
Total feed consumed (kg)	390.8 +/- 8.1	621.1 +/- 4.7	909.8 +/-13.5
Daily feed consumption (kg)	1.7 +/- 0.5	2.8 +-/ 0.3	4.1 +/- 0.1
Feed consumption (% total biomass)	1.7 +/- 0.2[a]	1.5 +/- 0.1[a]	1.5 +/- 0.2[a]
Feed conversion ratio (kg feed/kg biomass)	1.46 +/- 0.04[a]	1.44 +/- 0.06[a]	1.37 +/- 0.08[a]
Survival (%)	97 +/- 1.6[a]	98 +/- 0.3[a]	96 +/- 2.6[a]

All were significantly different. Overall feed conversion rates were not significantly different between treatments averaging 1.37, 1.44, and 1.46 at the high, medium and low densities. Therefore, the differences in growth rates were attributed to differences in daily consumption (1.7, 1.5, and 1.3 at the low, medium, and high rearing densities, respectively) expressed as a percentage of the total standing crop biomass.

The results of this study demonstrated the negative effect that rearing density (number fish/unit volume) can have on growth and production characteristics. It was observed growth rate (Figure 2) and final weight (Table 2) at harvest were negatively correlated with density, whereas, biomass accumulation was positively correlated with density. It should be noted that growth rates determined during the current study at all densities were within the range growth rates (0.59 to 2.61 g/d) previously observed over a variety of environmental conditions and culture systems (Woods et al. 1983; Smith et al. 1985; Kerby et al. 1987, Wolters and DeMay 1997). This wide variation in growth rate was attributed to decreasing water temperatures during the fall and winter months. Wolters and DeMay (1997) reported that growth rates fell to 0.59 g/d at temperatures approaching 15°C. Kerby et al. (1987) observed superior growth rates, 2.6 g/d, when temperatures exceeded 24°C. The ability to maintain consistent, near optimal temperatures for hybrid striped bass led to the high growth rates observed in the present study. This significantly reduced the production cycle time to less than 224 d (7.5 months) for the low and medium densities.

Negative growth and density interactions have been observed when culturing other fish species. Hengsawat et al. (1997), rearing African catfish (*Clarias gariepinus*) in cages at different densities observed that mean weights decreased with increasing density and biomass accumulation positively correlated with stocking density. Pond-reared red tilapia were observed to obtained the highest growth rate at the lowest stocking density (Zonneveld and Fadholi 1991). However, contradictory findings have been reported for hybrid striped bass (Kerby et al. 1987) and by Papoutsoglou et al. (1997) for the European sea bass (*Dicentrarchus labrax*). Kerby et al.(1987) observed that a doubling in rearing density improved mean fish weight by 24.5%. Similarly, Liu et al. (1999) reported that palmetto bass growth increased with increasing density. The authors suggested that interaction between growth and

density may be further complicated by social interactions and physical constraints of the culture vessel as density increases.

It was observed that while daily growth rate slowed as rearing density increased, harvested biomass was significantly higher. This suggests that such aggressive stocking strategies might be employed to increase annual production totals. However, additional studies are needed to evaluate the long-term impact of the increased production time required for fish to reach market size on the overall economic efficiency of such a strategy.

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