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Approaches to Golden Algae Control: In-Lake Mesocosm Experiments

Final Technical Report

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Abstract: *Prymnesium parvum*, a haptophyte alga, occurs worldwide. It is tolerant of large variations in temperature and salinity, and is capable of forming large fish-killing blooms. In the USA, the first recorded *P. parvum* bloom occurred in 1985 in a semi-arid region of the country (Pecos River, Texas). Since then, the incidence of *P. parvum* blooms dramatically increased in the USA, where the organism has invaded lakes and rivers throughout southern regions and most recently into northern regions. Fortunately, *P. parvum* population dynamics are influenced by several factors that may serve as tools for management. These include hydraulic flushing, pH and ammonia additions. Here we report on in-lake enclosure experiments conducted during periods of bloom initiation, and bloom development and decline. We demonstrated that all three approaches have promise to controlling blooms in localized areas of lakes. Most promising were our manipulations involving pulsed hydraulic flushing (30% water exchange once per week using water deeper in the lake); the neutralization of ambient waters (lowering pH to 7); and ammonia additions (elevating to 40 μM).

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Preface

This final technical report was developed to convey the experiments and results of the Testing Approaches to Golden Algae Control: In-lake Mesocosm Experiments project on Lake Granbury, Texas. This project was supported by Congress with funding through the U.S. Army Corps of Engineers.

The report was prepared by the project scientists, Drs. Daniel Roelke of Texas A&M University, Bryan Brooks of Baylor University, and James Grover of the University of Texas at Arlington. The Lake Granbury project was managed by the Texas Water Resources Institute (TWRI), part of Texas AgriLife Research, the Texas AgriLife Extension Service, the College of Agriculture and Life Sciences at Texas A&M University, and project manager Danielle Kalisek provided assistance in preparing the report. Dr. B.L. Harris, acting director of TWRI, was the principal investigator of the project.

Additional project collaborators included the Texas Parks and Wildlife Department, Texas Commission on Environmental Quality, and Brazos River Authority.

Unit Conversion Factors

Multiply	By	To Obtain
acres	4,046.873	square meters
acre-feet	1,233.5	cubic meters
degrees Fahrenheit	(F-32)/1.8	degrees Celsius
feet	0.3048	meters
gallons (U.S. liquid)	3.785412 E-03	cubic meters
hectares	1.0 E+04	square meters
inches	0.0254	meters
inches	2.54	centimeters
miles (U.S. statute)	1,609.347	meters
miles (U.S. statute)	1.609344	kilometers
ounces (mass)	0.02834952	kilograms
ounces (U.S. fluid)	2.957353 E-05	cubic meters
pints (U.S. liquid)	4.73176 E-04	cubic meters
pints (U.S. liquid)	0.473176	liters
square feet	0.09290304	square meters
square inches	6.4516 E-04	square meters
square miles	2.589998 E+06	square meters
square yards	0.8361274	square meters
yards	0.9144	meters

1 Introduction

Background

Inflows and salinity have long been recognized as factors influencing phytoplankton community dynamics and structure (Ketchum, 1951, 1954). The magnitude and timing of inflows produce nutrient pulse and flushing loss variations that select for species adapted for these conditions, which in turn influence plankton community composition and productivity (Roelke *et al.*, 2003; Buyukates and Roelke, 2005; Miller *et al.*, 2008). Nutrient pulses and flushing losses associated with inflows also have been linked to the incidence of harmful algal blooms (Seliger*et al.*, 1970; Anderson and Stolzenbach, 1985; Paerl, 1988; Jacoby *et al.*, 2000; Moustaka-Gouni*et al.*, 2006; Mitrovic*et al.*, 2008), including toxic blooms of *Prymnesium parvum* (Roelke *et al.*, 2010a, 2011).

P. parvum, a haptophyte alga, occurs worldwide. It is tolerant of large variations in temperature and salinity, and is capable of forming large fish-killing blooms (Lundholm and Moestrup, 2006; Baker *et al.*, 2007, 2009; Southard *et al.*, 2010). In the USA, the first recorded *P. parvum* bloom occurred in 1985 in a semi-arid region of the country (Pecos River, Texas) (James and De La Cruz, 1989). Since then, the incidence of *P. parvum* blooms dramatically increased in the USA, where the organism has invaded lakes and rivers throughout southern regions and most recently into northern regions (Fig. 1, from Roelke et al. 2011). *P. parvum* blooms typically occur in aquatic systems that are eutrophic and brackish (Kaartvedt*et al.*, 1991; Guo*et al.*, 1996; Roelke *et al*, 2007a, 2010a, b).

Many factors contribute to *P. parvum* bloom formation. They include production of chemicals toxic to grazers (Granéli and Johansson, 2003; Tillmann, 2003; Barreiro *et al.*, 2005; Michaloudi*et al.*, 2009; Brooks *et al.*, 2010), use of alternative energy and nutrient sources through mixotrophy and saprophytic nourishment (Nygaard and Tobiesen, 1993; Skovgaard and Hansen, 2003; Lindehoff*et al.*, 2009), suppression of competitors through allelopathy (Fistarol*et al.*, 2003, 2005; Granéli and Johansson, 2003; Roelke *et al.*, 2007a; Errera*et al.*, 2008), and resistance to the allelopathic effects of other algae (Suikkanen*et al.*, 2004; Tillmann*et al.*, 2007). Factors that negatively influence *P. parvum* population density might include grazing by toxin-resistant zooplankton and pathogenic effects of virus (Schwierzke*et al.*, 2010). In addition, some cyanobacteria

may inhibit *P. parvum* blooms (Grover *et al.*, 2010; Roelke *et al.*, 2010b; James et al., 2011).

In regards to inflow and salinity, both have been shown as important factors influencing *P. parvum* population dynamics and reproductive growth rates. A recent study documenting the entire seasonal *P. parvum* bloom cycle in a Texas lake found that cell loss through hydraulic flushing during a period of high inflow, along with cessation of toxin production associated with nutrient loading, was the primary mechanism terminating the bloom (Roelke *et al.*, 2010a). Another study employing data records spanning a decade for three lake systems of the Brazos River showed that inflow bloom thresholds exist, above which fish-killing, system-wide blooms were not possible (Roelke et al., 2011). In regards to salinity, using a Texas strain of *P. parvum* (UTEX LL 2797), the optimal salinity for reproductive growth was determined to be 22 psu. At 10°C, a temperature representative of winter conditions when blooms are most common in the region, growth rates decreased ~10-fold (from ~0.2 to 0.02 d^{-1}) as salinity decreased from the optimum to levels found in Texas lakes (Baker *et al.*, 2007, 2009). Baker *et al.* (2007, 2009) also suggested that small variations in salinity at low levels determine whether reproductive growth is possible.

Recent findings from our research group also identified a pH dependent influence on the magnitude of ambient *P. parvum* toxicity in Lakes Granbury and Whitney, and *P. parvum* laboratory cultures (Valenti et al. 2010). Specifically, Valenti et al. identified that higher pH levels of 8.5 result in greater toxicity than lower pH levels (6.5, 7.5), which suggests the toxins released by *P. parvum* may behave as weak bases in aqueous solutions. These results from field observations and laboratory experiments suggest that targeted reductions of pH can reduce the potency of *P. parvum* toxins and related impacts to fisheries.

Barkoh et al. (2003) experimentally manipulated ammonia to treat *P. parvum*. These observations, when accounting for the more toxic unionized form, indicated potential utility of employing ammonia amendments for managing *P. parvum* threats to aquatic life. More recent studies by our research team (Grover et al. 2007) further examined ammonia treatments in laboratory studies. These previous efforts provide a reasonable justification to perform field oriented experiments in confined regions of reservoirs (e.g., coves) experiencing *P. parvum* blooms to further test the effectiveness of ammonia pulses in bloom mitigation, while

also examining relative effects on other components of phytoplankton and zooplankton communities.

Objective

We performed in-lake enclosure experiments investigating potential management options for mitigation of *P. parvum* impacts on inland waters. Specifically, they were manipulations of hydraulic flushing, pH and ammonia. These are described below.

Approach

Study region

The Brazos River flows southeast across Texas (USA), spanning a rain gradient from the arid western regions of the state (averaging ~13-26 cm year^{-1}) to the moister eastern region, with an average of ~155 cm year^{-1}. This study was conducted in Lake Granbury (centered at 32.40° N, 97.76° W, construction completed in 1969), located in a region of the watershed receiving ~90 cm year^{-1} of rainfall (Figure 1). Lake Granbury is sinuous with shorelines that follow the submerged river channel. Its capacity is 189 x 10^6 m^3. Its surface area, average depth and total drainage area are 34 km^2, ~5.5 m and 41,732 km^2. Lake Granbury has experienced recurrent fish-killing, system-wide *P. parvum* blooms over the last decade (Roelke et al. 2011).

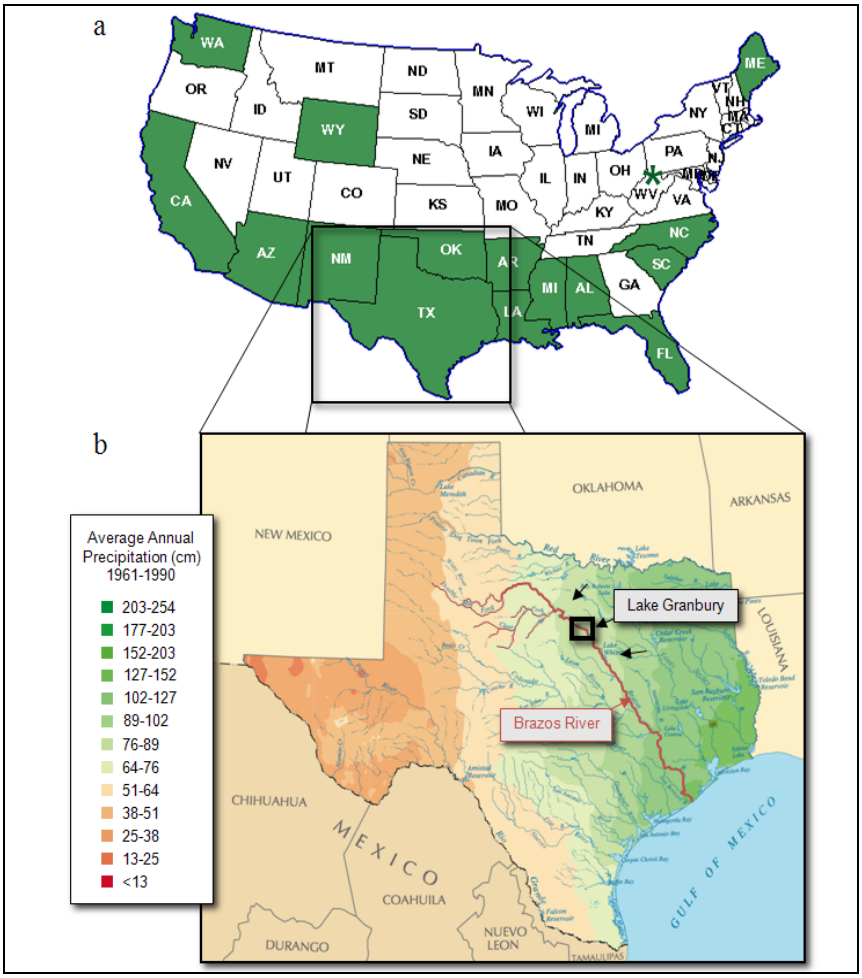

Figure 1. Distribution of P. parvum in the US, where states colored green have the organism (a), and the location of our in-lake experiments (b), which were conducted February and March, 2010.

Approach

We conducted collaborative research focused on testing the efficacy of various approaches to controlling *P. parvum* blooms. Our investigation assessed *P. parvum* bloom dynamics, and plankton community response as a whole, by measuring the response of the natural phytoplankton community to experimental manipulations and by assessing the toxicity response of *P. parvum* under these changed conditions.

Our results provide an understanding of how *P. parvum* populations respond to direct intervention (see experimental treatments below). Relative toxicity tests established linkages between toxicity and these approaches to bloom control. The research provides a better understanding of what approaches should be pursued as management tools in specific regions of Texas lakes, possibly serving as bloom control mechanisms.

Experiments were conducted at the mesocosm scale using pre-designed limnocorrals supplied by Aquatic Research Instruments. We constructed a limnocorral array that was comprised of 1.57 m^3 clear plastic containers that extended from the surface to a depth of 2 m, with an approximate diameter of 1 m. Each limnocorral had rigid supports at the surface and bottom, a closed bottom, and a separate flotation collar. Bags were open to the air during the deployments. This design was successfully employed previously in a Texas lake similar to Lake Granbury (Roelke et al. 2007). From our previous work with limnocorrals of this dimension we know that the contained volume was well-mixed. We've verified this by collecting profile data within previously deployed limnocorrals. A well-mixed state was maintained because wave energy experiences little dissipation as it passes through the flexible material of the limnocorral siding. Because limnocorrals of this dimension remains well-mixed, grab samples were taken from a single depth (0.5 meters).

Our treatments (see below) were implemented every seventh day of the 21-day experiments, i.e., at t0, t7 and t14. Sampling of the limnocorrals occurred on each of these days, as well as t21 (experiment conclusion). For t0, sampling occurred after treatment initiation. This enabled us to characterize the initial condition in each of the limnocorrals. For t7 and t14, sampling occurred just before the maintenance of the treatments. This enabled us to characterize the affect of the treatments during the first seven and 14 days of the experiments. Our first experiment was initiated on February 23, 2010, and our second experiment was started on March 31, 2010.

Our in-field experiments were designed to determine how *P. parvum* populations and the overall plankton community responded to manipulations that included:

Treatments (all performed in triplicate)

1. Hydraulic flushing
2. Reduced pH
3. Ammonia pulses

Hydraulic flushing was tested at three levels, which were 0.05, 0.1 and 0.3 d^{-1}, where the control enclosure received no inflows. Our controlled flushing events occurred every seventh day of the 21-day experiment (i.e., t0, t7 and t14), where days between flushing events experienced no inflow. Thus, this treatment represents a pulsed flushing condition. Flushing events were achieved by removal of a measured volume from targeted limnocor-

rals using calibrated buckets, followed by refilling using waters pumped from depth. Waters used for the flushing treatments were taken at a depth of ~7 meters. Temperature, pH, and salinity were very similar between surface and deeper waters, as this system is a shallow, warm-monomictic lake that during winter months is holomictic. We used multiple water pumps powered by an array of deep-cell 12 V batteries housed on our research vessel.

The influence of reduced pH was evaluated at three levels, 7.0 (neutral), 7.5 (slightly basic) and 8.0 (slightly reduced from natural basic condition), where the pH of the control enclosure was what naturally occurred in the lake, which was ~ 8.5 (Roelke et al. 2010a). We manipulated pH of limnocorrals in Lake Granbury to test the hypothesis that lowering pH decreases the magnitude of ambient toxicity to fish and other aquatic organisms, which extends our previous observations in the laboratory with cultures and pH manipulation of field samples to an experimental field study (Valenti et al 2010). To examine this question, we employed common US Environmental Protection Agency methods for Toxicity Identification Evaluations (US EPA 1991).

The pH of limnocorrals was adjusted by careful additions of 1 N sulfuric acid solution. We selected sulfuric acid to reduce pH rather than hydrochloric, nitric, phosphoric, or carbonic acid because SO_4^- is relatively biological benign compared to the other conjugate bases. A 1 N solution was prepared by adding 28 ml of ACS-grade concentrated sulfuric acid per 1 L of Lake Granbury water (APHA et al 1998). Chlorine can be highly toxic to aquatic life and this is particularly relevant for our experiments because free chlorine (the most toxic form) becomes more predominant in aquatic solutions at lower pH (Brungs 1973). Nitric and phosphoric acid were avoided because augmenting limnocorrals with these additions could alter the nutrient (N:P) stoichiometry of the system. Similarly, we choose not to use carbonic acid because it would introduce more inorganic carbon into pH adjusted systems.

For t0, the amount of solution required to reduce the pH to the desired treatment level was based on an extrapolation from a prior laboratory titration experiment with Lake Granbury water. Before any pH adjustment, the pH of each limnocorral was measured using YSI 600 XLM or YSI 6600 data sondes. Two point calibrations (pH 7 and pH 10) were completed for the data sondes prior to their use and immediately following their use as a post-calibration quality control measure. To adjust pH, a volume of 1 N

sulfuric acid solution was measured in a 500-ml graduated cylinder and then slowly added in small pulses of 10-20 ml. Concurrent with the addition, surface water in the limnocorrals were mixed continuously and the pH was continuously monitored and measured with a data sonde. To ensure uniform mixing while adding the acid solution, pH measurements were taken at various depths in the water column. Once the pH stabilized, additional sulfuric acid solution was added as previously described until the desired pH treatment level was reached. In addition to biweekly visits to each of the targeted limnocorrals for pH measurement and adjustment, data sondes were deployed in each of the pH treatment levels (low, medium, high, and control) to allow continuous monitoring of pH during the course of the experiment. Preliminary studies with pH manipulation of Lake Granbury water indicated that pH treatment levels remained relatively stable under field conditions.

Ammonia pulses were achieved through the weekly addition of NH_4Cl at prescribed levels, to triplicate limnocorrals. The two levels delivered achieved added concentrations similar to those used in previous laboratory experiments (Grover et al., 2007), levels of 10 and 40 μM. The high dose proved effective in lowering the toxicity of laboratory cultures of *P. parvum* grown at 20° C. During the time period of the proposed experiments, temperature was expected to rise from about 10° C to about 20° C, so effective reduction of toxicity in the second phase of experiments is expected. Moreover, the mechanism of toxicity reduction reported earlier (Grover et al., 2007) appeared to be related to a feedback cycle whereby growing populations of *P. parvum* raised the pH sufficiently to deprotonate NH_4 to free NH_3, to which *P. parvum* is highly susceptible. A similar feedback is anticipated in these field experiments, based on previous observations of high pH (> 8.5 at some stations) during a *P. parvum* bloom in the first quarter of 2007 on Lake Granbury.

Sampling of the enclosures occurred at the start of the experiment, then every seventh day thereafter. A second 21-day experiment was initiated shortly after the first experiment finished, employing the same treatments. The first experiment was conducted during a period of bloom initiation and the second experiment during a period of bloom development and decline. Response variables sampled for during each time of sampling included: *P. parvum* population density, phytoplankton biomass and composition, zooplankton biomass and composition, inorganic nutrients, toxicity and other water quality parameters. These are discussed in more detail in the following section. Samples for *P. parvum* population density,

phytoplankton biomass and composition, and inorganic nutrients were taken from a 1-liter grab sample. Samples for zooplankton biomass and composition were taken from a 12-liter grab sample, and grab samples for toxicity were 5 liters. Other water quality parameters were measured on site.

So that we can better interpret our experimental results within the context of the natural in-lake conditions, we continued to monitor Lake Granbury during the period of the experiment and shortly thereafter. As part of the monitoring, we sampled 10 fixed location stations in Lake Granbury, described previously (see Roelke et al. 2010a). All parameters measured in the enclosures were also measured at each of the in-lake stations. To complement our fixed-station characterizations of *P. parvum* population dynamics, and phytoplankton as a whole, we measured spatial patterns of chlorophyll *a* during each sampling trip with Dataflow, a high-speed, flow-through measurement apparatus developed for mapping physicochemical parameters in shallow aquatic systems (Madden and Day 1992). We used this integrated instrument system to concurrently measure multiple water quality parameters that included chlorophyll *a* (*in-vivo* fluorescence) from a boat following closely spaced transects (see Roelke et al. 2010a). Measurements were taken at 2-second intervals from ~20 cm below the surface. An integrated GPS was used to simultaneously plot sample locations. GPS and Dataflow information were then used to create detailed contour maps (Surfer v8.0). Our monitoring of in-lake conditions occurred monthly.

Method descriptions

Estimations of *P. parvum* population density in surface waters were achieved using a settling technique (Utermöhl, 1958), where a 100 mL phytoplankton sample was collected at ~0.5 m depth and preserved using glutaraldehyde, 5% v/v, and then a 1 mL subsample was settled for 24 h. Randomly selected fields-of-view were then counted until >200 *P. parvum* cells were enumerated (20 to 40 fields-of-view).

Estimates of total phytoplankton biomass (approximated using chlorophyll *a*) and biomass of taxonomically-aggregated phytoplankton groups (approximated as a fraction of the total chlorophyll *a*) were determined from phytopigment biomarker concentrations (Pinckney et al. 1998) and the use of CHEMTAX (Mackey et al. 1997, Wright et al. 1996). Briefly, filters containing phytopigments were sonicated in 100% acetone (3 ml) for 30 seconds and then extracted in the dark for 20-24 h at -20° C. Extracts

were filtered (0.2 μm) and injected (300 ul) into an HPLC system equipped with reverse-phase C_{18} columns in series (Rainin Microsorb-MV, 0.46 x 10 cm, 3mm, Vydac 201TP, 0.46 x 25cm, 5mm). A nonlinear binary gradient, adapted from Van Heukelem et al. (1994), was used for pigment separations. Solvent A consisted of 80% methanol and 20% ammonium acetate (0.5M adjusted to pH 7.2), and Solvent B was 80% methanol and 20% acetone. Absorption spectra and chromatograms were acquired using a Shimadzu SPD-M10av photodiode array detector, where pigment peaks were quantified at 440 nm.

Using the measured phytopigment concentrations, biomasses of higher phytoplankton taxa were estimated with CHEMTAX. CHEMTAX is a matrix factorization program that enables the user to estimate the abundances of higher taxonomic groups from concentrations of pigment biomarkers (Mackey et al. 1997, Wright et al. 1996). The program uses a steepest descent algorithm to determine the "best fit" of an unknown sample to an initial estimate of pigment ratios for targeted algal taxa. The taxa used in the analysis were cyanobacteria, euglenophytes, chlorophytes, prymnesiophytes, cryptophytes and diatoms, which were selected because of their prevalence in Lake Granbury at the time of the experiments.

Our sampling also included enumeration of zooplankton. Zooplankton were collected using a 12-liter Schindler trap, concentrated to 50 ml and preserved in 2.5% buffered formalin. Subsamples, 10-15 ml, were settled for a 24 h period, then counted using an inverted, phase-contrast, light-microscope (40x and 200x, Leica Microsystem Inc.). For each individual counted, geometric shapes were determined that best corresponded to the shape of the zooplankter and dimensions were measured that enabled calculation of the individual's biovolume (Wetzel and Likens 1991). Identification was to the taxonomic level of genus. For purposes of this paper, zooplankton were categorized into total copepod adults, copepod nauplii, total rotifers and protozoa. Our enumeration technique typically resulted in ~200 individuals counted per sample.

Samples for inorganic nutrients (nitrogen and phosphorus) were filtered through pre-combusted GF/F filters, and the filtrates were frozen until analysis. Inorganic nutrient concentrations were determined using autoanalyzer methodology (Armstrong and Sterns 1967, Harwood and Kuhn 1970). For this study, nitrate (NO_3), nitrite (NO_2) and ammonium (NH_4) were summed as dissolved inorganic nitrogen (DIN), and phosphorus was soluble reactive phosphorus (SRP).

Salinity, temperature and pH were determined with a water quality multi-probe (Quanta, Hydrolab) and light penetration was determined with a Secchi disk.

Since toxins produced by *P. parvum* under various physiological states are not fully understood, standards for measuring concentrations of toxins are not available at this time. Toxicity was estimated, however, using other methods. In previous studies, researchers commonly employed an *in vitro* hemolytic assay (Johannson and Granéli 1999, Barriero et al. 2005, Uronen et al. 2005) or non-standardized *in vivo* bioassays to assess biological effects of *P. parvum* cultures under nutrient limitation. In this study, ambient toxicity from each enclosure and field sample was evaluated rigorously using a standardized 24 h static acute toxicity assay with the fathead minnow (*P. promelas*) model and a standardized 10 day static renewal chronic toxicity test with a cladoceran (*D. magna*) model, generally following standardized aquatic toxicology methodology (US EPA 1994, 2002), which we've reported previously (Roelke et al. 2007, Brooks et al. 2010).

To evaluate toxicity relationships among treatment combinations, ambient samples were diluted using a 0.5 dilution series with reconstituted hard water (RHW), which was performed according to US EPA recommendations (US EPA 2002). This dilution approach is routinely used to evaluate water quality of surface waters because it allows for assessment of relative extracellular toxicity among samples if an undiluted ambient sample is acutely toxic.

For each *P. promelas* toxicity test sample from each experimental unit and field sample, three replicate chambers with 7 organisms per chamber were used to assess toxicity at each dilution level. *D. magna* bioassays followed established US EPA protocols (US EPA 1994). RHW, prepared according to standard methods (APHA 1998), was used as control treatment water for all toxicity assays. Alkalinity (mg/L as $CaCO_3$) and hardness (mg/L as $CaCO_3$) of RHW was measured potentiometrically and by colorimetric titration, respectively, before initiation of acute studies (APHA 1998). Specific conductance (µS/cm), pH and dissolved oxygen (mg/L) of RHW was also measured before toxicity testing. All toxicity tests were performed in climate controlled chambers at 25 ±1°C with a 16:8 hour light-dark cycle. Less than 48 h old fathead minnow larvae were fed newly hatched *Artemia*nauplii two hours before initiation of testing (US EPA 2002). *D. magna*were fed a Cerophyll®/green algae suspension daily, which was pre-

pared according to methods reported previously (Brooks et al. 2004, Dzialowski et al. 2006). LC50 values for fathead minnow toxicity tests were estimated as percentage of ambient sample using Probit (Finney 1971) or Trimmed Spearman Karber (Hamilton et al. 1977) techniques, as appropriate.

Differences in our response variables between experimental treatments were tested for significance using various general linear models (GLM, SPSS Inc.). For most variables, repeated-measures analysis of variance (ANOVA), using Wilk's multivariate F-test, was employed. To dissect treatment effects at different times during the experiment, univariate ANOVA was used, followed by Tukey's HSD test. A total of 12 enclosures were used as controls in these analyses (3 planned controls plus 9 untreated enclosures intended for other treatments that were not employed). Therefore, the HSD tests were adjusted for these differences in degrees of freedom. In the first experiment during bloom initiation, samples for testing acute toxicity to fish were not taken from the 9 untreated enclosures, so only 3 control enclosures were used in statistical analyses, and because nearly all enclosures were not detectably toxic on days 7 and 14, acute toxicity to fish was statistically analyzed only for day 21. In the second experiment during bloom development and decline, data on acute toxicity to fish were analyzed for days 7, 14, and 21 using repeated measures ANOVA. For this experiment, acute toxicity to fish was tested on all 12 control enclosures. For both experiments, non-toxic cultures were coded to LC50 of 100%, and LC50 data were analyzed on a percent dilution basis. Some response variables appeared to have heteroscedasticity, and were log-transformed and reanalyzed statistically, but because results were nearly always the same as results for raw data, only the latter are reported here.

2 Technical Reporting and Discussion

Lake conditions (extended record)

For the period of our monitoring, inflows into Lake Granbury were episodic, as is common in lakes of the south-central USA. From September 2006 through March 2007 inflows were barely discernable (Figure 2). In April 2007 a large inflow event occurred with peak flows attaining 80 x 10^6 m^3 d^{-1}. Episodic inflows of varying magnitude and duration persisted through June, where the largest inflow event reached ~120 x 10^6 m^3 d^{-1}. The lake then entered a period of low inflows, lasting until after the initiation of our second in-lake experiment in 2010.

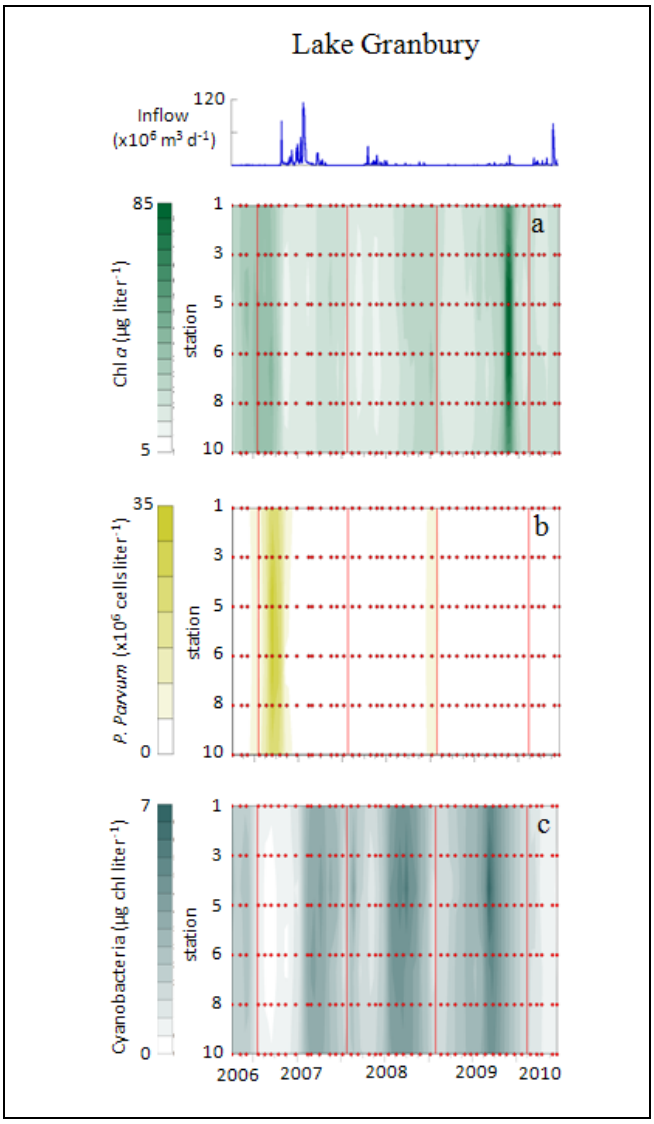

Figure 2. Time series data of chlorophyll a (a), *P. parvum* population density (b) and cyanobacteria biomass (c) from monthly sampling trips performed in Lake Granbury where trips spanned five years and locations of sampling stations encompassed the entire length of the lake, with station 10 being at the dam.

According to our pigment analyses, phytoplankton biomass peaked in March 2007 just prior to the first large inflow event of 2007, with highest biomass occurring towards the lower end of the lake (Figure 2a). The next prominent peak in phytoplankton biomass occurred in late 2009, where chlorophyll *a* concentrations reached ~90 µg liter^{-1}. During the period of the in-lake experiments in 2010, chlorophyll *a* throughout the lake was in the range of 10-20 µg liter^{-1}.

Nearly coincident with the first chlorophyll *a* concentration maxima, a system-wide *P. parvum* bloom reached its highest population densities of

~40 x 10^6 cells liter^{-1} in February 2007 with highest population densities occurring in the mid-reaches of the lake (91% of the phytoplankton biovolume). Cell densities >10 x 10^6 cells liter^{-1} are considered bloom proportions based on historical observations in lakes of the south-central USA (TPWD 2003, Roelke et al. 2007, Schwierzke et al. 2010). Average *P. parvum* densities for the lake declined ~27% by March 2007 (Fig. 2b). Measurements of ambient toxicity to fish were consistent with observed population densities, with LC_{50} values as low as 4% observed in February in the mid reaches of the lake, with toxicity to fish decreasing (LC_{50} increasing) by March (see Roelke et al. 2010). *P. parvum* population densities for the lake were obliterated after the first large inflow event to the lake in 2007, decreasing by 89% from the March to April sampling. In addition, waters were no longer toxic to fish. With the exception of the lower population density bloom at the end of 2008, *P. parvum* blooms were not system-wide for the remainder of this data record. Instead, blooms were localized and occurring at different times throughout the lake. During the 2010 period of study, *P. parvum* population densities remained below the defined bloom level of 10 x10^6 cells liter^{-1} throughout much of the lake.

Cyanobacteria were not abundant in Lake Granbury during the time of bloom development or termination in 2006-2007. Instead, cyanobacteria biomass was maximal during the months of July through September throughout this period of study (Fig. 2c). During the 2010 period when the in-lake experiments were conducted, cyanobacteria were lower, in the range of 1-1.5 µg-chl *a* liter^{-1}.

Dissolved inorganic nutrients sometimes showed a strong relationship with inflow. Immediately following the first large inflow event in April 2007 both DIN and SRP reached maxima of ~24 µM-N and ~0.55 µM-P (Figure 3a, b). Highest nutrient concentrations were measured in the lower reaches of the lake at this time. During January through March 2007, when *P. parvum* population densities were greatest and then started to decline, the DIN:SRP was ~30 with DIN concentrations ~1.35 µM-N and SRP ~0.05 µM-P. Except for December 2006, nutrient concentrations during the bloom of 2006-07 were similar to the months prior to the bloom. In December, SRP concentrations were at their lowest, ~0.03 µM-P, while DIN was ~3.55 µM-N (DIN:SRP ~122). Inorganic nutrient maxima were present during 2008, but they did not always correspond with inflow. Inorganic nutrient maxima were not apparent during 2009, nor were significant inflow events. During 2010, and at the time of our in-lake

experiments, inorganic nutrient concentrations were relatively high in the lake.

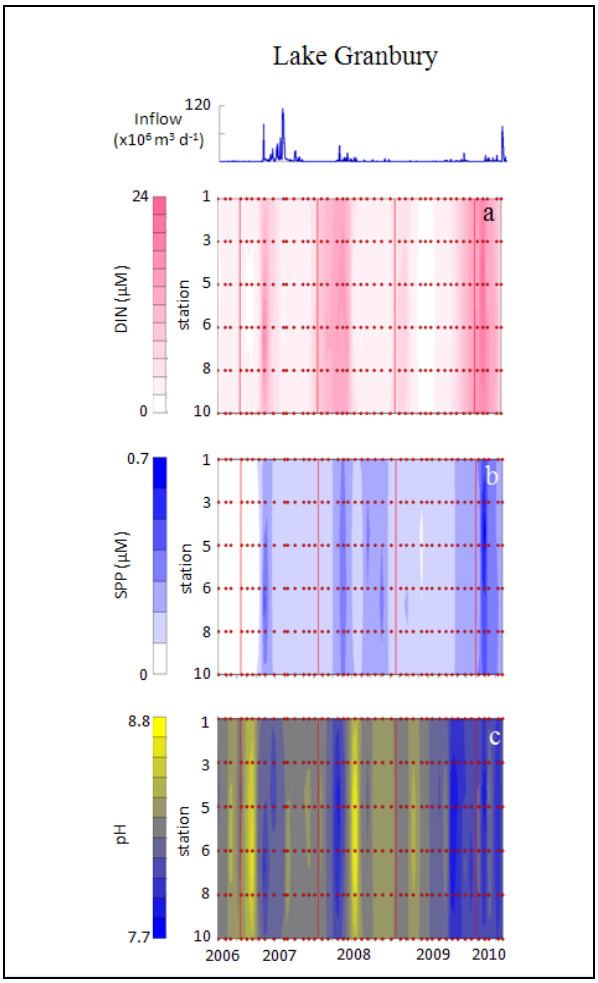

Figure 3. Time series data of dissolved inorganic nitrogen (a), soluble reactive phosphorus (b) and pH (c) from monthly sampling trips performed in Lake Granbury where trips spanned five years and locations of sampling stations encompassed the entire length of the lake, with station 1 being the lake's headwaters and station 10 being at the dam.

While pH varied spatially and temporally, a prominent feature was relatively high pH coinciding with the *P. parvum* bloom in 2006-07, while a rapid decrease in pH immediately followed the first large inflow event in April 2007 (Figure 3c). Large fluctuations in pH were apparent in the lake for the entire period of study.

As with pH, zooplankton populations varied spatially and temporally. Peak population densities, however, occurred later in the period of study when system-wide *P. parvum* blooms did not occur (Figure 4).

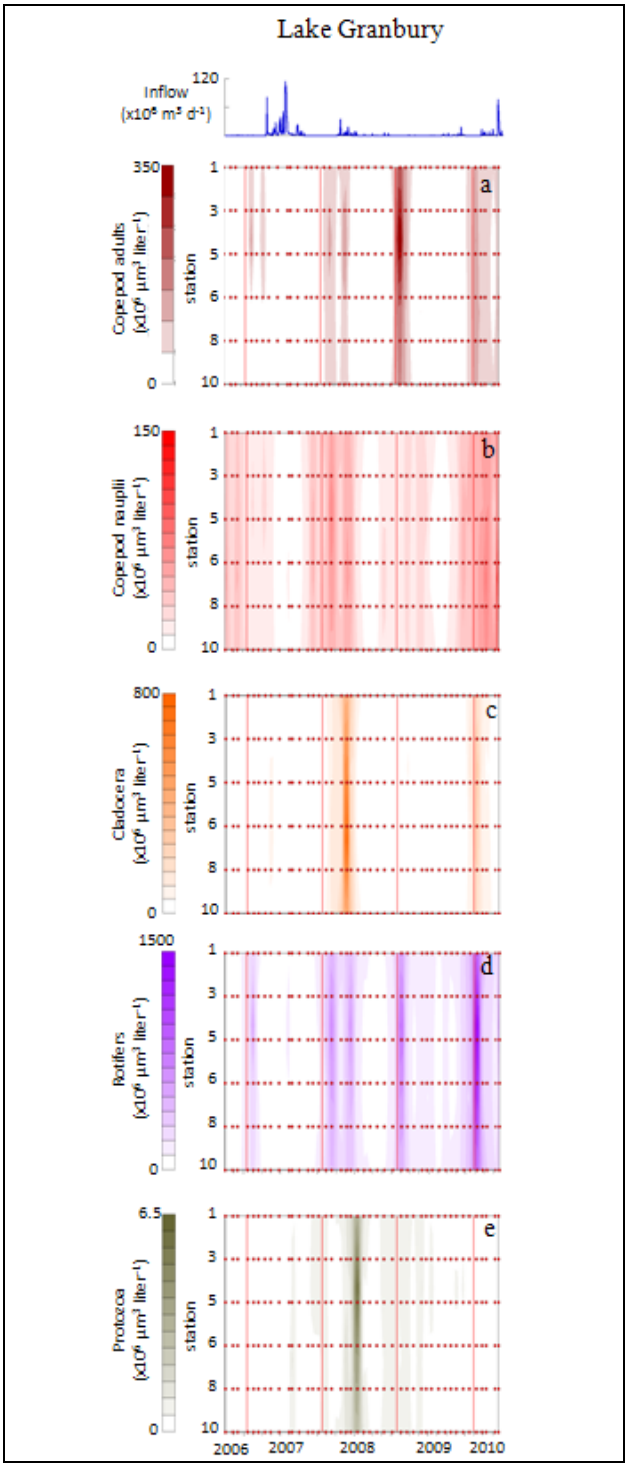

Figure 4. Time series data of zooplankton, which include adult copepods (a), copepod nauplii (b), cladocerans (c), rotifers (d), and protozoa (e) from monthly sampling trips performed in Lake Granbury where trips spanned five years and locations of sampling station encompassed the entire length of the lake, with station 1 being the lake's headwaters and station 10 being at the dam.

Cove conditions [spanning the period of in-lake experiments]

Phytoplankton biomass was accumulating in the cove during the 2010 period of in-lake experiments. At the start of the first experiment, February 23, chlorophyll *a* was 13.5 µg liter^{-1}. While at the start of the second experiment, March 31, it was 22.9 µg liter^{-1} (Figure 5a). The population of *P. parvum* mirrored this trend with densities of 0.76 and 1.1 x10^{6} cells liter^{-1} (Figure 5b). Toxicity in the cove was only observed on April 13, with an LC50 of 77% dilution (not shown), which occurred after the start of the second experiment.

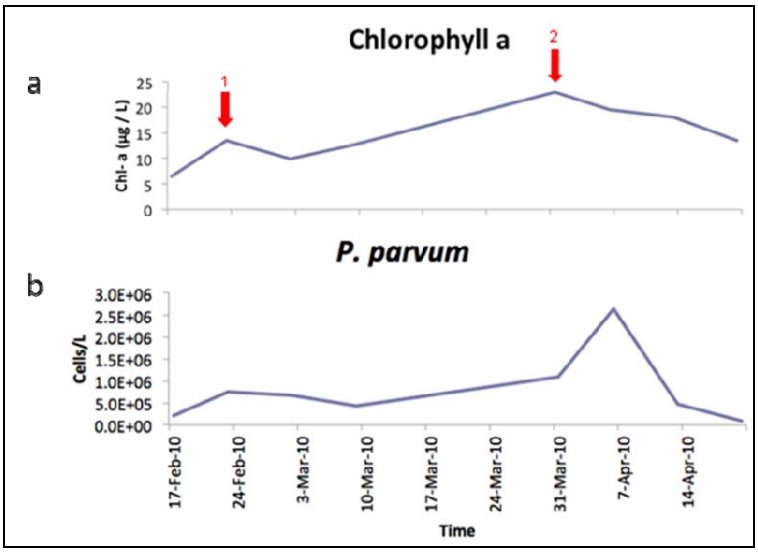

Figure 5. Time series data of chlorophyll a (a) and *P. parvum* population density (b) from weekly sampling trips performed in Lake Granbury where samples were collected in the cove where the experiments were deployed. Arrows indicate the start dates of the experiments were deployed. Arrows indicate the start dates of the experiments.

Inorganic nutrients showed an opposite trend with phytoplankton biomass. At the start of the first experiment DIN and SRP were 50 µM and 0.6 µM (N:P ~83, suggesting P-limiting conditions), while at the start of the second experiment DIN and SRP were 0.5 µM and 0.1 µM (N:P ~5, suggesting N-limiting conditions) (Figure 6a, b). The range over which pH varied during this period was small, with values of ~7.8 and ~7.7 at the start of the first and second experiments (Figure 6c).

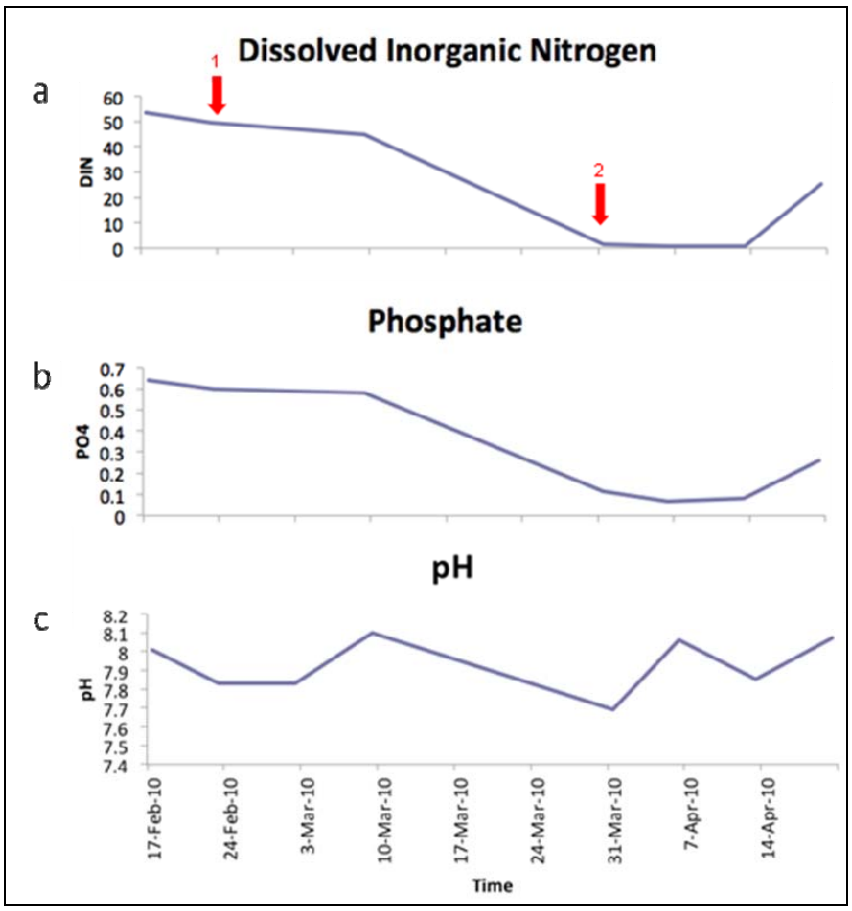

Figure 6. Time series data of dissolved inorganic nitrogen (a), soluble reactive phosphorus (b) and pH (c) from weekly sampling trips performed in Lake Granbury where samples were collected in the cove where the experiments were deployed. Arrows indicate the start dates of the experiments.

Zooplankton showed taxa-specific trends in the cove during the period of in-lake experiments. For example, copepod adults and nauplii increased in density, with populations nearly tripling from the start of the first experiment compared to the second (Figure 7 a, b). Rotifers increased nearly 5-fold at these times (Figure d). Cladocerans, on the other hand, decreased ~50% from the start of the first experiment compared to the second (Figure c). Protozoans showed a population maxima during the period of in-lake experiments (Figure 7e), but the accumulation of this population did not commence until after the start of the first experiment and its decline completing before the start of the second experiment.

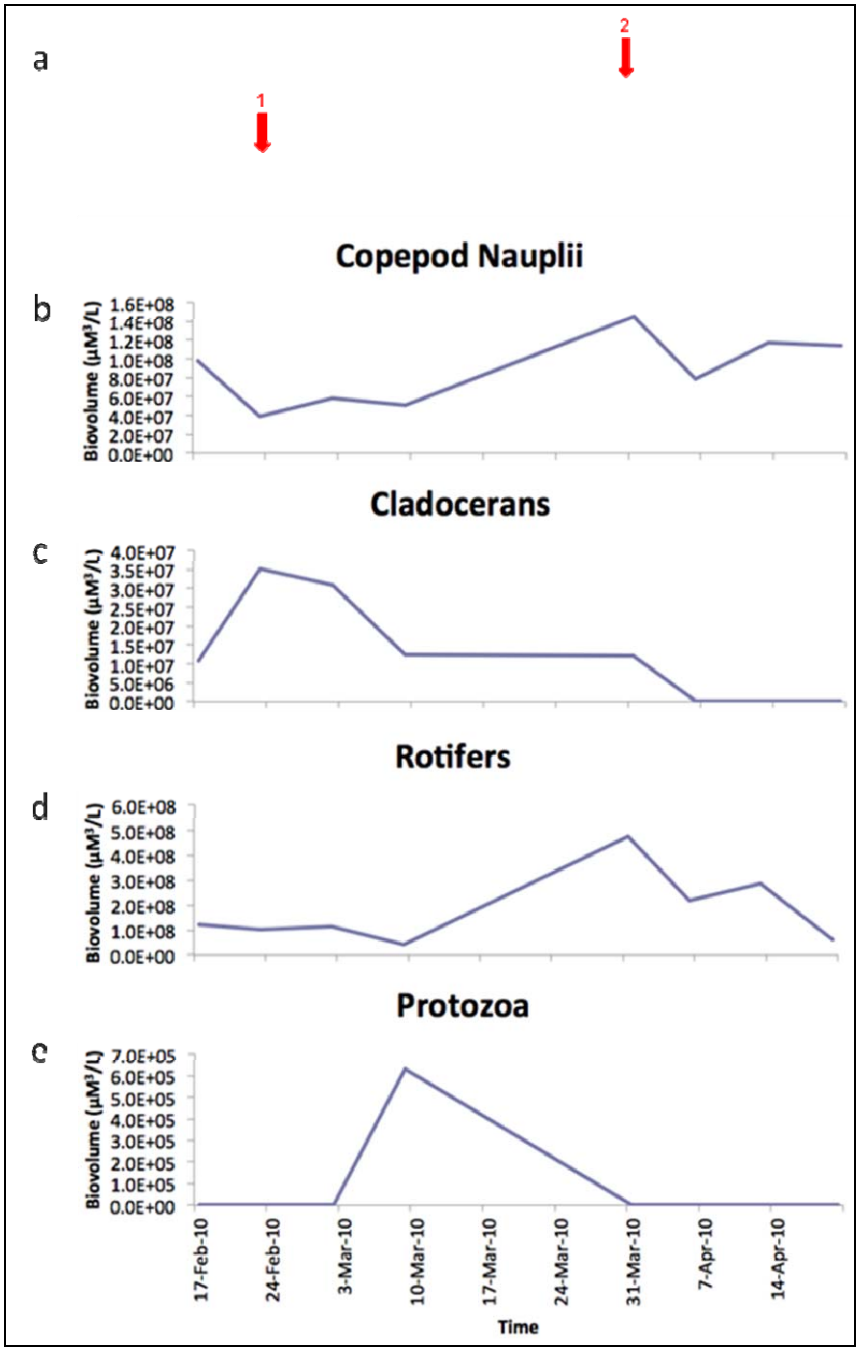

Figure 7. Time series data of zooplankton, which include adult copepods (a), copepod nauplii (b), cladocerans (c), rotifers (d), and protozoa (e) from weekly sampling trips performed in Lake Granbury where samples were collected in the cove where the experiments were deployed. Arrows indicate the start dates of the experiments.

In-lake experiments

Because our first experiment commenced before significant accumulation of *P. parvum* cells in the cove and because water conditions had not yet become toxic, we will refer to this period as "pre-bloom" in text below. We

will refer to the period when the second experiment commenced as "bloom development and decline" because *P. parvum* population density was higher in the cove and toxic water conditions were measured.

Statistical analysis

For most response variables in each experiment, treatment effects over the duration of the experiment were tested with a repeated-measures (RM) analysis of variance (ANOVA), using Wilk's multivariate F-test. For the flushing treatments, a one-way analysis was used for both experiments with four groups: control, low flushing (0.05 d^{-1}), intermediate flushing (0.1 d^{-1}) and high flushing (0.3 d^{-1}). For the pH manipulations, a one-way analysis was used with three groups for the first experiment: control (~8.5 during the experiment) and two lowered pH levels (7.5 and 7.0); and four groups for the second experiment: control (again, ~8.4) and three lowered pH levels (8.0, 7.5, 7.0). For the NH_4 additions, a one-way analysis was used for both experiments with three groups: controls, low NH_4 additions (10 μM), and high NH_4 additions (40 μM). To dissect treatment effects at different times during the experiment, univariate ANOVA was used, followed by Tukey's HSD test.

A total of 12 enclosures were used as controls in these analyses (3 planned controls plus 9 untreated enclosures intended for other purposes). Therefore, the HSD tests were adjusted for these differences in degrees of freedom. In the first experiment during bloom initiation, samples for testing acute toxicity to fish were not taken from the 9 untreated enclosures, so only 3 control enclosures were used in statistical analyses, and because nearly all enclosures were not detectably toxic on days 7 and 14, acute toxicity to fish was statistically analyzed only for day 21.In the second experiment during bloom development and decline, data on acute toxicity to fish were analyzed for days 7, 14, and 21 using RM-ANOVA. For this experiment, acute toxicity to fish was tested on all 12 control enclosures. For both experiments, non-toxic cultures were coded to LC50 of 100%, and LC50 data were analyzed on a percent dilution basis. For both experiments, effects on Daphnia reproduction were tested only on day 21, so univariate ANOVA was used. Three control cultures were tested in the first experiment, and 12 in the second experiment. Some response variables appeared to have heteroscedasticity, and were log-transformed and reanalyzed statistically, but because results were nearly always the same as results for raw data, only the latter are reported here.

First experiment – Pre-bloom conditions

Control

P. parvum population density was well below defined bloom levels at the start of the pre-bloom experiment (Figure 8). In addition, water was not toxic to fish (Figure 9). As the experiment progressed, however, *P. parvum* accumulated eventually exceeding bloom levels by the end of the experiment with waters becoming toxic. Total phytoplankton biomass peaked at T7 (Figure 10) with zooplankton biomass peaking at T14 (Figure 11). Both decreased after their respective peaks, as *P. parvum* accumulated and water became toxic.

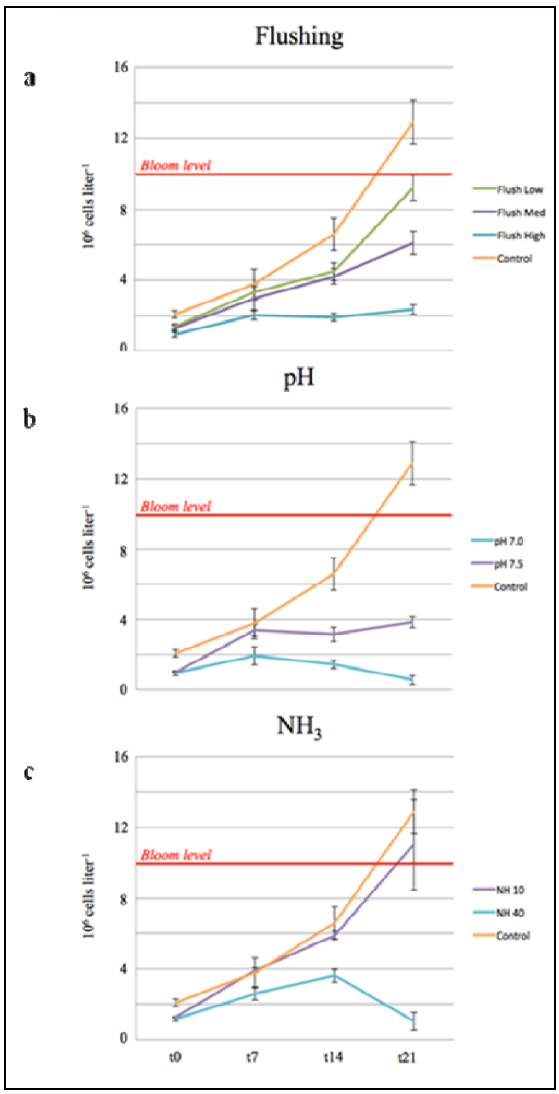

Figure 8. Time series data of *P. parvum* population density from weekly samplings of the pre-bloom experiment from enclosures that encompassed the flushing (a), pH (b) and NH4 (c) treatments.

Flushing

Flushing treatments during the pre-bloom experiment slowed the accumulation of *P. parvum* cells and the exchange magnitude had a strong correlation with *P. parvum* density. High magnitude flushing resulted in significantly lower *P. parvum* density (*P*-value<0.001). However, low flushing treatments reached higher population densities than the control, although this difference was not statistically significant. Similarly, medium treatments had a weak effect, in that *P. parvum* densities alternated between higher and lower concentrations compared to the control; again these minor differences were not statistically significant (Figure 8a).

Ambient toxicity was subsequently reduced by source water flushing. All mesocosms were non-toxic from initiation through T14 (Figure 9a); however, by the end of the experiment (T21), conditions in the control mesocosms had become extremely toxic (Figure 9b). Results from LC_{50} tests represented the acute toxicity to juvenile fathead minnows (*Pimephales promelas*) through % survivorship when exposed to sample water for 48-h. Toxicity levels in the control mesocosms resulted in ~17% survivorship of minnows, low flushing treatments had ~59% survivorship, medium treatments ~85% and were significantly lower than the control (*P* –value=0.024), and high level flushing resulted in 100% survivorship with no deviation in any of our replications (*P* –value=0.008).

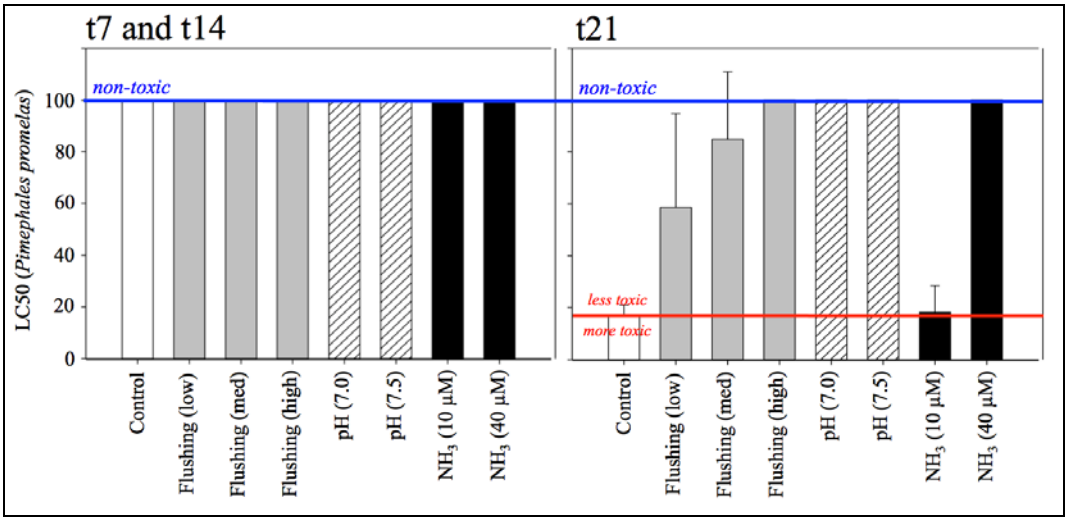

Figure 9. Water toxicity data from the pre-bloom experimental treatments during the 7th and 14th day of the experiment (a) and at day 21 (b).

Phytoplankton biomass increased during the pre-bloom experiment, peaking at T7, then decreasing for the remainder of the experiment (Figure 10a). Flushing had no observable effect.

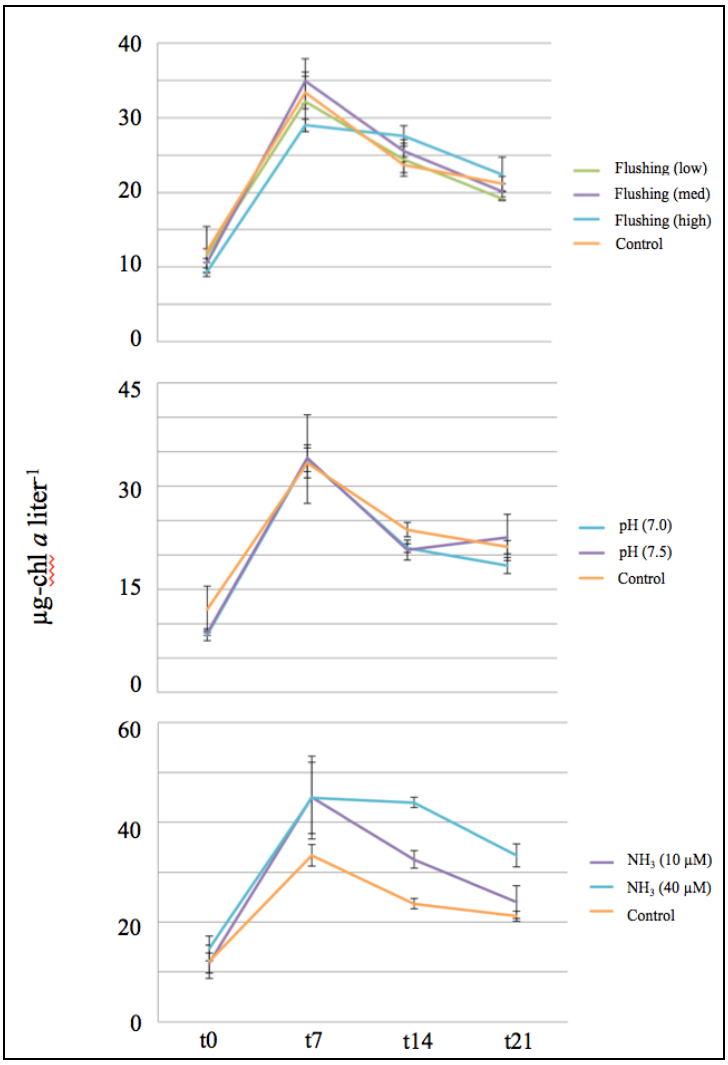

Figure 10. Time series data of total phytoplankton biomass from weekly samplings of the pre-bloom experiment from enclosures that encompassed the flushing (a), pH (b) and NH4 (c) treatments.

Zooplankton biomass increased during the pre-bloom experiment and peaked at T14, following an increase in phytoplankton, then decreased with their phytoplankton prey by T21 (Figure 11a). High flushing had a dilution effect, slowing the accumulation of zooplankton, although statistically non-significant.

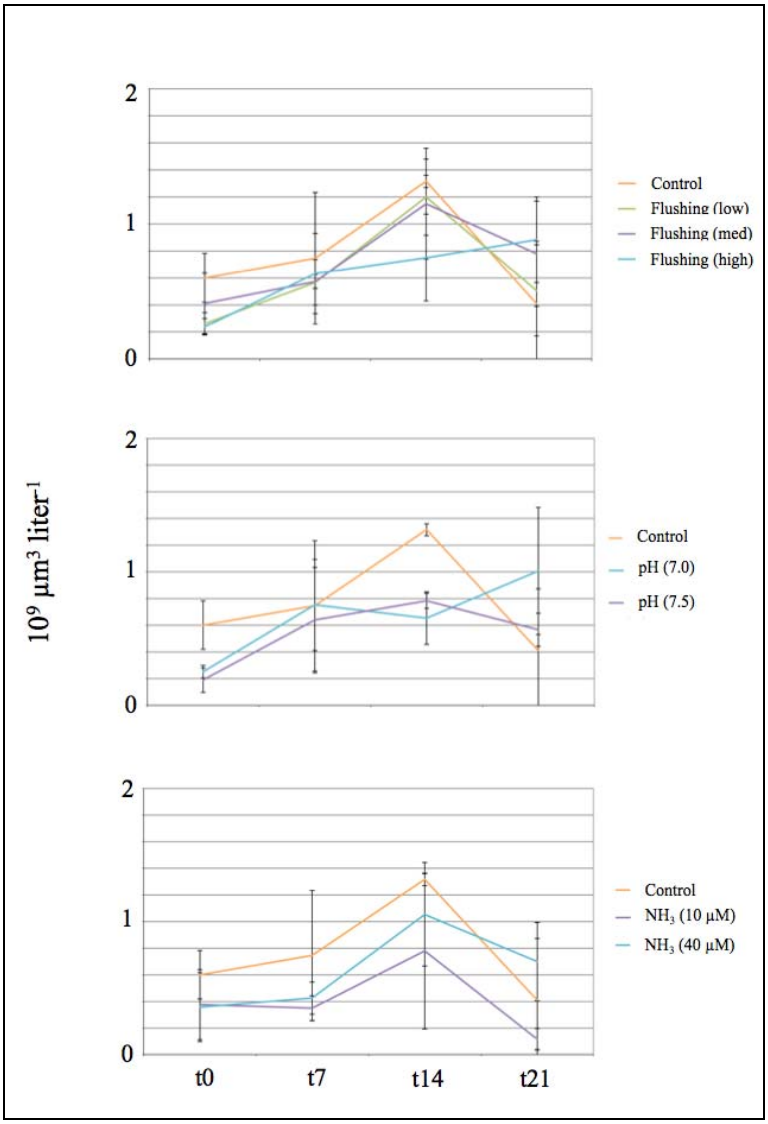

Figure 11. Time series data of total zooplankton biomass from weekly samplings of the pre-bloom experiment from enclosures that encompassed the flushing (a), pH (b) and NH4 (c) treatments.

pH manipulation

When the pre-bloom experiment was initiated on T0 there was no difference between cove pH and limnocorral pH values; however, by T21, pH in control limnocorrals were increased (mean pH = 8.86; n = 3) relative to the cove pH (8.1). As noted above, at T0, T7 and T14 during pre-bloom experiment 1, *P. parvum* cells were present in all of the limnocorrals, and increased in density in the control experimental units. By T21, *P. parvum* densities in both pH treatment levels (7, 7.5) were significantly reduced (ANOVA; $p < 0.05$) relative to controls, which exceeded HAB threshold levels (Figure 8).

Though a HAB had not formed and limnocorral samples were not acutely toxic to *P. promelas* (Figure 9a) or sublethally toxic to *D. magna* at T7 or T14, a highly toxic HAB to *P. promelas* had formed in control limnocorrals by T21 (Figure 9b). pH treatment levels (7, 7.5) were highly effective at ameliorating ambient toxicity (ANOVA; $p<0.05$); no *P. promelas* mortality was observed throughout the study in limnocorrals reduced to pH 7 or 7.5 (Figures 9a and 9b).

As noted above, phytoplankton biomass increased during the pre-bloom experiment 1, peaked at T7, then decreased for the remainder of the experiment (Figure 10b). However, pH treatment levels (7, 7.5) were not significantly different from controls ($p>0.05$).

Similarly, zooplankton biomass increased during the pre-bloom experiment, peaked at T14 in response to an increase in phytoplankton biomass, but decreased by T21 (Figure 11b). Similar to the phytoplankton biomass response variable, no pH effects were observed (Figure 11b).

NH_4 addition

Ammonium additions were intended to raise dissolved NH_4 concentrations by 10 and 40 μM. Determinations of dissolved NH_4 suggest that concentrations higher than these nominal levels were obtained. On day 0, immediately after treatment, NH_4 averaged 16.0 μM in low NH_4 enclosures, and 88.1 μM in high NH_4 enclosures. Accounting for NH_4 in control enclosures on day 0 (average of 8.0 μM), about 85% of the intended level was obtained in the low NH_4 addition enclosures, and 265% in the high NH4 addition enclosures. Although it is possible that enclosure volumes were smaller than intended, it is more likely that high NH_4 in samples taken after addition was a result of incomplete mixing to the depth of the enclosures. Dissolved NH_4 increased during the experiment, reaching an average of 38.2 μM in enclosures with low NH_4 additions, and an average of 121.1 μM in enclosures with high NH_4 additions. These concentrations are 127% and 101% of what would be achieved if NH_4 treatments were perfectly mixed and accumulated conservatively over the course of the experiment. Ammonium concentrations were significantly affected by treatments (RM-ANOVA, Wilk's $F_{8,24} = 351.65$, $P < 0.001$) on all days of the experiment (ANOVA, $P < 0.05$), and significantly higher on average for low NH_4 additions than controls, and significantly higher still for high NH_4 additions (Tukey's HSD, $P < 0.05$).

Temperature and pH were virtually identical in enclosures with low and high NH_4 additions. Temperature and pH both increased during the experiment in these enclosures, so that calculated percentages of unionized NH_4 were 1-3% for days 0 – 7, and 12-13% for days 14 – 21.

Nitrate data indicate that a modest amount of nitrification may have occurred. Nitrate concentrations decreased during the experiment in control enclosures, from 26.1 to 0.1 μM on average. In enclosures with low NH_4 additions, nitrate decreased from 19.1 to 12.6 μM on average, while enclosures with high NH_4 additions showed no obvious trend with average nitrate ranging 17.8 to 20.6 μM. Nitrate concentrations were significantly affected by treatments (RM-ANOVA, Wilk's $F_{8,24} = 81.52$, $P < 0.001$) on days 7, 14, and 21 (ANOVA, $P < 0.05$). On day 7, nitrate was significantly higher on average for high NH_4 additions than controls, and on days 14 and 21 nitrate was significantly higher on average in high NH_4 additions than in low NH4 additions, which were significantly higher than controls (Tukey's HSD, $P < 0.05$).

The abundance of *P. parvum* increased in all enclosures from day 0 to 14, though less rapidly in enclosures receiving high NH_4 additions (Fig. 8). These increases continued until day 21 in control enclosures and those with low NH_4 additions, reaching bloom levels exceeding 10×10^6 cells liter^{-1}. The abundance of *P. parvum* declined from day 14 to 21, however, in enclosures with high NH_4 additions and never exceeded bloom levels. Abundances of *P. parvum* were significantly affected by treatments (RM-ANOVA, Wilk's $F_{8,24} = 6.35$, $P < 0.001$) on days 7 and 21 (ANOVA, $P < 0.05$). On day 7, the abundance of *P. parvum* was significantly lower on average for high NH_4 additions than for low NH_4 additions, and on day 21 the abundance of *P. parvum* was significantly lower on average in high NH_4 additions than in low NH_4 additions or controls (Tukey's HSD, $P < 0.05$). Although the abundance of *P. parvum* was somewhat higher with low NH_4 additions than in controls, consistent with a weak fertilizing effect, this difference was not statistically significant.

No samples from any enclosures taken on days 7 or 14 displayed acute lethal toxicity to fish; on day 21 samples from enclosures with high NH_4 additions were also not acutely toxic to fish, but those from controls and enclosures with low NH_4 additions were toxic, with LC 50 ranging from about 10%-30% dilution (Fig. 9). On day 21, LC50 values were significantly affected by treatments (ANOVA, $F_{2,6} = 167.38$, $P < 0.001$). LC50 was sig-

nificantly higher (less toxic) on average for high NH_4 additions than for low NH_4 additions or controls (Tukey's HSD, $P < 0.05$).

Chlorophyll *a* increased from day 0 to 7 in controls and enclosures with low NH_4 additions, and then decreased until day 21; the increase continued in enclosures with high NH_4 additions until day 14, followed by a decrease to day 21 (Fig. 10). On days 14 and 21, chlorophyll *a* was higher on average in enclosures with high NH_4 additions than those with low NH_4 additions, which were higher than controls, consistent with a fertilization effect for total phytoplankton biomass. Chlorophyll *a* was significantly affected by treatments (RM-ANOVA, Wilk's $F_{8,24} = 27.25$, $P < 0.001$) on days 7 and 21 (ANOVA, $P < 0.05$). On day 14, chlorophyll *a* was significantly higher on average for high NH_4 additions than for low NH_4 additions, which were significantly higher than controls, and on day 21 chlorophyll *a* was significantly higher on average in high NH_4 additions than in low NH_4 additions or controls (Tukey's HSD, $P < 0.05$). *Daphnia* reproduction tested with samples from day 21 averaged 51 neonates (SD = 15), with significant differences among treatment (ANOVA, $F_{2,6} = 2.23$, $P = 0.19$).

The total abundance of zooplankton increased from days 0 to 14 in all enclosures, reaching about 800 individuals liter^{-1}, and then declined on day 21 to levels lower than those observed initially (Fig. 11). Total abundance of zooplankton was not significantly affected by treatments (RM-ANOVA, Wilk's $F_{8,24} = 1.06$, $P = 0.42$), nor did univariate tests detect significant treatment effects on any day (ANOVA, $P > 0.05$). The total biovolume of zooplankton also increased from days 0 to 14 in all enclosures, and then declined on day 21. However, for enclosures with high NH_4 additions, total biovolume of zooplankton on day 21 remained above the initial level, and was higher than in controls or enclosures with low NH_4 additions, suggesting that the fertilization effect on total phytoplankton biomass might have propagated to zooplankton biomass, albeit weakly. Total biovolume of zooplankton was not significantly affected by treatments overall (RM-ANOVA, Wilk's $F_{8,24} = 1.06$, $P = 0.42$), but on day 21 univariate analysis detected a significant effect (ANOVA, $P = 0.027$). On day 21, total biovolume of zooplankton was significantly higher on average in high NH_4 additions than in low NH_4 additions (Tukey's HSD, $P < 0.05$).

Second experiment – Bloom development and decline

Control

At the start of the bloom development and decline experiment *P. parvum* population density was just shy of the defined bloom level (Figure 12) with water still being non-toxic to fish. As the second experiment progressed, *P. parvum* accumulated eventually reaching a density 2-fold greater than the defined bloom level with waters becoming very toxic (Figure 13). Total phytoplankton biomass declined for much of this experiment, with a slight recovery at T21 (Figure 14). Zooplankton biomass declined for the duration of this experiment (Figure 15).

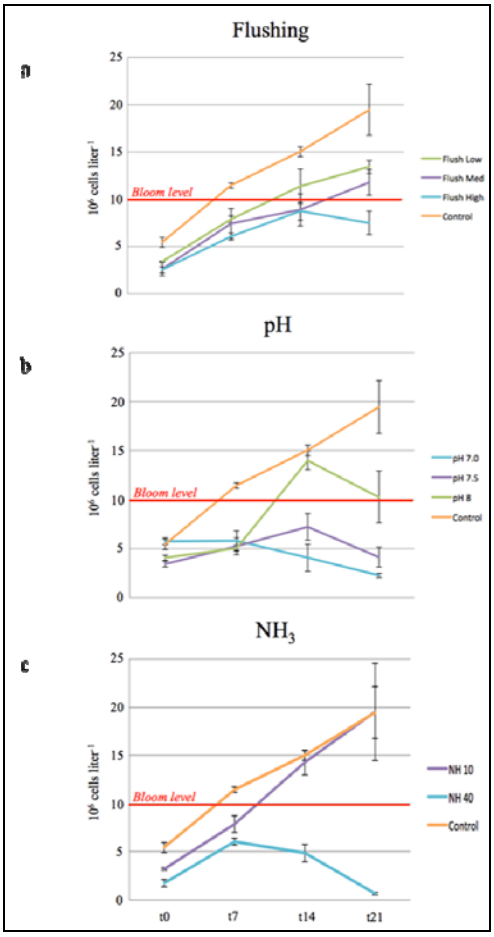

Figure 12. Time series data of P. parvum population density from weekly samplings of the bloom development and decline experiment from enclosures that encompassed the flushing (a), pH (b) and NH4 (c) treatments.

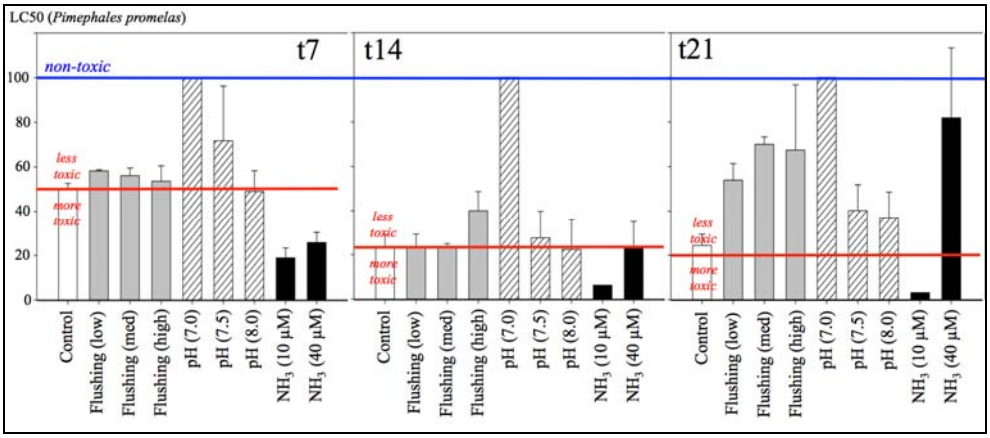

Figure 13. Water toxicity data from the bloom development and decline experimental treatments during the 7th (a), 14th (b) and 21st (c) day of the experiment.

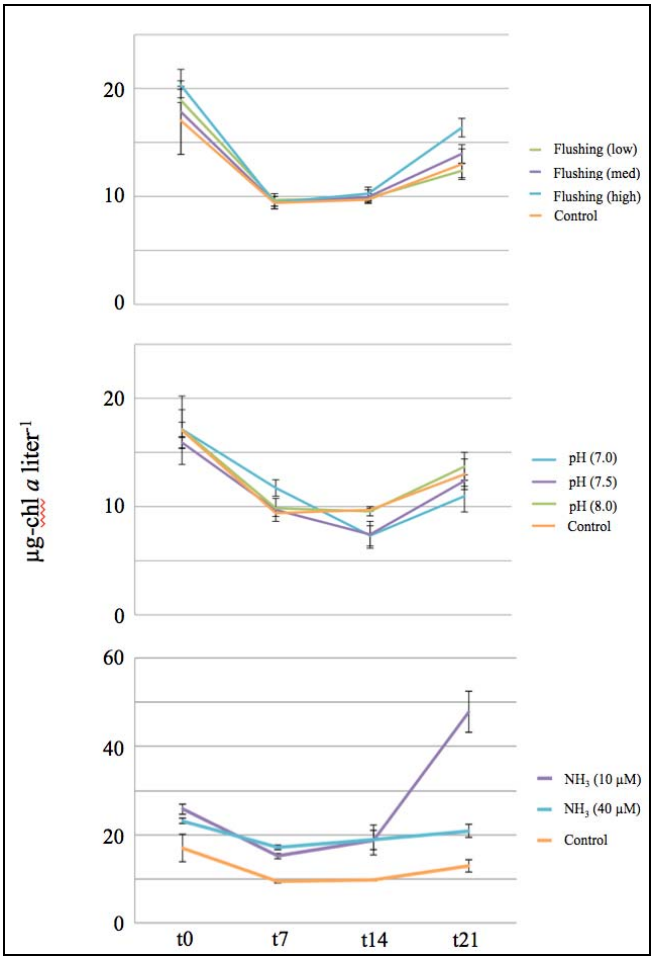

Figure 14. Time series data of total phytoplankton biomass from weekly samplings of the bloom development and decline experiment from enclosures that encompassed the flushing (a), pH (b) and NH4 (c) treatments.

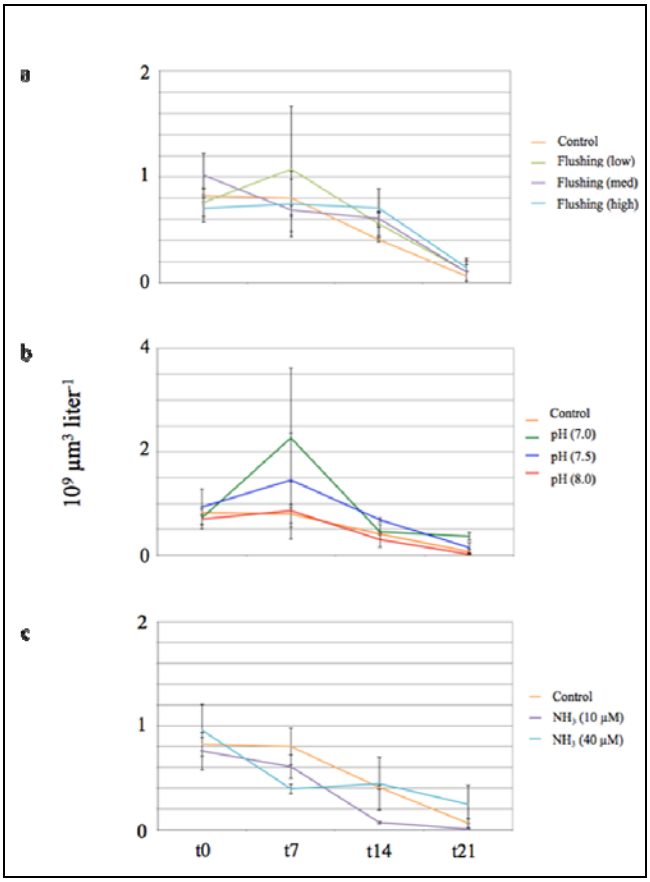

Figure 15. Time series data of total zooplankton biomass from weekly samplings of the bloom development and decline experiment from enclosures that encompassed the flushing (a), pH (b) and NH4 (c) treatments.

Flushing

Consistent with our first experiment, flushing treatments during the bloom development and decline experiment slowed the accumulation of *P. parvum* cells and the exchange magnitude had a strong correlation with *P. parvum* density. All three flushing levels produced *P. parvum* densities significantly lower than the control (P –value≤0.003), and the high level flushing circumvented bloom proportions (10 x 10^6 cells L^{-1}) from being reached throughout the length of the experiment (Figure 12a).

Also consistent with the first experiment, ambient toxicity was reduced by source water flushing. The same monotonic relationship observed during the first experiment was also illustrated in the bloom development and decline experiment (Figure 13). All mesocosms had at moderate toxicity through T7, resulting in ~50% survivorship of minnows (Figure 13a). Within the successive week (T14), control, low flushing, and medium

flushing conditions increased in toxicity at a similar rate resulting in ~25% survivorship. High flushing treatment results diverged from this trend and exhibited a minimal increase in toxicity (~40% survivorship, Figure 13b). At the conclusion of the experiment (T21), both the high and medium treatments were significantly less toxic than the control (high flushing *P* –value=0.004; medium flushing *P* –value=0.012). Control water yielded ~25% survivorship, the low flushing treatments decreased in toxicity to result in ~53% survival of fish, and both the medium and high flushing magnitudes had ~70% survival rate (Figure 13c).

Flushing had no observable effect on changes in total phytoplankton biomass, with chlorophyll *a* declining for much of this experiment and a slight recovery at T21 (Figure 14a).

Throughout the second experiment, zooplankton density was continually decreasing (Figure 15a). Slower rates of decline were witnessed in medium and high flushing treatments, especially at T14 and T21 when toxicity levels were significantly lower in these treatments and chlorophyll *a* levels were beginning to increase, representing grazing opportunities; however, these differences were statistically non-significant.

pH manipulation

During experiment 2, we included an additional pH treatment level (pH = 8) to the experimental design. At T0 pH of control limnocorrals were raised to levels consistent with control conditions of experiment 1 (mean pH = 8.58; n = 3). As such, these levels were thus higher than ambient cove conditions (pH = 7.69 at T0) and represented the highest pH among the pH treatment levels (7, 7.5, 8). As noted above, when experiment 2 was initiated *P. parvum* cells were present in all limnocorrals at T0 and increased in density in the control experimental units. By T14 and continuing through T21, *P. parvum* densities in controls and the highest pH treatment level (8) exceeded HAB thresholds (Figure 12b), but lower treatment levels (7, 7.5) were significantly reduced ($p < 0.05$) relative to controls (Figure 12b).

Over the 21 d study, control limnocorrals became increasingly acutely toxic to *P. promelas* (Figure 13). Similar to experiment 1, a statistically significant difference was observed between *P. promelas* LC_{50} values and pH treatment level ($p<0.05$); Figure 13). Acute toxicity to fish was significantly ameliorated by the pH 7 treatment level, but not the 7.5 or 8 treatment

levels, on T7, T14 and T21 (Figure 13). Though no statistically significant difference was observed between *D. magna* reproduction and pH treatments (p=0.217), mean reproduction increased from 16.2 neonates female^{-1} in controls to 34.7 neonates female^{-1} at pH 7, indicating a marked reduction of sublethal toxicity to cladocerans. A statistically significant relationship was also observed between *P. parvum* cell density and both *P. promelas* LC_{50} (r^2= 0.47, p<0.05) values and *D. magna* reproduction (r^2=0.32, p<0.05).

As noted above both phytoplankton biomass (Figure 14b) and zooplankton density (Figure 15b) continually decreased during experiment 2, but no significant effects of pH were observed on phytoplankton biomass and total zooplankton biomass.

Interestingly, on T21 cladocerans were only observed in two of the three low pH (7) limnocorrals, which corresponds to the observed decreased sublethal toxicity observed for *D. magna* reproduction responses at pH = 7 relative to controls and higher pH treatment levels.

NH_4

Determinations of dissolved NH_4 suggest that concentrations higher than the nominal levels of 10 and 40 μM were obtained. On day 0, immediately after treatment, NH_4 averaged 27.5 μM in low NH_4 enclosures, and 61.7 μM in high NH_4 enclosures. Accounting for NH_4 in control enclosures on day 0 (average of 1.0 μM), about 265% of the intended level was obtained in the low NH_4 addition enclosures, and 152% in the high NH4 addition enclosures. Although it is possible that enclosure volumes were smaller than intended, it is more likely that high NH_4 in samples taken after addition was a result of incomplete mixing to the depth of the enclosures. Dissolved NH_4 increased during the experiment, reaching an average of 28.0 μM in enclosures with low NH_4 additions, and an average of 90.3 μM in enclosures with high NH_4 additions. These concentrations are 93% and 75% of what would be achieved if NH_4 treatments were perfectly mixed and accumulated conservatively over the course of the experiment. Ammonium concentrations were significantly affected by treatments (RM-ANOVA, Wilk's $F_{8,24}$ = 2352.49, P < 0.001) on all days of the experiment (ANOVA, P < 0.05), and significantly higher on average for low NH_4 additions than controls, and significantly higher still for high NH_4 additions (Tukey's HSD, P < 0.05).

Temperature was virtually identical in enclosures with low and high NH_4 additions, while pH differed by less than 0.3 on average. Temperature was highest on days 7 and 14, while pH showed no trend during the experiment in these enclosures. Calculated percentages of unionized NH_4 were about 5-15% for all days.

Nitrate data indicate that a small amount of nitrification may have occurred in enclosures with high NH_4 additions. Nitrate concentrations in controls and enclosures with low NH_4 additions remained below 0.3 μM on average throughout the experiment. In enclosures with high NH_4 additions, average nitrate concentration increased over the course of the experiment from 0.2 to 1.6 μM. Nitrate concentrations were significantly affected by treatments (RM-ANOVA, Wilk's $F_{8,24} = 14.16$, $P < 0.001$) on all days (ANOVA, $P < 0.05$). On day 0, no pairwise differences between means were significant (Tukey's HSD, $P > 0.05$). On days 7 and 14, nitrate was significantly higher on average for high NH_4 additions than in controls or enclosures with low NH4 additions, and on day 21 nitrate was significantly higher on average in high NH_4 additions than in low NH4 additions, which were significantly higher than controls (Tukey's HSD, $P < 0.05$).

The abundance of *P. parvum* increased throughout the experiment in controls and enclosures with high NH_4 additions (Fig. 12). Control enclosures exceeded bloom levels of 10×10^6 cells liter^{-1} on day 14, while enclosures with low NH4 additions exceeded blooms levels on day 7. In contrast, the abundance of *P. parvum* increased from day 0 to 7 in enclosures with high NH_4 additions, and then declined until day 21, never exceeding bloom levels. Abundances of *P. parvum* were significantly affected by treatments (RM-ANOVA, Wilk's $F_{8,24} = 17.45$, $P < 0.001$) on all days (ANOVA, $P < 0.05$). On days 0 and 7, the abundance of *P. parvum* was significantly lower on average for high NH_4 additions than for controls, and on days 14 and 21 the abundance of *P. parvum* was significantly lower on average in high NH_4 additions than in low NH_4 additions or controls (Tukey's HSD, $P < 0.05$). Although the abundance of *P. parvum* was somewhat higher on day 21 with low NH_4 additions than in controls, consistent with a weak fertilizing effect, this difference was not statistically significant.

Acute lethal toxicity to fish displayed differing changes over time for different treatments (Fig. 13). On day 7, enclosures with low and high NH_4 additions (LC50 about 20-25%) were more toxic to fish than controls (LC50 about 50%). On day 14, controls and enclosures with high NH_4 additions had comparable toxicity to fish (LC50 about 20-30%), and enclo-

sures with low NH_4 additions were more toxic (LC50 about 6%). On day 21, enclosures with high NH_4 additions were considerably less toxic to fish (LC50 about 80%) than other treatments, controls had intermediate toxicity (LC50 about 30%), and enclosures with low additions of NH_4 were very toxic (LC50 about 3%). LC50 was significantly affected by treatments (RM-ANOVA, Wilk's $F_{6,24} = 18.05$, $P < 0.001$) on all days (ANOVA, $P < 0.05$). On day 14, no pairwise differences between treatments were significant (Tukey's HSD, $P > 0.05$), however. On day 7, LC 50 was significantly higher on average in controls than in high or low NH_4 additions, and on day 21 LC50 was significantly higher on average in high NH_4 additions than in low NH_4 additions or controls (Tukey's HSD, $P < 0.05$). Chronic sublethal toxicity to *Daphnia* was high in enclosures with low NH_4 additions, somewhat lower in controls, and lowest enclosures with high NH_4 additions. *Daphnia* reproduction tested with samples from day 21 averaged 16 neonates (SD = 15) for controls, was zero for all enclosures with low NH_4 additions, and averaged 50 neonates (SD = 7) for enclosures with high NH_4 additions, with significant differences among treatment (ANOVA, $F_{2,6} = 2.23$, $P = 0.19$). *Daphnia* reproduction in enclosures with high NH_4 additions was significantly higher than in controls or enclosures with low NH_4 additions.

Chlorophyll *a* decreased from day 0 to 7 in all enclosures, and changed little thereafter in controls and enclosures with high NH_4 additions, but increased from days 14 to 21 in enclosures with low NH_4 additions (Fig. 14). Throughout the experiment, chlorophyll *a* was higher on average in enclosures with NH_4 additions than in controls, consistent with a fertilization effect for total phytoplankton biomass. Chlorophyll *a* was significantly affected by treatments (RM-ANOVA, Wilk's $F_{8,24} = 59.78$, $P < 0.001$) on all days (ANOVA, $P < 0.05$). On day 0, chlorophyll *a* was significantly higher on average in low NH_4 additions than in controls, on days 7 and 14 chlorophyll *a* was significantly higher on average in low and high NH_4 additions than in controls, and on day 21 chlorophyll *a* was significantly higher on average in low NH_4 additions than in high NH_4 additions, which were significantly higher than controls (Tukey's HSD, $P < 0.05$).

The total abundance of zooplankton decreased throughout the experiment in all enclosures, somewhat more rapidly in those with NH_4 additions (Fig. 15). Total abundance of zooplankton was significantly affected by treatments (RM-ANOVA, Wilk's $F_{8,24} = 4.80$, $P = 0.001$) on day 7 (ANOVA, $P < 0.05$). On day 7, total abundance of zooplankton was significantly higher on average in low and high NH_4 additions than in controls (Tukey's HSD,

$P < 0.05$).The total biovolume of zooplankton also decreased throughout the experiment in all enclosures, at similar rates among treatments. Total biovolume of zooplankton was significantly affected by treatments (RM-ANOVA, Wilk's $F_{8,24} = 6.07$, $P < 0.001$) on days 14 and 21 (ANOVA, $P < 0.05$). On day 14, total biovolume of zooplankton was significantly higher on average in high NH_4 additions than in low NH_4 additions, and on day 21 total biovolume of zooplankton was significantly higher on average in high NH_4 additions than in controls and low NH_4 additions (Tukey's HSD, $P < 0.05$).

3 Conclusion

Conclusions

In our first experiment, conducted during a pre-bloom condition, all treatments proved effective against *P. parvum* accumulation and onset of toxicity (with the exception of the low NH_4 dosage). All three flushing levels prevented accumulation of cells to bloom proportions, as did both levels of lowered pH and the high NH_4 addition. Furthermore, these treatments were generally benign to other plankton. For example, flushing and lowered pH had no effect on total phytoplankton biomass. Addition of NH_4 stimulated phytoplankton biomass, however. Regarding zooplankton, increased flushing subtly caused lower biomass at the highest flushing level only, while lowering of pH had no effect. Addition of NH_4 led to reduced zooplankton biomass, although the variation in this data made it difficult to discern.

Similarly, all treatments proved effective against *P. parvum* accumulation and onset of toxicity in our second experiment, conducted during a period of bloom development and decline. All three flushing levels slowed the accumulation of *P. parvum* cells, with the highest level of flushing preventing bloom proportions from occurring. Similarly, lowered pH slowed the accumulation of *P. parvum* cells, with pH levels of 7.5 and 7.0 preventing bloom proportions from occurring. Addition of NH_4 showed mixed results. The high dosage was lethal to *P. parvum*, while the lower dosage showed no effect on the accumulation of cells. Flushing resulted in decreased toxicity, as did the high NH_4 dosage. Lowering of pH to 7.0 completely eliminated toxicity. Addition of low NH_4 resulted in greater toxicity. Paralleling the first experiment, these treatments were generally benign to other plankton. Flushing and lowered pH again had no effect on total phytoplankton biomass, and addition of NH_4 stimulated phytoplankton biomass. With the exception of the low NH_4 addition, where toxicity was enhanced, treatments generally had no effect on zooplankton biomass.

Previous research founded in theory and system-wide monitoring showed that the incidence of *P. parvum* blooms was sensitive to the magnitude and timing of inflow events (Roelke et al. 2010, 2011; Grover et al. 2011). *P. parvum* is sensitive to cell losses though hydraulic flushing because its growth rate at the time of year when it forms blooms is low, primarily due to temperature. Furthermore, toxin production is linked to nutrient avail-

ability (Roelke et al. 2007, Errera et al. 2008), where increased nutrient availability slows the toxin production rate. So, nutrients added during the process of hydraulic flushing would reduce the toxin production rate. In the absence of toxin production, ambient toxicity decreases, as these chemicals are highly photosensitive (James et al. 2011). Without the benefit of its toxins, *P. parvum* quickly losses its competitive advantage over other phytoplankton and is displaced. In the research reported here, which is based on in-lake experiments using natural assemblages at the mesocosm scale, we further underscore the importance of hydraulic flushing as it influences *P. parvum* blooms.

The magnitude of ambient *P. parvum* toxicity is sensitive to pH, as we demonstrated in our *P. parvum* laboratory experiments using a clone established from Texas waters (Valenti et al. 2010). The toxins released by *P. parvum* seem to behave as weak bases in aqueous solutions that at higher pH levels result in greater toxicity than lower pH levels. Our laboratory findings were supported by our in-lake mesocosm experiments, where not only was toxicity ameliorated but *P. parvum* population density was prevented from reaching bloom proportions. Again, without the benefit of its toxins, *P. parvum* quickly losses its competitive advantage over other phytoplankton and is displaced. Because changes in pH act at the chemical level and not the biological level, the impact of this manipulation occurs more quickly, with results observed only after 7 days (the first observation time).

P. parvum is sensitive to NH_4, specifically to the more toxic unionized form (Barkoh et al. 2003, Grover et al. 2007). This sensitivity is common in many phytoplankton taxa, where ammonia inhibits both reproductive growth rates and rates of photosynthesis. Our in-lake mesocosm experiments were consistent with these previous findings, where *P. parvum* population density and toxicity were lessoned at high NH_4 dosages. Inorganic nitrogen, however, is also a nutrient essential for phytoplankton. Many phytoplankton taxa are able to utilize NH_4 directly as a nutrient, and most taxa are able to use nitrate, a product of nitrification that decreases NH_4 concentrations. So our lower level of NH_4 addition was not great enough to bring about an acute toxicity effect on *P. parvum*, but instead increased nitrogen availability allowing for greater accumulation of biomass.

The pressing questions that our research findings bring to light involve what might have happened if our treatments were applied to an open sys-

tem, e.g., a cove of a lake, instead of a closed system like the limnocorrals employed in this research. If initiated during a pre-bloom period, would our treatments have circumvented a bloom? If initiated during a period of bloom development, would our treatments have suppressed a bloom? These questions remain unanswered and should be the focus of future research.

Recommendations

The incidence of *P. parvum* blooms has been linked to inflows, where system-wide fish-killing blooms only occur at times of low inflow (Roelke et al. 2010, 2011). Unfortunately, overland flows are predicted to decrease in the southcentral USA with climate change and human population growth. Without a mitigation response, it is likely that the devastation brought about from these blooms will be amplified. To increase our understanding of potential management strategies, in-field demonstration projects are needed. Findings from our in-lake experiments elucidate a direst for future research.

Manipulation of whole lake systems may not be practical. A focus on smaller subsystems of lakes, such as coves, seems more promising. Preventing blooms from forming or propagating into coves will create a refuge habitat for aquatic organisms, including the many recreationally important fish species in this region. The existence of refuge habitat during periods of bloom will accelerate the recovery of populations sensitive to *P. parvum* blooms.

We recommend in-lake demonstrations focus on flushing and pH neutralization. While NH_4 addition showed promise, there are as yet downstream effects of fertilization that have not been assessed. Flushing shows promise because water within the system free of *P. parvum* cells (deeper waters) can be used as the source water. So waters from upstream locations would not be needed. pH neutralization shows promise because it works quickly and can be implemented at minimal cost. Both flushing and pH neutralization were benign to other aquatic organisms and would likely have no downstream effects.

Lake Granbury would an ideal location for such an in-lake demonstration. The lake is characteristic of many coves from which appropriate locations could be selected. There is also a great understanding of the lake ecology generated from many years of study. In addition, *P. parvum* blooms are fairly predictable, so in-lake demonstrations could be timed well. Most

importantly, there is strong local support from the Granbury community, who continue to engage with the scientific community through several town hall meetings.

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