



Plankton community responses in earthen channel catfish nursery ponds under various fertilization regimes

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Abstract

We evaluated fertilizer effects on phytoplankton and zooplankton concentrations in channel catfish *Ictalurus punctatus* nursery ponds. In 2001, three ponds were not fertilized and three ponds were fertilized with inorganic (8.4 kg/ha N, 2.0 kg/ha P followed by twice a week applications at half the initial rate) and organic fertilizer (224 kg/ha cottonseed meal followed by once a week applications of 28 kg/ha) for 4 weeks. Total P and soluble reactive P (SRP) were significantly higher in fertilized ponds ($P < 0.05$), however no differences in algal populations resulted. Of the zooplankton, only copepod nauplii were higher in fertilized ponds. In 2002, 26 ponds were used. Sixteen ponds were newly constructed; 10 ponds were old (>10 years). Within each age group, treatments of no fertilizer (controls), organic fertilizer only, inorganic fertilizer only, or both organic and inorganic fertilizers were used for 3 weeks. In 2002, the same P but higher N rates were used (20.2 kg/ha N followed by twice a week at half rate). Several differences occurred among treatments ($P < 0.05$). Both total P and total N were significantly higher in ponds fertilized with inorganic fertilizer and both inorganic and organic fertilizers than in control ponds and organically fertilized ponds. Also, total P, total N, SRP, and nitrite were all significantly higher in old ponds than in new ponds. All phytopigment concentrations were also higher in old ponds than in new ponds. Chlorophyll *a* (total algal biomass), lutein (green algal biomass), and fucoxanthin (diatom biomass) were significantly higher in ponds fertilized with inorganic fertilizer or both inorganic and organic fertilizers than in control or organically fertilized ponds. Old ponds contained significantly more rotifers, copepod adults, and ostracods than new ponds. Rotifer and ostracod concentrations were not different among fertilizer treatments. Copepods adults and nauplii were significantly increased in

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ponds fertilized with both organic and inorganic fertilizers than in organically fertilized ponds. Cladocerans were significantly higher in inorganically fertilized ponds than in all others. Results indicated little benefit of organic fertilizer addition at the rates used. Also, ponds appear to be more N limited than P limited. Old ponds are more fertile than new ponds. Applying only inorganic fertilizer at an initial rate of ~ 20 kg/ha N and 2 kg/ha P, followed by subsequent applications of half the initial rate for 3–4 weeks increased zooplankton concentrations desirable for fry with larger mouth gapes.

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1. Introduction

Approximately 70% of United States channel catfish *Ictalurus punctatus* production occurs in the Yazoo-Mississippi River floodplain (locally called “the Delta”) on soils consisting of 50–60% montmorillonite clay, 30–40% silt, and less than 5% sand (Tucker, 1996). Farm sale of catfish to processors during the first 8 months of 2002 totaled 192.1 million kg with gross sales of \$242 million for growers (Commodity Economics Division, 2002). Large numbers of fry are required to supply stocking needs; in July 2002, fingerlings held on farms totaled 1.64 billion (Commodity Economics Division, 2002). Survival of fry after stocking into ponds is not well documented, and reported values range from about 55% (Hatch et al., 1987) to 80% (Moore and Waldrop, 1994).

Fertilization protocols specific to catfish nursery ponds in the Delta should improve production efficiency and may reduce survival variability. Addition of fertilizers to nursery ponds is common practice among all cultured species of fish. Nutrients in fertilizers are incorporated into biomass (algal and zooplankton) and, through a complex web of nutrient assimilation and recycling, ultimately incorporated into fish biomass. Several factors (location, source water, morphometry and sediments) can affect fertilizer application responses (Knud-Hansen, 1998). Additionally, different management practices associated with different species (e.g., feeding and stocking rates) may affect fertilization responses.

Although channel catfish have been farmed in the United States for over 40 years, research on fertilization practices specific to channel catfish nursery ponds in the Delta has not been conducted. Recommendations for fertilization of channel catfish nursery ponds vary widely. Tucker and Robinson (1990) recommended fertilizing fry ponds with 2.2–4.5 kg/ha of 10–34–0 or 13–38–0 (0.2–0.6 kg/ha N; 0.3–0.8 kg/ha P) liquid fertilizers every 2 days until a bloom develops, but did not mention organic fertilizer applications. General fry pond fertilization recommendations for ponds in the southeastern United States include much higher rates of inorganic fertilizer combined with organic applications. Ludwig et al. (1998) recommend an initial application of 54 kg/ha of liquid 10–30–0 (5.4 kg/ha N; 7.1 kg/ha P) and 280 kg/ha organic fertilizer (rice bran, cottonseed meal, or alfalfa pellets) followed by weekly applications of half the initial rate of inorganic and a fifth the organic rate.

The purpose of this study was first to evaluate phytoplankton and zooplankton responses to fertilization (addition of both organic and inorganic fertilizer) in channel

catfish nursery ponds before fish stocking. We also evaluated responses to organic, inorganic, and a combination of both fertilizer types in newly constructed versus established catfish nursery ponds.

2. Methods

Studies were conducted in 0.4-ha, 1.1-m deep earthen ponds constructed on alluvial clay soils of the Yazoo-Mississippi River floodplain at the Thad Cochran National Warmwater Aquaculture Center, Stoneville, Mississippi. Well water was supplied from the Mississippi River Alluvial Aquifer, the source of water for most catfish aquaculture in northwest Mississippi (Tucker, 1996; Tucker et al., 2001), which yielded total hardness and total alkalinity of 150–200 mg/l CaCO₃. In 2001, six ponds were filled 14 May. Afternoon water temperatures ranged from 28 °C at the beginning of the study to 31 °C at the end. Three ponds served as controls and did not receive fertilizer applications. The other three ponds received an initial application of 224 kg/ha cottonseed meal, 56 kg/ha calcium nitrate (8.4 kg/ha N), and 6 l/ha triple superphosphate (2.0 kg/ha P). After the initial application, fertilized ponds received once weekly applications of 28 kg/ha cottonseed meal and twice-weekly applications of 28 kg/ha calcium nitrate, and 1.7 l/ha triple superphosphate for 4 weeks. Although fertilization recommendations for catfish ponds in northwest Mississippi suggest adding less than 1 kg/ha of each N and P (Tucker and Robinson, 1990), anecdotal evidence from our research ponds suggests that higher rates, especially of N, provide better phytoplankton responses.

In 2002, 26 ponds were filled from 6 May to 13 May. Afternoon water temperatures ranged from 24 °C at the beginning of the study to 32 °C at the end. Sixteen ponds were newly constructed and had not been previously used for fish production; 10 ponds had been used for fish production for 16 years. Within each pond age group, treatments of no fertilizer additions, organic fertilizer only, inorganic fertilizer only, or a combination of organic and inorganic fertilizers were randomly assigned (Table 1). Ponds receiving organic fertilizer received an initial application of 84 kg/ha cottonseed meal followed 3 days later by 56 kg/ha. Thereafter, 28 kg/ha was added twice per week for 3 weeks. Ponds assigned to inorganic applications received an initial application of 44.8 kg/ha urea (20.2 kg/ha N), 6.2 l triple superphosphate (2.0 kg/ha P), followed by twice weekly applications of 22.4 kg/ha urea, and 2.5 l triple superphosphate for 3 weeks.

Table 1
Study design and treatment allocation for pond fertilization studies

Level of fertilizer	Level of "Age"		Total
	Old ponds	New ponds	
No additions	3 reps	4 reps	7 reps
Organic only	3 reps	4 reps	7 reps
Inorganic only	2 reps	4 reps	6 reps
Organic + Inorganic	2 reps	4 reps	6 reps
Total	10 reps	16 reps	

Old ponds were in use for 16 years; new ponds had not been previously used.

In both studies, sampling occurred twice per week. Water samples were collected by taking five tube samples (modified from Graves and Morrow, 1998) from the northeastern and southwestern corners of each pond (water depth = 1.3 m) and combined to obtain a total water volume of about 8 l. Chemical and biological variables from each pond were estimated from sub-samples of the composite sample.

The following water analyses were conducted according to methods described by APHA (1989): total ammonia (phenate method), nitrite (diazotization), nitrate (cadmium reduction and diazotization), total P (persulfate digestion and ascorbic acid finish), and soluble reactive P (SRP) (ascorbic acid method). Total N was determined by alkaline persulfate oxidation/digestion followed by cadmium reduction and diazotization (Koroleff, 1983).

Pigment analysis was used to assess phytoplankton community composition by using HPLC methodology (Zimba et al., 1999). Pigments (carotenoids and chlorophylls) were quantified with an HP1100 equipped with diode array and fluorescence detectors (Agilent Technologies, Palo Alto, CA). Identification of specific divisions of algae is possible by using taxon-specific pigment biomarkers (Zimba et al., 2002). A pigment library was used to identify samples; unknown samples were quantified by linear regression of known commercial standards.

Zooplankton samples were collected by taking oblique 2-m tows with a 63- μ m mesh Wisconsin-style net (Wildlife Supply, Saginaw, MI) at two locations in each pond. A mark was placed on the net's tow rope at 2 m. Samples were preserved in 240 ml of buffered formalin-sucrose solution prior to enumeration by light microscopy (Geiger and Turner, 1990). All organisms in three 1-ml subsamples from each pond were counted by using a Sedgwick-Rafter counting cell as described by Geiger and Turner (1990) and identified with the taxonomic keys of Thorp and Covich (1991).

In 2001, the analysis was for a completely randomized design with repeated measures taken on ponds twice per week for 4.5 weeks. In 2002, the analysis was for a 2×4 factorial (2 ages; 4 fertilizer types) design with repeated measures taken on ponds twice per week for 3.5 weeks. Data were analyzed with the MIXED procedure in SAS Version 8.02 software (SAS Institute, Cary, NC, USA) (Littell et al., 1996). The covariance structure, autoregressive of order 1, was used in the repeated measure

Table 2

Least-square means (SEM) of water quality variables (mg/l) in fertilized and control ponds from 18 May through 15 June 2001

	Un-fertilized control	Fertilized
Total Ammonia Nitrogen	0.054 (0.019)a	0.065 (0.019)a
Nitrite	0.009 (0.010)a	0.020 (0.010)a
Nitrate	0.062 (0.062)a	0.150 (0.062)a
Total Nitrogen	0.747 (0.136)a	1.075 (0.136)a
Soluble Reactive Phosphorus*	0.118 (0.046)a	0.338 (0.046)b
Total Phosphorus*	0.205 (0.040)a	0.426 (0.040)b

Variables within a row sharing the same letter are not significantly different ($P > 0.05$). Repeated measures mixed model, $N = 3$.

*Significant ($P < 0.05$) time \times treatment interaction.

Table 3

Least-square means (SEM) of phytopigment concentrations ($\mu\text{g/l}$) in fertilized and control ponds from 18 May through 15 June 2001

	Un-fertilized control	Fertilized
Chlorophyll <i>a</i>	38.64 (5.41)	54.14 (05.41)
Lutein	0.181 (0.050)	0.253 (0.050)
Fucoxanthin	1.742 (0.544)	3.256 (0.544)
Zeaxanthin	0.971 (0.169)	1.240 (0.169)
Myxoxanthin	0.011 (0.012)	0.015 (0.012)

No significant differences ($P>0.05$) or interactions were found among variables. Repeated measures mixed model, $N=3$.

model. Mean comparisons were made using an LSD test with a significance level of $P \leq 0.05$.

3. Results

Although we used higher rates of N and P than suggested by Tucker and Robinson (1990), the fertilization protocol used in 2001 still resulted in few significant differences between fertilized and non-fertilized ponds (Table 2). Total P and SRP were higher in fertilized ponds, whereas N species were not different. There was a significant interaction between treatment and time with P and SRP. Phosphorus concentrations increased in both treatments over time, but increased more in fertilized ponds. The increased P concentration did not increase algal populations as indicated by the phytopigments (Table 3). There was no significant treatment by time interaction with phytopigment or zooplankton concentrations. Only copepod nauplii were significantly higher in fertilized ponds than non-fertilized ponds (Table 4).

The increased N application rate in 2002 resulted in several differences not found in to the 2001 results (Fig. 1, Table 5). Although several interactions occurred, main effects are presented because F -values for main effects were generally several times greater than F -statistics for interactions; interactions with main effects are discussed in text.

Table 4

Least-square means (SEM) of zooplankton (#/l) in fertilized and non-fertilized ponds from 18 May through 15 June 2001

	Un-fertilized control	Fertilized
Rotifers	2576 (544)a	1799 (544)a
Copepod nauplii	104 (56)a	396 (56)b
Copepod adults	168 (15)a	205 (15)a
Cladocerans	53 (25)a	91 (25)a
Ostracods	14 (4)a	19 (4)a

Variables within a row sharing the same letter are not significantly different ($P>0.05$). Repeated measures mixed model, $N=3$.

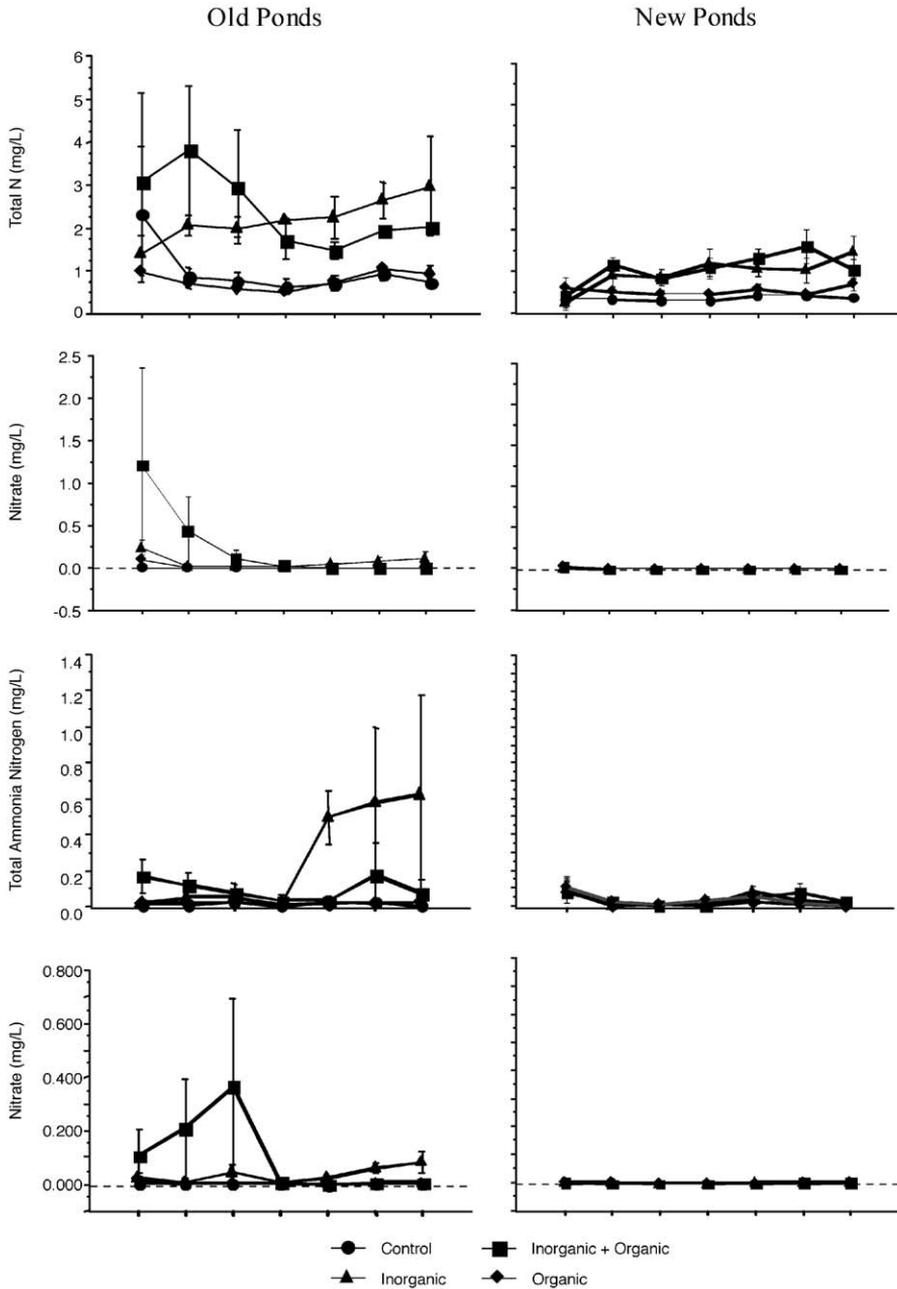


Fig. 1. Selected water quality variables (mg/l) in new and old ponds treated with various fertilization regimes from 13 May through 6 June 2002. Symbols represent means \pm SE (old $N=10$, new $N=16$, control $N=7$, inorganic $N=6$, organic $N=7$, inorganic+organic $N=6$). Error bars if not visible are smaller than the symbol.

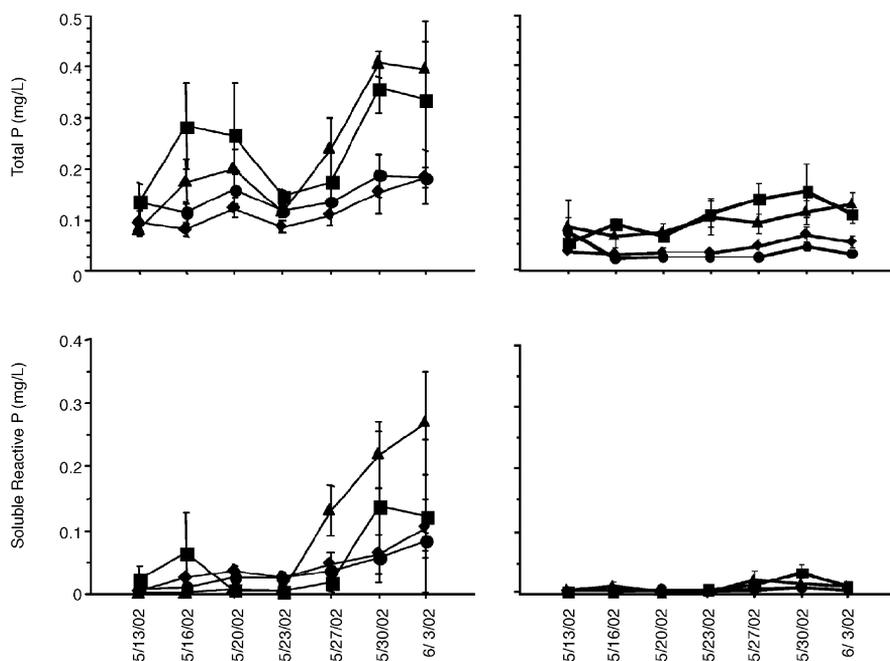


Fig. 1 (continued).

3.1. Nutrients

Total N was higher in old ponds than in new ponds (Fig. 1, Table 5). Total N was also higher in inorganic or inorganic plus organic fertilizer treatments than in control ponds or organic only treatments. A significant interaction occurred between pond age and time. New ponds, at filling, had low concentrations of total N. These values increased over time, whereas old ponds had higher concentrations at filling and decreased over time, but remained higher than new ponds throughout the study. There was also a significant interaction between fertilizer treatment and time. Non-fertilized control ponds decreased in N concentration over time. Ponds treated with inorganic fertilizer only increased in total N over time. Ponds treated with organic or both inorganic and organic fertilizers decreased in total N during the first half of the study, then increased over the second half.

Nitrate concentrations were not significantly different among main effects of pond age or fertilizer treatment, however NO_3 concentrations had a significant interaction among main effects. In both new and old control ponds, NO_3 levels were low and remained low throughout the study. New, inorganic fertilized ponds also remained low in NO_3 , but old inorganic fertilized ponds increased throughout the study. Old organic only fertilized ponds, at filling, were higher in NO_3 than new organically fertilized ponds, but NO_3 concentrations rapidly decreased in these old ponds and remained low and similar to new organically fertilized ponds throughout the remainder of the study.

Table 5

Least-square means (SEM) of water quality variables (mg/l) in new and old ponds treated with various fertilization regimes from 13 May through 6 June 2002

		Control	Inorganic	Organic	Both	Total
TAN ^{a,b,c}	New	0.024 (0.050)	0.038 (0.050)	0.039 (0.050)	0.039 (0.050)	0.035 (0.025)a
	Old	0.018 (0.058)	0.267 (0.070)	0.023 (0.058)	0.103 (0.070)	0.103 (0.032)a
	Total	0.021 (0.038)ab	0.153 (0.043)a	0.031 (0.038)b	0.071 (0.043)ab	
NO ₃ ^{a,b,c,d}	New	0.0001 (0.0137)	0.0012 (0.0137)	0.0011 (0.0137)	0.0013 (0.0137)	0.0011 (0.0068)a
	Old	0.0012 (0.0158)	0.0403 (0.0194)	0.0044 (0.0158)	0.1034 (0.0194)	0.0374 (0.0088)b
	Total	0.0010 (0.0105)a	0.0208 (0.0119)b	0.0028 (0.0105)a	0.0524 (0.0119)ab	
NO ₂ ^{a,b,c}	New	0.003 (0.093)	0.005 (0.093)	0.006 (0.093)	0.005 (0.093)	0.005 (0.046)a
	Old	0.001 (0.107)	0.076 (0.131)	0.017 (0.107)	0.252 (0.131)	0.087 (0.060)a
	Total	0.002 (0.071)a	0.040 (0.080)a	0.012 (0.071)a	0.129 (0.080)a	
TN ^{a,b}	New	0.367 (0.278)	0.947 (0.278)	0.563 (0.278)	1.040 (0.278)	0.729 (0.139)a
	Old	0.987 (0.320)	2.220 (0.392)	0.766 (0.320)	2.424 (0.392)	1.599 (0.179)b
	Total	0.677 (0.212)a	1.584 (0.240)b	0.664 (0.212)a	1.732 (0.240)b	
SRP ^{a,b,c}	New	0.004 (0.013)	0.008 (0.013)	0.004 (0.013)	0.009 (0.013)	0.006 (0.006)a
	Old	0.036 (0.014)	0.091 (0.018)	0.043 (0.014)	0.054 (0.018)	0.056 (0.008)b
	Total	0.020 (0.010)a	0.050 (0.011)a	0.023 (0.010)a	0.031 (0.011)a	
TP ^{a,b,c}	New	0.036 (0.018)	0.096 (0.018)	0.045 (0.018)	0.105 (0.018)	0.070 (0.009)a
	Old	0.147 (0.020)	0.230 (0.025)	0.119 (0.020)	0.243 (0.025)	0.185 (0.011)b
	Total	0.092 (0.013)a	0.163 (0.015)b	0.082 (0.013)a	0.174 (0.015)b	

Total values of main effects age or fertilization regime sharing the same letter are not significantly different ($P > 0.05$). Repeated measures mixed model, old $N = 10$, new $N = 16$, control $N = 7$, inorganic $N = 6$, organic $N = 7$, both $N = 6$.

^a Significant interaction between pond age and time.

^b Significant interaction between fertilizer treatment and time.

^c Significant pond age by fertilizer treatment by time interaction.

^d Significant interaction between pond age and fertilizer treatment.

Old ponds treated with both organic and inorganic fertilizers had the highest concentrations of NO₃ overall. Nitrate concentrations were high at the beginning, decreased over the first half of the study, and then were similar to new ponds for the second half of the study.

Nitrite concentrations remained below 0.5 mg/l in all ponds throughout the study. Nitrite concentrations were significantly higher in old ponds than in new ponds; ponds treated with inorganic fertilizer had higher concentrations than control ponds or ponds treated only with organic fertilizer. There were also several significant interactions among main effects with NO₂ concentrations. Nitrite concentrations were lowest throughout the study in control ponds. New ponds remained low and constant in NO₂ regardless of treatment. Highest NO₂ concentrations occurred in old ponds treated with both organic and inorganic fertilizers; concentrations were high during the first half of the study, but then were similar to new ponds treated with both fertilizers during the second half of the study.

Total ammonia nitrogen (TAN) was not different between old and new ponds, but was higher in inorganically fertilized ponds than in organically fertilized ponds. New and old control ponds were similar throughout the study in TAN. Old and new ponds fertilized with inorganic fertilizer were similar in TAN for the first half of the study, but were higher in the second half. Old and new ponds fertilized with both organic and inorganic decreased

initially in TAN concentrations, and then increased with old ponds reaching higher concentrations than new ponds. New and old organically fertilized ponds were similar in TAN throughout.

Total P was higher in old ponds than in new ponds. Total P increased in inorganically or inorganic and organically fertilized ponds when compared to control ponds or organically only fertilized ponds. In all other treatments, both old and new ponds increased in P over time, with old ponds increasing at a faster rate.

Soluble reactive P was higher in old ponds than in new ponds. All ponds increased in SRP during the study, with old ponds increasing faster.

3.2. *Phytopigments*

Many differences were also seen in 2002 among algal phytopigments (Fig. 2, Table 6). Considering the main effect of pond age, all measured phytopigments were significantly higher in old ponds than in new ponds.

Chlorophyll *a* (total algal biomass indicator) was higher in inorganic and inorganic plus organic treatments than in control or organic only treatments, but had significant interactions among main effects. Control ponds and organically fertilized ponds—both old and new—were similar in chlorophyll *a* concentrations and decreased over the study. Chlorophyll *a* in new ponds fertilized with either inorganic only or both inorganic and organic fertilizers was low throughout the study. Old ponds fertilized with either inorganic only or both inorganic and organic fertilizers increased initially in chlorophyll *a*, and then decreased throughout the second half of the study.

Lutein (green algal biomass) was also higher in inorganic and inorganic plus organic treatments than in control and organic only treatments, and showed a significant interaction between treatment and time. Control ponds had nearly constant lutein concentration throughout the study; organically treated ponds decreased in lutein throughout the study. Pond fertilized with inorganic or both organic and inorganic fertilizers increased in lutein initially, and then decreased throughout the second half of the study.

Fucoxanthin (diatom biomass) was significantly higher in inorganic and inorganic plus organic fertilized ponds than in organic fertilized or control ponds. There were significant interactions between pond age by time and treatment by time. Both new and old ponds increased in fucoxanthin for the first week and decreased the last week of the study, with old ponds reaching a higher peak 1 1/2 weeks into the study. Control ponds and organically fertilized ponds were low and constant throughout the study, whereas inorganic and inorganic plus organic fertilized ponds increased in fucoxanthin for the first 10 days, then decreased the last half of the study.

Zeaxanthin (cyanobacteria) was significantly higher in ponds fertilized with both inorganic and organic material than in organic fertilized ponds, and showed significant interactions among main effects. New ponds, regardless of treatment, remained low and consistent in zeaxanthin concentration. Old ponds treated with organic fertilizer decreased throughout the study; inorganic and inorganic plus organic fertilized old ponds had an initial increase in zeaxanthin, but then decreased throughout the rest of the study.

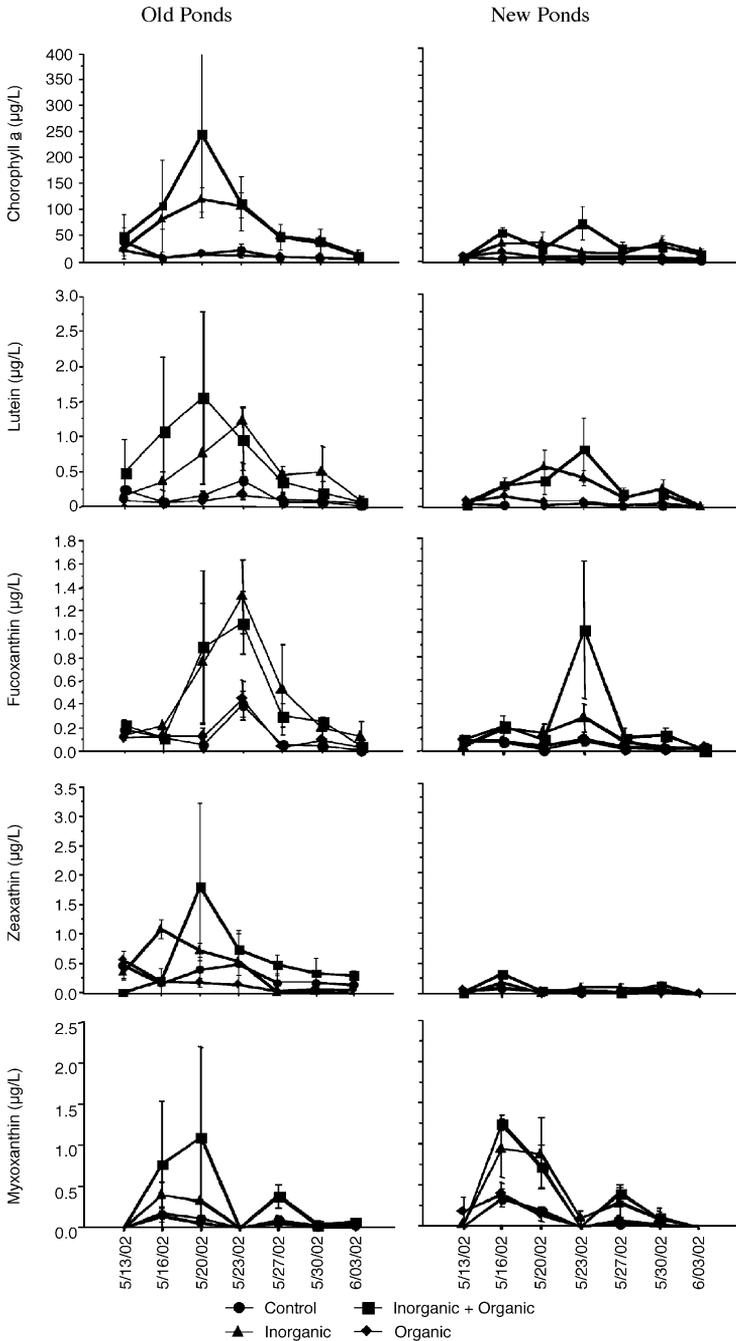


Fig. 2. Selected algal pigments (ng/ml) in new and old ponds treated with various fertilization regimes from 13 May through 6 June 02. Symbols represent means \pm SE (old $N=10$, new $N=16$, control $N=7$, inorganic $N=6$, organic $N=7$, inorganic+organic $N=6$). Error bars if not visible are smaller than the symbol.

Table 6

Least-square means (SEM) of phytopigment concentrations ($\mu\text{g/l}$) in new and old ponds treated with various fertilization regimes

		Control	Inorganic	Organic	Both	Total
Chlorophyll <i>a</i> ^{a,b,c,d}	New	5.4 (9.0)	24.2 (9.0)	9.8 (9.0)	32.9 (9.0)	18.1 (4.5)a
	Old	15.9 (10.4)	63.9 (12.7)	12.2 (10.4)	87.8 (12.7)	44.9 (5.8)b
	Total	10.7 (6.9)a	44.0 (7.8)b	11.0 (6.9)a	60.3 (7.8)b	
Lutein ^b	New	0.03 (0.09)	0.26 (0.09)	0.75 (0.09)	0.028 (0.09)	0.16 (0.04)a
	Old	0.14 (0.10)	0.51 (0.13)	0.09 (0.10)	0.67 (0.13)	0.35 (0.06)b
	Total	0.09 (0.07)a	0.38 (0.08)b	0.08 (0.07)a	0.47 (0.08)b	
Fucoxanthin ^{a,b}	New	0.05 (0.05)	0.12 (0.05)	0.06 (0.05)	0.25 (0.05)	0.12 (0.03)a
	Old	0.12 (0.06)	0.47 (0.08)	0.14 (0.06)	0.42 (0.08)	0.29 (0.04)b
	Total	0.09 (0.04)a	0.29 (0.05)b	0.10 (0.04)a	0.33 (0.05)b	
Zeaxanthin ^{a,b,d}	New	0.04 (0.06)	0.08 (0.06)	0.06 (0.06)	0.10 (0.06)	0.07 (0.03)a
	Old	0.30 (0.07)	0.40 (0.08)	0.18 (0.07)	0.57 (0.08)	0.36 (0.04)b
	Total	0.17 (0.05)ab	0.24 (0.05)ab	0.12 (0.05)a	0.38 (0.05)b	
Myxoxanthin ^{a,b}	New	0.02 (0.05)	0.07 (0.05)	0.02 (0.05)	0.07 (0.05)	0.04 (0.02)a
	Old	0.06 (0.05)	0.12 (0.07)	0.03 (0.05)	0.34 (0.07)	0.14 (0.03)b
	Total	0.04 (0.04)a	0.09 (0.04)ab	0.03 (0.04)a	0.21 (0.04)b	

Comparisons made between age or among fertilization regime sharing the same letter are not significantly different ($P > 0.05$). Repeated measures mixed model, old $N = 10$, new $N = 16$, control $N = 7$, inorganic $N = 6$, organic $N = 7$, both $N = 6$, 13 May through 6 June 2002.

^a Significant interaction between pond age and time.

^b Significant interaction between fertilizer treatment and time.

^c Significant Interaction between pond age and fertilizer treatment.

^d Significant pond age by fertilizer treatment by time interaction.

Myxoxanthin (cyanobacteria) concentrations were higher in ponds fertilized with both organic and inorganic fertilizers than in control ponds and organically only fertilized ponds. There were significant interactions among main effects. Old ponds had a higher initial increase in myxoxanthin than new ponds. All ponds had an initial peak in myxoxanthin at about 7–10 days and a second peak at about 17 days. The old ponds and ponds treated with inorganic fertilizer had higher peaks for myxoxanthin.

3.3. Zooplankton

Zooplankton concentrations were different among treatments in 2002 (Fig. 3, Table 7). Rotifer concentrations were significantly higher in old ponds than in new ponds but not different among fertilizer treatments. Old ponds had a peak in rotifer populations 7 days after filling; new ponds had a peak in rotifer populations 14 days after filling.

Old and new ponds were not different in concentrations of copepod nauplii, but nauplii were more abundant in ponds fertilized with both organic and inorganic fertilizer than in ponds fertilized with organic only. Old ponds had high concentrations of nauplii by the time they were filled and concentrations remained high throughout the study, whereas new ponds did not reach similar concentrations of nauplii until 14 days after filling and reached peak nauplii concentrations about 17 days after filling. In all treatments, new ponds reached peak concentrations of nauplii 17–21 days after filling, with ponds fertilized with inorganic or both inorganic and organic fertilizers reaching the highest peak concentrations.

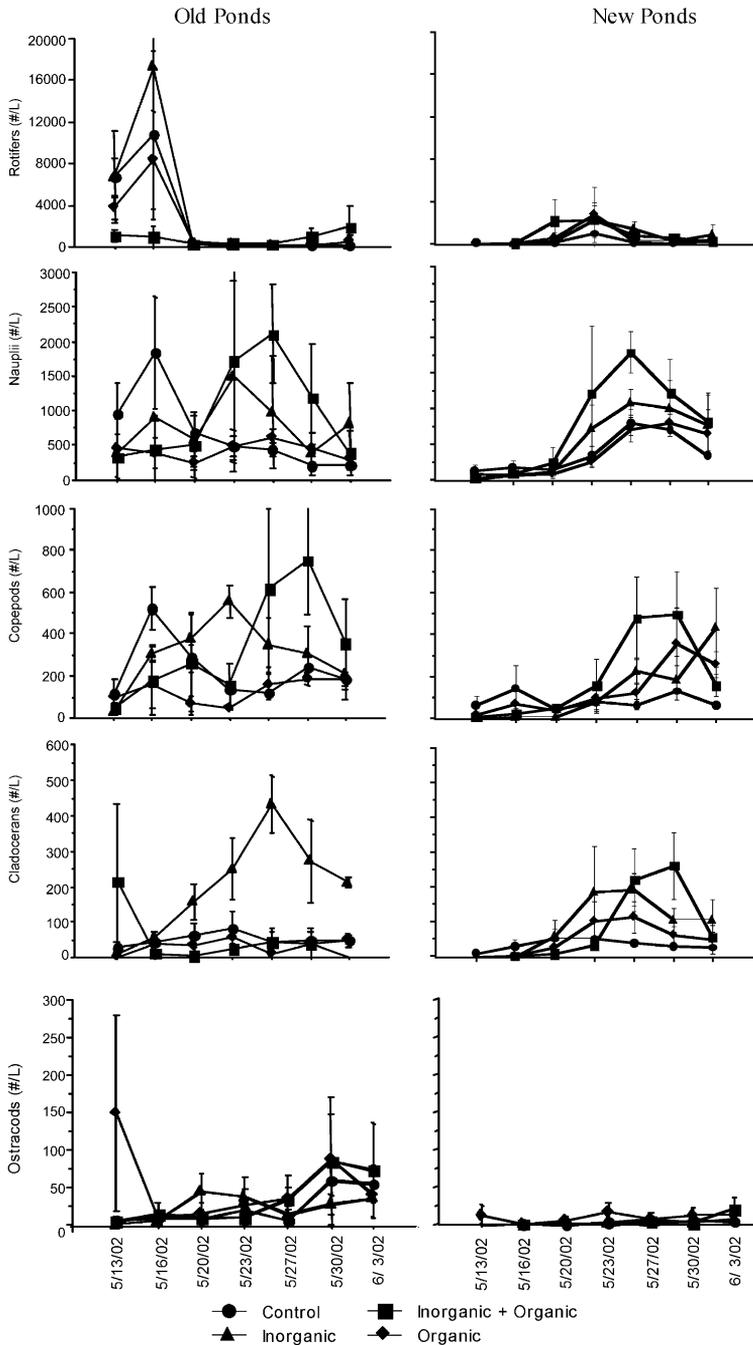


Fig. 3. Selected zooplankton (#/l) in new and old ponds treated with various fertilization regimes from 13 May through 6 June 2002. Symbols represent means \pm SE (old $N=10$, new $N=16$, control $N=7$, inorganic $n=N$, organic $N=7$, inorganic + organic $N=6$). Error bars if not visible are smaller than the symbol.

Table 7

Least-square means (SEM) of zooplankton (#/l) in ponds of different ages and different fertilization regimes

		Control	Inorganic	Organic	Both	Total
Rotifers ^a	New	310 (523)	785 (523)	668 (523)	921 (523)	671 (261)a
	Old	2551 (604)	3492 (739)	1912 (604)	810 (739)	2191 (337)b
	Total	1431 (399)a	2138 (453)a	1290 (399)a	865 (453)a	
Copepod nauplii ^{a,b}	New	386 (170)	551 (170)	385 (170)	780 (170)	526 (85)a
	Old	684 (197)	788 (241)	417 (197)	953 (241)	710 (110)a
	Total	535 (130)ab	670 (147)ab	401 (130)a	866 (147)b	
Copepod adults ^b	New	87 (48)	137 (48)	139 (48)	200 (48)	141 (24)a
	Old	229 (55)	303 (68)	129 (55)	337 (68)	249 (31)b
	Total	158 (36)ab	220 (41)ab	134 (36)a	268 (41)b	
Cladocerans ^{b,c}	New	35 (24)	93 (24)	51 (24)	84 (24)	66 (12)a
	Old	51 (27)	199 (33)	33 (27)	49 (33)	83 (15)a
	Total	43 (18)a	146 (20)b	42 (18)a	66 (20)a	
Ostracods ^b	New	3 (14)	3 (14)	10 (14)	4 (14)	5 (7)a
	Old	25 (16)	25 (19)	54 (16)	35 (19)	35 (9)b
	Total	14 (10)a	14 (12)a	32 (10)a	20 (12)a	

Comparisons made between age or among fertilization regime sharing the same letter are not significantly different ($P>0.05$). Repeated measures mixed model, old $N=10$, new $N=16$, control $N=7$, inorganic $N=6$, organic $N=7$, both $N=6$, 13 May through 6 June 2002. ^d Significant interaction between pond age and fertilizer treatment.

^a Significant interaction between pond age and time.

^b Significant interaction between fertilizer treatment and time.

^c Significant pond age by fertilizer treatment by time interaction.

Copepod adult concentrations were higher in old ponds than in new ponds and higher in ponds fertilized with both organic and inorganic fertilizer than in ponds fertilized only with organic material. In all fertilizer treatments, copepod concentrations tended to increase during the study with peak concentrations reached from 14–24 days after ponds were filled. Peak concentrations in ponds fertilized with organic and inorganic material reached about 600/l, compared to 300–350/l in ponds receiving only organic or inorganic fertilizer.

Over the course of the study, cladoceran samples were made up primarily (~ 65%) of *Moina* spp. Other genera included *Daphnia* (~ 26%)>*Chydorus* (~ 3%)>*Ceriodaphnia* (~ 1%)>*Bosmina* (~ 1%)>*Sida* (~ 1%)>*Alona* (<1%). Cladoceran concentrations were not significantly different between old and new ponds but were significantly higher in ponds fertilized with inorganic fertilizer than in all other treatments. Cladoceran population peaks were reached about 10 d after filling in both new (50/l) and old (80/l) control ponds. In inorganically fertilized ponds, cladocerans reached peak concentrations 17 days after ponds were filled in both new (200/l) and old (450/l) ponds. Ponds fertilized with both organic and inorganic fertilizer reached peak concentrations 21 days after being filled in new (25/l) and old (250/l) ponds. New ponds treated with organic fertilizer reached peak cladoceran concentrations (120/l) 17 days after filling, old ponds reached 60 cladocerans/l 14 and 24 days after filling.

Ostracod concentrations were greater in old ponds than in new ponds. Control ponds had peak concentrations of ostracods (30/l) 21 days after filling. Inorganically fertilized ponds reached the highest ostracods concentrations (20/l) 24 days after being filled. Ponds fertilized with both organic and inorganic fertilizer reached 40 ostracods/l 24 days after

being filled. In organically fertilized ponds, ostracods concentrations were highest just after ponds were filled (75/l) and 21 days after filling (50/l).

4. Discussion

Reasons to fertilize catfish nursery ponds are to (1) rapidly obtain a phytoplankton bloom to prevent growth of macrophytes and (2) increase zooplankton concentrations for fry forage. Although catfish fry are offered commercial feeds when stocked, fry readily consume zooplankton and selectively forage on larger zooplankton such as copepods, cladocerans, and ostracods but do not consume rotifers or copepod nauplii (Mischke et al., 2003). Fry older than two weeks consume cladocerans, ostracods, and chironomid larvae (Bonneau et al., 1972). When fry are 5 weeks old, prepared feeds comprise the majority of ingested feed (Bonneau et al., 1972). Although stocking rates have increased from about 70 000 fry/ha in 1972 to about 250 000 fry/ha today, fry behavior still seems to indicate reliance on natural food organisms during the first 3–4 weeks of culture. No evidence is available that fry actually consume prepared feeds added to the ponds during the initial weeks of culture, and it is assumed that initially added feed serves as a fertilizer (Tucker and Robinson, 1990). Therefore, the best fertilization protocol for catfish fry would be one that quickly establishes a phytoplankton bloom to prevent macrophyte growth and produces the greatest number of large zooplankton at the time of fry stocking. High concentrations of copepods, cladocerans and ostracods would be desirable from the time of stocking through about 5 weeks of production.

The fertilization rates used in 2001 had little affect on phytoplankton concentrations and, consequently, little affect on zooplankton. Nutrient enrichment bioassays indicated that nitrogen may be limiting in the ponds (unpublished data); therefore, the nitrogen levels were increased for the 2002 study. After increasing the nitrogen rates, several significant differences in phytoplankton standing stocks were detected between fertilized and non-fertilized ponds. This result indicates these ponds are primarily nitrogen limited and not phosphorus limited. This is in contrast to Tucker and Robinson's (1990) contention that phosphorus is the key ingredient in fertilizer and recommendation to use a fertilizer with three times as much P_2O_5 as N (i.e., 0.7:1 N/P ratio). Most catfish ponds are constructed on soils of Alligator or Sharkey clays that are moderately acidic (pH 5–6.5) with low organic matter content (Tucker, 1996). These soils historically have medium to high soil test levels of P. Although P precipitation is usually a concern in water with high hardness, total P and soluble reactive P increased in all ponds throughout the study in both years. In this study we used four times as much N as P_2O_5 (i.e., 9:1 N/P ratio); Culver et al. (1993) recommend using 15 times as much N as P_2O_5 (i.e., 34:1 N/P ratio) for walleye *Stizostedion vitreum* nursery ponds. Although the 2002 protocol resulted in significant differences among treatments, even higher nitrogen application may be beneficial, especially in new ponds. In all treatments, nitrate levels remained low and were essentially zero in the new ponds. Total nitrogen and phosphorus concentrations increased in all ponds receiving inorganic fertilizer, although not as much in new ponds, indicating new ponds may require several times the amount of nitrogen and phosphorus required in old ponds. Phytoplankton and zooplankton concentrations were dropping to

low levels in all fertilization treatments at the end of the study. Because no fish were present, this may also indicate a need for increased fertilizer application rates.

The addition of cottonseed meal was expected to have a direct effect on zooplankton populations; organic fertilizer provides substrate for bacterial growth, can be consumed by zooplankton directly, and may decompose and provide nutrients for phytoplankton (Knud-Hansen, 1998). However, organic fertilizer application had little effect on phytoplankton or zooplankton concentrations. Our initial application was substantially less than that recommended by Ludwig et al. (1998), and the weekly rate was approximately half the recommended rate. Using higher rates of organic fertilizer may eventually produce responses in phytoplankton or zooplankton, but Qin et al. (1995) and Soderberg et al. (1997) also found little response to organic fertilizers (alfalfa meal) in walleye *S. vitreum* ponds and recommend not fertilizing with organic fertilizers because of the potential dissolved oxygen reduction. Although channel catfish fry ponds typically have emergency aeration available, aerating ponds is expensive and unnecessarily risking dissolved oxygen depletion would be unwise. During a 6-week trial, Ludwig (2002) was able to increase zooplankton populations with increased organic fertilizer (rice bran; 472–1888 kg/ha) and inorganic fertilizer, but increased fertility was detrimental to water quality and adversely affected fish survival.

Old ponds contained higher nutrient levels, higher algal biomass, and higher concentrations of rotifers, copepods, and ostracods. This would be expected from reported temporal succession patterns in channel catfish ponds (Zimba et al., 2003). As ponds age, nutrients accumulate for the first 3 years. It is also likely that resting stages of algae and zooplankton also increase. This suggests that older, mature ponds should be used as nursery ponds; phytoplankton blooms should respond more quickly because of the higher ambient nutrient levels and zooplankton populations should increase more quickly because of the established seed populations.

Several authors of fertilization studies are proponents of managing ponds as individual systems rather than using traditional fertilization protocols where every pond is treated in a similar manner. Anderson (1993) contends that adjacent ponds have individual “personalities”, and that using a systems approach to pond fertilization will improve the quantity and quality of fish produced and improve ecological and economic efficiencies. Culver et al. (1993) recommend fertilizing walleye fry ponds in Ohio with weekly nutrient restoration to 600 µg N/l and 30 µg P/l with inorganic fertilizers only. Systems approaches to pond fertilization may be ecologically more efficient than the traditional approach. This approach would be appropriate at public hatcheries or farms with few ponds to manage. However, commercial catfish aquaculture has over 10000 ha in fry and fingerling production. Continuous monitoring of nutrient concentrations and individual pond fertilization rates would be labor intensive and cost prohibitive. Most of the catfish industry is also located in northwest Mississippi; farm-to-farm responses to fertilization should be similar given similar sediment, climatology, and water source.

Zooplankton responses to fertilization were also studied in Texas ponds without fish (Parmley and Geiger, 1985). In that study, at 20 °C, small cladocerans reached maximum densities on day 15, copepod nauplii and adults on day 26, and *Daphnia* spp. on day 30. Based on these zooplankton succession patterns, we previously recommended filling fry

ponds 2–3 weeks before stocking (Mischke et al., 2003). However, in the present study, zooplankton populations reached peak concentrations earlier and began increasing at an accelerating rate 7–10 days after filling. Temperatures over the course of our study were greater and may be the reason for faster zooplankton establishment.

5. Conclusion

Zooplankton populations desired in catfish culture in the old ponds began increasing at an accelerating rate about 7–10 days after filling. Copepod and cladoceran populations in inorganically fertilized old ponds reached peak concentrations at 14 and 17 days, respectively. Phytoplankton biomass increased more quickly in old ponds and ponds receiving inorganic fertilizer. The rates of organic fertilizer used in this study did not appear to be beneficial in bloom production or concentrations of larger-sized zooplankton. We suggest using only established ponds for fry culture and filling ponds 7–10 days before stocking. Applying inorganic fertilizer at an initial rate of ~ 20 kg/ha N and 2 kg/ha P, followed by subsequent applications of half the initial rate for 3–4 weeks increased zooplankton concentrations desirable for fry with larger mouth gapes. If newly constructed ponds are used, higher fertilizer rates are probably necessary to achieve the same response.

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