North American Journal of Aquaculture 72:97–106, 2010
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DOI: 10.1577/A09-020.1

[Article]

Effect of Channel Catfish Stocking Rate on Yield and Water Quality in an Intensive, Mixed Suspended-Growth Production System

BARTHOLOMEW W. GREEN*[1]

U.S. Department of Agriculture, Agricultural Research Service, Aquaculture Systems Research Unit, Aquaculture/Fisheries Center, University of Arkansas at Pine Bluff, Pine Bluff, Arkansas 71601, USA

Abstract.—This study was conducted to determine the effect of stocking rate on yield of channel catfish *Ictalurus punctatus* and water quality in a mixed suspended-growth (biofloc) production system with zero water exchange. Channel catfish (National Warmwater Aquaculture Center 103 strain; average fish weight = 13 g) were stocked into nine 35-m^2 tanks (28 m^3/tank) at a rate of 2.9, 5.7, or 8.5 fish/m^2 for a 238-d grow-out period. One 1.865-kW blower for every three tanks supplied air continuously through a diffuser grid (constructed of 2.5-cm-diameter polyvinyl chloride pipe) on the bottom of each tank. Well water was added only periodically to replace evaporative losses. Fish in each tank were fed daily as much floating catfish feed (32% protein, extruded pellets) as they could consume in a 20-min period. Channel catfish net yield ranged from 0.99 to 3.71 kg/m^3 and increased linearly with stocking rate ($r^2 = 0.87$). At harvest, mean individual weight (0.54 kg/fish), survival (62.1%), specific growth rate (1.54% per day), and net feed conversion ratio (1.9) did not differ significantly among stocking rates. Cumulative feed addition averaged 6.66 kg/m^3 for the 8.5-fish/m^2 treatment, significantly greater than the 4.04 and 2.96 kg/m^3 for the 5.7- and 2.9-fish/m^2 treatments, respectively, which did not differ. Mean nitrate-nitrogen concentration was significantly higher and mean pH was significantly lower in the 8.5-fish/m^2 treatment compared with the other two treatments. There were no other differences in water quality among treatments. Total ammonia-nitrogen concentration was low throughout the experiment because of nitrification and phytoplankton uptake. This study demonstrated that high yields of channel catfish could be obtained by stocking up to 8.5 fish/m^2 in a mixed suspended-growth production system.

Aquaculture species retain only a portion of the nitrogen (N) consumed as feed. For example, channel catfish *Ictalurus punctatus* reared in earthen ponds retained 26% of feed N (Boyd 1985) and Pacific white shrimp *Litopenaeus vannamei* grown in earthen ponds retained 27.2–40.6% of feed N (Teichert-Coddington et al. 2000; Casillas-Hernández et al. 2006). Unassimilated feed N is excreted generally as ammonia-N, which can deteriorate water quality as its concentration increases. Management of total ammonia-N concentration in the culture unit increases in importance as feeding rate increases to maintain un-ionized ammonia concentrations below toxic or growth-limiting levels (Hargreaves and Kucuk 2001).

Ammonia-N concentration in earthen ponds is controlled primarily by phytoplankton uptake (Brune et al. 2003; Tucker and Hargreaves 2004). However, in addition to the role of phytoplankton, microbial processes that occur in the water column also are active in controlling water quality, and this assemblage of living organisms and particulate organic matter is referred to as a mixed suspended-growth system (Hargreaves 2006). This system also currently is referred to as a "biofloc" system but has had other names in the past; the term biofloc refers to the assemblage of living organisms and particulate organic matter (Burford et al. 2004; Hargreaves 2006; Avnimelech 2007). Hargreaves (2006) uses the term photosynthetic suspended-growth system to describe a system where phytoplankton uptake of N is the predominant process controlling water quality.

Ammonia-N is utilized by chemoautotrophic bacteria for the nitrification process and by heterotrophic bacterial biomass production (Brune et al. 2003; Ebeling et al. 2006). Ammonia-oxidizing bacteria oxidize ammonia-N to nitrite-N (NO_2-N) and nitrite-oxidizing bacteria oxidize NO_2-N to nitrate-N (NO_3-N) during nitrification. Heterotrophic bacterial biomass production in aquacultural systems immobilizes ammonia-N and is stimulated by increasing the organic carbon (C): N ratio through addition of a carbon source (e.g., wheat flour, cellulose, or molasses) with a high C:N ratio (Avnimelech et al. 1992; Avnimelech 1999; Ebeling et al. 2006). The increased heterotrophic

* Corresponding author: bart.green@ars.usda.gov

[1] Present address: U.S. Department of Agriculture, Agricultural Research Service, Stuttgart National Aquaculture Research Center, Post Office Box 1050, Stuttgart, Arkansas 72160, USA

Received March 20, 2009; accepted July 24, 2009
Published online December 21, 2009

bacterial biomass, in addition to immobilizing ammonia-N, can serve as a protein source for aquaculture species whose feeding habits allow consumption of the biofloc (Avnimelech 2007). Tilapia *Oreochromis* spp. (Avnimelech 2007; Azim and Little 2008; Avnimelech and Kochba 2009) and Pacific white shrimp (Burford et al. 2004; Wasielesky et al. 2006) derive nutrition from consumed biofloc.

Fish that do not consume the biofloc also can be grown in a mixed suspended-growth system. Production of hybrid striped bass (striped bass *Morone saxatilis* × white bass *M. chrysops*) did not differ significantly in tanks with continuous water exchange or managed to establish a mixed suspended-growth production system (Milstein et al. 2001). There are no reports in the literature of producing channel catfish in a mixed suspended-growth system, although Brune et al. (2001) reported producing channel catfish in a photosynthetic suspended-growth system. In a preliminary trial, growth and yield of hybrid catfish (channel catfish × blue catfish *I. furcatus*) were low in tanks using a mixed suspended-growth production system because the hybrid catfish apparently did not adapt well to the tank environment (author's unpublished data).

The objective of the present research was to evaluate the effect of stocking rate on channel catfish (National Warmwater Aquaculture Center 103 [NWAC103] strain) yield and water quality in a mixed suspended-growth production system with zero water exchange.

Methods

This research was conducted at the U.S. Department of Agriculture, Agricultural Research Service, Aquaculture Systems Research Unit, University of Arkansas, Pine Bluff. Nine wood-framed tanks (surface area, 35 m^2; volume, 28 m^3) with semicircular ends and equipped with a center divider were fitted with a 30-mil, high-density, polyethylene liner and used for this research. One 1.865-kW blower for every three tanks supplied air continuously through a diffuser grid (constructed of 2.5-cm-diameter polyvinyl chloride pipe) on the bottom of each tank. Two days after filling with well water (total alkalinity, 50.46 mg/L as $CaCO_3$), each tank was fertilized with 0.32 kg of 18-46-0 (18% N, 46% phosphate, 0% potash) fertilizer; the fertilizer was dissolved in a bucket of tank water before application. Sodium chloride was added to each tank to ensure that the chloride concentration exceeded 100 mg/L. One application of agricultural limestone (250 mesh) at 4.5 kg/tank was made to increase total alkalinity in recently filled tanks. Beginning in early September, agricultural limestone was added to tanks as needed to mitigate low water pH. Well water was added only periodically to replace evaporative losses.

A completely randomized design was used to test the effect of stocking 100, 200, or 300 channel catfish/tank (2.9, 5.7, or 8.5 fish/m^2; 3.6, 7.0, or 10.7 fish/m^3). Tanks were stocked on 22 March 2007 with fingerling NWAC103-strain channel catfish (Wolters et al. 2000; Bosworth et al. 2004) that had been vaccinated against the pathogen *Edwardsiella ictaluri* (AQUAVAC-ESC; Intervet/Schering-Plough Animal Health, Millsboro, Delaware) and purchased from an Arkansas catfish producer. At stocking, fish weight averaged 13 g. Beginning on 28 March 2007, fish were fed a 32%-protein, floating, extruded feed daily to apparent satiation (20 min) and the amount of feed used was recorded. The weight of dead fish recovered was also recorded. All tanks were harvested by draining 238 d after stocking. At harvest, fish from each tank were counted and weighed individually. Gross yield was calculated as the sum of individual weights of harvested fish; net yield was calculated as gross yield minus the biomass of fish stocked. Feed conversion ratio (FCR) was calculated per tank as the total quantity of feed fed divided by the sum of the net total channel catfish yield. Specific growth rate (SGR) was calculated as:

$$\mathrm{SGR} = 100 \times \frac{\log_e W_{t+1} - \log_e W_t}{T_{t+1} - T_t},$$

where W represents mean individual weight (g) at time $t + 1$ or time t, and T represents time $t + 1$ or t in days.

Water samples were collected around 0700 hours from each tank on a weekly basis during March through November. In each tank, a clean, empty, uncapped, 1-L sample bottle was submerged in inverted position to approximately 30 cm depth and turned upright to collect the sample. Samples were capped, stored in a cooler on ice, and transported to the laboratory, where analyses began immediately. Nitrite-N (diazotization), NO_3-N (cadmium reduction), total ammonia-N (TAN, salicylate method), and soluble reactive phosphorus (ascorbic acid method) were analyzed using flow-injection analysis according to manufacturer instructions (FIAlab 2500; FIAlab Instruments, Bellevue, Washington). Chlorophyll *a* was extracted in 2:1 chloroform : methanol from phytoplankton, which were filtered from samples using a glass fiber filter (0.45-μm pore size); the chlorophyll-*a* concentration in the extract was determined by spectroscopy (Lloyd and Tucker 1988). Settleable solids were quantified beginning 23 May 2007 using an Imhoff cone (Eaton et al. 2005). Total alkalinity was measured on five occasions throughout the experiment by titration (Eaton et al. 2005). Dissolved oxygen (DO) concentration and water temperature in each tank were monitored continuously beginning in mid-April by a

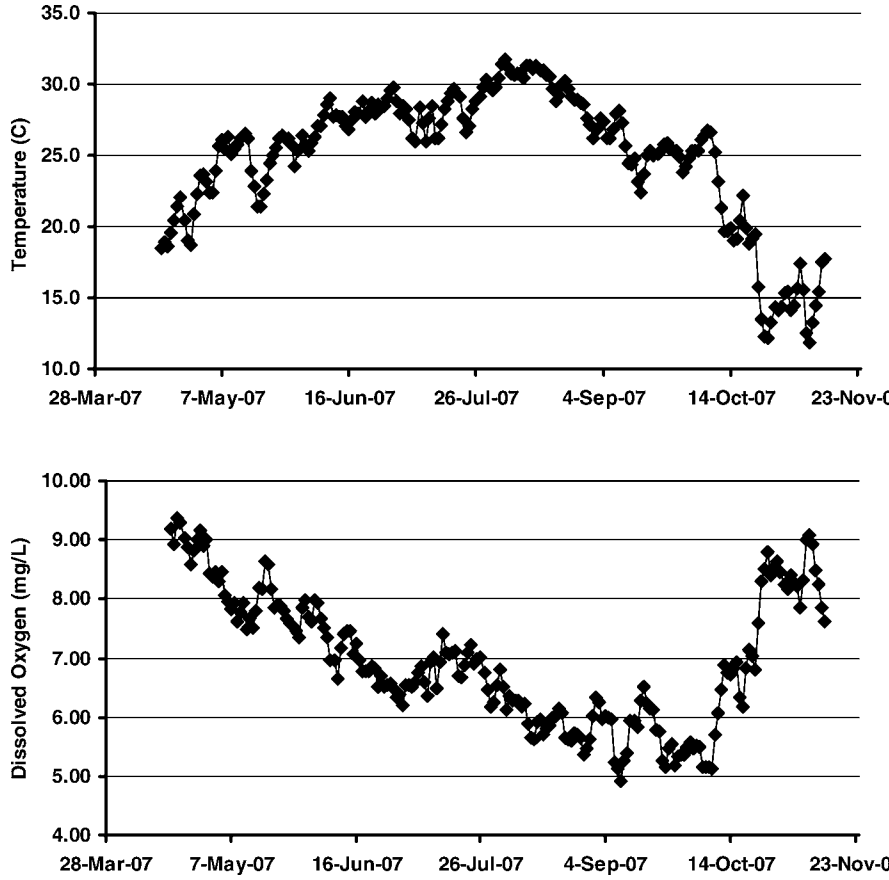

FIGURE 1.—Mean daily water temperature (°C; upper panel) and dissolved oxygen concentration (mg/L; lower panel) in mixed suspended-growth (biofloc) production system tanks stocked with channel catfish at 2.9–8.5 fish/m^2.

galvanic DO probe (Sensorex, Garden Grove, California) and a thermistor (Model 109; Campbell Scientific, Logan, Utah) connected to a data logger (Model CR10X; Campbell Scientific). The nitrification rate in tanks was estimated from the NO_3-N accumulation rate.

In mid-June, most of the fish in one replicate of the 5.7-fish/m^2 treatment died overnight. The cause of the fish mortality was unknown but may have been a toxic algal bloom since the chlorophyll-*a* concentration increased from 715.4 mg/m^3 on 13 June to 1,378.1 mg/m^3 on 20 June; concentrations of DO and water quality variables were within acceptable limits during the week before the die-off. Data from this replicate were excluded from analyses and reporting. Survival (%) data were arcsine transformed before analysis (Sokal and Rohlf 1995). Data were analyzed by mixed-models analysis of variance and linear regression analysis using the Statistical Analysis System version 9.1.3 (SAS Institute, Inc., Cary, North Carolina). Significance level was set at 0.05.

Results

Mean daily DO concentration in tanks generally exceeded 5.0 mg/L, and average DO percent saturation ranged from 62% to 100% (Figure 1). No significant difference among treatments was detected for mean DO concentrations, which averaged 7.1, 7.0, and 6.8 mg/L for the 2.9-, 5.7-, and 8.5-fish/m^2 treatments, respectively. Minimum DO concentrations also did not differ significantly among treatments and averaged 6.1, 5.9, and 5.8 mg/L for the 2.9-, 5.7-, and 8.5-fish/m^2 treatments, respectively. Tank water temperature varied seasonally (Figure 1). Mean maximum tank water temperature during the hottest part of the summer did not exceed 33.5°C. The average difference between the maximum and minimum tank daily water temperatures was 3.2°C.

Although channel catfish net yield for the 8.5-fish/m^2 treatment (3.71 kg/m^3) was significantly greater

TABLE 1.—Least-squares means (±SE) of individual weight at harvest, gross and net yields, net daily yield, survival, specific growth rate (SGR), and feed conversion ratio (FCR) for channel catfish stocked at 2.9–8.5 fish/m^2 in mixed suspended-growth (biofloc) production system tanks. Means followed by the same letter within a column are not significantly different ($P > 0.05$).

Stocking rate (fish/m^2)	Individual weight (kg/fish)	Yield (kg/m^3)		Net daily yield (g•m^{-3}•d^{-1})	Survival (%)	SGR (%/d)	FCR
		Gross	Net				
2.9	0.57 ± 0.02 z	1.04 ± 0.32 z	0.99 ± 0.32 z	4.1 ± 1.3 z	48.2 ± 1.0 z	1.58 ± 0.02 z	3.6 ± 0.6 z
5.7[a]	0.47 ± 0.03 z	2.15 ± 0.39 z	2.04 ± 0.39 z	8.6 ± 1.6 z	65.9 ± 1.6 z	1.50 ± 0.03 z	2.0 ± 0.8 z
8.5	0.50 ± 0.02 z	3.87 ± 0.32 y	3.71 ± 0.32 y	15.6 ± 1.3 y	72.7 ± 1.0 z	1.52 ± 0.02 z	1.8 ± 0.6 z

[a] $N = 2$, $N = 3$ for other two treatments.

than for the two lower stocking rates, which did not differ significantly (Table 1), net yield (y, kg/m^3) increased linearly with increased stocking rate (x, fish/m^2) and was described by the equation $y = 0.4838x - 0.4801$ ($r^2 = 0.87$). Net daily yield (g•m^{-3}•d^{-1}) differed significantly among treatments (Table 1) and increased linearly with increased stocking rate ($r^2 = 0.87$). Mean individual weight at harvest did not differ significantly among treatments (Table 1), although a weak inverse relationship between individual weight at harvest and stocking rate was observed ($y = -0.0745 \log_e x + 0.6433$; $r^2 = 0.4378$). No significant differences among treatments were detected for survival, SGR, and FCR (Table 1).

Feed consumption increased (Figure 2) beginning in late April, when water temperatures remained above 20°C consistently (Figure 1). Sustained high feeding rates were observed from mid-July through early October and averaged 24.2, 30.0, and 49.1 g•m^{-3}•d^{-1} for the 2.9-, 5.7-, and 8.5-fish/m^2 treatments, respectively. The mean daily feed loading by week for the entire culture period was significantly greater for the 8.5-fish/m^2 treatment (28.8 g•m^{-3}•d^{-1}) than for the 5.7-fish/m^2 treatment (17.4 g•m^{-3}•d^{-1}) and 2.9-fish/m^2 treatment (12.7 g•m^{-3}•d^{-1}), which did not differ significantly. Cumulative feed addition averaged 6.66 kg/m^3 for the 8.5-fish/m^2 treatment, significantly greater than the 4.04 and 2.96 kg/m^3 for the 5.7- and

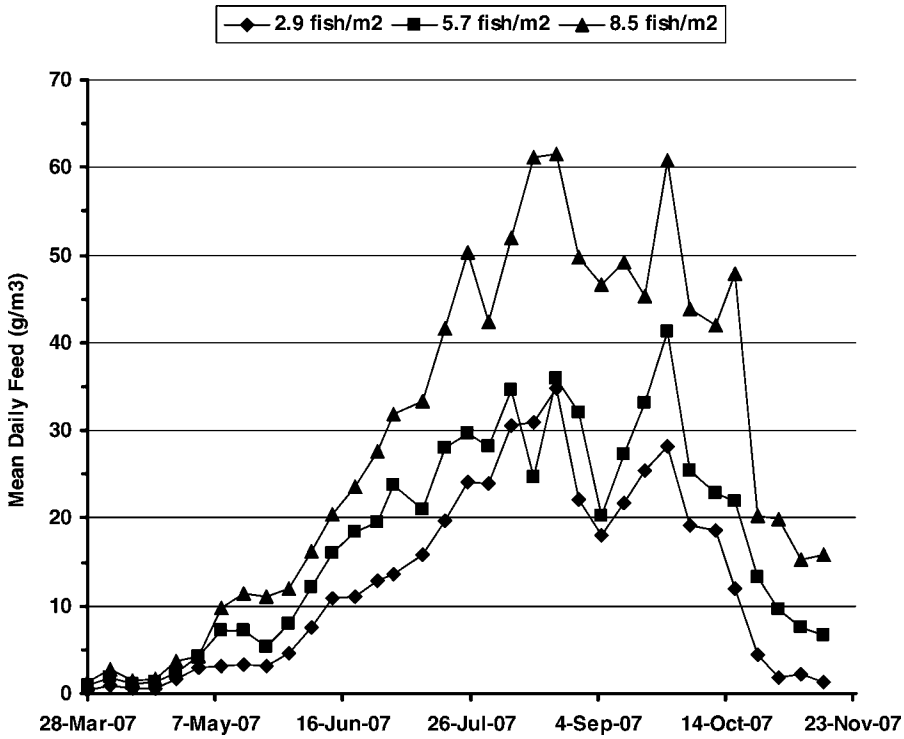

FIGURE 2.—Mean daily feed consumption (g/m^3; by week) by channel catfish stocked at 2.9–8.5 fish/m^2 in mixed suspended-growth (biofloc) production system tanks.

TABLE 2.—Least-squares means (±SE) for water quality variable concentrations (TAN = total ammonia nitrogen; SRP = soluble reactive phosphorus; chl *a* = chlorophyll *a*) in mixed suspended-growth (biofloc) production system tanks stocked with channel catfish at 2.9–8.5 fish/m^2. Means followed by the same letter within a column are not significantly different ($P > 0.05$).

Stocking rate (fish/m^2)	pH	Total alkalinity (mg/L as $CaCO_3$)	TAN (mg/L)	Nitrite-N (mg/L)	Nitrate-N (mg/L)	SRP (mg/L)	Chl *a* (mg/m^3)	Settleable solids (mL/L)
2.9	7.77 ± 0.04 z	63.5 ± 4.1 z	0.30 ± 0.07 z	0.25 ± 0.09 z	5.98 ± 1.24 z	3.1 ± 0.4 z	690.8 ± 122.5 z	20.4 ± 3.2 z
5.7[a]	7.72 ± 0.05 z	66.6 ± 5.0 z	0.25 ± 0.08 z	0.23 ± 0.12 z	7.59 ± 1.52 z	4.1 ± 0.5 z	1,014.5 ± 150.0 z	31.6 ± 4.0 z
8.5	7.51 ± 0.04 y	47.8 ± 4.1 z	0.49 ± 0.07 z	0.42 ± 0.09 z	19.20 ± 1.24 y	5.7 ± 0.4 y	1,041.3 ± 122.5 z	34.2 ± 3.2 z

[a] $N = 2$, $N = 3$ for other two treatments.

2.9-fish/m^2 treatments, respectively, which did not differ. Feed consumption decreased in mid- to late October as water temperature declined to 20°C and below.

Treatment means of water quality variables are shown in Table 2. Mean concentrations of dissolved inorganic N species by treatment across sampling dates are shown in Figure 3. Mean TAN was less than 0.5 mg/L and did not differ among treatments (Table 2). There was a weak ($r^2 = 0.23$), positive linear relationship between mean TAN concentration and feed input. Mean NO_2-N concentration also did not differ significantly among treatments, whereas mean NO_3-N concentration for the 8.5-fish/m^2 treatment was significantly greater than those for the 5.7- and 2.9-fish/m^2 treatments (Table 2). There was a positive linear relationship ($r^2 = 0.76$) between mean NO_3-N concentration and cumulative feed input. The nitrification rate averaged 0.10, 0.15, and 0.28 mg $N \cdot L^{-1} \cdot d^{-1}$for the 2.9-, 5.7-, and 8.5-fish/m^2 treatments, respectively, and increased linearly with daily feed input ($r^2 = 0.62$).

Mean chlorophyll-*a* concentration did not differ significantly among treatments (Table 2); however, treatment means increased as feed input increased ($r^2 = 0.48$). Chlorophyll-*a* concentration in all treatments consistently exceeded 500 mg/m^3 beginning 30 May (Figure 4). Mean settleable solids concentration did not differ significantly among treatments (Table 2); however, 58.8% of the variation among tank means was accounted for by mean daily feed rate. The trends in mean settleable solids concentrations for each treatment throughout the experiment are shown in Figure 4. The drop in mean settleable solids for all treatments between 5 and 12 September may have been weather related: two cold fronts associated with cooler air temperatures and increased winds penetrated the region during this period, and 5.6 cm of rain fell on 11 September. Mean pH in all treatments began to decrease beginning in mid-June, and the greatest decline occurred in the 8.5-fish/m^2 treatment (Figure 4). Mean pH in the 8.5-fish/m^2 treatment was significantly less than mean pH values for the other two treatments (Table 2). Although total alkalinity did not differ significantly among treatments (Table 2), mean total alkalinity in the 8.5-fish/m^2 treatment declined by 65.6 mg/L as $CaCO_3$ between May (prenitrification) and September, whereas the decrease in the other two treatments ranged from 35.4 to 39.2 mg/L as $CaCO_3$.

Discussion

Environmental conditions in the tank were suitable for channel catfish culture. Mean daily tank water temperatures and the seasonal variation in tank water temperature were similar to those observed in ponds in Arkansas (Green and Popham 2008). The level of aeration provided to the tanks was sufficient to maintain the mean DO concentration at greater than 60% saturation and minimum DO concentration above 42% saturation in all tanks, even at the high feeding rates used in this study. Torrans (2008) reported reductions in channel catfish feed consumption and growth when mean DO concentration was less than 2.68 mg/L, or about 33% of saturation.

Channel catfish net yield from tanks in the present study ranged from 0.99 to 3.71 kg/m^3 (9,900–37,100 kg/ha), and net daily yield ranged from 4.1 to 15.6 $g \cdot m^{-3} \cdot d^{-1}$ (41–156 $kg \cdot ha^{-1} \cdot d^{-1}$). The low end of this range approximated results reported for channel catfish production in earthen ponds, which represents a fundamental level of a photosynthetic suspended-growth production system because algal primary productivity is the dominant process affecting pond water quality (Hargreaves 2006). On average, 4,143 kg/ha or 0.30 kg/m^3 of food-sized channel catfish were harvested in 2002 from the typical commercial channel catfish production pond, which averaged 1.37 m deep (USDA 2003). Other reports representative of channel catfish net yield from earthen ponds included 6,273 kg/ha (~0.51 kg/m^3; Green et al. 2009), 6,467 kg/ha (~0.47 kg/m^3; Torrans 2008), and 8,995 kg/ha (~0.66 kg/m^3; Li et al. 2005). Net daily yields for these studies ranged from 3.5 to 4.2

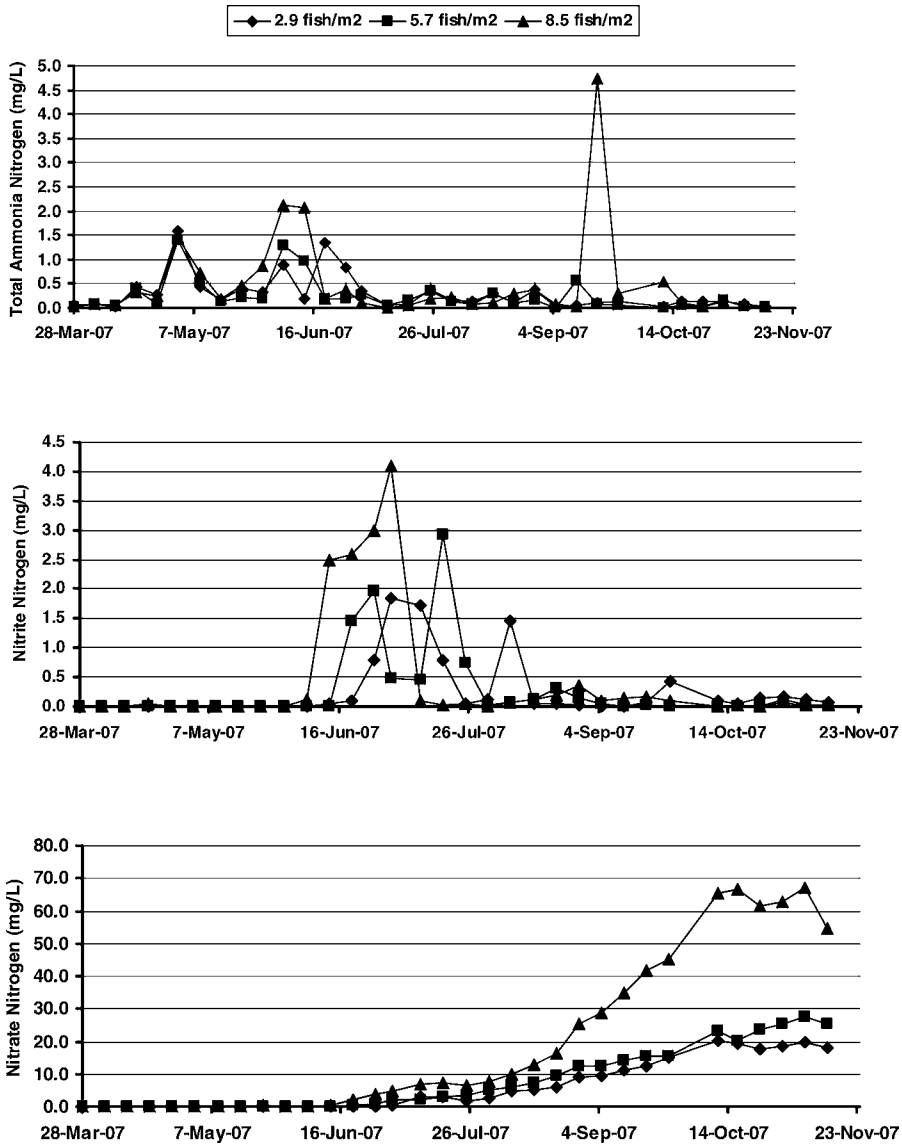

FIGURE 3.—Changes in concentrations (mg/L) of dissolved inorganic nitrogen species during the channel catfish culture cycle in mixed suspended-growth (biofloc) production system tanks stocked at 2.9–8.5 fish/m^2.

$g \cdot m^{-3} \cdot d^{-1}$. Torrans (2005) reported channel catfish net yields of 10,830 and 23,547 kg/ha (0.90–1.96 kg/m^3) from intensively aerated and circulated ponds. The corresponding net daily yields were 3.6 and 7.8 $g \cdot m^{-3} \cdot d^{-1}$.

Pond water quality variable concentrations reported by Torrans (2005) ranged from 342 to 439 mg/m^3 for chlorophyll *a*, from 1.20 to 1.26 mg/L for TAN, from 0.30 to 0.53 mg/L for NO_3-N, from 113 to 140 mg/L for total suspended solids (TSS), and from 42 to 45 mg/L for volatile suspended solids (VSS). Phytoplankton productivity appeared to be the dominant process controlling water quality in Torrans' (2005) ponds, and heterotrophic bacterial productivity probably had a subordinate role in controlling water quality. The 40–45-mg/L VSS concentration range was similar to that reported for the partitioned aquaculture system (PAS; Brune et al. 2003). The low NO_3-N concentrations and moderate TAN concentrations reported by Torrans (2005) indicated that nitrification played a minor role in controlling pond water quality. The continuously operating circulator in ponds in that study probably

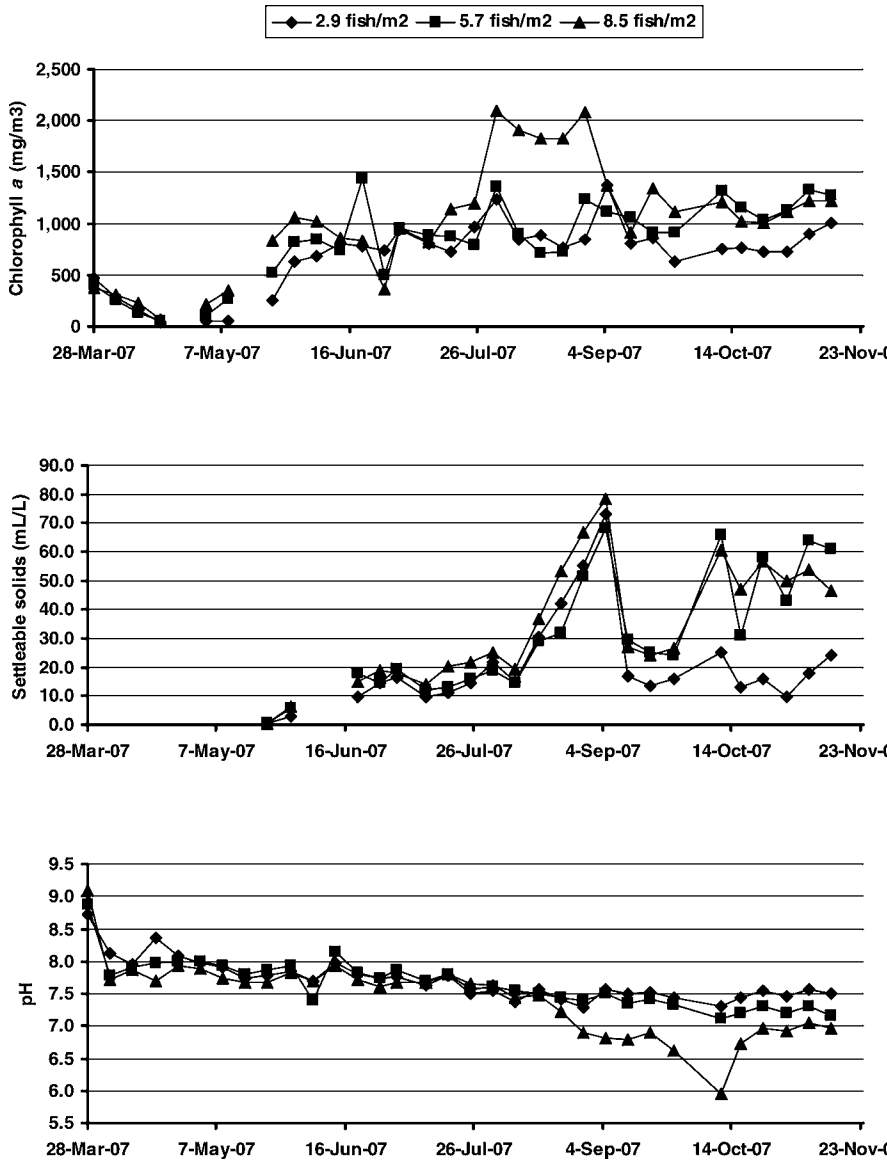

FIGURE 4.—Mean chlorophyll-*a* concentration (mg/m^3), settleable solids concentration (mL/L), and pH throughout the channel catfish production season in mixed suspended-growth (biofloc) production system tanks stocked at 2.9–8.5 fish/m^2. A gap in data among dates indicates that an analysis was not done. Settleable solids analysis was initiated on 23 May 2007.

promoted the suspended-growth system relative to ponds without continuous circulation.

Fish in all treatments in the present experiment were fed at high rates (24.2–49.1 g•m^{-3}•d^{-1}) through most of the growing season. The decrease in mean daily feed ration observed in late August–early September probably was related to decreased tank water temperatures caused by the arrival of the first of the autumnal cold fronts into the region. For comparison, channel catfish in food-fish production ponds were fed 110–135 kg•ha^{-1}•d^{-1}, on average, during the production season (Robinson et al. 2004), which was equivalent to 8.0–9.9 g•m^{-3}•d^{-1}. Daily feeding rates for food-fish ponds could be as high as 168–224 kg/ha (12.3–16.4 g•m^{-3}•d^{-1}), but these rates would not be sustained. Torrans (2005) reported that the mean feeding rate for a 252-d study was 168 kg•ha^{-1}•d^{-1} (14.0 g•m^{-3}•d^{-1}) for channel catfish in ponds where DO concentration

was maintained above 50% saturation. Torrans (2005) maintained an average feeding rate of 328 $kg \cdot ha^{-1} \cdot d^{-1}$ (27.3 $g \cdot m^{-3} \cdot d^{-1}$) in those ponds during August. Mean FCR values for the present study were consistent with those reported for other channel catfish production studies (e.g., Li et al. 2005; Torrans 2005).

The PAS is a higher-level photosynthetic suspended-growth system in which channel catfish productivity is greater than in earthen ponds and more similar to the mixed suspended-growth production system tested in this experiment than to conventional pond culture. In the PAS, the phytoplankton population is managed to sustain high primary productivity, which uses N from excreted feed and supplies much of the DO required (Brune et al. 2001, 2003). Channel catfish are the primary culture species, are confined to about 3.5% of the system volume (Brune et al. 2001, 2003; Baumgarner et al. 2005), and are fed intensively. Tilapia are co-stocked into the system but are confined to a separate compartment (~1.4% of system volume) and graze on the algal population. Brune et al. (2004) reported channel catfish net yield as high as 4.2 kg/m^3 and total net fish yield (channel catfish plus Nile tilapia *Oreochromis niloticus*) as high as 5.7 kg/m^3 during the 200-d growing season, with net daily channel catfish yield of 21.1 $g \cdot m^{-3} \cdot d^{-1}$ and net daily total fish yield of 26.6 $g \cdot m^{-3} \cdot d^{-1}$. The average daily feed rate was 28.0 $g \cdot m^{-3} \cdot d^{-1}$, and the maximum feed rate was 63.5 $g \cdot m^{-3} \cdot d^{-1}$ (Brune et al. 2004). The channel catfish average and maximum feeding rates, net yield, and net daily yield for the 8.5-fish/m^2 treatment in the present experiment are comparable with results reported for the PAS.

Other than the initial application of fertilizer, formulated feed was the sole managed nutrient source for tanks. As the feeding rate began to increase in late April, mean TAN concentrations began to increase, reaching a maximum of 2.1 mg/L, then decreasing to low levels for the remainder of the season (Figure 3). Un-ionized ammonia concentrations in tanks were calculated from TAN concentration, pH, and water temperature (Emerson et al. 1975) and consistently were less than the threshold of 0.91-mg/L NH_3-N reported to affect channel catfish growth (Hargreaves and Kucuk 2001). The TAN spike observed in mid-September resulted from a sudden transitory spike in one tank; this TAN spike probably resulted from sample contamination because during the preceding week, the chlorophyll-*a* concentration in that tank increased from 884 to 1,164 mg/m^3, the settleable solids concentration was stable at 21 mL/L, and daily feed rate averaged 32 g/m^3. Un-ionized ammonia concentration during the TAN spike was not of concern because at the prevailing water temperature and pH (23–27°C and 6.75, respectively), the un-ionized ammonia level ranged from 0.32% to 0.44% (Emerson et al. 1975).

The onset of nitrification in this study was judged to occur when NO_3-N concentrations increased from low levels (~0.025-mg/L NO_3-N) to levels consistently above 0.100-mg/L NO_3-N. Populations of nitrifying bacteria appeared to become active in all treatments beginning in mid-June. Increased NO_2-N concentrations persisted through late July in all treatments, overlapped somewhat with the increased TAN concentrations in mid-June, and then decreased to low levels (Figure 3). The NO_3-N concentration increased in all treatments beginning in mid-June, and the increase continued through mid-October, when concentrations stabilized (Figure 3). Although the nitrification rate increased with increased feeding rate, the calculated nitrification rate may underestimate the actual nitrification rate because phytoplankton utilize NO_3-N when TAN concentration is low (Burford et al. 2003). The estimated nitrification rate in a mixed suspended-growth system averaged 3.2 mg $N \cdot L^{-1} \cdot d^{-1}$ for Nile tilapia culture (Rakocy et al. 2005) and averaged 0.1–0.6 mg $N \cdot L^{-1} \cdot d^{-1}$ for Pacific white shrimp culture (Burford et al. 2003). The higher nitrification rate reported by Rakocy et al. (2005) probably resulted from the higher average and maximum feeding rates (127 and ~200 $g \cdot m^{-3} \cdot d^{-1}$, respectively). Mean treatment pH began to decrease concurrently with the increase in NO_3-N concentration as a result of nitrification (Figure 4), and this decrease was mitigated by the addition of agricultural limestone.

There was a substantial lag between the initiation of feeding and the onset of nitrification. Ammonia-N is considered to be the growth-limiting nutrient for the nitrifying bacteria *Nitrosomonas* spp. (Sharma and Ahlert 1977). Phytoplankton are thought to compete more effectively for ammonia-N than nitrifying bacteria when concentrations during the growing season are low in channel catfish ponds (Hargreaves 1997). Phytoplankton biomass, as indicated by chlorophyll-*a* concentration, began to increase in May as feeding rates increased; by 30 May 2007, mean chlorophyll *a* exceeded 500 mg/m^3 in all treatments. The ammonia-N concentration began to increase once the rapid increase in chlorophyll-*a* concentration slowed. It was at this point in early to mid-June that nitrification began and TAN decreased. The cumulative feed N addition at the onset of nitrification (calculated based on 91.5% feed dry matter [Boyd 1985] and feed N content [% protein in feed/6.25]) did not differ significantly among treatments and averaged 22.5, 30.2, and 29.5 g N/m^3 for the 2.9-, 5.7-, and 8.5-fish/m^2 treatments, respectively. However, the onset of

nitrification in the 8.5-fish/m^2 treatment occurred after 79 feed-days, significantly sooner than the 97 feed-days for the 2.9-fish/m^2 treatment. Onset of nitrification for the 5.7-fish/m^2 treatment occurred after 90 feed-days, which was not significantly different from the other treatments. Thus, it appears that a threshold ammonia-N concentration had to be attained before nitrification could begin.

Total alkalinity was destroyed by nitrification. Although the decline in total alkalinity between May (prenitrification) and September did not differ significantly among treatments, the decrease in the 8.5-fish/m^2 treatment was 67–85% higher and was related to the greater nitrification rate in response to the greater feed input.

High feeding rates were sustained in all treatments during mid-July through early October. Channel catfish retained only 26.8% of feed N, with the remainder excreted primarily as ammonia-N (Boyd 1985). However, TAN concentrations remained low in all treatments during this period. The sustained high concentrations of chlorophyll *a* in all treatments beginning in early June indicated a persistent high rate of primary productivity that was contemporaneous with nitrification. High rates of primary productivity were sustained throughout the experiment, probably because phytoplankton used NO_3-N as a source of N, particularly when TAN concentrations were low and NO_3-N concentrations were high (Burford et al. 2003).

In summary, the results of this experiment demonstrated that channel catfish could be reared in a zero-exchange, mixed suspended-growth (biofloc) production system at stocking rates of up to 8.5 fish/m^2 and at average daily feeding rates that were approximately five times higher than those for food-fish production ponds. Channel catfish productivity at the highest stocking rate approximated that reported for the PAS. Excreted feed N appeared to be used predominantly by phytoplankton during the first third of the production cycle was apparently used by nitrifying bacteria primarily (as TAN) and by phytoplankton secondarily (as NO_3-N) during the remainder of the experiment. As a result, TAN concentrations remained low in all treatments throughout the production cycle.

Acknowledgments

George Huskey, Joshua McNeely, and James Mauldin are thanked for their assistance during this study. I thank Jerry Ludwig, Les Torrans, and Peter Perschbacher for reviewing a draft of this manuscript. The Southern Regional Aquaculture Center Innovative Technologies and Methodologies for Commercial-Scale Pond Aquaculture Project provided partial funding for this research. Mention of trade names or commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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