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Effect of Stocking Large Channel Catfish in a Biofloc Technology Production System on Production and Incidence of Common Microbial Off-Flavor Compounds

Bartholomew W Green[1]* and Kevin K Schrader[2]

[1]US Department of Agriculture, Agriculture Research Service, Harry K. Dupree Stuttgart National Aquaculture Research Center, Stuttgart, Arkansas USA
[2]US Department of Agriculture, Agricultural Research Service, Natural Products Utilization Research Unit, Thad Cochran National Center for Natural Products Research, University, Mississippi, USA

Abstract

Density-dependent production and incidence of common microbial off-flavors caused by geosmin and 2-methylisoborneol were investigated in an outdoor biofloc technology production system stocked with stocker-size (217 g/fish) channel catfish at 1.4, 2.1, or 2.8 kg/m^3. Individual weight at harvest ranged from 658-829 g/fish and was inversely related to stocking density. Net fish yield ranged from 3.8-5.4 kg/m^3, and increased linearly as stocking density increased. The percentage of sub-marketable fish (<0.57 kg/fish) increased linearly with increasing stocking rate. Mean total feed consumption increased linearly with stocking density, but feed consumed per fish was inversely related to stocking density. Feed conversion ratio did not differ significantly among treatments. Concentrations of geosmin and 2-methylisoborneol in biofloc water were low throughout the study. All sampled fillets contained low concentrations of geosmin and 2-methylisobornel, but these fillets likely would not be deemed as having objectionable "earthy" or "musty" off-flavors when evaluated by trained processing plant flavor testers because of the low concentrations present. Data from this study combined with data from our two previous studies provide strong evidence that the incidence of geosmin- and 2-methylisoborneol-related off-flavor episodes is low in the BFT production system.

Keywords: Biofloc technology; Channel catfish; Ictalurus Punctatus; Stockers; Density; Geosmin; 2-Methylisoborneol; MIB; Market-Size fish

Introduction

High yields are obtained from the biofloc technology (BFT) production system in response to high stocking and feeding rates because the biofloc, which is maintained in suspension by continuous aeration, metabolizes excreted feed nitrogen [1,2]. Net yield of market-size channel catfish (*Ictalurus punctatus*) as high as 9.3 kg/m^3 has been reported for BFT production [3]. In addition to channel catfish, the BFT production system is used to grow the Pacific white shrimp (*Litopenaeus vannamei*) [4] and [5] and Nile tilapia (*Oreochromis niloticus*) [2] and [6].

Stocking rate is known to affect channel catfish production at different life stages and in a variety of production environments, including the BFT production system [7-9]. Although, individual fish growth and final fish size are inversely related to stocking rate, yield can increase with stocking rate because of the greater number of fish. In investigating density effects on channel catfish production in BFT production, stocking rate was inversely related to individual weight at harvest, but positively related to net fish yield [3,9,10]. Stocker-size catfish (115-150 g/fish) are being stocked increasingly by farmers in food-fish ponds so that harvested fish are within the 0.57-2.04 kg/fish size range preferred by processing plants. In 2009, 56.6% of fish stocked into production ponds by farmers were stocker catfish [11].

The effect of stocking rate on rearing stocker-size catfish to market size in BFT production has not been researched. Only one study addresses production of market size catfish in ponds stocked with stocker catfish. In that study, up to 98.5% of the channel catfish population was within the preferred size range when ponds were stocked with 0.26 kg/fish average-size stocker catfish [12]. Thus, it is important to determine how stocking rate of stocker-size catfish affects production of market size fish in BFT production.

Geosmin and 2-methylisoborneol (MIB) are the compounds responsible for the "earthy" and "musty" off-flavors, respectively, and these compounds can accumulate in fish flesh and temporarily render them unmarketable [13] and [14]. Harvest delays caused by off-flavor episodes are a persistent problem for catfish farmers: 69.6% of operations and 53.3% of food-fish ponds experienced delayed harvest in 2002, and 80.7% of operations and 48.1% of food-fish ponds experienced delayed harvest in 2009 [11,15]. Geosmin and MIB have been detected in channel catfish BFT culture units, but aqueous concentrations generally are low and in preceding studies only 11% of culture units contained fish that would be judged as having "earthy" or "musty" off-flavors when evaluated by trained processing plant flavor testers [3,10]. In contrast, concentrations of geosmin and MIB in food-fish pond waters can exceed 2,000 ng/L and 700 ng/L, respectively [16-18], and as many as 76% of ponds may contain off-flavored fish from July-September [19]. Thus, reduced incidence of episodes of "earthy" or "musty" off-flavors is a potential advantage of the BFT production system compared to static-water pond systems.

In this study we sought to determine the effect of initial biomass of stocker-size channel catfish on production characteristics, water

***Corresponding author:** Green BW, US Department of Agriculture, Agricultural Research Service, Harry K. Dupree Stuttgart National Aquaculture Research Center, USA, Tel: 870-673-4483; E-mail: bart.green@ars.usda.gov

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quality, and microbial off-flavor compounds in an outdoor BFT production system.

Materials and Methods

Biofloc technology production system

This study was carried out in nine 15.6-m^3 tanks located outdoors at the USDA Agricultural Research Service, Harry K. Dupree Stuttgart National Aquaculture Research Center (HKDSNARC), Stuttgart, AR, USA. Triplicate tanks (described in detail by Green et al. [10]) were assigned using a completely randomized design to initial fish biomass treatments of 1.4, 2.1, or 2.8 kg/m^3 (5.4, 8.1, or 10.8 fish/m^2; designated LO, MED, HI, respectively). Animal care and experimental protocols were approved by the HKDSNARC Institutional Animal Care and Use Committee and conformed to ARS Policies and Procedures 130.4 and 635.1.

Between 23 April-9 May 2012, tanks were filled with well water (total alkalinity=228.4 mg/L as $CaCO_3$), and each was seeded with 2.3 m^3 of water from a HKDSNARC pond containing a phytoplankton bloom, fertilized with 1.5 kg 11-37-0 (N-P-K) and 2.0 kg dried molasses (Sweet45, Westway Feed Products, New Orleans, LA, USA), and treated with 4.5 kg stock salt to ensure that chloride concentration exceeded 100 mg/L. Pond water was added to the tanks to expedite development of a phytoplankton bloom to aid in the removal of total ammonia-N (TAN). An additional mean of 2.4, 2.0, and 4.7 kg dried molasses was added to LO, MED, and HI treatment tanks, respectively, from 15 May-1 June. Dried molasses was added to tanks as a carbon source to stimulate bacterial transformation of TAN [20,21]. No water was exchanged, but well water was added as needed to replace evaporative loss and losses to draining settling chambers. Sodium bicarbonate (1.13 kg/tank) was added as needed to maintain pH above pH 7.0; mean total sodium bicarbonate added was 5.7, 7.6, and 8.8 kg/tank for the LO, MED, and HI treatments, respectively.

Each tank was equipped with a 130-L (117-L working volume) conical-bottom settling chamber; a 2.5-cm diameter air lift moved water to the settling chamber at 5.6 L/min. Settling chambers were operated, on average, 5.9 h/d on 11 d during the period 26 July-14 August in order to reduce TSS concentration to approximately 300-400 mg/L as recommended by Green et al. [10].

Catfish stocking and feeding rates

Stocker channel catfish were harvested from a holding pond, and fish from the population retained by a No. 70 bar grader (217 ± 74 g/fish; mean ± SD; coefficient of variation, CV,=34.1%) were stocked randomly into tanks on 10 May 2012. A random sample of 310 fish from the initial population was weighed individually (Figure 1). Fish were fed once daily with a commercially produced 32% protein floating extruded feed (premium formulation, Delta Western Feed Mill, Indianola, Mississippi) to apparent satiation (10 min) and the amount was recorded. Feed conversion ratio (FCR) was calculated for each tank as the total quantity of administered feed (wet weight) divided by the net total yield. The weight of dead fish recovered was recorded, but not all mortalities were recovered; weight of dead fish was not included in FCR calculation. All tanks were harvested by draining; two replicate tanks per treatment were harvested on 11 October and the remaining replicate per treatment was harvested on 12 October. At harvest, all fish per tank were weighed individually.

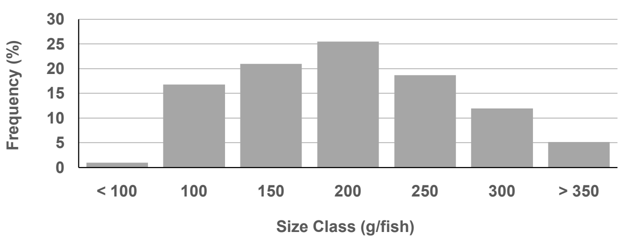

Figure 1: Mean size class distribution of the population of stocker channel catfish stocked into an outdoor biofloc technology production system. Mean (± SD) initial weight was 217 ± 74 g/fish.

Water quality analyses

Water samples were collected weekly from each tank. Sample pH was measured electrometrically. Total alkalinity (titration to pH 4.5), settleable solids (SS), total suspended solids (TSS), and total volatile solids (TVS) in raw samples were measured using methods given by Eaton et al. [22]. Water was filtered through 0.2-um pore size membrane filter and analyzed for nitrite-nitrogen (NO_2-N, diazotization), nitrate-nitrogen (NO_3-N, cadmium reduction), and soluble reactive phosphorus (PO_4-P, ascorbic acid method) using flow injection analysis according to manufacturer instructions (FIAlab 2500; FIAlab Instruments, Bellevue, WA, USA). Flow injection analysis also was used to quantify total ammonia-nitrogen (TAN) fluorometrically in filtered samples using the *o*-phthaldialdehyde method of Genfa and Dasgupta [23]. Water samples were filtered through a 0.45-um pore size glass fiber filter for chlorophyll *a* analysis. Chlorophyll *a* was extracted in 2:1 chloroform:methanol from the phytoplankton (planktonic algae and cyanobacteria as well as those associated with the biofloc) retained on the filter, and the chlorophyll *a* concentration in the extract was determined by spectroscopy [24].

Dissolved oxygen (DO) and temperature in each tank were monitored continuously (10-sec scan rate) by a galvanic oxygen sensor (Type III, Oxyguard, Birkerød, Denmark) and a thermister (Model 109, Campbell Scientific, Logan, UT, USA) connected to a datalogger (Model CR206 or CR1000, Campbell Scientific, Logan, UT, USA).

Determination of microbial off-flavor compounds

Water samples were collected from each tank on 20 June and at approximately 4-wk intervals thereafter through 10 October for analysis of geosmin and 2-methylisoborneol (MIB). A sample of the biofilm that accumulated on the tank liner at the water surface also was collected from each tank on 20 June. Sample handling and shipment to the USDA-ARS Natural Products Utilization Research Unit (NPURU), Oxford, MS, USA, for analysis followed Schrader et al. [3]. Four samples from 20 June were lost accidently during shipment due to vial breakage.

Five catfish were selected at random from each tank at harvest, euthanized by cranial percussion, and filleted. Catfish fillets (one fillet/fish) were placed in individual plastic bags, vacuum sealed, and immediately frozen until overnight shipment to the USDA-ARS-NPURU for analysis. Fish fillets were stored frozen until further processing to obtain microwave distillates. For analysis of each fillet, a single 20-g sample was resected from the anterior end of the fillet by cutting 1-cm wide portions (2-3 portions per fillet) vertically from the dorsal to ventral side of the fillet and then each 1-cm wide sample was cut into approximately 1-cm cube-like pieces to undergo microwave distillation according to the method of Lloyd and Grimm [25].

Prior to analysis, water samples and microwave distillates of catfish fillet samples were processed by placing 0.6-mL aliquots into separate

2-mL glass crimp-top vials each containing 0.3 g sodium chloride. The methodology of Lloyd et al. [26] as modified by Schrader et al. [27] was used to quantify geosmin and MIB using solid phase microextraction and gas chromatography-mass spectrometry (SPME-GC-MS). Samples were analyzed using an Agilent 6890 gas chromatograph (Agilent, Palo Alto, CA, USA) and Agilent 5973 mass selective detector with attached CombiPal autosampler and solid phase microextraction assembly (LEAP Technologies, Inc., Carrboro, NC, USA). The GC-MS conditions were the same as those outlined by Schrader et al. [28] and each sample was run in triplicate. The instrumental detection limit for each compound was 1 part per trillion

Data analysis

Datasets were analyzed using the mixed models analysis of variance (MIXED), frequency (FREQ), and regression (REG) procedures of SAS version 9.4 (SAS Institute, Cary, NC, USA). Mean geosmin and MIB concentrations in water and geosmin concentration in fillets were not normally distributed and an appropriate data transformation was not found; therefore, data were analyzed by nonparametric one-way analysis of variance (NPAR1WAY).

Results

Fish production and feed consumption

Stocker-size channel catfish attained mean final weights that ranged from 658-829 g/fish (Table 1) and decreased linearly with increased stocking density (R^2=0.617, P=0.012). Final weight CV averaged 31.7, 32.6, and 33.4% for the LO, MED, and HI treatments, respectively, and did not differ significantly among treatment (P=0.515). Additionally, no significant difference (P>0.05) was detected between initial and final weight CV within each treatment. Chi-square analysis indicated a significant (P<0.001) association between stocking rate and fish size classes (Figure 2). Specifically, there were fewer fish in the <0.57-0.57 kg/fish size classes and more fish in the 0.79 kg/fish and larger size classes than expected in the LO treatment. In the MED treatment, there were fewer fish in the 0.68 kg/fish and smaller size classes, and more fish in the 0.79-1.02 kg/fish size classes than expected. There were more fish in the <0.57-0.68 kg/fish size classes and fewer fish in the 0.79 kg/fish and larger size classes than expected in the HI treatment. The percentage of sub-marketable fish (<0.57 kg/fish) increased linearly with increasing stocking rate (R^2=0.699, P=0.005). The percentage of fish larger than 0.57 (R^2=0.699, P=0.005) decreased linearly with increased stocking rate. Fish growth differed significantly among treatments and was linearly related to stocking density (R^2=0.698, P=0.005).

Gross and net yields ranged from 5.2-8.2 and 3.8-5.4 kg/m^3, respectively, and increased linearly as initial biomass increased (R^2= 0.900, P<0.001, and R^2=0.715, P=0.004, respectively). Catfish survival was not affected by stocking rate and averaged 97.2% across treatments.

Feed consumption was affected significantly by treatment (Table 1). Following a period of rapid increase that occurred during the first month following stocking, consistently high daily feed consumption was sustained from 10 June-15 September. Mean daily feed consumption during this peak feeding period increased linearly with increased

Initial	Individual			Fish		Feed			
Biomass	weight	Yield (kg/m^3)		growth	Survival	Daily	Peak†	Total	
(kg/m^3)*	(g/fish)	Gross	Net	(g/d)	(%)	(g/m^3/d)		(kg/m^3)	FCR††
1.4	828.9^a	5.2^b	3.8^b	4.0^a	97.3	50^b	60^b	7.7^b	1.5
2.1	771.0^{ab}	7.1^a	5.0^a	3.6^{ab}	96.7	69^a	82^a	10.6^a	1.5
2.8	658.4^b	8.2^a	5.4^a	2.9^b	97.7	76^a	92^a	11.7^a	1.4
Pooled SE	34.1	0.3	0.2	0.2	0.01	3	3	0.4	0.1
ANOVA, P>F	0.032	0.001	0.001	0.022	0.658	0.001	0.001	0.001	0.615

*n=3 replicates per treatment.
†Period of peak feed consumption, weeks 24-37 (10 June-15 September).
††FCR=wet weight of feed/net fish yield.

Table 1: Least squares means (± SE) for individual weight at harvest, gross and net yields, net daily yield, survival, daily and peak feed consumption, total feed, and feed conversion ratio (FCR) for stocker-size (217 g/fish) channel catfish stocked at 1.4-2.3 kg/m^3 in an outdoor biofloc technology production system and grown for 154 d.

Treatment	NH_4-N^a	NO_2-N^a	NO_3-N^a	PO_4-P^a	pH	T Alk^a	Chl *a*^{a}	SS^a	TSS^a
1.4 kg/m^3									
Initial	1.01	0.02	0.00	7.85	8.7	217.1	661.8	3	105.4
Final	0.02	0.03	94.91	23.58	7.5	84.4	1,694.0	57	667.8
Pooled SE	0.38	0.01	5.54	0.63	0.0	4.5	197.9	1	16.5
Pr > *F*	0.139	0.671	0.007	0.002	0.002	<0.001	0.026	<0.001	<0.001
2.1 kg/m^3									
Initial	0.84	0.03	0.00	8.32	8.7	214.1	675.9	4	114.8
Final	0.02	0.02	112.60	28.35	7.3	76.8	1,471.5	75	760.0
Pooled SE	0.37	0.01	11.51	2.01	0.1	4.4	231.0	7	41.4
Pr > *F*	0.260	0.895	0.020	0.002	<0.001	<0.001	0.072	0.002	0.005
2.8 kg/m^3									
Initial	0.41	0.03	0.00	7.48	8.7	218.4	602.8	4	102.2
Final	0.02	0.03	130.88	32.67	7.3	81.6	1,048.2	68	753.3
Pooled SE	0.14	0.01	6.45	0.70	0.1	6.8	234.9	1	25.0
Pr > *F*	0.128	0.982	0.005	<0.001	<0.001	0.002	0.251	<0.001	<0.001

^{a} Total ammonia nitrogen (mg/L NH_4-N), nitrite-nitrogen (mg/L NO_2-N), nitrate-nitrogen (mg/L NO_3-N), soluble reactive phosphorus (mg/L PO_4-P), total alkalinity (mg/L as $CaCO_3$ T Alk), chlorophyll *a* (mg/m^3 Chl *a*), settleable solids (mL/L SS), and total suspended solids (mg/L TSS).

Table 2: Within treatment comparison of least squares mean (± SE) initial and final water quality variable concentrations for outdoor biofloc technology tanks stocked with large (217 g/fish) channel catfish at 1.4–2.8 kg/m^3.

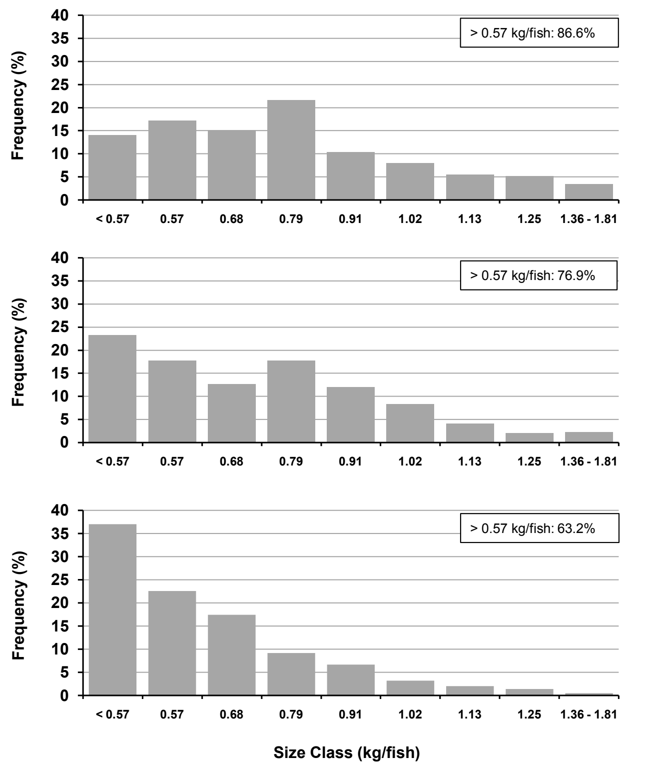

Figure 2: Mean size class distribution of channel catfish harvested from an outdoor biofloc technology production system. Stocker catfish (217 g/fish) were stocked at 1.4 kg/m^3 (top), 2.1 kg/m^3 (middle), or 2.8 kg/m^3 (bottom). Fish < 0.57 kg/fish are sub-marketable.

stocking biomass (R^2=0.850, P<0.001). Daily feed consumption during the peak feeding period was 18% higher, on average, than mean daily feed consumption for the entire experiment. Mean total feed consumption was linearly related to initial biomass (R^2=0.822, P<0.001). However, feed consumed per fish decreased linearly (R^2=0.659, P=0.008) with increased stocking density and averaged 1.23, 1.15, and 0.94 kg/fish for the LO, MED, and HI treatments, respectively. Feed conversion ratio did not differ significantly among treatments.

Water quality

Mean daily DO concentrations did not differ significantly among treatments, averaging (± SE) 6.4 ± 0.2 mg/L (P=0.697) (79.5 ± 2.6% saturation, P=0.758). Mean daily water temperature ranged from 13.9 –32.0°C over the course of the experiment, did not differ significantly among treatments (P=0.364), and averaged 26.7 ± 0.2°C.

No significant differences (P>0.05) were detected among treatments in initial concentration of any water quality variable. Mean final PO4-P concentration differed significantly (P=0.020) among treatments, and increased linearly (R^2=0.728, P=0.003) as stocking rate increased. No other significant differences (P>0.05) among treatments were detected for final water quality variable concentration. Significant differences were detected between mean initial and final concentrations of all water quality variables within treatment except for TAN and NO_2-N (Table 2).

Microbial off-flavor compounds

Geosmin and MIB concentrations in water were low in all tanks, and ranged from 0-11 and 0-31 ng/L, respectively (Table 3). Geosmin concentration did spike in one tank on one sample date, but decreased to near zero by the next sample date. Concentrations of geosmin and MIB were at or below the instrument detection threshold of 1 ng/L in 59% and 61%, respectively, of the water samples analyzed. No significant treatment differences were detected for mean geosmin (P=0.415) or mean MIB (P=0.125) concentration in water. Geosmin (P=0.169) and MIB (P=0.726) concentrations in the biofilm did not differ significantly among treatments. No significant treatment differences were detected for geosmin (P=0.866) or MIB (P=0.283) concentrations in fillets. Mean aqueous and fillet concentrations of geosmin and MIB consistently have been low during three consecutive years of research on catfish production using the BFT production system (Table 4).

	Sampling Date						
	6/20		7/11	8/15	9/12	10/10	10/11-12
Tank†	Water	Biofilm	Water	Water	Water	Water	Fillet
Geosmin							
R2	0 (0)	2 (0)	0 (0)	0 (0)	10 (0)	1 (0)	5.7 (1.6)
R3	0 (0)	467 (150)	0 (0)	1 (0)	2 (0)	2 (1)	66.6 (13.7)
R8	*	542 (28)	0 (0)	0 (0)	7 (0)	1 (0)	23.9 (5.2)
R4	0 (0)	97 (3)	0 (0)	7 (1)	1 (0)	2 (0)	52.9 (20.2)
R6	*	1 (0)	0 (0)	0 (0)	8 (1)	2 (2)	13.5 (2.4)
R7	*	78 (4)	0 (0)	0 (0)	11 (0)	1 (1)	22.1 (4.6)
R1	0 (0)	34 (2)	0 (0)	1 (0)	10 (1)	4 (2)	25.6 (9.3)
R5	4	4 (1)	0 (0)	482 (26)	4 (0)	1 (0)	19.4 (2.1)
R9	*	152 (8)	3	0 (0)	5 (0)	1 (0)	23.1 (6.2)
MIB							
R2	0 (0)	0 (0)	0 (0)	0 (0)	6 (1)	1 (0)	10.4 (3.5)
R3	0 (0)	78 (35)	0 (0)	4(1)	0 (0)	1 (0)	25.8 (6.2)
R8	*	0 (0)	0 (0)	0 (0)	0 (0)	2 (1)	18.0 (2.2)
R4	7 (1)	13 (2)	0 (0)	31 (5)	0 (0)	1 (0)	19.8 (4.7)
R6	*	0 (0)	0 (0)	15 (3)	0 (0)	5 (1)	16.3 (5.8)
R7	*	0 (0)	5 (1)	0 (0)	0 (0)	2 (1)	17.1 (4.0)
R1	0 (0)	0 (0)	6 (1)	0 (0)	0 (0)	2 (0)	12.6 (3.7)
R5	6 (1)	0 (0)	0 (0)	19 (1)	0 (0)	6 (1)	9.4 (3.0)
R9	*	69 (8)	3 (3)	10 (3)	0 (0)	2 (0)	13.9 (6.3)

†Initial biomass: 1.4 kg/m^3 (R2, R3, R8); 2.1 kg/m^3 (R4, R6, R7); 2.8 kg/m^3 (R1, R5, R9).

*Sample lost during shipment.

Table 3: Mean (±SD) geosmin and 2-methylisoborneol (MIB) concentrations during 2012 in water (ng/L), biofilm (ng/L), and fillet (ng/kg) in freshwater biofloc technology production system tanks stocked with channel catfish at three densities.

	Initial	Aqueous		Fillet		
	Biomass	Geosmin	MIB	Geosmin	MIB	
Year	(kg/m^3)	(ng/L)		(ng/kg)		Source
2012	1.4	1.7 (0.3)	1.0 (0.0)	32.1 (18.1)	18.1 (4.4)	Present experiment
	2.1	2.7 (0.3)	5.0 (1.7)	29.5 (12.0)	17.7 (1.0)	
	2.8	34.3 (31.8)	4.0 (1.2)	22.7 (1.8)	12.0 (1.3)	
2011	1.4	64.5 (33.7)	53.0 (25.0)	123.3 (35.3)	18.5 (2.3)	Green et al. [10]
	1.8	5.4 (41.3)	12.7 (30.7)	29.3 (2.6)	55.6 (10.1)	
	2.3	34.1 (33.7)	27.1 25.0)	85.9 (10.3)	56.6 (14.5)	
2010	0.4	6.5 (1.6)	21.2 (15.9)	216.1 (137.1)	243.4 (200.6)	Schrader et al. [3]
	0.9	9.1 (1.2)	18.3 (10.0)	17.7 (13.3)	30.9 (5.3)	
	1.4	6.2 (2.2)	5.9 (1.5)	35.8 (4.6)	25.0 (1.4)	
	2.5	58.8 (36.9)	15.1 (4.7)	60.8 (11.4)	31.6 (4.6)	

Table 4: Comparison of mean (± SE) treatment concentrations of geosmin and 2-methyisoborneol (MIB) in water and fillet meat of channel catfish reared in an outdoor biofloc technology production system in the current and previous studies.

Discussion

Density-dependent effects were observed for channel catfish growth and yield in the BFT production system. Although mean individual weight at harvest was inversely related to stocking rate, fish yield increased linearly because of the increasing number of fish. However, the proportion of market-size fish decreased linearly as fish yield increased, which will impact production economics. The common size range accepted by processing plants without penalty is 0.57-2.04 kg/fish. Despite a 10-15% decrease in numbers of market-size fish and a 7% decrease in mean final individual weight compared to the LO treatment, the MED treatment yield of market-size fish was 21% higher. Fish in the HI treatment were 14.6% smaller, the proportion of harvested fish within the two processing plant size ranges decreased 17-32%, and yield of market-size fish decreased as much as 14% in comparison to the MED treatment. Thus, it appears that MED treatment performed the best. However, an economic analysis, which was beyond the scope of this study, would be required to identify the best stocking density.

Stocker catfish (217 g/fish) were grown in the BFT production system for the first time in the current study. No data exist for performance of stocker catfish raised to market size in the BFT production system, and only one study reports on earthen pond production. Stocker catfish (0.26 kg/fish) stocked into earthen ponds at approximately 0.22 kg/m^3 (11,115/ha) grew at 4.0 g/d, achieved a mean final weight of 0.91 kg/fish in 164 d, and consumed 1.17 kg feed/fish [12]. This performance is similar to that of fish in the LO treatment of the current study. However, because of the increased stocking density, the 3.8 kg/m^3 NFY in the LO treatment was substantially higher that the estimated 0.43 kg/m^3 NFY in the pond study.

Channel catfish exhibit density-dependent growth during different phases of production and in multiple production environments [3,7] and [29-33]. Social interactions can affect growth of individual fish within the population [34] and [35]. Competition for food among individuals of a fish population is the most-common form of social interaction and results in growth dispensation [36]. If food is assumed to be distributed according to a size hierarchy, then size variation (as measured by the CV) would be exacerbated as competition intensifies. Increased stocking rate could be one factor that increases competition and variation in final fish size. Although stocking rate in the present study affected fish growth, variability in final fish size did not differ significantly among treatments and did not differ from variability in initial fish size. Few studies have examined the impact of stocking rate on variation in channel catfish final individual weight. Kilambi et al. [37] report that growth of channel catfish stocked in cages at 2.5-6.5 kg/m^3 is unaffected by stocking rate as is variability in final individual weight, with CVs ranging from 26.5-41.1%. In a large study on growth variation of channel catfish reared in cages, Konikoff and Lewis [38] report that final individual weight CVs converge towards 30-40%. In another BFT study [3], channel catfish individual weight CV decreased from 43.2% at stocking to 29.0-36.9% at harvest, whereas in a flow-through system, individual weight CV decreased from 45.5% at stocking to 40.7% at harvest [39]. Results of the present experiment were consistent with these reports in that final weight CVs decreased only slightly from the initial weight CV and were within the 30-40% range.

Competition for food in the current experiment likely was negligible because fish were fed daily to apparent satiation. Consequently, final weight CVs at the different stocking rates would not be expected to differ. However, despite the linear increase in feed consumption in response to increased stocking density, feed consumption per fish and fish growth decreased linearly as stocking density increased, but feed conversion was unaffected. This inverse individual fish feed consumption-stocking rate relationship also was observed for channel catfish in an earlier BFT production system study [3] and in earthen pond studies [40] and [41]. Despite being fed to apparent satiation a diet formulated to meet nutritional needs, feed consumption was restricted by increased stocking rate. Differential mortality cannot explain these differences because fish survival in the current study was high and did not differ significantly among treatments. Water quality variable concentrations all were within ranges considered acceptable for rapid fish growth and would not be expected to affect individual fish feed consumption or growth.

Water quality in the static water BFT tanks was driven by feed input in response to high fish biomass. High phytoplankton biomass (as indicated by chlorophyll *a* concentration) and nitrification (as indicated by high NO_3-N concentrations) converted the excreted feed nitrogen. As the amount of feed application increased in response to increasing fish biomass, NO_3-N, PO_4-P, and TSS increased, which is consistent with results from other studies on BFT production systems dominated by photo- and chemo-autotrophic processes [3,5,10,42]. Nitrification caused a significant reduction in pH, but this was moderated by periodic addition of sodium bicarbonate. Increased TSS limited light penetration, which inhibited phytoplankton growth more in the MED and HI treatments. Thus, phytoplankton uptake of TAN likely was greater in the LO treatment. The absence of soil, which is a major sink for P [43], explains the high PO_4-P concentrations in all treatments.

Aqueous concentrations of geosmin and MIB consistently were low throughout the present study, and these results were consistent with those for other BFT culture of channel catfish [3,10]. Based on results of previous BFT studies [3,10], the presence of off-flavor-producing microorganisms likely was transitory because the turbulent mixing of the water in BFT tanks favors faster-growing diatoms and chlorophytes over cyanobacterial bloom-forming genera, which lose the cell buoyancy regulation competitive advantage they enjoy in quiescent waters [13,44].

Catfish fillets sampled in the current study all had analytical instrument detectable concentrations (≥ 1 ng/kg) of geosmin and MIB, but no sampled fillet exceeded the previously reported sensory threshold detection levels for geosmin (250-500 ng/kg) and MIB (100-200 ng/kg) of trained catfish processing plant flavor testers [45]. It is unlikely that fish in the present study would be classified as having objectionable "earthy" or "musty" off-flavors when evaluated by trained processing plant flavor testers because aqueous geosmin and MIB concentrations to which they had been exposed were low.

Three consecutive years (including the present study) of aqueous and fillet geosmin and MIB data [3,10] provides strong evidence of a reduced incidence of common microbial off-flavor episodes (i.e., "earthy," "musty") for channel catfish grown in the BFT production system compared to earthen ponds. Additionally, other types of off-flavors that can occur in catfish related to their foraging for food (e.g., "grassy," "vegetable") [46] are unlikely to occur in the BFT production units lined with high-density polyethylene. In the two earlier studies [3,10], 11.1% of tanks each year contained off-flavored fish, whereas in the current study no tank contained off-flavored fish. In catfish pond waters in the southern U.S., in contrast, geosmin and MIB concentrations that exceed 2,000 ng/L and 700 ng/L, respectively, are observed [16-18] and episodes of off-flavored (gesomin- or MIBtainted) fish can be correlated with the presence of an off-flavor-producing cyanobacterium [19]. Geosmin and MIB off-flavors are prevalent in commercial catfish ponds from July-September, during which time up to 76% of ponds can contain off-flavored fish [19]. Only one report was found in which the incidence of off-flavor episodes for pond-raised channel catfish approached levels we observed in the BFT production system. Torrans and Lowell [47] report that 0-20% of channel catfish ponds co-stocked with blue tilapia (*Oreochromis aureus*) contained off-flavored catfish whereas 58-67% of catfish ponds in monoculture contained off-flavored fish.

In summary, density-dependent growth of stocker catfish was observed, but did not lead to increased variability in fish final weights at the different stocking rates. The highest yield of market-size fish was obtained by stocking fish at 2.1 kg/m^3. However, an economic analysis would be required to identify the best stocking density. Although competition for food likely was negligible because fish were fed to apparent satiation, feed consumption per fish and fish growth were inversely related to stocking rate. The reason as to why individual fish feed intake decreased as stocking rate increased remains unknown, but likely is related to some aspect of social interaction. Concentrations of geosmin and MIB in tank waters were low throughout the study and while fillets from sampled fish contained analytically detectable geosmin and MIB concentrations, no fish would be classified as having "earthy" or "musty" off-flavors when evaluated by trained processing plant flavor testers. Data from this study combined with data from our two previous studies provide strong evidence that the incidence of common microbial off-flavor episodes is low in the BFT production system. However, there remains a continued need to identify the microbial sources of and elucidate the dynamics of geosmin and MIB production in the BFT production system.

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