

## Channel Catfish Polyculture with Fathead Minnows or Threadfin Shad: Effects on Pond Plankton Communities and Catfish Fillet Flavor, Color, and Fatty Acid Composition

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### Abstract

Threadfin shad, *Dorosoma petenense*, or fathead minnows, *Pimephales promelas*, were co-cultured with channel catfish, *Ictalurus punctatus*, in earthen ponds to determine the effects of planktivory on plankton community dynamics and catfish fillet quality. Fathead minnows had no effect on the plankton community structure or catfish fillet flavor, color, and fatty acid composition. Fillet color was also unaffected by the presence of threadfin shad. Small differences were found in fillet fatty acid composition for catfish from ponds with shad, but these differences probably have no biological significance. Threadfin shad did, however, have important impacts on the plankton community structure and catfish flavor. Size-selective filter-feeding by shad reduced cyanobacterial abundance relative to ponds with catfish-only and fathead minnows. Relative abundance of smaller phytoplankton in the groups Chlorophyta, Cryptophyta, Bacillariophyceae, and Euglenophyta increased in ponds with shad. Relative abundance of small zooplankton (rotifers) also increased in shad ponds. Reduced abundance of large, colonial cyanobacteria that are known to produce odorous metabolites caused a corresponding reduction in off-flavor prevalence and intensity in catfish from ponds with threadfin shad when sampled in September. Although threadfin shad dramatically reduced catfish off-flavor prevalence during the warm season, they apparently caused a high prevalence of “fishy” off-flavors in the February sample. This undesirable flavor appeared to be caused by catfish foraging on shad killed during a preceding period of exceptionally cold water temperatures. Use of threadfin shad for phytoplankton biomanipulation therefore presents a dilemma: catfish–shad polyculture reduces prevalence of cyanobacteria-related off-flavors in warm months but may cause undesirable forage-related off-flavors in the colder months. Catfish farmers must consider these benefits and risks when deciding to use threadfin shad as a management tool.

Uncontrolled phytoplankton growth is the natural consequence of culture practices in channel catfish, *Ictalurus punctatus*, ponds. Large amounts of manufactured feed are fed to promote rapid fish growth. Fish wastes contain nitrogen, phosphorus, and other plant nutrients that stimulate development of dense phytoplankton communities. Nutrient loading rates are often so great that phytoplankton growth is no longer nutrient limited, but is rather restricted by light availability (Tucker and van der Ploeg 1993). These conditions promote phytoplankton communities dominated by colonial, gas-vacuolate cyanobacteria (blue-green algae),

which have a competitive advantage over other phytoplankton in hypereutrophic environments (Paerl and Tucker 1995). Common species of *Planktothrix* (*Oscillatoria*), *Anabaena*, and *Microcystis* are undesirable in catfish ponds because they are poor oxygenators of the water and may produce ichthyotoxins or odorous compounds that make fish off-flavor.

Reducing phytoplankton biomass in hypereutrophic ecosystems is difficult. The most dependable approach to phytoplankton management is to reduce nutrient loading rates, which is not possible in aquaculture ponds with feeding (Tucker et al. 2008). Catfish farmers currently rely on chemical algicides to manage noxious phytoplankton communities (Tucker

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et al. 2001; Zimba et al. 2002; Schrader et al. 2005), but the use of algicide is not always effective and has several disadvantages. As such, there has been interest in managing phytoplankton communities using “top-down” ecological influences. Top-down control of phytoplankton communities is based on the trophic cascade hypothesis (Carpenter et al. 1985; McQueen et al. 1989), which posits that predation from higher trophic levels can affect the structure and biomass of communities at lower trophic levels. The use of top-down effects to control plankton abundance and water quality is called biomanipulation.

Most studies of biomanipulation in aquaculture have focused on using plankton-feeding fish to alter the phytoplankton community structure. Silver carp, *Hypophthalmichthys molitrix*, and blue tilapia, *Oreochromis aureus*, have been proposed as candidates for biomanipulation of catfish pond plankton communities (Cremer and Smitherman 1980; Smith 1985; Burke et al. 1986; Torrains and Lowell 1987; Tucker 2006). Both fish are not native to the United States, have certain traits that are undesirable in polyculture with catfish, and their impacts on cyanobacterial communities have been equivocal (summarized by Tucker et al. 2004).

In this study, we investigate the use of two native North American fishes as candidates for biomanipulation of catfish pond phytoplankton communities. Threadfin shad, *Dorosoma petenense*, and fathead minnows, *Pimephales promelas*, are relatively small, planktivorous fishes that appear to have few traits that would interfere with channel catfish pond aquaculture. We evaluated the impact of these fish on plankton communities and the prevalence of off-flavors in channel catfish. Both fish can also serve as forage for adult channel catfish and consumption may affect other fillet quality attributes. We therefore assessed the effect of polyculture on fillet color and fatty acid profiles.

### Methods and Materials

The study was conducted from the spring of 2009 through the winter of 2010 at the Thad

Cochran National Warmwater Aquaculture Center in Stoneville, Mississippi. Thirty levee-type ponds were used in the study. Ponds were 0.4 ha in area and 1.1 m average depth, and were constructed on alluvial clay soils (the Sharkey series of very fine, smectitic, thermic Chromic Epiaquerts) of the Yazoo–Mississippi River floodplain in northwest Mississippi. Water was supplied from a well pumping from the Mississippi River Alluvial Aquifer, which is the water source for all catfish aquaculture in northwest Mississippi. Pond water levels were maintained below the tops of standpipes so that no water was discharged from ponds during the sampling period. Total alkalinity and total hardness of pond waters varied between 100 and 200 mg/L as CaCO<sub>3</sub>, with about 70% of the hardness contributed by calcium. Each pond was equipped with a 2.2 kW electric paddlewheel aerator that was used when dissolved oxygen concentrations were less than 3 mg/L. Pond construction, soil type, water supply, water management, and aeration practices were typical of those used in channel catfish aquaculture in northwest Mississippi (Tucker 1996).

Ponds were stocked in April 2009 with stocker-size channel catfish (0.15 kg/fish) at 15,000 fish/ha. The study used a 2 × 3 factorial design with two feeding rates (daily or every-other-day) and three forage species treatments (no forage species, threadfin shad, and fathead minnows). The effect of biomanipulation was tested across two common feeding practices because phytoplankton community structure is affected by nutrient loading derived from fish feeds, and the two feeding protocols used here represent two discrete levels of feed input. For the forage fish treatments, respective forage fish were stocked in early April 2009 at a rate of 11 kg/ha. Shad and fathead minnows were stocked early to allow time for reproduction. Catfish were fed a 28% protein diet to satiation when water temperatures exceeded about 15 C (April through October). During the cooler months of the year, fish were fed according to winter feeding schedules recommended by Robinson et al. (2004). Catfish were harvested in February 2010.

We were unable to quantify final populations of either threadfin shad or fathead minnows. This problem has been encountered in other studies using small forage fish in catfish ponds (Green et al. 2010). In our study, complications also arose because a period of unusually cold weather in January 2010 caused a complete kill of threadfin shad a month before catfish were harvested in February. This incident, which was important to the outcome of this study, is discussed further below. However, we sampled each pond in August using a 30-foot minnow seine and recovered abundant shad or minnows in appropriate ponds, indicating establishment of vigorous planktivore populations.

Water samples were collected every 2 wk from each pond with a tube sampler (modified from Graves and Morrow 1998), which samples the entire water column. Samples were taken in the mornings and subsamples (50 mL) were immediately preserved with Lugol's solution and stored at 4 C for microscopic examination of phytoplankton communities. Phytoplankton were identified and counted as "natural units" (colonies, filaments, or unialgal cells) in a Sedgewick-Rafter counting chamber at 300 $\times$  magnification (Eaton et al. 2005).

Zooplankton samples were collected by taking an oblique 2-m tow with an 80- $\mu$ m mesh, Wisconsin-style net (Wildlife Supply, Saginaw, MI, USA). A mark was placed on the net's tow rope at 2 m. Samples were preserved in 240 mL buffered formalin before organisms were counted under a light microscope (Geiger and Turner 1990). All zooplankton in 1-mL subsamples were counted using a Sedgewick-Rafter counting cell as described by Geiger and Turner (1990) and were identified with taxonomic keys of Thorp and Covich (1991).

Comparisons of fish flavor quality were conducted at two predetermined dates, one in early autumn and one in midwinter. Comparisons made at these times are particularly instructive in studies of catfish off-flavors because incidence of off-flavored fish is generally highest in late autumn and lowest in midwinter (van der Ploeg and Tucker 1993; Tucker 2000). In September and again at harvest (February), two

fish were caught from each pond. One fillet from each fish was used for flavor analysis and the other for color scoring and fatty acid analysis. Flavor testing methods were similar to those used by commercial operations. Type of off-flavor was described using common flavor descriptors (Johnsen et al. 1987; van der Ploeg and Tucker 1993) and the intensity of off-flavor was rated on a hedonic scale of 0–5, where 0 = no detectable off-flavor and 5 = extremely strong off-flavor.

For fillet color comparisons, digital pictures were taken of one fillet from each fish using an EOS ID Mark II digital SLR camera (Cannon USA Inc., Lake Success, NY, USA). Yellow intensity values (Commission Internationale de l'Eclairage [CIE]  $b^*$ ) were determined from the digital picture of the fillet at three locations along the dorsal line of the fillet using an Adobe Photoshop CS3 image editing software (Adobe Systems Inc., San Jose, CA, USA) as described by Li et al. (2011). After measurements were taken, fillets were immediately placed in black plastic bags and stored at  $-80$  C for subsequent fatty acid analysis. Fatty acid composition of pre-extracted fat (Folch et al. 1957) from fillet samples was analyzed by gas chromatography with a capillary column and flame ionization detection (AOAC 2000).

Phytoplankton and zooplankton abundances were analyzed in a 2  $\times$  3 factorial design (two feeding regimens; three forage fish treatments) with repeated measures taken on ponds. Data were analyzed with the MIXED procedure in SAS Version 9.2 software (SAS Institute, Cary, NC, USA); the covariance structure, autoregressive of order 1, was used in the repeated measure model. (Littell et al. 1996). Mean comparisons were made using a least significant difference (LSD) test with a significance level of  $P < 0.05$ . Remaining data, without repeated measures, was analyzed with one-way analysis of variance (ANOVA) using StatView Version 5.0.1 (SAS Institute). Fisher's protected least significant difference (PLSD) was used to test the differences among individual means. The difference was regarded as significant when  $P < 0.05$ .

TABLE 1. Repeated measure analysis of phytoplankton abundance (natural units per taxonomic group) in channel catfish ponds with catfish-only or catfish co-cultured with fathead minnows or threadfin shad and with feed treatments of every-other-day (EOD) or daily feeding to satiation. Numbers represent mean  $\pm$  SEM.

Treatment	Phytoplankton taxonomic group				
	Chlorophyta	Cryptophyta	Bacillariophyceae	Euglenophyta	Cyanophyta
Catfish-only	10,065 (3891)	167 (1174)	1075 (430)	91 (90)	15,597 (2933)
Fathead minnow	2463 (3891)	311 (1174)	1579 (430)	108 (90)	16,503 (2933)
Threadfin shad	2617 (3891)	5698 (1174)	3034 (430)	625 (90)	4913 (2933)
EOD	7843 (3177)	1727 (959)	2559 (351)	373 (74)	6726 (2395)
Daily	2253 (3177)	2390 (959)	1233 (351)	176 (74)	17,950 (2395)
Type 3 tests of fixed effects ( $P > F$ )					
Forage	0.3053	0.0035	0.0101	0.0003	0.0173
Feed	0.2255	0.6293	0.0133	0.0703	0.0029
Date	0.1359	<0.0001	<0.0001	0.3319	<0.0001
Forage $\times$ Date	0.1616	<0.0001	0.3970	0.2405	0.2816
Feed $\times$ Date	0.0947	0.9965	0.0124	0.5722	0.0387
Forage $\times$ Feed	0.3323	0.7396	0.3235	0.2773	0.1891
Forage $\times$ Feed $\times$ Date	0.1180	1.0000	0.6111	0.8898	0.3313

## Results and Discussion

### Plankton Communities

The presence of threadfin shad had significant effects on the phytoplankton community structure (Table 1). Ponds with shad had greater relative abundance of cryptomonads (Cryptophyta), diatoms (Bacillariophyceae), and euglenophytes (Euglenophyta). Abundance of plants within these groups was similar for catfish-only ponds and ponds with fathead minnows. The most common genera in those groups were small plants, including *Cryptomonas* sp., small pennate diatoms (e.g., *Navicula* sp.), small centric diatoms (e.g., *Stephanodiscus* sp.) and small euglenophytes, such as species of *Trachelomonas*. Abundance of green algal species (Chlorophyta) was not affected by the presence of shad or fathead minnows.

Ponds with shad had significantly lower abundance of blue-green algae (cyanobacteria) than the other two treatments. The most common cyanobacterial species found in ponds were *Planktothrix agardhii*, *Planktothrix perornata*, *Anabaena* spp., *Microcystis* sp., and *Raphidiopsis brookii*. All are relatively large, colonial, gas-vacuolate species that are common during summer months in catfish

ponds throughout the Yazoo–Mississippi River floodplain (Tucker and Lloyd 1984; Paerl and Tucker 1995, Tucker 2000).

There was a significant interaction of cryptomonad abundance with treatment over time: cryptomonads were relatively rare in the fathead minnow and catfish-only treatments throughout the study, but increased dramatically in the winter in ponds containing threadfin shad. Previous surveys have also found cryptomonads (i.e., *Cryptomonas ovata*) to be primarily a cool-season plant in Mississippi catfish ponds (Tucker 1985). Diatom populations interacted significantly with feed treatments over time, being relatively constant over time in the daily fed ponds but having a large peak in numbers in July and October in ponds with every-other-day feeding. Euglenophytes were significantly more abundant in ponds with every-other-day feeding. Cyanobacterial abundance increased more over time in ponds fed daily compared to ponds with every-other-day feeding. Increased cyanobacterial abundance in ponds with daily feeding is consistent with observations that cyanobacteria have an increasingly competitive advantage over other phytoplankton as ecosystem nutrient loading increases (Paerl and Tucker 1995).

TABLE 2. Repeated measure analysis of zooplankton abundance (by major taxonomic group) in channel catfish ponds with catfish-only or catfish co-cultured with fathead minnows or threadfin shad and with feed treatments of every-other-day (EOD) or daily feeding to satiation. Numbers represent mean  $\pm$  SEM.

Treatment	Zooplankton group				
	Rotifers	Copepods	Copepod nauplii	Cladocerans	Ostracods
Catfish-only	23 (17)	60 (8)	120 (14)	71 (8)	5 (1)
Fathead minnow	54 (17)	51 (8)	118 (14)	54 (8)	2 (1)
Threadfin shad	101 (17)	34 (8)	80 (14)	63 (8)	7 (1)
EOD	55 (14)	49 (6)	105 (11)	63 (6)	5 (1)
Daily	63 (14)	47 (6)	106 (11)	63 (6)	5 (1)
Type 3 tests of fixed effects ( $P > F$ )					
Forage	0.0131	0.0668	0.0920	0.2737	0.0420
Feed	0.6646	0.8381	0.9486	0.9568	0.8371
Date	0.0135	<0.0001	<0.0001	<0.0001	<0.0001
Forage $\times$ Date	0.1148	0.9750	0.9971	0.0014	0.3202
Feed $\times$ Date	0.2244	0.0579	0.9072	0.9136	0.9769
Forage $\times$ Feed	0.6731	0.4799	0.4391	0.1965	0.3206
Forage $\times$ Feed $\times$ Date	0.2278	0.5561	0.8385	0.1613	0.5663

Copepod, copepod nauplii, and cladoceran abundances were not affected by the presence of shad or fathead minnows (Table 2). This result agrees with a past study (Ludwig 1996) that found no difference in these groups of zooplankton when fathead minnows were cultured with channel catfish. Rotifer populations, however, were greater in ponds with threadfin shad compared with the other fish treatments. Ostracods were also most abundant in threadfin shad ponds, although numbers of ostracods were very low in all ponds. Cladoceran abundance peaked in all ponds in the spring and had a smaller peak again in December in catfish-only ponds and ponds with fathead minnows. Shad ponds did not have a second wintertime peak in cladoceran abundance.

#### Off-flavor Prevalence

Prevalence of off-flavored catfish in September was high in catfish-only and fathead minnow ponds (Table 3). Off-flavored catfish were present in 7 of 10 catfish-only ponds and 8 of 10 fathead minnow ponds. In both treatments, all off-flavors were described as either musty (2-methylisoborneol) or earthy (geosmin). These two off-flavors are known to be of cyanobacterial origin (Tucker 2000).

TABLE 3. Number of ponds (out of 10 replicate ponds per treatment) on two sampling dates with populations of the odor-producing cyanobacteria *Planktothrix perornata* (Pla) or *Anabaena* spp. (Ana) in channel catfish ponds with catfish-only or catfish co-cultured with fathead minnows or threadfin shad.

Treatment	September			February		
	Pla	Ana	Total	Pla	Ana	Total
Catfish-only	4	4	7	0	0	0
Fathead minnows	5	7	9	0	0	0
Threadfin shad	2	2	3	0	0	0

Off-flavor prevalence was markedly lower in ponds with threadfin shad: only 3 of 10 ponds contained off-flavored fish, and 2 of those 3 ponds contained catfish with musty or earthy off-flavors. Concomitant with reduced prevalence, off-flavor intensity was also lower in catfish from ponds with shad. Mean flavor scores for fish sampled in September were 1.2 for fish from catfish-only ponds and 1.4 for catfish from fathead minnow ponds. Of the ponds with off-flavored fish in those two treatments, seven of seven catfish-only ponds contained catfish with distinct off-flavors (flavor score of  $\geq 2$ ) and five of eight fathead minnow ponds contained distinctly off-flavored catfish. On the other hand, mean flavor score was 0.5 in catfish from ponds with shad and of the three ponds

TABLE 4. Numbers of ponds (out of 10 replicate ponds per treatment) on two sampling dates with off-flavored channel catfish in ponds with catfish-only or catfish co-cultured with fathead minnows or threadfin shad. Prevalence is indicated for all off-flavors (All) and for off-flavors of cyanobacterial origin (Cya). Hedonic flavor scores range from 0 = no off-flavor to 5 = extreme off-flavor. Individual hedonic off-flavor scores are provided only for ponds with discernable off-flavors (i.e., scores of 0 are not tabulated). Individual scores are the average of four scores for each pond population: scores from two trained flavor testers for one fillet from each of two fish per pond. Mean flavor scores are the average of all 40 individual flavor scores on each date for each treatment (4 scores from each pond and 10 ponds per treatment).

Treatment	September			
	All	Cya	Scores	Mean (+/- SEM)
Catfish-only	7	7	2,2,2,2,2,2	1.2 (0.30) <sup>b</sup>
Fathead minnows	8	8	1,2,2,2,2,1,1,3	1.4 (0.30) <sup>b</sup>
Threadfin shad	3	2	1,1,2	0.5 (0.17) <sup>a</sup>
February				
Catfish-only	3	3	1,2,3	0.6 (0.34) <sup>a</sup>
Fathead minnows	8	7	2,2,1,2,2,1,2,2	1.3 (0.23) <sup>a</sup>
Threadfin shad	5	0	2,1,2,1,3	1.4 (0.42) <sup>a</sup>

with off-flavored catfish, fish from two of those ponds had hedonic flavor scores of 1, which is considered to be very mild.

Trends in off-flavor prevalence were supported by findings of known odor-producing cyanobacteria in all ponds with fish tainted by either 2-methylisoborneol or geosmin (Table 4). In the Yazoo-Mississippi River floodplain, the musty off-flavor in pond-raised catfish caused by 2-methylisoborneol is always associated with *P. perornata* and the earthy off-flavor associated with geosmin is nearly always associated with certain species of *Anabaena* (van der Ploeg et al. 1992; Tucker 2000; Schrader and Dennis 2005). Seven ponds in the catfish-only treatment contained fish tainted by either 2-methylisoborneol or geosmin, and all seven ponds contained populations of either *P. perornata* or species of *Anabaena*. Similarly, all eight ponds with off-flavored fish in the fathead minnow treatment were tainted with either 2-methylisoborneol or geosmin and all eight contained populations of either *P. perornata* or species of *Anabaena*. The association between musty or earthy off-flavors and the presence of known odor-producing cyanobacteria also held true for ponds in the shad treatment, confirming that the reduction in September off-flavor prevalence in ponds with shad was caused by absence of odor-producing cyanobacteria from most ponds.

Trends in catfish off-flavor prevalence and intensity were markedly different in February than in September (Table 3). None of the 30 ponds in our study had populations of odor-producing cyanobacteria in February (Table 4), which agrees with past observations that odor-producing cyanobacteria are uncommon in Mississippi catfish ponds when water temperatures are low (van der Ploeg et al. 1995). Nevertheless, 3 of 10 catfish-only ponds and 7 of 10 fathead minnow ponds contained catfish with musty or earthy off-flavors. This apparent anomaly – the presence of cyanobacterial off-flavors in the absence of odor-producing cyanobacteria – is not uncommon during the wintertime in Mississippi (Tucker and van der Ploeg 1999). The tainting compounds, 2-methylisoborneol and geosmin, are produced during warm-weather periods and then, as the water temperatures decrease with the onset of winter, the odor-producing cyanobacterial populations disappear yet the compounds remain in fish tissue because depuration rates are very slow in cold water (Johnsen et al. 1996).

In contrast to catfish from catfish-only and fathead minnow ponds, cyanobacterial off-flavors were absent from catfish in ponds with threadfin shad in February. Absence of off-flavors caused by 2-methylisoborneol or geosmin was likely due to the low incidence and intensity of those off-flavors in the

preceding months (as in the September sampling; Table 3). However, catfish in 5 of 10 ponds with threadfin shad had an off-flavor described as “fishy,” and fish from 3 of those ponds contained fish that were distinctly off-flavored (flavor scores  $\geq 2$ ). Fishy off-flavor is a common wintertime off-flavor in Mississippi pond-raised catfish, and is attributed to catfish foraging on dead or live fish during winter months when manufactured feed is offered infrequently (Tucker and van der Ploeg 1999). Similar “fishy” off-flavors have been described from catfish sampled in the winter from ponds with tilapia (Tucker 2000). In that instance, catfish developed pronounced fishy off-flavors when they fed on carcasses of tilapia that died from hypothermia. Threadfin shad are also cold-sensitive and often die in large numbers when water temperatures are below 10 C (Griffith 1978). In January 2010, all ponds were frozen for 10 d with up to 5 cm of ice during an exceptional period of low air temperatures. Dead shad could be seen under the ice and along the shore after the ice melted. Furthermore, no shad were seen when catfish were harvested a month later in February, indicating a complete low temperature-related kill of shad population. The fishy off-flavors found in catfish from ponds with shad in February were probably caused by foraging on inactive or dead shad during the cold water period prior to sampling. Lack of similar off-flavors in fish from the other treatments supports this assumption.

#### *Fillet Color*

There have been speculations that catfish consuming forage fish may accumulate yellow pigments that may affect the marketability of the product. In the present study, low levels of xanthophyll (lutein + zeaxanthin) were detected in threadfin shad (2.90 mg/kg) and fathead minnows (1.72 mg/kg). Channel catfish fillet color was not affected by forage treatment or feeding treatment in the present study. Average CIE  $b^*$  value (yellowness) was 16.6 (+/- 0.63) in September and 15.3 (+/- 2.13) in February. These values are much below the

TABLE 5. *Fatty acid profiles of channel catfish from ponds with catfish-only or catfish co-cultured with fathead minnows or threadfin shad. Values within a row without a letter in common are significantly different ( $P < 0.05$ ).*

	Catfish-only	Fathead minnows	Threadfin shad
Saturated	25.0 (0.27)	24.7 (0.37)	24.4 (0.30)
Mono-unsaturated	41.6 (0.69) <sup>a,b</sup>	40.8 (1.05) <sup>a</sup>	43.8 (0.75) <sup>b</sup>
18:2 n-6	15.9 (0.10) <sup>a</sup>	14.9 (0.29) <sup>b</sup>	14.0 (0.32) <sup>c</sup>
18:3 n-6	0.7 (0.05)	0.6 (0.04)	0.6 (0.05)
18:3 n-3	0.8 (0.08)	0.6 (0.09)	0.8 (0.16)
18:4 n-3	0.5 (0.02)	0.6 (0.06)	0.5 (0.04)
20:2 n-6	1.4 (0.03)	1.5 (0.08)	1.2 (0.20)
20:3 n-6	1.8 (0.07) <sup>a</sup>	1.8 (0.07) <sup>a</sup>	1.6 (0.04) <sup>b</sup>
20:4 n-6	2.3 (0.16)	2.6 (0.14)	2.2 (0.14)
20:4 n-3	0.8 (0.11)	0.8 (0.11)	0.6 (0.09)
20:5 n-3	0.3 (0.04) <sup>a</sup>	0.2 (0.04) <sup>a</sup>	0.5 (0.04) <sup>b</sup>
22:4 n-6	0.3 (0.08)	0.3 (0.08)	0.3 (0.08)
22:5 n-6	1.5 (0.08) <sup>a</sup>	1.5 (0.06) <sup>a</sup>	1.2 (0.08) <sup>b</sup>
22:5 n-3	0.3 (0.04) <sup>a</sup>	0.4 (0.05) <sup>a,b</sup>	0.5 (0.05) <sup>b</sup>
22:6 n-3	1.7 (0.11)	1.8 (0.13)	1.9 (0.03)
Total n-3	4.3 (0.20)	4.5 (0.39)	4.9 (0.29)
Total n-6	23.9 (0.44) <sup>a</sup>	23.3 (0.44) <sup>a</sup>	21.0 (0.44) <sup>b</sup>
Total n-3 LCPUFA <sup>1</sup>	3.0 (0.14)	3.3 (0.29)	3.5 (0.14)
Total n-6 LCPUFA	7.3 (0.36) <sup>a</sup>	7.8 (0.26) <sup>a</sup>	6.4 (0.17) <sup>b</sup>
Total Fat	7.5 (0.50)	8.0 (0.79)	8.1 (0.15)

<sup>1</sup>Long-chain polyunsaturated fatty acids (LCPUFA;  $\geq 20$  carbon atoms;  $\geq 2$  double bonds).

levels that are considered unmarketable for the catfish product (Li et al. 2011).

#### *Fillet Fatty Acid Profiles*

The presence of threadfin shad and fathead minnows has significant effects on catfish fillet fatty acid composition (Table 5). In general, catfish co-cultured with threadfin shad had slightly higher levels of mono-unsaturated fatty acids and slightly lower levels of total n-6 and total n-6 long-chain polyunsaturated fatty acids than fish from the catfish-only and fathead minnow ponds. The differences in fatty acid composition were small (differing by  $< 10\%$ ) and do not appear to be of biological significance. Apparently, fish in all treatments fed predominantly on manufactured feeds which reflected in the relatively consistent fatty acid compositions in fish across all treatments. Although the development of fishy off-flavors in catfish from some

threadfin shad ponds indicates that catfish were foraging on shad during the winter, the extent or duration of that foraging does not appear sufficient to markedly affect fillet fatty acid composition, especially total n-3 and n-3 long-chain polyunsaturated fatty acid concentrations.

#### *Threadfin Shad for Off-flavor Management*

Threadfin shad are primarily omnivorous filter feeders and consume small food particles (<0.39 mm) by filtration, but also use particulate feeding to eat larger prey items (Holanov and Tash 1978). Size-selective grazing by threadfin shad reduces the biomass of zooplankton (especially larger forms, such as cladocerans and cyclopoid copepods) and large, colonial phytoplankton (Baca and Drenner 1995; Lo Giudice et al. 2004; Green et al. 2010). Smaller phytoplankton species then dominate the plankton community because of reduced competition and zooplankton grazing pressure. These observations were confirmed in our study (Tables 1 and 2) and extended to include the associated impacts on fish flavor quality.

Threadfin shad reduced populations of large, filamentous cyanobacteria, thereby reducing the prevalence of cyanobacterial off-flavors in the September sampling. This has important implications. Preharvest off-flavors are common in pond-raised catfish and are a serious management problem. If off-flavored fish are marketed, first-time buyers may assume that the objectionable flavor is inherent in the product and shun future purchases. To prevent off-flavored fish from reaching the marketplace, fish samples from each crop of harvest-sized fish are taste-tested by trained personnel before the crop is accepted for processing. If fish are found to be off-flavor, the crop from that pond is not accepted for processing, and farmers must wait until the odorous compounds are eliminated from fish either by passive diffusion or metabolism. Harvest delays associated with off-flavors in pond-raised catfish cost the industry millions of dollars annually (Engle et al. 1995; Tucker 2000).

Off-flavors of cyanobacterial origin are the most common flavor problems encountered in

catfish farming during warmer months (van der Ploeg and Tucker 1993) and catfish farmers currently attempt to eradicate odor-producing cyanobacteria using chemical algicides. Our findings indicate that the use of threadfin shad to reduce the incidence of cyanobacteria-related off-flavors may be an alternative to algicide use.

Although threadfin shad reduced catfish off-flavor incidence during the warm season by reducing the abundance of odor-producing cyanobacteria, catfish in half the ponds with shad had “fishy” off-flavors in the February sample. This undesirable flavor was probably caused by catfish foraging on dead or moribund shad. Threadfin shad are known to be cold-intolerant and cold water temperatures (ponds were iced over) in the month preceding the February sampling caused a complete kill of shad in all ponds. Anecdotal reports indicate similar losses in ponds with shad in Mississippi and Alabama. In fact, threadfin shad were difficult to purchase in the spring of 2010 due to wintertime losses suffered by commercial shad producers. Shad survival in shallow catfish ponds probably varies greatly depending on winter weather patterns, but water temperatures less than 10 C are common in December through February in Mississippi catfish ponds (Wax et al. 1987). Cold-related shad die-offs of varying severity can therefore be expected in most winters.

Channel catfish markets demand fish year-round, with peak demand in February and March, coinciding with the Lenten season (Hargreaves and Tucker 2004). As such, off-flavors encountered in the winter are at least as problematic as those encountered in the warmer months. Using threadfin shad for phytoplankton biomanipulation therefore presents a dilemma: catfish-shad polyculture appears to reduce catfish off-flavor incidence in warm months but causes off-flavors in the colder months. Catfish farmers must consider these benefits and risks when deciding to use threadfin shad as a management tool.

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