Improving farm pond aquaculture water quality using zooplankton and long-term monitoring

by

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Abstract

Eutrophication and degradation of water quality place a strain on freshwater resources and call for best management strategies. In freshwater farm pond aquaculture, water quality and health of fish are important players. Due to excess waste and feed recirculating in these systems, eutrophic conditions are common and long-lasting. This thesis examined a three year data set to determine both trends of water quality and the use of zooplankton to improve water quality in west Alabama farm pond aquaculture. Chapter 1 examined planktonic community interactions, where high zooplankton ponds were found to effectively graze upon and decrease phytoplankton and cyanobacteria biovolume compared to low zooplankton ponds. Chapter 2 looked at long term water quality trends, with chlorophyll, phycocyanin, and microcystin as response variables to pick the models that best fit the data. These studies will help to improve and predict water quality conditions in hypereutrophic freshwater aquaculture systems.

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Chapter 1

Zooplankton improve water quality in farm pond aquaculture

ABSTRACT

Warming temperatures and extended growing seasons conducive for algal blooms are further exacerbated in shallow aquaculture ponds due to high nutrient loading from fish feed and nitrogenous waste. 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. Although effective, such treatments 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 from August 2018 to June 2020 examined zooplankton and phytoplankton communities, cyanobacterial toxins, and nutrients, in nine catfish aquaculture farm-ponds in west Alabama, USA. During this project, farm managers reduced planktivorous 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. 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 30ºC, the effect of zooplankton on phytoplankton was most pronounced during the non-growing season (November to 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 biomanipulation may be an efficient method to control algal blooms in farm-pond aquaculture.

INTRODUCTION

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 & 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 respiration of phytoplankton 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-Taeeab et al., 2019). Consequently, daily aeration of aquaculture ponds is often required to maintain suitable water quality for farmed fish.

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

Cyanobacteria are often 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) and toxin production (Paerl & Paul, 2012; Walsby & McAllister, 1987). Cyanobacterial genera, such as *Oscillatoria* and *Dolichospermum* (previously called *Anabaena*), 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 & Schrader, 2020). Off-flavors affect aquaculture by prolonged holding and extended feeding costs until off-flavors are not detected (delayed harvest), delays in stocking, and increased health issues (Engle et al., 1995).

Controlling Algal Blooms

Phytoplankton can be controlled by a variety of approaches, including filtering, shading, limiting nutrients, or applying algaecides (Donaghay & Osborn, 1997). Currently, the only chemical control treatments approved for use in catfish aquaculture in the US are copper sulfate ($CuSO_4$) and under special circumstances diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) (EPA, 2003). Although both algaecides 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 (García-Villada et al., 2004; Rouco et al., 2014), and short-lived treatment effectiveness (Buley et al., In press). Therefore, pursuing alternative methods for controlling algal blooms in aquaculture may aid in long-term bloom management.

The use of zooplankton as a biological control of phytoplankton in natural systems has been reported for decades (Brooks & Dodson, 1965; Porter, 1973; Hanson & 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 as a means to control plankton, both phytoplankton and zooplankton, in eutrophic systems (Guo et al., 2014, Wang et al., 2016). Most relevant studies altered food webs by removing planktivorous fish (directly with chemical or physical methods or by adding piscivorous fishes) to promote large-bodied zooplankton (i.e., trophic cascade hypothesis (Shapiro, 1985; 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, edibility, and biochemical properties (de Bernardi & Guissani, 1990; Borges et al*.,* 2010), recent studies have shown that large-bodied zooplankton, namely *Daphnia,* effectively graze on cyanobacteria, including toxic strains (Chislock et al*.,* 2013b; Chislock et al., 2019a; Chislock et al., 2019b). 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 planktivorous fishes, the zooplankton communities are enhanced to promote a top-down biological control of phytoplankton.

Application to aquaculture and purpose

Although studies have shown success of biomanipulation in shallow eutrophic systems (Kasprzak et al., 2002; Peretyatko et al., 2009), the purpose of this observational experiment was to apply the findings of Brooks and Dodson (1965) and Shapiro et al. (1985) 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 & Beaver, 1990). The fingerlings 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; therefore, biomanipulation in these systems may be successful. We hypothesize that ponds displaying a reduction in planktivorous fish will have higher concentrations of zooplankton leading to lower phytoplankton abundance and improved water quality.

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 7,000-8,000/acre. Feeding rates and aeration ranged from 143-262 pounds/acre and 9.6-13.3 horsepower/acre, respectively (Table 1). Ponds B5-9 at the second farm were stocked with channel catfish (*I. punctatus*) at a density of 7,500-8,000/acre and fed until satiation, up to 150 pounds/day. Aeration for these ponds ranged from 4.5-10 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 planktivorous fish in some ponds by preventing small fish from entering their ponds when filling using small (44.5 mm) mesh nets (B5 and B9) or a fine screen sock (A1). After ponds were refilled, the planktonic communities established themselves from naturally occurring communities. A long, rigid integrated plastic tube sampler (inside diameter = 51 mm)

was used near-shore (~2m) to collect water at the same location in each pond from the surface to ~0.5 m deep that was stored in plastic containers. 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 (NO_2-N), total ammonia nitrogen (TAN), and soluble reactive phosphorus (SRP)), cyanobacterial toxins (microcystin), 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 hours in the dark at 4^{o} C (Sartory & 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 with persulfate digestion (Gross & Boyd, 1998). Nitrite-N, TAN (salicylate method), and SRP were measured colorimetrically on a spectrophotometer (Reardon et al. 1966; Murphy and Riley, 1962). Intracellular 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 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 hours, 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 taxa 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 50*i* 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., 1985). All zooplankton data were grouped into major taxa prior to statistical analyses (Edmondson, 1959).

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.

RESULTS

Zooplankton biomass

Two years of quarterly sampling nine ponds showed significant differences of zooplankton biomass between ponds with low or high zooplankton biomass ($p < 0.0000001$; Figures 1A,B). On average across 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 ≥ 31% more abundant ($p < 0.000014$) based on dry biomass, in low zooplankton ponds than high zooplankton ponds (Figure 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 (Figure 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.

Phytoplankton abundance

Due to the highly productive nature of these aquaculture ponds, phytoplankton are generally abundant 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, Franceia*)*,* 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 (3A-B, F). High zooplankton ponds had 51% less chlorophyll ($p = 0.00449$; Figure 3A), 58% less phytoplankton biovolume ($p = 0.00609$; Figure 3C), 30% less phycocyanin ($p < 0.001$; Figure 3B), and 81% less cyanobacterial biovolume ($p = 0.0742$; Figure 3D), on average, than low zooplankton ponds. Although the significance was a 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 ($p < 0.0001$; Figure 3F). Cyanobacteria generally dominated all ponds during the growing season when near surface water temperatures were ≥ 28°C (late April to late October; Figures 3B, D). Microcystin concentrations were 88% lower in high zooplankton ponds than low zooplankton ponds ($p = 0.0305$; Figure 3E) with expected peaks during the growing season when toxigenic cyanobacteria are present (Figures 3B, D)

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 (Figure 4). High zooplankton ponds had 17% more TN ($p = 0.0254$; Figure 4A) and 68% more TAN ($p = 0.000363$; Figure 4C) than low zooplankton ponds. Interestingly, high and low zooplankton ponds did not statistically differ in concentrations of TP ($p = 0.499$; Figure 4B), SRP ($p = 0.184$; Figure 4D), or NO_2-N ($p = 0.753$; Figure 4E).

DISCUSSION

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

Zooplankton abundance

Foodweb manipulations (namely, excluding planktivorous fish) led to large (18x), sustained differences in ambient zooplankton biomass between high (planktivorous fish excluded) and low zooplankton (planktivorous fish not excluded) ponds (Figures 1A, B, $p<0.0000001$). Zooplankton are successful in controlling algae when they are not being predated upon by planktivorous fish (Brooks & 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., 2013, 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, reflecting similar environments to those found in southeastern catfish aquaculture ponds and when cyanobacterial blooms are common. 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 (Figures 2A,B). This finding is most likely mediated by planktivorous fish that tend to favor larger bodied prey (Brooks & Dodson 1965). These differences may also be due to zooplankton feeding strategies, where copepods feed discriminately and cladocerans are generalists (Teegarden, 1999; Geller & Müller, 1981). Regardless of the mechanism, it is clear that high abundances of zooplankton lead to large reductions in phytoplankton, including cyanobacteria (Figure 3), in hypereutrophic aquaculture ponds.

Phytoplankton abundance

Our results showed a decrease in total phytoplankton and cyanobacteria, with the presence of higher zooplankton abundance, especially during the winter months (Figures 3A, 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 & Dodson, 1965; Carpenter et al., 1985; Porter, 1973; Hanson & Butler, 1994, Shapiro et al., 1985). 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 (Figure 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 (Figures 3B, 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 (Filatova et al., 2020). 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.

Nutrient availability

Most nutrients measured (TN, TP, TAN, and SRP) tended to be higher in ponds with more zooplankton (Figure 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 (Figure 4E; $p=0.753$). Low zooplankton ponds had lower measured values for TP, SRP, and NO_2-N; however, there were no statistically significant differences in nutrient values with respect to high and low zooplankton (Figure 4B, C, 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 & 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 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 & 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).

Management implications

In whole-lake studies, like Brooks & 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 algaecides 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.

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 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.

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TABLE 1.1.

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	2
B6	10.0			7500	4.50	10
B7	5.00			7500	6.00	6
B8	4.00			8000	6.00	6
B9	2.00			8000	10.0	1

FIGURE LEGENDS

Figure 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.

Figure 2. Relative quarterly abundance of (A) copepods and (B) cladocerans measured as dry biomass (µg/L) for high (black) and low (grey) zooplankton biomass ponds. Error bars represent one standard error.

Figure 3. Mean quarterly water quality data associated with phytoplankton for both pond types (black = high zooplankton biomass, grey = low zooplankton biomass), including (A) chlorophyll-*a* concentration (µg/L) representing all phytoplankton, (B) phycocyanin concentration (µg/L) representing cyanobacteria, (C) total phytoplankton biovolume (μm^3/ml), (D) cyanobacterial biovolume (μm^3/ml), (E) particulate microcystin concentrations (µg/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.

Figure 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 phosphorus (mg/L), and (E) nitrite-nitrogen (mg/L). Error bars represent one standard error.

Figure 1.1.

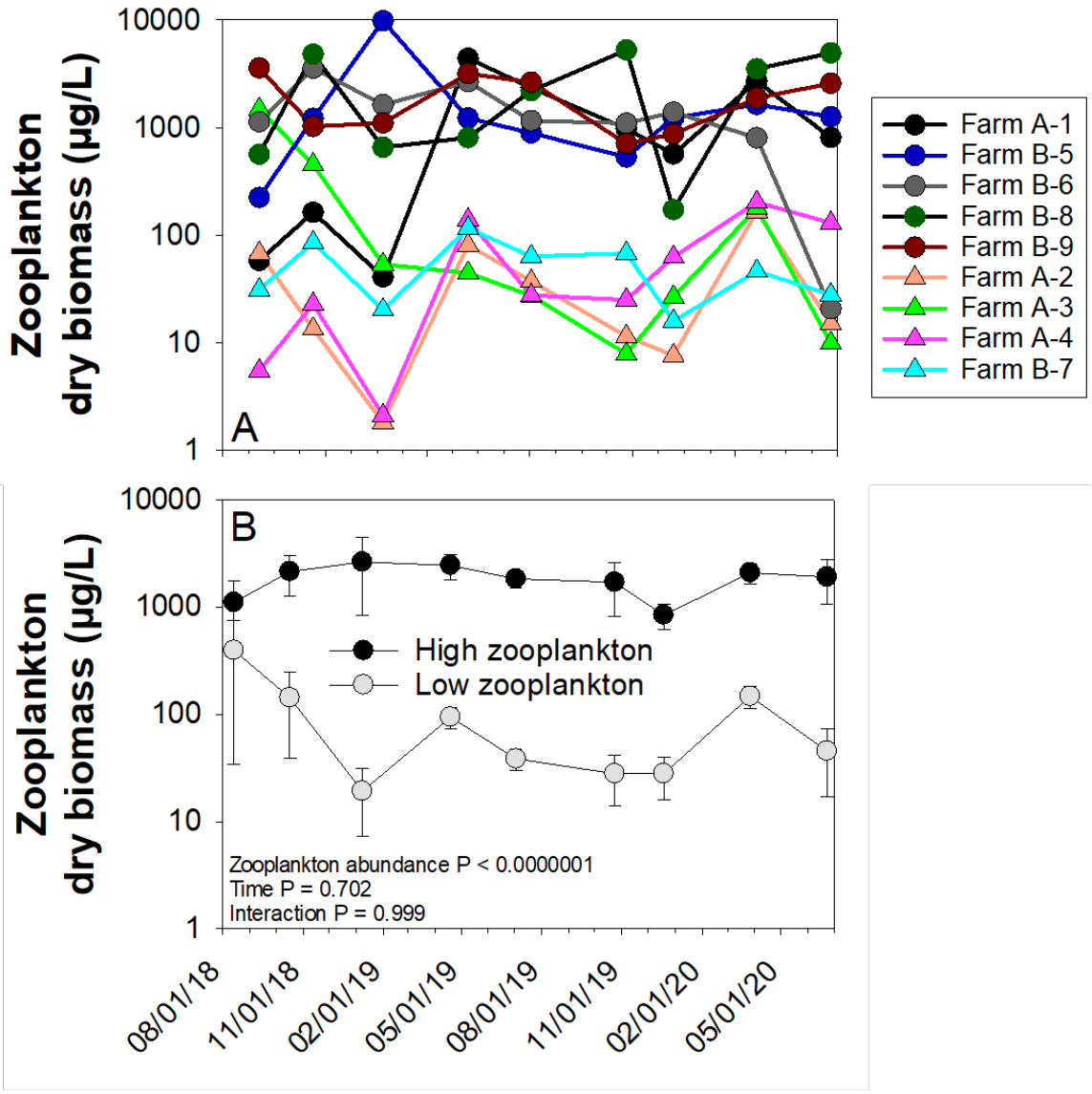

Zooplankton dry biomass (µg/L)
10000
1000
100
10
1
A
Farm A-1
Farm B-5
Farm B-6
Farm B-8
Farm B-9
Farm A-2
Farm A-3
Farm A-4
Farm B-7
B
High zooplankton
Low zooplankton
Zooplankton abundance P < 0.0000001
Time P = 0.702
Interaction P = 0.999
08/01/18
11/01/18
02/01/19
05/01/19
08/01/19
11/01/19
02/01/20
05/01/20

Figure 1.2.

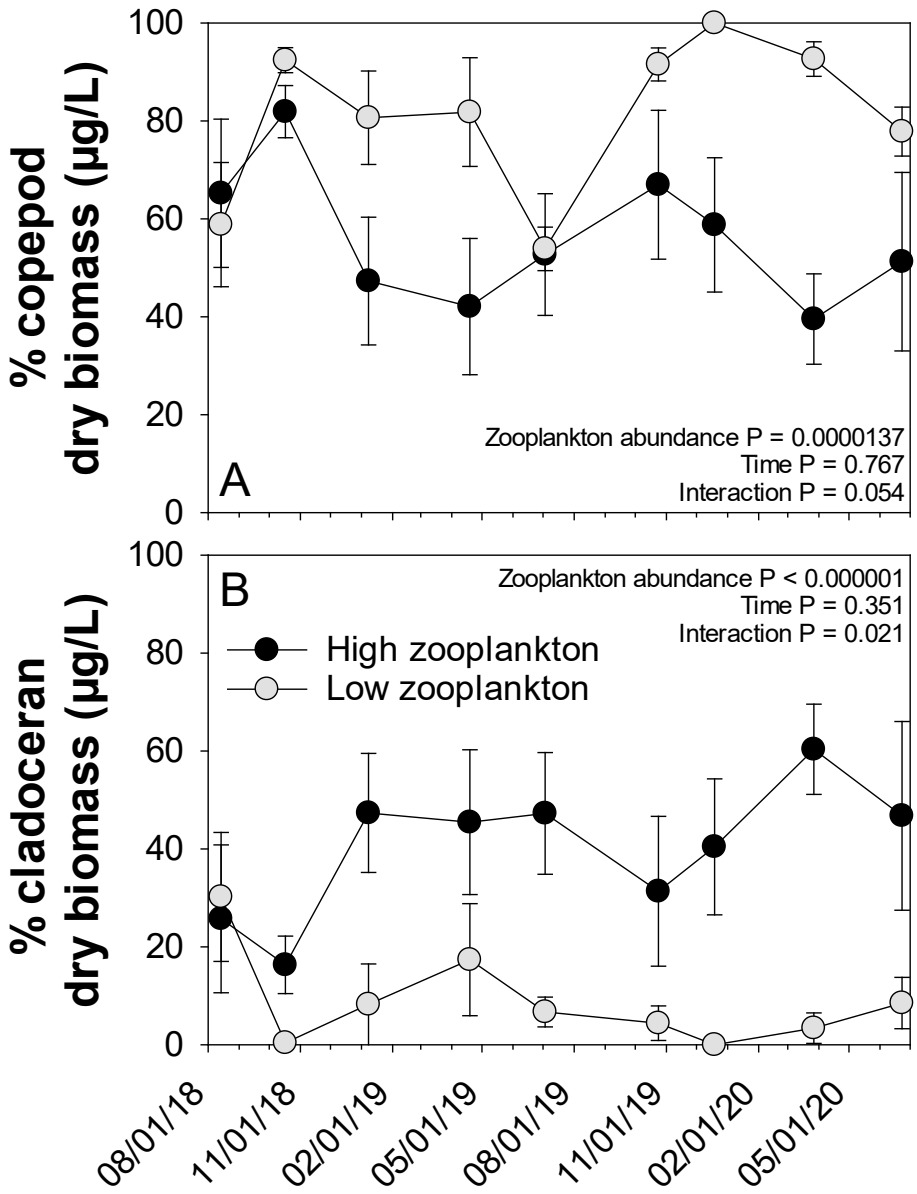

% copepod dry biomass (µg/L)
100
80
60
40
20
0
A
Zooplankton abundance P = 0.0000137
Time P = 0.767
Interaction P = 0.054
% cladoceran dry biomass (µg/L)
B
Zooplankton abundance P < 0.000001
Time P = 0.351
Interaction P = 0.021
High zooplankton
Low zooplankton
08/01/18
11/01/18
02/01/19
05/01/19
08/01/19
11/01/19
02/01/20
05/01/20

Figure 1.3.

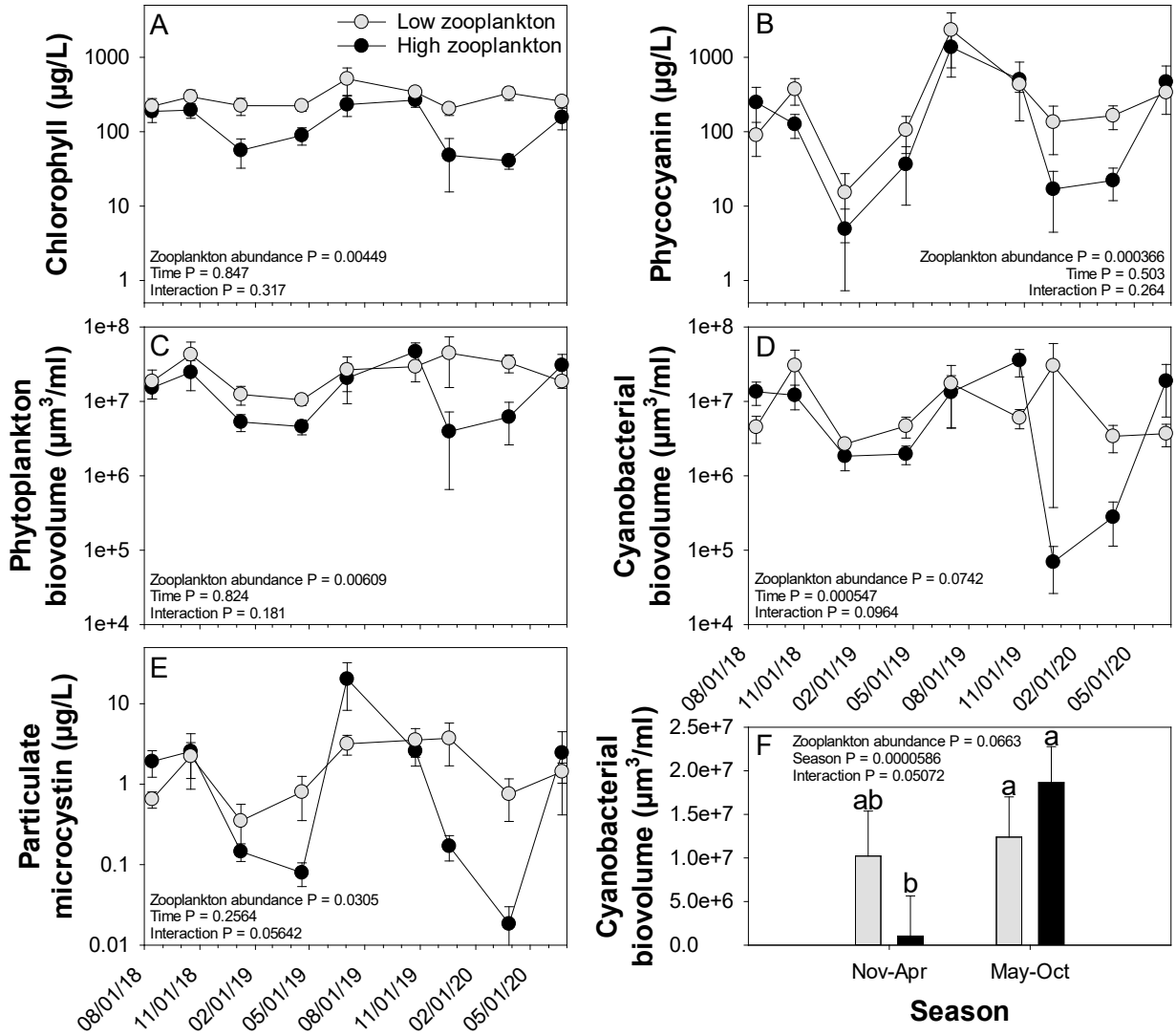
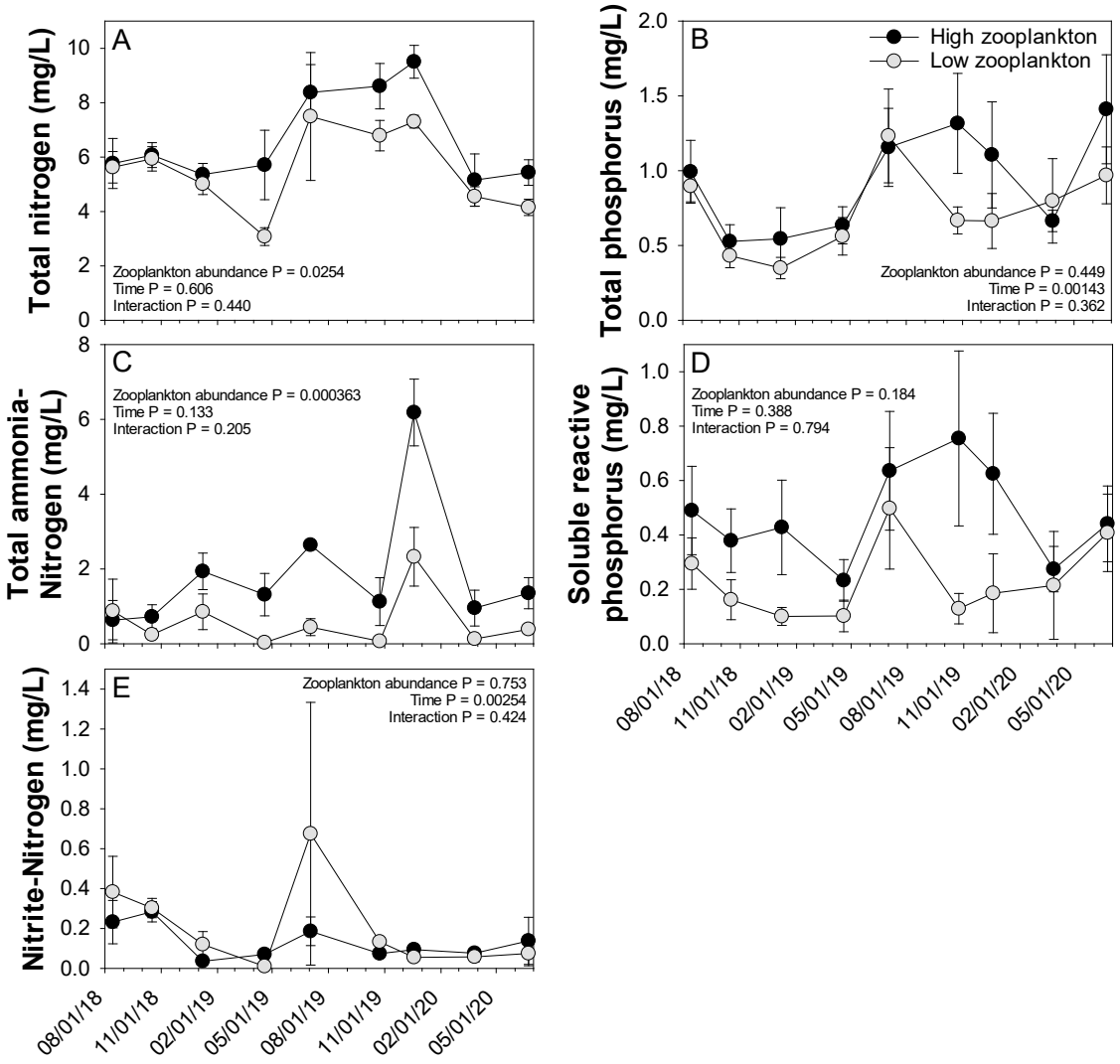

A
Low zooplankton
High zooplankton
Chlorophyll (µg/L)
1000
100
10
1
Zooplankton abundance P = 0.00449
Time P = 0.847
Interaction P = 0.317
B
Phycocyanin (µg/L)
1000
100
10
1
Zooplankton abundance P = 0.000366
Time P = 0.503
Interaction P = 0.264
C
Phytoplankton biovolume (µm3/ml)
1e+8
1e+7
1e+6
1e+5
1e+4
Zooplankton abundance P = 0.00609
Time P = 0.824
Interaction P = 0.181
D
Cyanobacterial biovolume (µm3/ml)
1e+8
1e+7
1e+6
1e+5
1e+4
Zooplankton abundance P = 0.0742
Time P = 0.000547
Interaction P = 0.0964
E
Particulate microcystin (µg/L)
10
1
0.1
0.01
Zooplankton abundance P = 0.0305
Time P = 0.2564
Interaction P = 0.05642
08/01/18
11/01/18
02/01/19
05/01/19
08/01/19
11/01/19
02/01/20
05/01/20
F
Cyanobacterial biovolume (µm3/ml)
2.5e+7
2.0e+7
1.5e+7
1.0e+7
5.0e+6
0.0
Zooplankton abundance P = 0.0663
Season P = 0.0000586
Interaction P = 0.05072
ab
b
a
a
Nov-Apr
May-Oct
Season

Figure 1.4.


A
Total nitrogen (mg/L)
Zooplankton abundance P = 0.0254
Time P = 0.606
Interaction P = 0.440
B
Total phosphorus (mg/L)
High zooplankton
Low zooplankton
Zooplankton abundance P = 0.449
Time P = 0.00143
Interaction P = 0.362
C
Total ammonia-Nitrogen (mg/L)
Zooplankton abundance P = 0.000363
Time P = 0.133
Interaction P = 0.205
D
Soluble reactive phosphorus (mg/L)
Zooplankton abundance P = 0.184
Time P = 0.388
Interaction P = 0.794
E
Nitrite-Nitrogen (mg/L)
Zooplankton abundance P = 0.753
Time P = 0.00254
Interaction P = 0.424
08/01/18
11/01/18
02/01/19
05/01/19
08/01/19
11/01/19
02/01/20
05/01/20

Chapter 2

Leveraging water quality monitoring data to guide algaecide use in aquaculture ponds

ABSTRACT

The importance of aquaculture farming has increased over time as escalating commercial fishing efforts threaten natural fish populations in an attempt to feed our growing world population. Although hypereutrophic conditions are common in aquaculture systems because of regular nutrient additions through feeding, the resulting blooms of phytoplankton can be harmful to fish and lead to tainted products through the production of off-flavor compounds. In response to algal blooms, diverse algaecides are routinely applied to control phytoplankton. However, such algaecide applications may harm unintended phytoplankton, including chlorophytes. This study uses three years of physical, chemical, and biological water quality data collected across 21 aquaculture ponds in west Alabama, USA, from July 2018 to April 2021. Three response variables were used to indicate eutrophic and potentially harmful conditions, including chlorophyll-*a* (pigment in all phytoplankton), phycocyanin (pigment in cyanobacteria), and microcystin (hepatotoxin produced by some cyanobacteria). Statistical models guided by AIC and all subsets model selection were used to determine which model best explained each response variable. As expected, season (i.e., warm growing vs. cool non-growing) had large effects on most parameters, including phycocyanin, microcystin, physical measurements, and dissolved nutrient concentration. Interestingly, chlorophyll and total nutrients were less affected by season showing that these ponds tend to be algal and nutrient rich all year. The best model to explain algal abundance (as chlorophyll-*a*) contained physical parameters, including dissolved oxygen, Secchi depth, temperature, and season. For cyanobacteria (measured as phycocyanin), the AICc score for the chemical model were lower, including nitrate, total ammonia nitrogen,

total nitrogen, total phosphorous, and season. For the algal toxin microcystin, the chemical model was the best fit and included nitrate-N, total ammonia nitrogen, and total nitrogen, with season. In summary, long-term monitoring of aquaculture ponds showed clear seasonality in important parameters that can negatively influence water quality and can guide algaecide treatment periods to manage toxigenic and off-flavor producing cyanobacteria.

INTRODUCTION

Water quality in aquaculture

The practice of aquaculture has become widespread and vital for sustaining wild populations and feeding the growing global population (Brummett, 2003). Freshwater aquaculture systems face challenges as resources become more limited and under higher demand, concurrent with the threat of a changing climate (De Silva, 2013). As the productivity of the system increases with regular additions of fertilizers and feed, the system's water quality deteriorates and induces stress on fish (Boyd & Tucker, 2012). These hypereutrophic conditions along with increasing temperatures exacerbate the harmful effects of water quality on fish and calls for the establishment of best management practices (Tucker et al., 2008). Although a variety of algaecides exist (Yang et al., 2018; Buley et al. 2021), there are only two approved chemicals available to control blooms of phytoplankton in aquaculture ponds, including copper sulfate (being the only approved algaecide for unregulated use) and diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) in certain circumstances (Federal Joint Subcommittee on Aquaculture, 1994, EPA 2003). The adverse and unintended effects of repeated chemical treatments have been studied (Boyd & Massaut, 1999; Jacob et al., 2016) suggesting that utilizing a more informed chemical application strategy may be useful when determining if phytoplankton in systems are harmful or beneficial. By understanding water quality trends and

determining what conditions promote harmful versus beneficial phytoplankton, farmers can save on treatment costs, improve production, and promote ecosystem health.

Characteristics influencing water quality

Physical parameters

Physical elements of a system can indicate poor water quality, either by the visible state (e.g., clarity or turbidity) or measurements of the properties, such as temperature and dissolved oxygen (DO) concentration, which can influence fish growth and health. Secchi depth is a simple, fast, centuries long used measure of transparency that indicates light visibility in the water column (Carlson, 1977). Changes in pH and DO over a 24 hr period are often attributed to phytoplankton photosynthesis during the day and aerobic respiration at night (Boyd, 2015), although pond aeration can supplement DO when needed, such as under hypoxia or anoxia that can directly cause fish die-offs or make fish more susceptible to diseases and other health concerns (Schreck et al., 2001). Since the growth of aquatic organisms, including phytoplankton and fish, is often mediated by ambient water temperature, seasonality will have large impacts on water quality. For example, warmer temperatures during the growing season will promote higher metabolic activity of all organisms in the system. Increasing temperatures often indicate higher growth rates of phytoplankton, while extreme and sustained increases often favor cyanobacterial species (Paerl & Huisman, 2008).

Chemical parameters

Nutrients are a primary factor influencing water quality and eutrophication, specifically the organic and inorganic forms of nitrogen and phosphorus (Conley et al., 2009). The total forms of nitrogen and phosphorus often indicate trophic state conditions in freshwater systems and are often the target of management. However, in aquaculture systems, nutrient management

is challenging since nitrogen and phosphorus can account for up to 5% of the total percent of feed that are regularly supplied in large quantities and quickly contribute to bioavailable nutrient sources as excreted waste (Boyd & Queiroz, 2001). Dissolved forms of phosphorus, like the bioavailable orthophosphate, are quickly used by phytoplankton or bound in sediment (Massik & Costello, 1995). Dissolved forms of nitrogen, such as nitrate and ammonium, are commonly available forms of nitrogen that phytoplankton can utilize, except for diazotrophic cyanobacteria that can fix atmospheric nitrogen (Suttle & Harrison, 1988; Vincent, 1992). Although important for growth, ammonia and nitrite can be toxic to aquatic species and are therefore important to monitor in aquaculture (Boyd, 2015). Understanding these factors can allow for better pond management and tracking when unfavorable conditions might occur.

Response variables of poor water quality

The measurement of the pigment chlorophyll-*a* includes all photosynthetic phytoplankton without taxonomic discrimination, albeit some taxa may contain more pigment per cell than others (Felip & Catalan, 2000; Alvarez, 2017). While some algae, including chlorophytes that are often present in these systems, are not toxic, they can still cause issues with oxygen availability (Paerl et al., 2001). Moreover, a variety of phytoplankton taxa, such as dinoflagellates, chlorophytes, and cyanobacteria, can produce taste and odor compounds (also called off-flavors) (Smith et al., 2008) that may not harm fish but can cause poor taste of fish fillets and/or increase holding times resulting in a 30% loss of revenue for farmers annually (Engle et al., 1995; Tucker, 2010). Lastly, some phytoplankton, including a variety of cyanobacterial genera, can be toxic (Carmichael et al., 2001; Paerl et al., 2001).

Phycocyanin is an accessory pigment found in cyanobacteria, that are often abundant in aquaculture systems, as well as cryptophytes, which are often rare to absent in these systems.

Cyanobacteria produce nuisance secondary metabolites, including taste and odor compounds and toxins, which can be detrimental to the fish and costly for the aquaculture industry. Common cyanobacterial toxins include a long list of secondary metabolites, including hepatotoxins, cytotoxins, and neurotoxins, that can be harmful to fish and their regulatory functions (Smith et al., 2008). Microcystin is one of the most widely-studied cyanobacterial toxins in systems around the world (Buley et al., in press) and can be produced by many cyanobacterial genera, including well-studied, colonial *Microcystis* that is commonly found in farm pond aquaculture systems. Cyanobacterial toxins can affect fish species during all life stages, impacting their liver, kidney, growth, and osmoregulation to name a few (Malbrouck & Kestemont, 2006). Long term exposure to high levels of cyanobacterial toxins can ultimately be fatal, even though fish are likely more tolerant to the toxins than other organisms (Smith et al., 2008). Known incidents of fish kills from this hepatotoxin have been reported (Zimba et al., 2001). Being able to understand what conditions favor cyanobacterial toxins may allow farmers to predict when the best time is to treat ponds to avoid the toxins adverse effects.

In this study, three years of monthly monitoring data of catfish aquaculture ponds were used to forecast concentrations of three variables of interest, including phytoplankton measured at chlorophyll-*a*, cyanobacteria measured as phycocyanin, and microcystin toxin. Physical or chemical measurements were used as the predictors to find the likelihood of what parameters best explained variation in the targeted response variables. We hypothesize that as the response variable becomes more specialized in other words from algal bloom (measured as chlorophyll-*a*) to cyanobacterial bloom (measured as phycocyanin) to toxic cyanobacterial bloom (measured as microcystin), parameter selection will also be more specific to the function of that response variable.

METHODS AND MATERIALS

Twenty-one aquaculture ponds across five farms in west Alabama were sampled monthly from July 2018 to April 2021. Whole water samples were collected with an integrated tube sampler at ~0.5 m depth in the morning from the same location during the entirety of the project. A multi-probe sonde recorded temperature, pH, specific conductivity, and dissolved oxygen (DO) *in situ* at ~0.2 m depth. Water transparency was measured with a Secchi disk. Upon arrival to the laboratory, samples were immediately processed for chlorophyll-*a*, phycocyanin, total nitrogen (TN), total phosphorus (TP), nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), total ammonia nitrogen (TAN), soluble reactive phosphorus (SRP), and particulate microcystin (i.e., collected on a filter).

Algal pigments, including chlorophyll-*a* (all phytoplankton) and phycocyanin (cyanobacteria), were collected on Pall A/E filters and measured fluorometrically. Chlorophyll-*a* samples were extracted for 24 hrs in the dark at 4ºC with 90% aqueous ethanol (Sartory & Grobbelaar, 1984). Phycocyanin was measured by extracting filters in a 50 mM phosphate buffer in the dark for four hours after grinding (Kasinak et al. 2015). Total phosphorus and nitrogen were measured using a spectrophotometer with persulfate digestion (Gross & Boyd, 1998). Nitrate-N was measured using the second derivative of the recorded wavelength to calculate absorbance on a spectrophotometer (Crumpton et al., 1992), while the other dissolved nutrients, including NO_2-N, TAN (salicylate method), and SRP, were measured colorimetrically on a spectrophotometer, according to Boyd and Tucker (1992), Reardon et al. (1966) and Murphy and Riley (1962) respectively. Intracellular microcystin toxins were measured using enzyme-linked immunosorbent assay (ELISA; Abraxis-ADDA) kits after extracting samples collected on Pall A/E filters with acidified 75% aqueous methanol (Yang et al., 2018).

Statistical Analyses

Data were analyzed using a linear mixed effects model with a maximum likelihood in the *lme4* package in R for variable selection and significance to the response variables (chlorophyll, phycocyanin, and microcystin) (Bates et al., 2014 version 4.0.2). For each response variable, two types of models were created to test if one grouping of measurements, physical or chemical, would better explain it. Six total models were created, two global models (physical and chemical) for each response variable (chlorophyll-*a*, phycocyanin, and microcystin) (Table 2.1). The first global model contained all physical measurements recorded, including DO, temperature, and Secchi depth as fixed continuous variables. The second global model included all chemical measurements, containing TP, TN, TAN, SRP, NO_2-N, and NO_3-N as fixed continuous effects. Both global models also included season as a categorical variable (2=growing season, 1=non-growing season) describing either growing (May-October) or non-growing (November-April) season, and each pond was added as a random effect to avoid pseudoreplication. Chemical parameters and response variables were log transformed to help with scaling. Prior to running the models, variables were tested for correlation using the variance inflation factor (*VIF*) function and the correlation function (*cor*). *VIF* scores were <2.0 or <4.2 for the chemical or physical model parameters, respectively, and were less than 5.0 indicating low variance inflation and no collinearity. Correlations were less than 0.5, indicating weak correlation between variables. These signify predictors can be used in the models. QQ-plots tested for residual normality.

The *dredge* function from the *MuMIn* package was used to run an all subset analysis nested within the global model. Akaike Information Criterion (AIC) in the *MuMIn* package was used to compare the physical and chemical parameter model sets, with three different model

selection approaches, using chlorophyll, phycocyanin, and microcystin as the response variable (Barton, 2020). AIC are unitless values that allow comparisons to determine which model best fits the data for those that have the same response variable. The model that has the lowest value is the best model in the set of candidate models. We used AICc values, which are AIC values that correct for sample size (Anderson et al., 2000). Model weights are a measure of weight to determine if the top model is the best of the set, calculated from the relative likelihood of the model divided by the sum of all values across all models (Anderson et al., 2000). Variable weights were calculated from the sum of model weights over all models, where the higher the proportion signifies its importance relative to other variables (Burnham & Anderson, 2003, p.168). Low weights signified weak evidence that those will be included in the top model. The full model-average was used to determine estimates, meaning estimates were averaged not just from the top models but from all models considered weighted by AIC weight (Anderson et al., 2000).

RESULTS

Water quality data trends

Chlorophyll showed that catfish aquaculture ponds tend to be productive throughout the year with median values of 225.7 µg/L, including the non-growing season that had 22% relatively less chlorophyll compared to the growing season, when fish are fed less frequently (Figure 1A). However, long-term trends show strong seasonal effects on cyanobacteria (as phycocyanin, Figure 1B) and their toxins (as microcystin, Figure 1C). Phycocyanin and microcystin concentrations are both 430% higher during the growing season (Figure 1B-C). As expected, temperature and DO showed inverse relationships (i.e., warm water has lower DO, Figure 2A-B). Secchi depth was generally shallow and fairly consistent over time, with an

average 2% decrease in depth in the growing season (Figure 2C). Trends in total and dissolved forms of specific nutrients varied (Figure 3). For example, TP and TN were 46% and 8% more abundant in the growing season (Figure 3A, C), respectively. Nitrate-N was 2.2% less abundant in the growing season and NO_2-N was 1.48x more abundant (Figure 3B, D). Soluble reactive phosphorus was 82% higher in the growing season (Figure 3E). Total ammonia nitrogen was 39% less abundant in the growing season, with peaks up to 8 mg/L in the non-growing season (Figure 3F).

When comparing trends over time from 2018 to 2021, some parameters increase in abundance over time, while other decrease. Phycocyanin and TP increased over time, by 6% and 11% respectively in the growing and 210% and 37% respectively in non-growing over three years (Figure 2.1B, 2.3A). From 2018 to 2021, TN, NO_3-N, NO_2-N, SRP, and microcystin all decreased in growing season by 17%, 11%, 24%, 15% and 55% respectively and 49%, 7%, 52%, and 30% respectively in the non-growing season (Figure 2.3A-B, 2.3D-E, 2.1C). Secchi and temperature trends show a 4% and 10% decrease in the growing season and a decrease of 1.3% and 21% in the non-growing (Figure 2.2 A, C). Other trends saw differences in response between seasons, like chlorophyll, with decreased in the growing season by 5% but increased in the non-growing season by 30%. Total ammonia nitrogen had the inverse relationship, increasing by 13% in the growing season and decreased by 9% in the non-growing season (Figure 2.3F). However, either increase or decrease in measurements suggest they are still present throughout the entire year and should be monitored as such.

Model variable selection

Chlorophyll as the response variable

The physical model for chlorophyll, selected DO, Secchi depth, temperature, and season from the *dredge* function in the top model (Table 2.2, Formula 1). AICc was -131 and the model weight suggests there is a 90% chance that this model is the best fit out of those models considered. The sum of the variable weights found that Secchi (cm), DO (mg/L), and temperature (°C) have a 100% chance of being in the best model and season has a 90% chance. For the chemical model, TP (mg/L), TN (mg/L), TAN (mg/L), SRP (mg/L), and season were selected, leaving out NO_2-N (mg/L) and NO_3-N (mg/L) (Table 2.2, Formula 2). The AICc was -38.8 and the model weight indicates that there is a 47% chance that this model is the best fit out of all considered. The sum of variable weights for this model has a 100% chance that TAN (mg/L) and season were included in the true best model, 98% for SRP (µg/L), 97% for TN (mg/L), and 95% for TP (mg/L).

Phycocyanin as the response variable

The physical model selected DO (mg/L), Secchi (cm), temperature (°C), and season as parameters in the top model for predicting phycocyanin concentration (Table 2.2, Formula 3). The AICc was 851.73 and the model weight suggests that there is a 94% chance that this model is the best out of all considered. The sum of the variable weights showed a 100% chance that season, Secchi (cm), and DO (mg/L) are in the true best model and a 94% chance that temperature is. The top chemical model for phycocyanin selected TP (mg/L), TN (mg/L), NO_3-N (mg/L), TAN (mg/L), and season parameters, leaving out SRP (mg/L) and NO_2-N (mg/L) (Table 2.2, Formula 4). The AICc was 843.28 and the model weight suggests that there is a 66% chance that this is the top model out of all considered. Total ammonia nitrogen (mg/L), TP (mg/L), and season have a 100% of being in the true best model, TN (mg/L) had a 94% chance and NO_3-N (mg/L) had a 82% chance based on the sum of the weights.

Microcystin as the response variable

The physical model for explaining microcystin concentration selected Secchi depth (cm) and season as variables for the top model, leaving out DO (mg/L) and temperature (ºC) (Table 2.2, Formula 5). The AICc for this model was 886 and the top model had a 91% chance of being the best model out of those considered from the dataset. Variable weights suggest season and Secchi have a 100% of being in this model with temperature and DO have only 8% and 2% chance, respectively. The chemical parameters for the top microcystin model included TN (mg/L), NO_3-N (mg/L), TAN (mg/L), and season, where SRP (mg/L) TP (mg/L) and NO_2-N (mg/L) were removed (Table 2.2, Formula 6). The AICc was 857 and the model weight suggests there is a 46% chance this model is the top model of all considered. While this likelihood suggests some uncertainty, the next best model adds TP as a significant parameter, being 40% likely to be the best model. Sum of weights show that season, TN (mg/L), TAN (mg/L), and NO_3-N (mg/L) have a 100% chance of being included in the true best model. Total phosphorus (mg/L) had a 47% chance of being in the true best model, NO_2-N (mg/L) 8%, and SRP (mg/L) 7%, although not in the final model.

DISCUSSION

In this study, water quality of 21 aquaculture ponds was monitored monthly across three years. From these data, models were built to determine which parameters (chemical or physical) best explained the three response variables, including chlorophyll, phycocyanin, and microcystin. Determining these parameters can assist farmers on algaecide treatment of their farms and under what conditions they might expect harmful algal growth and toxins to be present.

Water Quality Trends

Water quality trends across the study show consistent and predictable levels of phytoplankton (based on two algal pigments), microcystin toxin, and chemical and physical parameters. As expected, phycocyanin and microcystin concentrations were greater in the growing season (Sommer et al., 1986; Jöhnk et al., 2008). Total nutrients were greater in the growing season mirroring increases in feed additions and fish waste. Nitrate-N and TAN were generally low in the growing season likely due to increases in nutrient uptake by phytoplankton. However, interestingly NO_2-N and SRP were more abundant in the growing season. In reduced conditions, NO_2-N may be more abundant in the growing season as DO levels decrease (Boyd & Tucker, 2012). The abundance of SRP in the growing season may be explained by its release from sediments during the warmer months (Jensen & Andersen, 1992) or through the release from cells following algaecide application (Boyd & Tucker, 2012).

Trends from 2018 to 2021 suggest phycocyanin has increased in abundance in both growing and non-growing season over time, chlorophyll-*a* is decreasing in the growing season and microcystin is decreasing in both, especially in warmer conditions, similar to what other long-term studies have found (Reynolds et al., 2012). Other nutrients have decreased over time in warmer conditions, like TN, NO_3-N, and NO_2-N, that may be the result of warmer temperatures increasing rate of removal (Zheng et al., 2016); whereas the increase in TAN over time in the growing season may be due to the general decrease in chlorophyll over time, as ammonia is the preferred nitrogen substrate for phytoplankton, followed by NO_3-N (Hargreaves, 1998). In instances when nutrients increased in abundance over time, like TP in both seasons, suggest that phytoplankton may be decreasing over time, supported by data that chlorophyll-*a* is decreasing. Although chlorophyll-*a* is decreasing in the growing season, phycocyanin is increasing in both, maintaining biovolume levels of phytoplankton that can efficiently assimilate

nutrients that causes them to decrease over time and season (Lenard et al., 2019). In the growing season, phycocyanin levels are increasing while chlorophyll-*a* is decreasing, suggesting that cyanobacteria has been outcompeting other species during these warmer months over the past three years (Paerl & Paul, 2012)

Chlorophyll

Based on AICc and model selection when chlorophyll was the response variable, the physical parameter model was better able to predict and fit the data (Table 2.1, Formula 1). The implications of this model can assist farmers when blooms of all algae may occur, with parameters that are often easy to measure *in situ*. The model of best fit included temperature, DO, Secchi, and season. Temperature is an important driver for growth of all aquatic organisms, including all phytoplankton, so including it in the model is sound, especially with respect to season. Season contributes significantly to phytoplankton growth, especially in the growing season, as temperature is a key driver for the bioavailability of some nutrients, like phosphorus (Philips et al., 2017). Because Secchi measures light availability in the water column, it's relationship to chlorophyll-*a* values can indicate bloom conditions when the systems are not as clear (Carlson, 1977). Dissolved oxygen is an important variable when determining chlorophyll-*a* values because it is used when phytoplankton respire at night and is produced when phytoplankton photosynthesize. Typically, DO becomes an issue when a population crashes or respires at higher rates than they can photosynthesize. These aquaculture systems are aerated when needed (usually every evening during the growing season), resolving some DO issues.

Phycocyanin

For the models explaining the phycocyanin response variable, the chemical parameter model AICc was 843.28 (Table 2.2, Formula 4). The parameters selected for this model includes

NO_3, TAN, TN, TP, and season. Total ammonia nitrogen had one of the highest likelihood of being included in the model, which is supported by the literature, as it is most readily taken up by cyanobacteria and would therefore promote their growth (Masuda & Boyd, 1994; Hargreaves, 1998). Total phosphorous is also absorbed very quickly by a dense bloom of cyanobacteria, removing on average 41% in 24h, and subsequently promoting their growth (Boyd & Musig, 1981). In hypereutrophic ponds, TN is a product from excess fish feed and nitrogenous waste (Boyd & Tucker, 2012). The debate of nitrogen versus phosphorus as indicators of cyanobacterial blooms has been ongoing for decades (Conley et al., 2009; Schindler et al., 2008), but for these systems, both seem to play a role in bolstering cyanobacterial biovolume. The seasonal element shows a clear selection for the growing season, that often come hand in hand with warmer temperatures where cyanobacteria can outcompete other species (Paerl & Paul, 2012). The dominance of cyanobacteria compared to other phytoplankton species are expected to continue to shift into the coming years, as seen with these data trends, causing more extreme conditions in shallow, wind-exposed, and high-water retention systems, like aquaculture ponds (Markensten et al., 2010).

Microcystin

The chemical model for explaining microcystin was of better fit (lower AICc score by 29) than the physical parameter model (Table 2.2, Formula 6). The model included NO_3-N, TAN, TN, and season, suggesting nitrogen may have a larger effect than phosphorus or physical parameters on microcystin levels in these systems. While physical parameters are important (e.g., temperature and light) for microcystin production, nutrients are essential for the growth of phytoplankton and the production of the toxin itself (Dai et al., 2016). As it is the main component of the microcystin molecule (14% of molecular weight of microcystin-LR), nitrogen

should be included as a significant parameter (Dai et al., 2016). Total nitrogen can promote both the growth of *Microcystis* (Davis et al., 2009) and toxicity of blooms (Monchamp et al., 2014). Studies have found excess nitrogen can support microcystin production (Dait et al., 2016) and be the focus for higher-than-normal N levels. Inorganic forms of nitrogen (e.g., NO_3-N and TAN) have shown a strong relationship with microcystin production. Nitrate-N was shown to directly correlate with microcystin genes after multiple blooms in Lake Mikata, but there were no significant relationships between the genes and temperature or phosphate (Yoshida et al., 2007). Total ammonia nitrogen has been shown to increase under rapid decomposition of organic matter, like during a bloom crash and release toxins after cell lysis (Yang et al., 2012).

Conclusions

This study explained chlorophyll, phycocyanin, and microcystin measurements by a multitude of water quality measurements, both physical and chemical. Data trends across time and season illuminate the variability of seasonal effects on water quality, within the year and over time. Chlorophyll-*a* was best explained by physical parameters, whereas phycocyanin and microcystin were both explained by the chemical model parameters. While trends of microcystin are decreasing, phycocyanin is increasing over time, increasing harmful conditions. Models and water quality data trends can assist farmers on best management practices when blooms occur, especially given the time of year. Information and models can procure an estimation of chlorophyll-*a,* phycocyanin, and microcystin given either physical or chemical measurements taken by farmers, once values have been $\log_{10}$ transformed to fit the model. Using this tool may assist farmers prior to treating, to determine when a bloom is either harmful (high phycocyanin and microcystin) or a bloom of non-toxic green algae (chlorophyll-*a*) and avoid treating when nuisance algae is not present.

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Table 2.1

Response variable	Model Parameters	AICc	AIC Weights
Chlorophyll-a	1. TP + TN + TAN + SRP +NO2 + NO3 + Season	-38.78	0.46
	2. DO + Secchi + Temp + Season	-131.31	0.9
Phycocyanin	3. TP + TN + NO3 + NO2 + TAN + SRP + Season	843.28	0.66
	4. DO + Secchi + Temp + Season	851.73	0.94
Micocystin	5. TP + TN + TAN + SRP +NO2 + NO3 + Season	846.76	0.46
	6. DO + Secchi + Temp + Season	874.62	0.91

TABLE 2.2

Formula 1: Log_{10} (Chlorophyll (µg/L)) = -0.0164 Secchi (cm)+ 0.012 Temp (ºC)+ 0.04 DO (mg/L)+ 0.119 Season

Formula 2: Log_{10} (Chlorophyll (µg/L)) = -0.123 TAN (mg/L)+ 0.228 TP (mg/L)+ 0.362 TN (mg/L)+ -0.133 SRP (mg/L)+ 0.139 Season

Formula 3: Log_{10} (Phycocyanin (µg/L)) = -0.0244 Secchi (cm) + 0.268 Temperature (ºC) + 0.671 DO (mg/L) + 0.739 Season

Formula 4: Log_{10} (Phycocyanin (µg/L)) = -0.165 NO_3-N (mg/L) -0.277 TAN (mg/L) + 0.836 TN (mg/L) + 0.581 TP (mg/L) +0.726 Season

Formula 5: Log_{10} (Microcystin (µg/L)): -0.0224 Secchi (cm) + 0.906 Season

Formula 6: Log_{10} (Microcystin (µg/L)) = -0.25 NO_3-N (mg/L)+ -0.191 TAN (mg/L) + 1.78 TN (mg/L) + 0.834 Season

FIGURE LEGENDS

Figure 2.1. Chlorophyll-*a* (A, µg/L), phycocyanin (B, µg/L), and particulate microcystin (C, µg/L) concentrations in 21 aquaculture ponds sampled monthly from July 2018 to April 2021. Data used in figures were raw measurements of each response variable.

Figure 2.2. Temperature (A, ^{o}C), dissolved oxygen (B, mg/L), and Secchi depth (C, cm) concentrations in 21 aquaculture ponds sampled monthly from July 2018 to April 2021. Data used in figures were raw measurements of each response variable. Data in these figures were raw *in situ* measurements.

Figure 2.3. Total nitrogen (A, mg/L), nitrate-nitrogen (B, mg/L), total phosphorus (C, mg/L), nitrite-nitrogen (D, mg/L), soluble reactive phosphorus (E, mg/L), and total ammonia nitrogen (F, mg/L) concentrations in 21 aquaculture ponds sampled monthly from July 2018 to April 2021. Data used include raw data collected from analyses.

Figure 2.1.

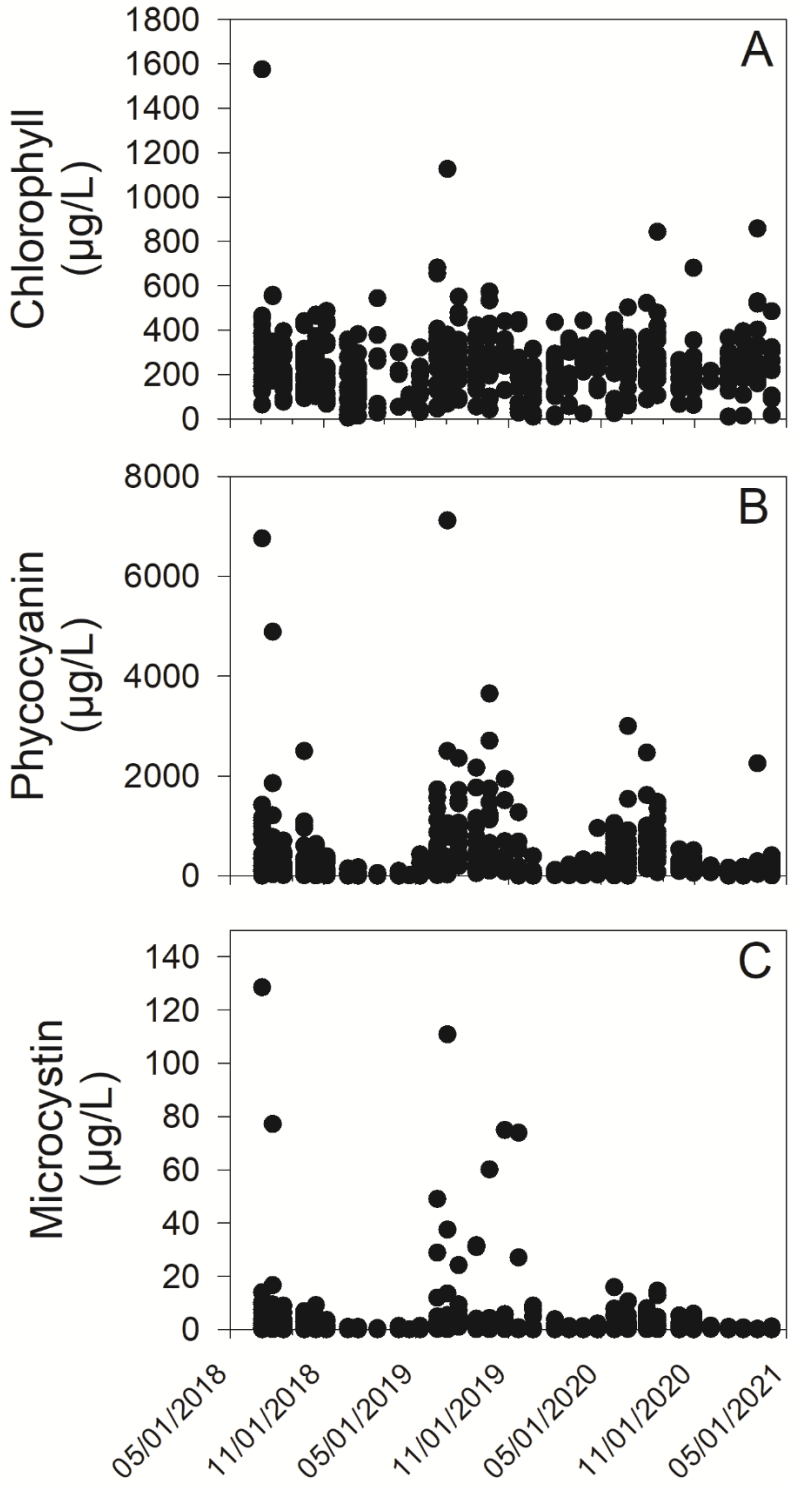

A
Chlorophyll (µg/L)
1800
1600
1400
1200
1000
800
600
400
200
0
B
Phycocyanin (µg/L)
8000
6000
4000
2000
0
C
Microcystin (µg/L)
140
120
100
80
60
40
20
0
05/01/2018
11/01/2018
05/01/2019
11/01/2019
05/01/2020
11/01/2020
05/01/2021

Figure 2.2.

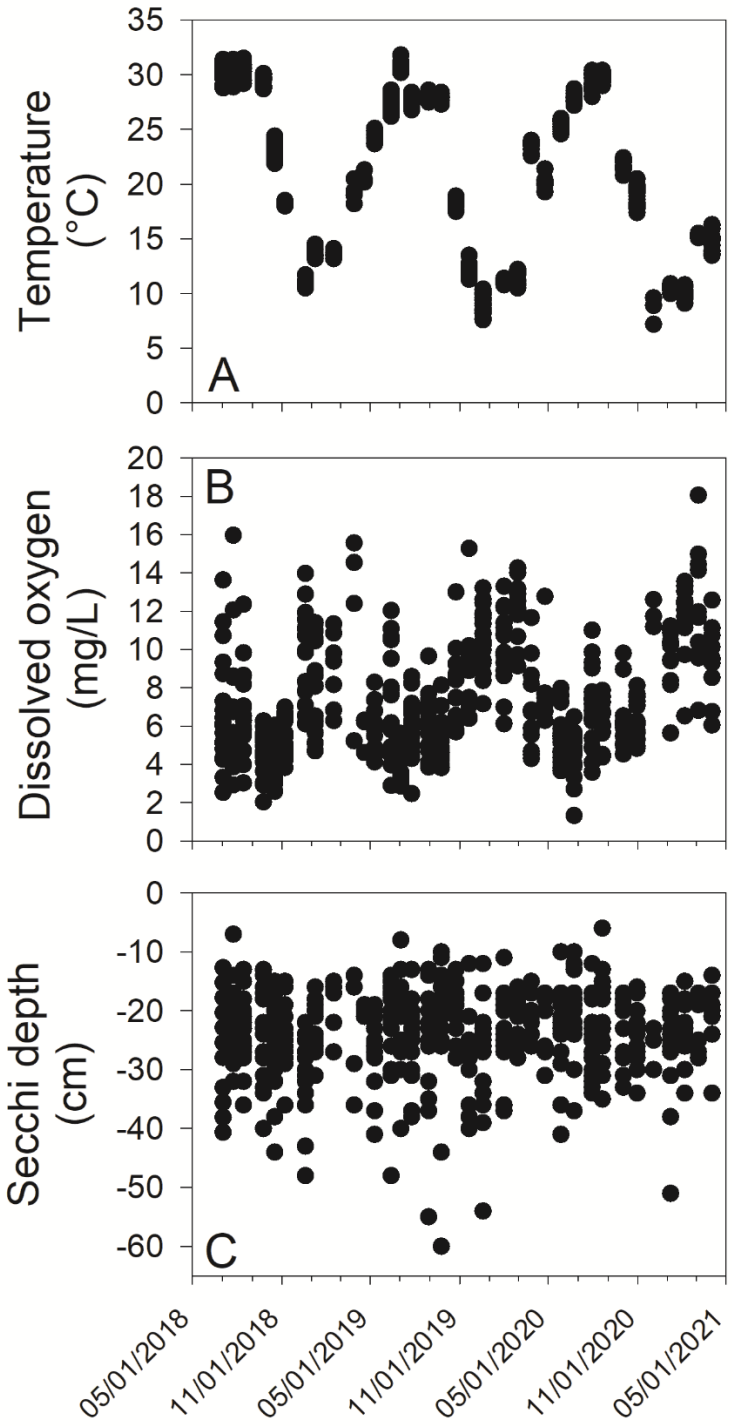
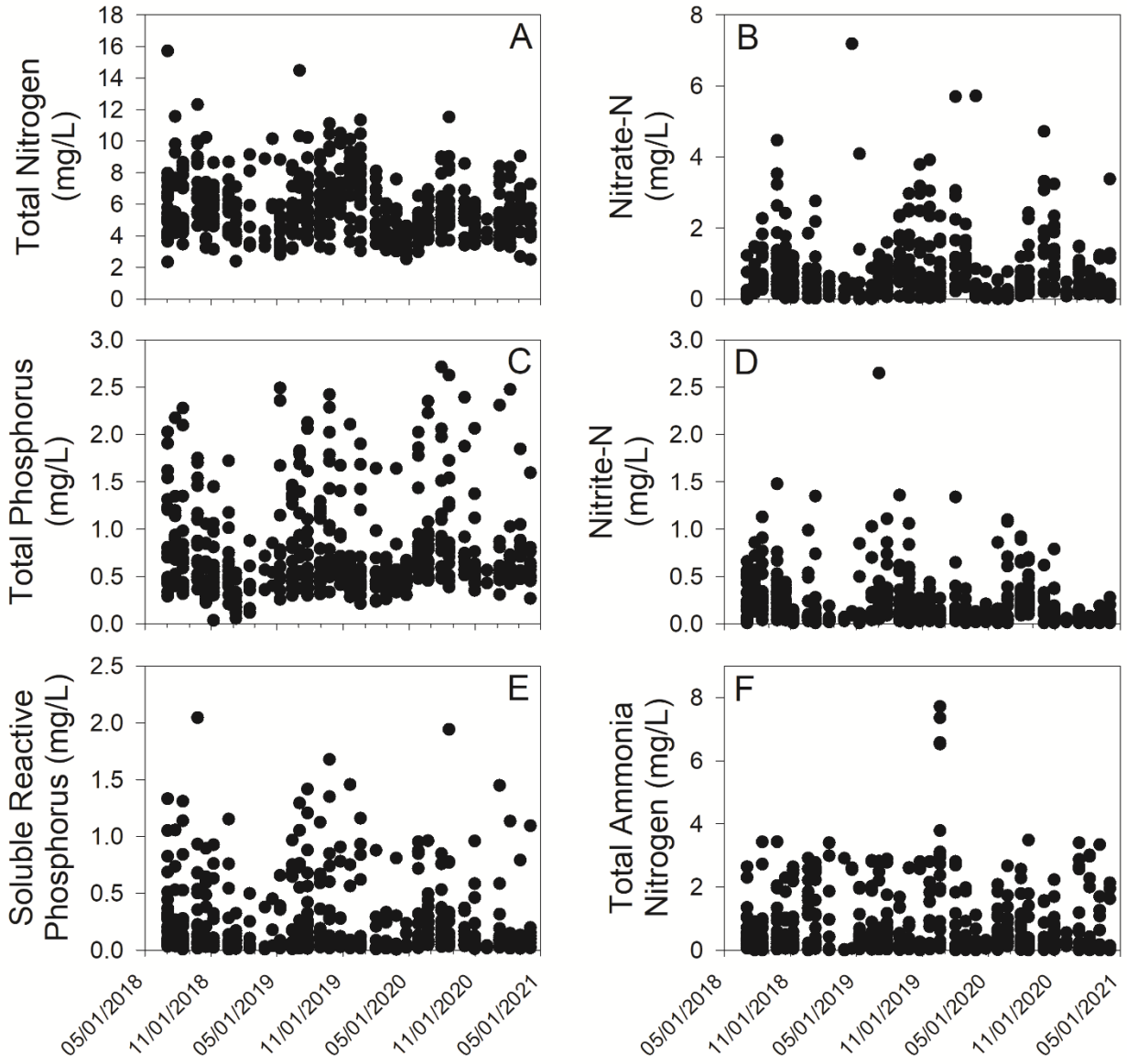

Temperature (°C)
35
30
25
20
15
10
5
0
A
Dissolved oxygen (mg/L)
20
18
16
14
12
10
8
6
4
2
0
B
Secchi depth (cm)
0
-10
-20
-30
-40
-50
-60
C
05/01/2018
11/01/2018
05/01/2019
11/01/2019
05/01/2020
11/01/2020
05/01/2021

Figure 2.3.


A
Total Nitrogen (mg/L)
18
16
14
12
10
8
6
4
2
0
B
Nitrate-N (mg/L)
8
6
4
2
0
C
Total Phosphorus (mg/L)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
D
Nitrite-N (mg/L)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
E
Soluble Reactive Phosphorus (mg/L)
2.5
2.0
1.5
1.0
0.5
0.0
F
Total Ammonia Nitrogen (mg/L)
8
6
4
2
0
05/01/2018
11/01/2018
05/01/2019
11/01/2019
05/01/2020
11/01/2020
05/01/2021