



# Zooplankton as an alternative method for controlling phytoplankton in catfish pond aquaculture

Angelea P. Belfiore, Riley P. Buley, Edna G. Fernandez-Figueroa, Matthew F. Gladfelter, Alan E. Wilson<sup>\*</sup>

Auburn University, School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn, AL 36849, USA

## ARTICLE INFO

### Keywords:

Cyanobacterial control  
Biomaniipulation  
*Daphnia*  
Eutrophication  
Harmful algal bloom  
Trophic cascade

## ABSTRACT

In pond aquaculture, production of toxins and off-flavor compounds by cyanobacteria can negatively affect fish health and production. Studies have explored chemical or physical methods for controlling algal blooms in aquaculture ponds, which although effective, may be short-lived and can negatively impact non-target organisms, including aquaculture species. Food web manipulations have a long history in lake and fisheries management to improve water quality, but have been rarely considered in aquaculture. This study examined zooplankton and phytoplankton communities, cyanobacterial toxins, and nutrients in nine catfish aquaculture farm-ponds in west Alabama, USA. The goal of this project was to track phytoplankton and zooplankton abundances with respect to each other, with and without efforts to reduce zooplanktivorous fish in some of the ponds. During this project, farm managers reduced zooplanktivorous fish abundance in select ponds to create a large-scale field experiment that addressed the role of zooplankton control of phytoplankton in hypereutrophic catfish aquaculture ponds when zooplanktivorous fish were or were not excluded. There was a strong negative effect of zooplankton on phytoplankton, including cyanobacteria, despite high nutrient concentrations. Although high zooplankton ponds sustained elevated zooplankton biomass during much of this study, including when pond temperatures exceeded 30 °C, the effect of zooplankton on phytoplankton was most pronounced during the non-growing season (November–April). In addition, total ammonia nitrogen was significantly higher in high zooplankton ponds, which could lead to ammonia toxicity in fish at elevated temperature and pH. Our findings suggest that zooplankton biomanipulation may be an efficient method to control algal blooms in farm-pond catfish aquaculture.

## 1. Introduction

### 1.1. Algae in aquaculture

As human expansion continues, freshwater resources will be further limited while supporting a growing population (Rodell et al., 2018) that often leads to excessive nutrient loading and pollution creating eutrophic systems that promote the growth of phytoplankton (Carpenter et al., 1999; Heisler et al., 2008). In some aquaculture systems, phytoplankton serve as the base of the food web and aid in important nutrient cycling processes while ultimately increasing aquaculture yield (Paerl and Tucker, 1995). However, some phytoplankton, including toxic taxa, can pose a threat to fish health at high abundances (Manning and Nobles, 2017). Moreover, hypoxia promoted by excessive nocturnal planktonic respiration or through bacterial decomposition of decaying

algal blooms can cause fish kills (Boyd, 2019). Long-term stress leading to fish mortality can be attributed to hypoxic or anoxic conditions if dissolved oxygen (DO) is below the species threshold (Abdel-Tawwab et al., 2019). Consequently, daily aeration of aquaculture ponds is often required to maintain suitable water quality for farmed fish.

Nutrient concentrations can be high in outdoor, pond-based aquaculture systems due to regular feeding and fish waste. This is true for the US catfish industry, which is located predominately in the southeastern US and uses outdoor earthen ponds as the primary production unit. Once remineralized by zooplankton and fish, fish feed can contribute substantially to the pool of available nutrients for phytoplankton depending on the time of year, feed management strategies, and rate of ingestion (Bosma and Verdegem, 2011). These factors, along with stable, shallow ponds and high temperatures, allow for regular and persistent algal blooms. These blooms are often dominated by cyanobacteria (commonly

<sup>\*</sup> Corresponding author.

E-mail address: [wilson@auburn.edu](mailto:wilson@auburn.edu) (A.E. Wilson).

<https://doi.org/10.1016/j.aqrep.2021.100897>

Received 14 July 2021; Received in revised form 31 August 2021; Accepted 4 October 2021

Available online 13 October 2021

2352-5134/© 2021 The Authors.

Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

called blue-green algae), but also can include major taxa such as chlorophytes, haptophytes, euglenophytes, and dinoflagellates (Lopez et al., 2008).

Cyanobacteria can be the dominant photosynthetic organism found in freshwater algal blooms because of their competitive abilities (e.g., pseudovacuoles to aid in buoyancy regulation, nitrogen fixation, high thermal tolerance; Paerl and Paul, 2012). Some cyanobacterial species produce microcystin toxins that can affect animal health (Chen et al., 2009; Li et al., 2021; Walsby and McAllister, 1987; Wang et al., 2021). They also have the ability to produce off-flavors, such as geosmin and 2-methylisborneol (MIB), which can be especially detrimental for aquaculture farms causing muddy tasting fish fillets (Tucker and Schrader, 2020) that can prolong holding time and extend feeding costs until off-flavors are not detected (delayed harvest), delays in stocking, and increased health issues (Engle et al., 1995).

### 1.2. Controlling algal blooms

Phytoplankton can be controlled by a variety of approaches, including filtering, shading, limiting nutrients, or applying algacides (Donaghay and Osborn, 1997). Currently, the only algacides approved for use in catfish aquaculture in the US are copper sulfate ( $\text{CuSO}_4$ ) and under special circumstances diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) (United States Environmental Protection Agency (EPA), 2003). Although both algacides are effective for controlling algal blooms in aquaculture, there have been negative results associated with these kinds of chemicals, including excessive zooplankton mortality (Mischke et al., 2009), phytoplankton resistance to the chemical (Garcia-Villada et al., 2004; Rouco et al., 2014), and short-lived treatment effectiveness (Buley et al., 2021). Therefore, pursuing alternative methods for controlling algal blooms in aquaculture may aid in long-term bloom management.

The use of zooplankton in biomanipulation of phytoplankton in natural systems has been reported for decades (Brooks and Dodson, 1965; Porter, 1973; Hanson and Butler, 1994). These controls, like the proposed use of grass carp to reduce cyanobacteria, have been limited to macrophytes and filamentous algae (Kasinak et al., 2015a). Other biomanipulations have used carp to control plankton, both phytoplankton and zooplankton, in eutrophic systems (Guo et al., 2015; Wang et al., 2016; Xie and Liu, 2001). Most relevant studies altered food webs by removing zooplanktivorous fish (directly with chemical or physical methods or by adding piscivorous fishes) to promote large-bodied zooplankton (i.e., trophic cascade hypothesis (Shapiro et al., 1975; Carpenter et al., 1985)). Filter-feeding zooplankton, such as cladocerans (i.e., *Daphnia* and *Bosmina*), have been shown to control phytoplankton leading to clear-water states in eutrophic systems (Triest et al., 2016). Although studies suggest that zooplankton are unable to control cyanobacteria because of their size, abundance, edibility, and biochemical properties (De Bernardi and Giussani, 1990; Borges et al., 2010), recent studies have shown that large-bodied zooplankton, namely *Daphnia* (Chislock et al., 2013b, 2019a, 2019b) and smaller-bodied cladocerans (Guo and Xie, 2006), can effectively graze on cyanobacteria, including toxic strains as well. Unlike natural lake systems where there may be a regular fluctuation of nutrients entering and leaving the system, aquaculture ponds are relatively closed systems with much of the nutrient cycling happening between algae and bacteria (Moriarty, 1997). Therefore, by removing non-harvested zooplanktivorous fishes, the zooplankton communities are enhanced to promote a top-down biological control of phytoplankton.

### 1.3. Application to aquaculture and purpose

Studies have shown both success of biomanipulation in shallow eutrophic systems (Kasprzak et al., 2002; Peretyatko et al., 2009) and failures (Peretyatko et al., 2012) especially with high cyanobacterial biovolume (Gliwicz, 1990). The purpose of this observational

experiment was to apply the findings of Brooks and Dodson (1965) and Shapiro et al. (1975) to test if large-bodied zooplankton can control phytoplankton abundance in an aquaculture system. Most biomanipulation experiments have occurred in temperate lakes, differing from those conducted in small southeastern US ponds (Crisman and Beaver, 1990). The fingerling catfish in those aquaculture ponds are dependent on zooplankton as their food source, more so than the adults (Ludwig, 1999). However, catfish in this study are stocked at a size not dependent on zooplankton as their primary food source given their poor eyesight and routine manufactured feed additions; therefore, biomanipulation in these systems may be successful. We hypothesized that ponds with reduced zooplanktivorous fish will have higher concentrations of zooplankton leading to lower phytoplankton abundance and improved water quality.

## 2. Materials and methods

Nine catfish aquaculture ponds split between two farms (Farm A1–4 ponds; Farm B5–9 ponds) in west Alabama were sampled on the same day each quarter for two years (August 2018, October 2018, January 2019, April 2019, July 2019, October 2019, December 2019, March 2020, and June 2020). Ponds A1–4 stocked hybrid catfish (*Ictalurus punctatus* X *I. furcatus*) at 7000–8000/acre. Feeding rates ranged from 143 to 262 pounds/acre and aeration from 9.6 to 13.3 horsepower/acre (Table 1). Ponds B5–9 at the second farm were stocked with channel catfish (*I. punctatus*) at a density of 7500–8000/acre and fed until satiation, up to 150 pounds/day. Aeration for these ponds ranged from 4.5 to 10 horsepower/acre (Table 1). No dissolved oxygen (DO) crashes were observed in the 9 ponds. Both farms applied copper treatment to their ponds as needed (up to four times a week) during the growing season (May–October). Ponds B6–8 were copper-treated at much higher frequency than ponds B5 and B9.

To test the effects of biomanipulation on water quality in catfish aquaculture ponds, farm managers reduced zooplanktivorous fish (i.e., *Dorosoma* sp.) in some ponds by using small (44.5 mm) mesh nets (B5 and B9) or a fine screen sock (A1) when refilling ponds. After ponds were refilled, the planktonic communities established themselves from naturally occurring taxa. A long, rigid integrated plastic tube sampler (inside diameter = 51 mm) was used near-shore (~2 m) to collect 4 L of water at the same location in each pond from the surface to ~0.5 m deep. Temperatures ranged from 7.6 to 23.6 °C in the non-growing season and 17.5–31.2 °C in the growing season. Samples were processed immediately upon return to the laboratory for two algal pigments (chlorophyll-a and phycocyanin), nutrients (total nitrogen (TN), total phosphorus (TP), nitrite-nitrogen ( $\text{NO}_2\text{-N}$ ), total ammonia nitrogen (TAN), and soluble reactive phosphorus (SRP)), cyanobacterial toxins (microcystin),

**Table 1**

Details on production, species, stocking rates, aeration, feeding rates, and treatment rates for ponds A1–4 and B5–9.

Pond ID	Size (acre)	Catfish species	Feeding rate (lbs/acre)	Stocking rate per acre	Aeration (HP/acre)	Chemical treatment (A1–4: pounds of treatment/day; B5–9: times/week)
A1	3.75	Hybrid	262	8000	13.30	2.5–30
A2	6.25		147	7000	9.60	5.0–25
A3	6.50		161	7000	12.30	25–40
A4	6.50		143	7000	10.76	5.0–30
B5	4.00	Channel	To satiation	7500	5.00	2x
B6	10.00			7500	4.50	10x
B7	5.00			7500	6.00	6x
B8	4.00			8000	6.00	6x
B9	2.00			8000	10.0	1x

phytoplankton biovolume, and zooplankton dry biomass.

Algal pigments, including chlorophyll-a (all phytoplankton) and phycocyanin (cyanobacteria), were measured fluorometrically from samples collected on Pall A/E filters. Chlorophyll extraction was done with 90% aqueous ethanol and stored for 23 h in the dark at 4 °C (Sartory and Grobbelaar, 1984). Phycocyanin was measured by extracting filters in a phosphate buffer in the dark for four hours (Kasinak et al., 2015b). Total phosphorus and TN were measured using a spectrophotometer after persulfate digestion (Gross and Boyd, 1998). Nitrite-N, TAN (salicylate method), and SRP were measured colorimetrically on a spectrophotometer (Reardon et al., 1966; Murphy and Riley, 1962). Particulate microcystin toxins were measured using enzyme-linked immunosorbent assay (ELISA; Abraxis-ADDA) kits after extracting samples collected on Pall A/E filters with 75% aqueous acidified methanol (Yang et al., 2018).

Phytoplankton samples were preserved with 1% Lugol's iodine solution in glass bottles. To process phytoplankton samples, preserved samples were mixed and settled in Hydro-Bios™ chambers, each holding 10 ml total (1 ml preserved sample + 9 ml deionized water), where some additional Lugol's solution was added to maintain the preservation. Once samples had settled for at least 24 h, all phytoplankton were enumerated in 25 fields on a Nikon Eclipse Ti2 inverted microscope and measured at 200x–400x (Yang et al., 2018). Identifications were made to genus (Edmondson, 1959). Measurements of cells were shape-dependent, including length and width, diameter, or cell depth for each species for each sample to calculate mean cell biovolume for each taxon that was multiplied by cell density to estimate cell biovolume for each species. All phytoplankton data were grouped into major taxa prior to statistical analyses.

Zooplankton samples were concentrated onto a 100 µm sieve and preserved in 95% ethanol. Zooplankton samples were counted in a 1 ml Sedgwick-Rafter chamber, either counting all organisms in the sample or taking a subsample if not all organisms could fit in the chamber. The chamber was enumerated on a Nikon Eclipse 50i compound microscope and measured at 40x–100x magnification (Yang et al., 2018). Zooplankton length (µm) was measured from top of the head to the base of the body to estimate total biomass (µg) by multiplying dry biomass by the average length of the animal (Culver et al., 2011). All zooplankton data were grouped into major taxa prior to statistical analyses (Edmondson, 1959).

### 2.1. Statistical analysis

Repeated measures analysis of variance (ANOVA) using a restricted maximum log-likelihood (REML) method was used in the *nlme* R package (Pinheiro et al., 2020; R version 4.0.2) to determine if response variables (e.g., plankton, pigment, and nutrient concentrations) were affected by zooplankton pond type, time, and their interaction across the duration of the experiment. A Tukey's post-hoc test was done when parameters were less than 0.05 significance to determine which groups differed. We determined high and low zooplankton ponds by graphically examining trends and averaging groupings. These groups were then tested statistically verifying the significance of the separations using a RM-ANOVA. We included farm as a random factor in the analyses, nesting pond within farm. To determine if farm was a significant term to keep in the model, we tested models with and without it. There was no significant difference between the model with the pond nested in farm versus analysis where pond was used as a random factor.

## 3. Results

### 3.1. Zooplankton biomass

Two years of quarterly sampling nine ponds showed significant differences of zooplankton biomass between ponds with low or high zooplankton biomass ( $p < 0.0000001$ ; Fig. 1A and B). On average across

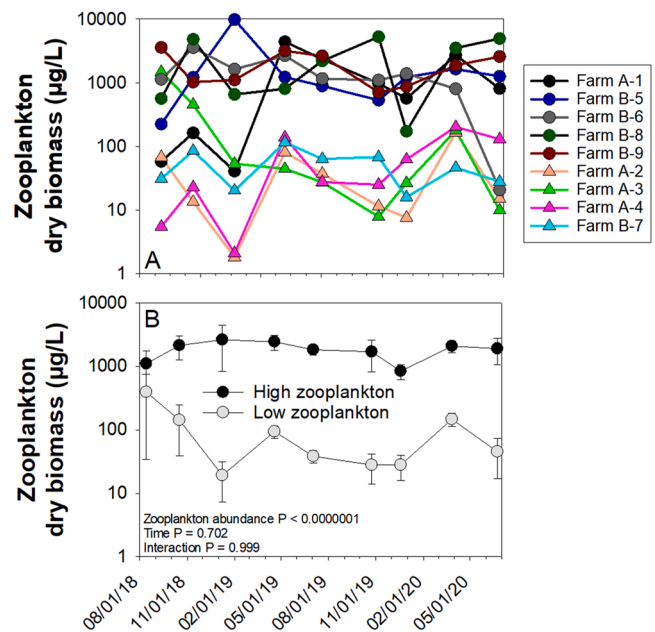


Fig. 1. (A) Quarterly zooplankton dry biomass (µg/L) for nine ponds with either high (dark circles) or low (light triangles) zooplankton abundance. (B) Mean quarterly zooplankton dry biomass (µg/L) for both pond types (black = high zooplankton biomass, grey = low zooplankton biomass). Error bars in panel B represent one standard error.

the entire study, high zooplankton ponds had 18 times more zooplankton dry biomass than low zooplankton ponds. Zooplankton in high zooplankton ponds included copepods (e.g., cyclopoid, calanoid, nauplii) and cladocerans (e.g., *Diaphanosoma*, *Ceriodaphnia*, *Daphnia* spp., and *Bosmina*). Copepods were common during the entire study in both types of zooplankton ponds, but were  $\geq 31\%$  more abundant ( $p < 0.000014$ ) based on dry biomass, in low zooplankton ponds than high zooplankton ponds (Fig. 2A). Cladocerans generally accounted for 30–89% of the zooplankton biomass in individual high zooplankton ponds and were four times relatively more abundant than in low zooplankton ponds across the entire study (Fig. 2B). Rotifers, namely *Asplanchna* spp., were generally low in abundance ( $< 10\%$  of total zooplankton biomass), if present at all, and were not included in statistical analysis.

### 3.2. Phytoplankton abundance

Due to the highly productive nature of these aquaculture ponds, phytoplankton are present year-round because of high nutrient inputs. A large phytoplankton diversity was observed across the study ponds, including major taxa, such as cyanobacteria (*Microcystis*, *Dolichospermum*, *Oscillatoria*), green algae (*Scenedesmus*, *Chlorella*, *Francia*), diatoms (*Synedra*, *Cyclotella*), euglenoids (*Euglena*, *Trachelomonas*, *Phacus*), and cryptophytes (*Cryptomonas*, *Rhodomonas*), but phytoplankton species composition and abundance varied slightly throughout the year with respect to growing and non-growing season. There was a considerable decrease in both total phytoplankton and cyanobacteria in the non-growing season (Fig. 3A–B and F). High zooplankton ponds had 51% less chlorophyll ( $p = 0.00449$ ; Fig. 3A), 58% less phytoplankton biovolume ( $p = 0.00609$ ; Fig. 3C), 30% less phycocyanin ( $p < 0.001$ ; Fig. 3B), and 81% less cyanobacterial biovolume ( $p = 0.0742$ ; Fig. 3D), on average, than low zooplankton ponds. Although the significance was marginal for the effect of zooplankton abundance on cyanobacterial biovolume ( $p = 0.0742$ ), there was a statistically significant effect when considering season and zooplankton abundance ( $p < 0.0001$ ; Fig. 3F). Cyanobacteria generally dominated all ponds during the growing season when near surface water temperatures were  $\geq 28$  °C (late April to late

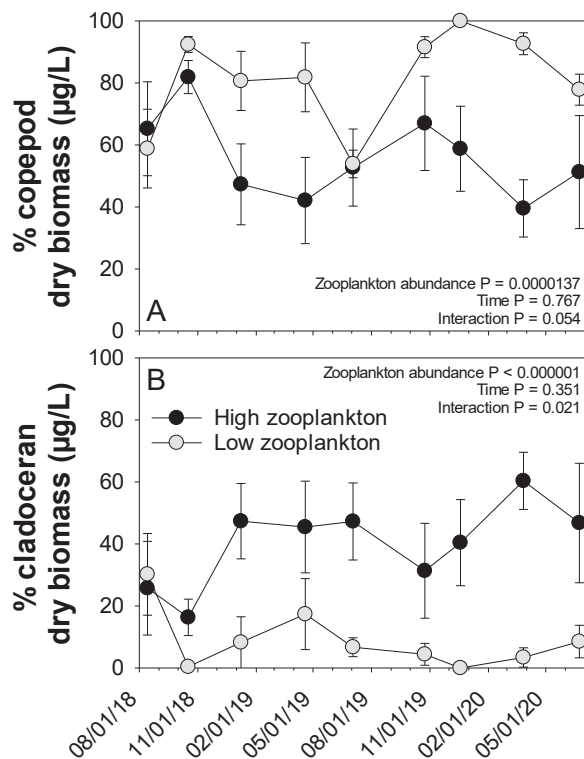


Fig. 2. Relative quarterly abundance of (A) copepods and (B) cladocerans measured as dry biomass ( $\mu\text{g/L}$ ) for high (black) and low (grey) zooplankton biomass ponds. Error bars represent one standard error.

October; Fig. 3B and D). Microcystin concentrations were 88% lower in high zooplankton ponds than low zooplankton ponds ( $p = 0.0305$ ; Fig. 3E) with expected peaks during the growing season when toxigenic cyanobacteria are present (Fig. 3B and D).

### 3.3. Nutrient availability

Ponds that had lower abundances of phytoplankton (i.e., high zooplankton ponds) had greater amounts of some dissolved nutrients given that less phytoplankton were present to assimilate available nitrogen and/or phosphorus (Fig. 4). High zooplankton ponds had 17% more TN ( $p = 0.0254$ ; Fig. 4A) and 68% more TAN ( $p = 0.000363$ ; Fig. 4C) than low zooplankton ponds. Interestingly, high and low zooplankton ponds did not statistically differ in concentrations of TP ( $p = 0.499$ ; Fig. 4B), SRP ( $p = 0.184$ ; Fig. 4D), or  $\text{NO}_2\text{-N}$  ( $p = 0.753$ ; Fig. 4E).

## 4. Discussion

This study highlighted interesting dynamics between large-bodied zooplankton and phytoplankton, including cyanobacteria, in hyper-eutrophic aquaculture ponds that suggests a sustainable tool for managing algal blooms. Although the abundances of zooplanktivorous fish in the studied ponds are not available, we report that minor management efforts (e.g., screen inflows to remove zooplanktivorous fish when filling ponds) are associated with significant improvements in water quality that were likely mediated by increases in ambient zooplankton communities.

### 4.1. Zooplankton abundance

Food web manipulations (namely, excluding zooplanktivorous fish) led to large (18x), sustained differences in ambient zooplankton biomass between high (zooplanktivorous fish excluded) and low zooplankton

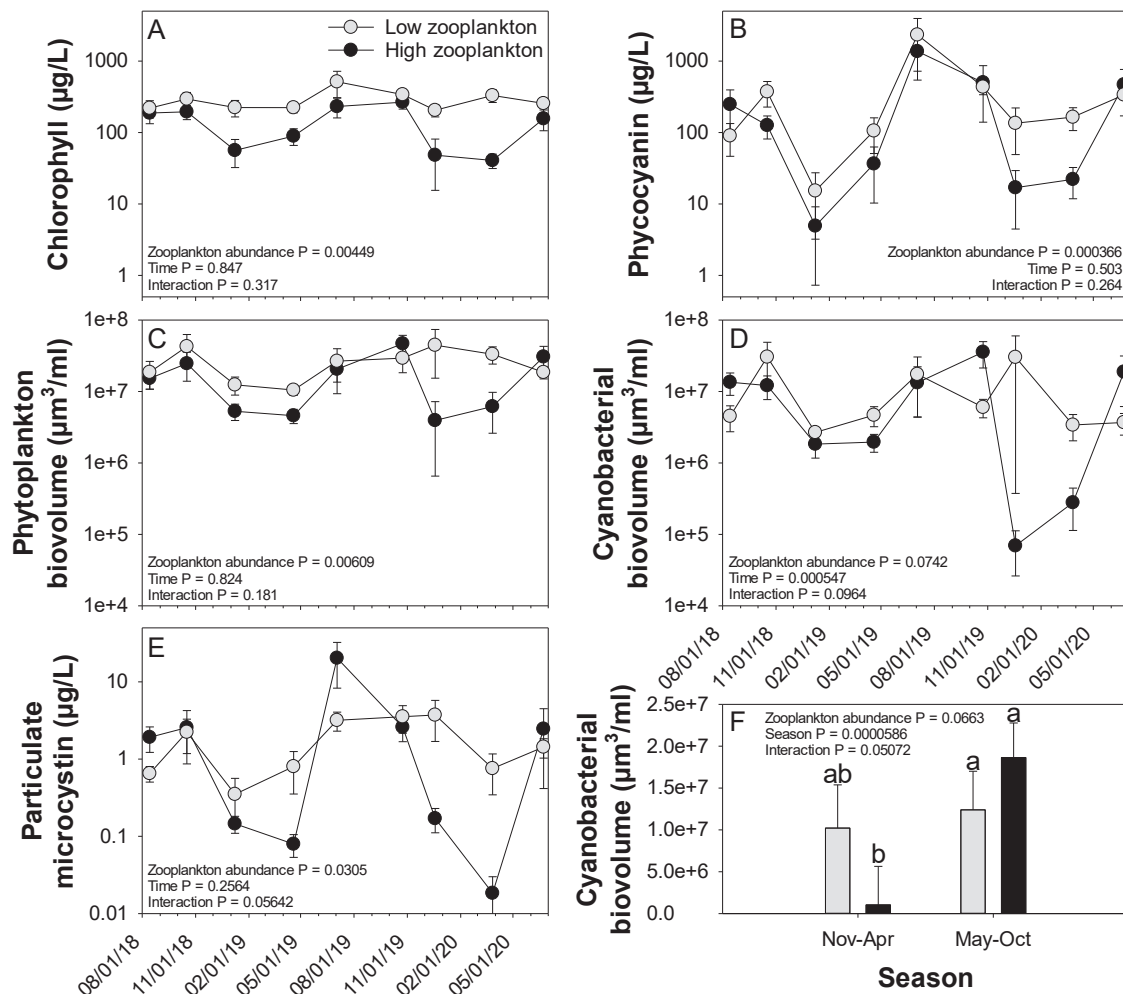
(zooplanktivorous fish not excluded) ponds (Fig. 1A and B,  $p < 0.0000001$ ). Zooplankton are successful in controlling algae when not being preyed upon by zooplanktivorous fish (Brooks and Dodson, 1965). Once zooplankton populations are established, evolutionary adaptation within zooplankton species may promote increased survivorship, as seen with rapid evolution of cladocerans to toxic *Microcystis* (Jiang et al., 2016), both found in our study ponds. *Daphnia* sp., similar to those found this study, have also been shown to reduce cyanobacterial biomass, even when cyanobacterial toxins were present and high, demonstrating their ability to survive and graze upon toxic cyanobacteria (Chislock et al., 2013a, 2013b, 2019a, 2019b). Cladoceran tolerance to toxins may also explain their ability to survive in these systems, similar to a study by Lyu et al. (2017) that showed cladoceran offspring had a higher tolerance for toxins if their mother was in warmer temperature systems ( $30^\circ\text{C}$ ), reflecting similar environments to those found in southeastern catfish aquaculture ponds. Zooplankton community structure varied between the two zooplankton pond types. Larger cladocerans were more abundant in high zooplankton ponds and smaller copepods were more abundant in low zooplankton ponds (Fig. 2A and B). This finding is most likely mediated by zooplanktivorous fish that tend to favor larger bodied prey (Brooks and Dodson, 1965). These differences may also be due to zooplankton feeding strategies where some copepods feed discriminately, although some do filter feed, and cladocerans are generalists (Teegarden, 1999; Geller and Müller, 1981). Regardless of the mechanism, it is clear that high abundances of zooplankton lead to large reductions in phytoplankton, including cyanobacteria (Fig. 3), in hypereutrophic aquaculture ponds.

### 4.2. Phytoplankton abundance

Our results showed a decrease in total phytoplankton and cyanobacteria, with the presence of higher zooplankton abundance, especially during the winter months (Fig. 3A and B). During the non-growing season when phytoplankton growth is slowed due to lower temperatures, light, and nutrient inputs, large-bodied zooplankton can establish themselves in pond communities and slow or delay the rise in phytoplankton blooms. This result has been observed in other successful biomanipulations, but in less productive systems (Brooks and Dodson, 1965; Carpenter et al., 1985; Porter, 1973; Hanson and Butler, 1994; Shapiro et al., 1975). Similar systems, as seen in Ji et al. (2016), observed changes in phytoplankton communities, especially cyanobacteria, after the cessation of aquaculture. The effect of zooplankton on phytoplankton, including cyanobacteria, was less pronounced during the growing season (Fig. 3A–D), similar to Ekvall et al. (2014). When small differences were observed for phytoplankton between zooplankton pond types (i.e., growing season), the effect could be attributed to the abundance of phytoplankton particles being too high for zooplankton, even large-bodied species, to control (Porter et al., 1982).

During the growing season with higher temperatures, extended day length, and high nutrient inputs, cyanobacteria dominated (especially colonial *Microcystis* spp.). Particulate microcystin concentrations tracked trends in cyanobacteria (Fig. 3B and D) and showed large fluxes during this study in high zooplankton ponds likely due to strong grazing on phytoplankton. Moreover, Filatova et al. (2020) saw positive correlations between water temperature and toxins for some cyanobacterial species. High concentrations of *Microcystis* do not necessarily indicate high levels of microcystin because not all strains of cyanobacteria, including *Microcystis*, produce the toxin (Lee et al., 2015). Because of the threat toxins have on fish in aquaculture, a reduction in these compounds improves water quality in those systems. Further, Ekvall et al. (2014) demonstrated that even though toxin levels were not reduced, they were transferred to a reduced, extracellular phase that is more readily degradable by microbes.





**Fig. 3.** Mean quarterly water quality data associated with phytoplankton for both pond types (grey = low zooplankton biomass, black = high zooplankton biomass), including (A) chlorophyll-*a* concentration ( $\mu\text{g/L}$ ) representing all phytoplankton, (B) phycocyanin concentration ( $\mu\text{g/L}$ ) representing cyanobacteria, (C) total phytoplankton biovolume ( $\mu\text{m}^3/\text{ml}$ ), (D) cyanobacterial biovolume ( $\mu\text{m}^3/\text{ml}$ ), (E) particulate microcystin concentrations ( $\mu\text{g/L}$ ), and (F) seasonal (growing season vs. non-growing season) cyanobacterial biovolume. Error bars represent one standard error. Letters in panel F denote results from Tukey's tests where different letters represent statistically different groups.

#### 4.3. Nutrient availability

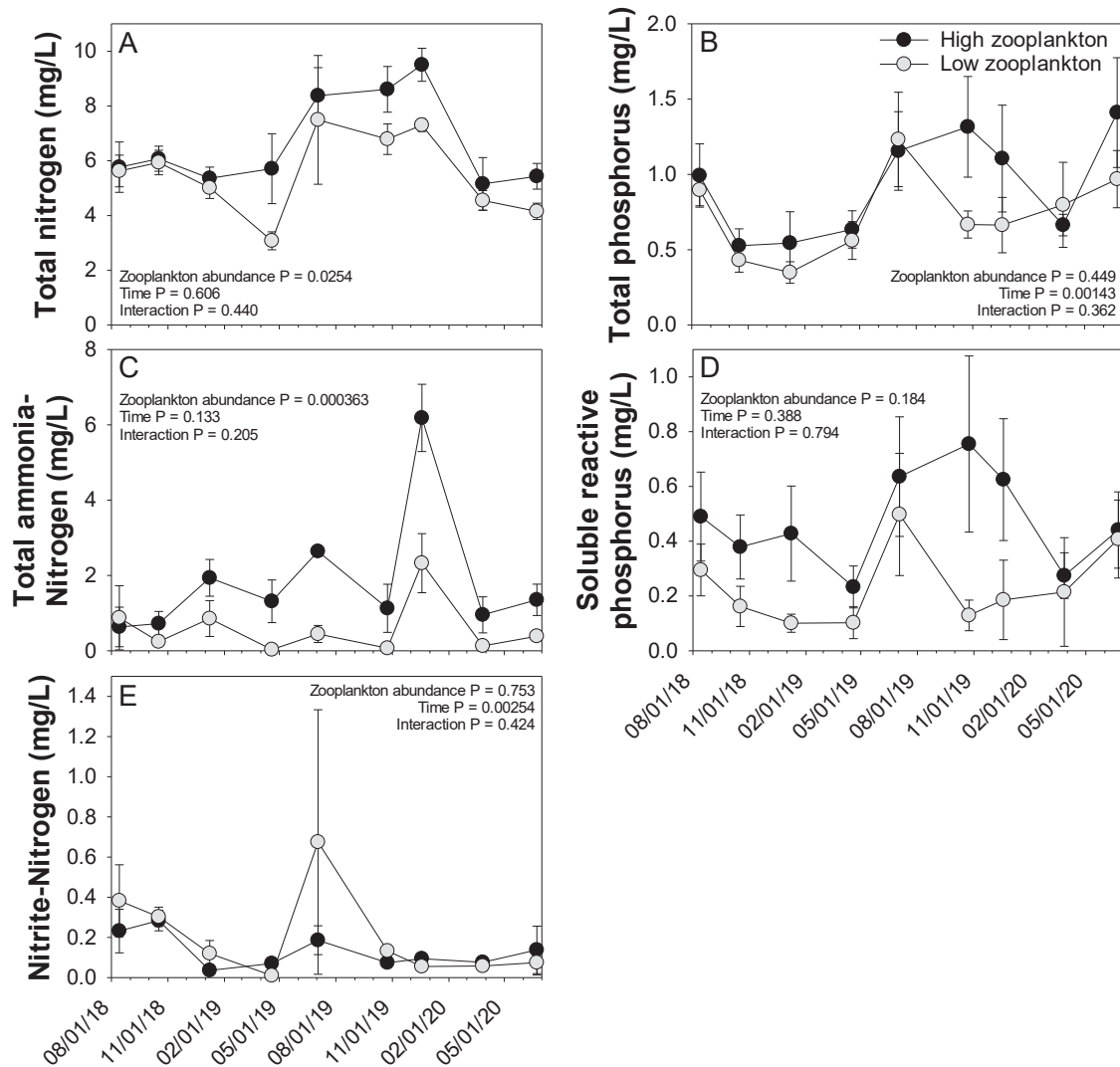
Most nutrients measured (TN, TP, TAN, and SRP) tended to be higher in ponds with more zooplankton (Fig. 4). Because grazing effects were greater and decreased the amount of phytoplankton, nutrient storage and removal decreased in the high zooplankton ponds. Nitrite-N was the only nutrient that had a peak in low zooplankton ponds, although the difference between zooplankton groups was not statistically significant (Fig. 4E;  $p = 0.753$ ). Low zooplankton ponds had lower measured values for TP, SRP, and  $\text{NO}_2\text{-N}$ ; however, there were no statistically significant differences in nutrient values with respect to high and low zooplankton (Fig. 4B–D). Therefore, the implications associated with higher nutrient levels are not directly related to zooplankton abundance or scarcity. Excess phosphorus in the system, if not used by plants or microorganisms, can be bound in the sediment to other minerals (Zhou and Boyd, 2015). Nitrite measured mostly around and below 0.6 mg/L, with a peak at 1.3 mg/L. At high levels it can be toxic to aquatic organisms, but with most values falling below 0.6 mg/L ( $> 1 \text{ mg/L}$  in polluted waters), it is not a threat (Boyd, 2019).

Total nitrogen and TAN had significant differences with respect to zooplankton abundance. Both were greater in high zooplankton ponds on average than low zooplankton ponds. Without a way to cycle nutrients in the system, ponds can quickly accumulate high levels of

ammonia and have the potential to be toxic and lethal especially when temperatures and pH are elevated (Randall and Tsui, 2002; Thurston et al., 1981). Some suggest that a prevention method for high ammonia is less feed and/or increased aeration, but each farmer should make those decisions based on the health and productivity of their pond (Durborow et al., 1997). The effects of increased TN can create eutrophic systems; however, zooplankton in these ponds are so abundant that they can prevent overgrowth of phytoplankton (Boyd, 2019).

#### 4.4. Management implications

In large-scale lake studies, like Brooks and Dodson (1965), zooplankton control of phytoplankton oftentimes leads to increased light penetration and enhanced growth of macrophytes. For farm pond aquaculture, ponds are managed to reduce nuisance species, like cyanobacteria and macrophytes, with algacides and herbicides, respectively. However, the use of such chemicals may have harmful effects on non-target species (Chislock et al., 2013a), such as limiting zooplankton growth (León et al., 2014) or may lead to phytoplankton resistance (García-Villada et al., 2004; Rouco et al., 2014). The implementation of these strategies may be observed in a relatively short time, as seen in Dulić et al. (2014), who observed changes in planktonic communities in less than a year when water source changed.



**Fig. 4.** Mean quarterly water quality data associated with nutrients for both pond types (black = high zooplankton biomass, grey = low zooplankton biomass), including (A) total nitrogen (mg/L), (B) total phosphorus (mg/L), (C) total ammonia-nitrogen (mg/L), (D) soluble reactive phosphorous (mg/L), and (E) nitrite-nitrogen (mg/L). Error bars represent one standard error.

## 5. Conclusions

In this study, we demonstrated that high zooplankton abundances in nutrient-rich catfish aquaculture ponds can lead to large, significant reductions in phytoplankton, including cyanobacteria. Part of this outcome may be attributed to the feeding strategy of catfish, who receive most of their diet from feed and only acquire a small percentage (0.8–2.5%) of their diet from natural food organisms (Robinson et al., 2001). High zooplankton ponds also had higher concentrations of TN and TAN than low zooplankton ponds, where the latter could be potentially toxic with a pH of 8 or higher during the warm, growing season. Although the decrease in biovolume of phytoplankton from zooplankton were observed all year, stronger improvements in water quality were observed during the non-growing season when cyanobacteria were low in abundance. Consequently, ambient zooplankton could be used to delay the onset of cyanobacterial blooms in hypereutrophic catfish aquaculture ponds.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

## Acknowledgments

The authors would like to thank the owners and managers of the participating farms for access to their land, advice, insights about aquaculture pond management, and feedback about this project. We would like to thank Drs. Luke Roy and Anita Kelly and the Alabama Fish Farming Center for technical support. We also thank members of the WilsonLab at Auburn University for assisting with sample processing. This study was supported by U.S. Department of Agriculture grants 2017-70007-27132 and 58-6010-0-006, U.S. National Science Foundation grant DEB-1831094, the Alabama Agricultural Experiment Station, and the Hatch program of the National Institute of Food and Agriculture, U.S. Department of Agriculture.

## References

- Abdel-Tawwab, M., Monier, M.N., Hoseinifar, S.H., Faggio, C., 2019. Fish response to hypoxia stress: growth, physiological, and immunological biomarkers. *Fish Physiol. Biochem.* 45, 997–1013. <https://doi.org/10.1007/s10695-019-00614-9>.
- Borges, P.A.F., Train, S., Dias, J.D., Bonecker, C.C., 2010. Effects of fish farming on plankton structure in a Brazilian tropical reservoir. *Hydrobiologia* 649, 279–291. <https://doi.org/10.1007/s10750-010-0271-2>.

- Bosma, R.H., Verdegem, M.C.J., 2011. Sustainable aquaculture in ponds: principles, practices and limits. *Livest. Sci. Spec. Issue.: Assess. Sustain. Dev. Anim. Prod. Syst.* 139, 58–68. <https://doi.org/10.1016/j.livsci.2011.03.017>.
- Boyd, C.E., 2019. *Water Quality: An Introduction*. Springer Nature.
- Brooks, J.L., Dodson, S.I., 1965. Predation, body size, and composition of plankton. *Science* 150, 28–35. <https://doi.org/10.1126/science.150.3692.28>.
- Buley, R.P., Adams, C., Belfiore, A.P., Fernandez-Figueroa, E.G., Gladfelter, M.F., Garner, B., Straus, D.L., Wilson, A.E., 2021. Field evaluation of seven products to control cyanobacterial blooms in aquaculture. *Environ. Sci. Pollut. Res.* 28, 29971–29983. <https://doi.org/10.1007/s11356-021-12708-0>.
- Carpenter, S.R., Kitchell, J.F., Hodgson, J.R., 1985. Cascading trophic interactions and lake productivity. *BioScience* 35, 634–639. <https://doi.org/10.2307/1309989>.
- Carpenter, S.R., Ludwig, D., Brock, W.A., 1999. Management of eutrophication for lakes subject to potentially irreversible change. *Ecol. Appl.* 9, 751–771. <https://doi.org/10.2307/2641327>.
- Chen, J., Xie, P., Li, L., Xu, J., 2009. First identification of the hepatotoxic microcystins in the serum of a chronically exposed human population together with indication of hepatocellular damage. *Toxicol. Sci.* 108, 81–89. <https://doi.org/10.1093/toxsci/kfp009>.
- Chislock, M.F., Doster, E., Zitomer, R.A., Wilson, A.E., 2013a. Eutrophication: causes, consequences, and controls in aquatic ecosystems. *Nat. Educ. Knowl.* 4, 10.
- Chislock, M.F., Sarnelle, O., Jernigan, L.M., Wilson, A.E., 2013b. Do high concentrations of microcystin prevent *Daphnia* control of phytoplankton? *Water Res.* 47, 1961–1970. <https://doi.org/10.1016/j.watres.2012.12.038>.
- Chislock, M.F., Kaul, R.B., Durham, K.A., Sarnelle, O., Wilson, A.E., 2019a. Eutrophication mediates rapid clonal evolution in *Daphnia pulex*. *Freshw. Biol.* 64, 1275–1283. <https://doi.org/10.1111/fwb.13303>.
- Chislock, M.F., Sarnelle, O., Jernigan, K.A., Anderson, V.R., Abebe, A., Wilson, A.E., 2019b. Consumer adaptation mediates top-down regulation across a productivity gradient. *Oecologia* 190, 195–205. <https://doi.org/10.1007/s00442-019-04401-4>.
- Crisman, T.L., Beaver, J.R., 1990. Applicability of planktonic biomanipulation for managing eutrophication in the subtropics. In: *Biomanipulation Tool for Water Management*. Springer, pp. 177–185. <https://doi.org/10.1007/BF02530338>.
- Culver, D.A., Boucherle, M.M., Bean, D.J., Fletcher, J.W., 2011. Biomass of freshwater Crustacean zooplankton from length-weight regressions. *Can. J. Fish. Aquat. Sci.* <https://doi.org/10.1139/f85-173>.
- De Bernardi, R., Giussani, G., 1990. Are blue-green algae a suitable food for zooplankton? An overview. *Hydrobiologia* 200, 29–41.
- Donaghay, P.L., Osborn, T.R., 1997. Toward a theory of biological-physical control of harmful algal bloom dynamics and impacts. *Limnol. Oceanogr.* 42, 1283–1296. [https://doi.org/10.4319/lo.1997.42.5\\_part\\_2.1283](https://doi.org/10.4319/lo.1997.42.5_part_2.1283).
- Dulić, Z., Marković, Z., Živić, M., Ćirić, M., Stanković, M., Subakov-Simić, G., Živić, I., 2014. The response of phytoplankton, zooplankton and macrozoobenthos communities to change in the water supply from surface to groundwater in aquaculture ponds. *Ann. Limnol. – Int. J. Limnol.* 50, 131–141. <https://doi.org/10.1051/limn/2014005>.
- Durborow, R.M., Crosby, D.M., Brunson, M.W., 1997. Ammonia in fish ponds. *South. Reg. Aquac. Cent. Publ.* 463, 1–2.
- Edmondson, W.T., 1959. *Freshwater Biology*. Wiley, New York.
- Eklav, M.K., Urrutia-Cordero, P., Hansson, L.-A., 2014. Linking cascading effects of fish predation and zooplankton grazing to reduce cyanobacterial biomass and toxin levels following biomanipulation. *PLoS One* 9, e112956. <https://doi.org/10.1371/journal.pone.0112956>.
- Engle, C.R., Pounds, G.L., Ploeg, M. van der, 1995. The cost of off-flavor. *J. World Aquac. Soc.* 26, 297–306. <https://doi.org/10.1111/j.1749-7345.1995.tb00258.x>.
- Filatova, D., Picardo, M., Núñez, O., Farré, M., 2020. Analysis, levels and seasonal variation of cyanotoxins in freshwater ecosystems. *Trends Environ. Anal. Chem.* 26, e00091 <https://doi.org/10.1016/j.teac.2020.e00091>.
- García-Villada, L., Rico, M., Altamirano, M., Sánchez-Martín, L., López-Rodas, V., Costas, E., 2004. Occurrence of copper resistant mutants in the toxic cyanobacteria *Microcystis aeruginosa*: characterisation and future implications in the use of copper sulphate as algacide. *Water Res.* 38, 2207–2213. <https://doi.org/10.1016/j.watres.2004.01.036>.
- Geller, W., Müller, H., 1981. The filtration apparatus of cladocera: filter mesh-sizes and their implications on food selectivity. *Oecologia* 49, 316–321. <https://doi.org/10.1007/BF00347591>.
- Gliwicz, Z.M., 1990. Why do cladocerans fail to control algal blooms? In: Gulati, R.D., Lammens, E.H.R.R., Meijer, M.-L., van Donk, E. (Eds.), *Biomanipulation Tool for Water Management, Developments in Hydrobiology*. Springer Netherlands, Dordrecht, pp. 83–97. [https://doi.org/10.1007/978-94-017-0924-8\\_8](https://doi.org/10.1007/978-94-017-0924-8_8).
- Gross, A., Boyd, C.E., 1998. A digestion procedure for the simultaneous determination of total nitrogen and total phosphorus in pond water. *J. World Aquac. Soc.* 29, 300–303. <https://doi.org/10.1111/j.1749-7345.1998.tb00650.x>.
- Guo, L., Wang, Q., Xie, P., Tao, M., Zhang, J., Niu, Y., Ma, Z., 2015. A non-classical biomanipulation experiment in Gonghu Bay of Lake Taihu: control of *Microcystis* blooms using silver and bighead carp. *Aquac. Res.* 46, 2211–2224. <https://doi.org/10.1111/arc.12375>.
- Guo, N., Xie, P., 2006. Development of tolerance against toxic *Microcystis aeruginosa* in three cladocerans and the ecological implications. *Environ. Pollut.* 143, 513–518. <https://doi.org/10.1016/j.envpol.2005.11.044>.
- Hanson, M.A., Butler, M.G., 1994. Responses of plankton, turbidity, and macrophytes to biomanipulation in a shallow prairie lake. *Can. J. Fish. Aquat. Sci.* 51, 1180–1188. <https://doi.org/10.1139/f94-117>.
- Heisler, J., Glibert, P.M., Burkholder, J.M., Anderson, D.M., Cochlan, W., Dennison, W. C., Dortch, Q., Gobler, C.J., Heil, C.A., Humphries, E., Lewitus, A., Magnien, R., Marshall, H.G., Sellner, K., Stockwell, D.A., Stoecker, D.K., Suddleson, M., 2008. Eutrophication and harmful algal blooms: a scientific consensus. *Harmful Algal Blooms Eutrophication* 8, 3–13. <https://doi.org/10.1016/j.hal.2008.08.006>.
- Ji, G., Xu, L., Wang, L., Xu, Z., Dai, X., Zhang, R., 2016. The response of phytoplankton in a subtropical lake to the cessation of aquaculture: a case study of Lake Dianshan, China. *Ann. Limnol. – Int. J. Limnol.* 52, 109–121. <https://doi.org/10.1051/limn/2015037>.
- Jiang, X., Gao, H., Zhang, L., Liang, H., Zhu, X., 2016. Rapid evolution of tolerance to toxic *Microcystis* in two cladoceran grazers. *Sci. Rep.* 6, 25319. <https://doi.org/10.1038/srep25319>.
- Kasinak, J.-M.E., Bishop, C.J., Wright, R.A., Wilson, A.E., 2015a. Grass carp do not consume the nuisance benthic cyanobacterium, *Lyngbya wollei*. *J. Aquat. Plant Manag.* 7.
- Kasinak, J.-M.E., Holt, B.M., Chislock, M.F., Wilson, A.E., 2015b. Benchtop fluorometry of phycocyanin as a rapid approach for estimating cyanobacterial biovolume. *J. Plankton Res.* 37, 248–257. <https://doi.org/10.1093/plankt/fbu096>.
- Kasprzak, P., Benndorf, J., Mehner, T., Koschel, R., 2002. Biomanipulation of lake ecosystems: an introduction. *Freshw. Biol.* 47, 2277–2281. <https://doi.org/10.1046/j.1365-2427.2002.01001.x>.
- Lee, T.A., Rollwagen-Bollens, G., Bollens, S.M., Faber-Hammond, J.J., 2015. Environmental influence on cyanobacteria abundance and microcystin toxin production in a shallow temperate lake. *Ecotoxicol. Environ. Saf.* 114, 318–325. <https://doi.org/10.1016/j.ecoenv.2014.05.004>.
- León, D., Peñalver, P., Casas, J., Juan, M., Fuentes, F., Gallego, I., Santillana, J.T., 2014. Effects of copper sulphate on zooplankton communities in ponds submitted to agricultural intensification. *Bol. Biodivers. Chile* 1–10.
- Li, B., Liu, Yipeng, Zhang, H., Liu, Ying, Liu, Yong, Xie, P., 2021. Research progress in the functionalization of microcystin-LR based on interdisciplinary technologies. *Coord. Chem. Rev.* 443, 214041 <https://doi.org/10.1016/j.ccr.2021.214041>.
- Lopez, C.B., Jewett, E.B., Dortch, Q., Walton, B.T., Hudnell, H.K., 2008. Scientific assessment of freshwater harmful algal blooms. Interagency Working Group on Harmful Algal Blooms, Hypoxia, and Human Health of the Joint Subcommittee on Ocean Science and Technology.
- Ludwig, G.M., 1999. Zooplankton succession and larval fish culture in freshwater ponds. *Southern Regional Aquaculture Center Stoneville, Mississippi* 700, 1–6.
- Lyu, K., Zhang, L., Gu, L., Zhu, X., Wilson, A.E., Yang, Z., 2017. Cladoceran offspring tolerance to toxic *Microcystis* is promoted by maternal warming. *Environ. Pollut.* 227, 451–459. <https://doi.org/10.1016/j.envpol.2017.04.095>.
- Manning, S.R., Nobles, D.R., 2017. Impact of global warming on water toxicity: cyanotoxins. *Curr. Opin. Food Sci. Food Toxicol.* • Food Saf. 18, 14–20. <https://doi.org/10.1016/j.cofs.2017.09.013>.
- Mischke, C.C., Wise, D.J., Zimba, P.V., 2009. Impact of copper sulfate on plankton in channel catfish nursery ponds. *J. World Aquac. Soc.* 40, 122–128. <https://doi.org/10.1111/j.1749-7345.2008.00225.x>.
- Moriarty, D.J., 1997. The role of microorganisms in aquaculture ponds. *Aquaculture* 151, 333–349. [https://doi.org/10.1016/S0044-8486\(96\)01487-1](https://doi.org/10.1016/S0044-8486(96)01487-1).
- Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* 27, 31–36. [https://doi.org/10.1016/S0003-2670\(00\)88444-5](https://doi.org/10.1016/S0003-2670(00)88444-5).
- Paerl, H.W., Paul, V.J., 2012. Climate change: links to global expansion of harmful cyanobacteria. *Water Res.* 46, 1349–1363.
- Paerl, H.W., Tucker, C.S., 1995. Ecology of blue-green algae in aquaculture ponds. *J. World Aquac. Soc.* 26, 109–131. <https://doi.org/10.1111/j.1749-7345.1995.tb00235.x>.
- Peretyatko, A., Teissier, S., De Backer, S., Triest, L., 2009. Restoration potential of biomanipulation for eutrophic peri-urban ponds: the role of zooplankton size and submerged macrophyte cover. In: *Pond Conservation in Europe*. Springer, pp. 281–291.
- Peretyatko, A., Teissier, S., De Backer, S., Triest, L., 2012. Biomanipulation of hypereutrophic ponds: when it works and why it fails. *Environ. Monit. Assess.* 184, 1517–1531. <https://doi.org/10.1007/s10661-011-2057-z>.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Core Team, R., 2020. nlme: linear and nonlinear mixed effects models. R. Package Version 3, 1–151. (<https://CRAN.R-project.org/package=nlme>).
- Porter, K.G., 1973. Selective grazing and differential digestion of algae by zooplankton. *Nature* 244, 179–180. <https://doi.org/10.1038/244179a0>.
- Porter, K.G., Gerritsen, J., Orcutt, J.D., 1982. The effect of food concentration on swimming patterns, feeding behavior, ingestion, assimilation, and respiration by *Daphnia*. *Limnol. Oceanogr.* 27, 935–949. <https://doi.org/10.4319/lo.1982.27.5.0935>.
- Randall, D.J., Tsui, T.K.N., 2002. Ammonia toxicity in fish. *Mar. Pollut. Bull.* 45, 17–23. [https://doi.org/10.1016/S0025-326X\(02\)00227-8](https://doi.org/10.1016/S0025-326X(02)00227-8).
- Reardon, J., Foreman, J.A., Searcy, R.L., 1966. New reactants for the colorimetric determination of ammonia. *Clin. Chim. Acta* 14, 403–405. [https://doi.org/10.1016/0009-8981\(66\)90120-3](https://doi.org/10.1016/0009-8981(66)90120-3).
- Robinson, E.H., Li, M.H., Manning, B.E., 2001. *A Practical Guide to Nutrition, Feeds, and Feeding of Catfish*. Office of Agricultural Communications, Division of Agriculture, Forestry and Veterinary Medicine, Mississippi State University, pp. 1–24.
- Rodell, M., Famiglietti, J.S., Wiese, D.N., Reager, J.T., Beaudoin, H.K., Landerer, F.W., Lo, M.-H., 2018. Emerging trends in global freshwater availability. *Nature* 557, 651–659. <https://doi.org/10.1038/s41586-018-0123-1>.
- Rouco, M., López-Rodas, V., González, R., Emma Huertas, I., García-Sánchez, M.J., Flores-Moya, A., Costas, E., 2014. The limit of the genetic adaptation to copper in freshwater phytoplankton. *Oecologia* 175, 1179–1188. <https://doi.org/10.1007/s00442-014-2963-1>.

- Sartory, D.P., Grobbelaar, J.U., 1984. Extraction of chlorophyll a from freshwater phytoplankton for spectrophotometric analysis. *Hydrobiologia* 114, 177–187. <https://doi.org/10.1007/BF00031869>.
- Shapiro, J., Lamarra, V., Lynch, M., 1975. Biomanipulation, an ecosystem approach to lake restoration. In: Brezonik, P., Fox, L. (Eds.), *Proceedings of a Symposium on Water Quality Management Through Biological Control*. University Press of Florida, Gainesville, pp. 85–96.
- Teegarden, G., 1999. Copepod grazing selection and particle discrimination on the basis of PSP toxin content. *Mar. Ecol. Prog. Ser.* 181, 163–176. <https://doi.org/10.3354/meps181163>.
- Thurston, R.V., Russo, R.C., Vinogradov, G.A., 1981. Ammonia toxicity to fishes. Effect of pH on the toxicity of the unionized ammonia species. *Environ. Sci. Technol.* 15, 837–840. <https://doi.org/10.1021/es00089a012>.
- Triest, L., Stiers, I., Van Onsem, S., 2016. Biomanipulation as a nature-based solution to reduce cyanobacterial blooms. *Aquat. Ecol.* 50, 461–483. <https://doi.org/10.1007/s10452-015-9548-x>.
- Tucker, C.S., Schrader, K.K., 2020. Off-flavors in pond-grown ictalurid catfish: Causes and management options. *J. World Aquac. Soc.* 51, 7–92. <https://doi.org/10.1111/jwas.12672>.
- United States Environmental Protection Agency (EPA), 2003. Reregistration eligibility decision (RED) for Diuron. ([https://archive.epa.gov/pesticides/reregistration/web/pdf/diuron\\_red-2.pdf](https://archive.epa.gov/pesticides/reregistration/web/pdf/diuron_red-2.pdf)).
- Walsby, A.E., McAllister, G.K., 1987. Buoyancy regulation by *Microcystis* in Lake Okaro. *N. Z. J. Mar. Freshw. Res.* 21, 521–524. <https://doi.org/10.1080/00288330.1987.9516249>.
- Wang, H., Xu, C., Liu, Y., Jeppesen, E., Svenning, J.-C., Wu, J., Zhang, W., Zhou, T., Wang, P., Nangombe, S., Ma, J., Duan, H., Fang, J., Xie, P., 2021. From unusual suspect to serial killer: cyanotoxins boosted by climate change may jeopardize megafauna. *Innovation* 2, 100092. <https://doi.org/10.1016/j.xinn.2021.100092>.
- Wang, Y., Gu, X., Zeng, Q., Mao, Z., Wang, W., 2016. Contrasting response of a plankton community to two filter-feeding fish and their feces: an in situ enclosure experiment. *Aquaculture* 465, 330–340. <https://doi.org/10.1016/j.aquaculture.2016.08.014>.
- Xie, P., Liu, J., 2001. Practical success of biomanipulation using filter-feeding fish to control cyanobacteria blooms: a synthesis of decades of research and application in a subtropical hypereutrophic lake. *Sci. World J.* 1, 337–356. <https://doi.org/10.1100/tsw.2001.67>.
- Yang, Z., Buley, R.P., Fernandez-Figueroa, E.G., Barros, M.U.G., Rajendran, S., Wilson, A. E., 2018. Hydrogen peroxide treatment promotes chlorophytes over toxic cyanobacteria in a hyper-eutrophic aquaculture pond. *Environ. Pollut.* 240, 590–598. <https://doi.org/10.1016/j.envpol.2018.05.012>.
- Zhou, L., Boyd, C.E., 2015. Bluegill yield in response to nitrogen and phosphorus versus phosphorus-only fertilization in ponds at different times since sediment removal. *Aquaculture* 446, 7–11. <https://doi.org/10.1016/j.aquaculture.2015.04.019>.