Vol. 46: 125–140, 2007 | AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol | Published February 2

Effects of nutrient enrichment on *Prymnesium parvum* population dynamics and toxicity: results from field experiments, Lake Possum Kingdom, USA

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ABSTRACT: Large fish kills associated with toxic populations of the haptophyte *Prymnesium parvum* occur worldwide. In the past 5 yr, incidences of *P. parvum* blooms in inland water bodies of Texas (USA) have increased dramatically, where cell densities in excess of 1×10^7 cells l^{-1} are typically observed. We conducted field experiments (Lake Possum Kingdom) during the fall and early spring of 28 d duration using 24 enclosures of 1.57 m^3 each. The experiments investigated the effect of nutrient enrichment, immigration of *P. parvum* and addition of barley straw extract on phytoplankton biomass and assemblage structure, *P. parvum* population density, zooplankton biomass and assemblage structure, bacteria, and toxicity. Nutrient enrichment stimulated *P. parvum* population growth beyond bloom proportions ($>1 \times 10^7$ cells l^{-1}). However, *P. parvum* did not dominate the assemblage under these conditions, as it does during natural blooms. Instead, euglenophytes and chlorophytes dominated. Toxicity, estimated using fish (*Pimephales promelas*) and cladoceran (*Daphnia magna*) bioassays and which is linked to *P. parvum*'s allelopathic and mixotrophic effectiveness, was greatly reduced (eliminated in many cases) under conditions of nutrient enrichment. The suppression of toxicity by nutrient addition suggested that targeted and time-limited nutrient manipulations might be used to mitigate the effects of *P. parvum* blooms. Immigration of *P. parvum* into natural assemblages and addition of barley straw extract had no significant effect on plankton dynamics.

KEY WORDS: Harmful algal bloom · HAB · Management · Mitigation · Immigration · Barley straw extract

INTRODUCTION

Harmful algal blooms (HABs) have increased in frequency, duration, and magnitude worldwide (Smayda 1990, Hallegraeff 1993), and produce deleterious effects that range from fish kills to various risks to human health (Van Dolah et al. 2001). Unfortunately, the environmental conditions leading to HABs are complex and often species specific, making it difficult to create universal approaches to management (see Roelke 2000, Roelke & Buyukates 2001, 2002).

The haptophyte *Prymnesium parvum* is a HAB-causing species that occurs worldwide and is responsible for large fish kills in coastal and inland water bodies

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(Moestrup 1994, Edvardsen & Paasche 1998). Scientists in Denmark and Holland first identified it in the late 1930s (see Shilo & Aschner 1953, McLaughlin 1958). More recent studies have revealed mechanisms by which *P. parvum* might gain a selective advantage over other phytoplankton, thereby allowing populations to accumulate biomass. These included inhibition of competitors through production of allelopathic chemicals (Fistarol et al. 2003, 2005, Granéli & Johansson 2003a, Barreiro et al. 2005, Uronen et al. 2005), mortality or grazing inhibition to predators through production of toxic chemicals (Granéli & Johansson 2003b, Rosetta & McManus 2003, Tillmann 2003, Barreiro et al. 2005, Calliari & Tiselius 2005), mixotrophic feeding strategies (Nygaard & Tobiesen 1993, Skovgaard & Hansen 2003), and resistance to allelopathic chemicals produced by some cyanobacteria (Suikkanen et al. 2004).

In 1985, the state of Texas (USA) confirmed a *Prymnesium parvum* bloom along the Pecos River. Since then, *P. parvum* blooms, where cell densities typically exceed 1×10^7 cells l^{-1} and surface waters take on a golden color, have affected over 19 lakes in 5 river basins in Texas. *P. parvum* blooms have resulted in fish kills exceeding 17.5 million fish valued at $6.5 million, most of this occurring over the last 5 yr (TPWD 2003).

The factors controlling the appearance of *Prymnesium parvum* blooms in these lakes have yet to be determined. From the literature, there is an apparent link between eutrophication, salinization and the appearance of *P. parvum* blooms. For example, blooms in Europe (Holdway et al. 1978, Kaartvedt et al. 1991, Amsinck et al. 2005), the Middle East (Rijn & Shilo 1989), and Asia (Krasnotshchek & Abramowitsch 1971, Guo et al. 1996) have all occurred in aquatic systems that were eutrophic and brackish. The observed locations of *P. parvum* blooms in Texas are consistent, in part, with these findings in that they appeared only in lakes with a higher salt content (i.e. salinities ranging from 2 to 4; TPWD 2003). *P. parvum* is present in Texas lakes with lower salt content (i.e. salinity <1), but it has not bloomed in those systems (D. L. Roelke & R. Kiesling unpubl. data).

Interestingly, while observed *Prymnesium parvum* blooms were mostly from eutrophic systems, toxicity-related interactions between *P. parvum* and other components of the plankton community appear to be stronger when cells become nutrient limited. In laboratory experiments using *P. parvum* cultures, toxicity (estimated using an *in vitro* hemolytic assay) increased when cells became nutrient limited and when the allelopathic effect on a cryptomonad (*Rhodomonas salina*) was enhanced (Uronen et al. 2005). In addition, mixotrophy increased under conditions of nutrient limitation because cells were suggested to release chemicals that allowed more efficient capture of prey (Barreiro et al. 2005). This same study also showed that mortality to predators grazing directly on *P. parvum* cells increased when nutrients were limited.

The likely mechanism(s) leading to the formation of HABs, in general, are many and diverse (see Paerl 1988, Anderson & Garrison 1997, Roelke & Buyukates 2001). In addition, the functioning of bloom-initiating mechanisms may be even more complex when the structure of the plankton community is considered, e.g. the influence of initial conditions on succession (see Huisman & Weissing 2001, Roelke et al. 2003, Buyukates & Roelke 2005). Immigration certainly influences community structure and can alter plankton dynamics (Leibold & Norberg 2004, Leibold et al. 2004). Immigration is especially important when considering mobile phytoplankton species with benthic resting stages, such as *Prymnesium parvum*, and habitats where inflowing waters carry substantial phytoplankton populations, such as reservoirs. With regards to *P. parvum* blooms in Texas lakes, it may be that in addition to the factors influencing cell toxicity, the structure of the early-season phytoplankton assemblage is also an important determinant, and that immigration of *P. parvum* cells during this time influences whether a bloom will initiate.

From a management perspective, some processes are known to strongly influence phytoplankton assemblage structure and might serve as tools for mitigation of HABs. These processes include nutrient loading magnitude and ratio (Tilman et al. 1986, Paerl 1988, Roelke et al. 1997, 1999, Grover et al. 1999), hydraulic flushing (Paerl 1988, Roelke et al. 1997, 1999), and particle flocculation onto clay particles (Hagström & Granéli 2005, Sengco et al. 2005). Another potential mitigation technique, used frequently in aquaculture, involves addition of barley straw extract (BSE). In this technique, BSE (lignin- and tannin-rich) can sometimes suppress growth of many algal taxa, thereby preventing excessive accumulation of biomass (Ridge & Pillinger 1996, Everall & Lees 1997, Schrader et al. 1998).

In the present study, we report on findings from field experiments conducted in Lake Possum Kingdom (Texas, USA), one of the systems plagued by *Prymnesium parvum* blooms in the last 5 yr. Our experiments investigated the role of nutrients, immigration, and BSE, as they influenced plankton dynamics early in the growing season. Specifically, our response variables included the accumulation of phytoplankton biomass and shifts in assemblage structure, *P. parvum* demographics, zooplankton biomass and assemblage structure, bacterial concentration, and ambient toxicity. Our experimental approach was novel because it employed in-lake, large-volume enclosures to investigate *P.*

parvum population dynamics in natural plankton communities. Furthermore, we measured toxicity of field samples using fish (*Pimephales promelas*) and cladoceran (*Daphnia magna*) bioassays, instead of using the *in vitro* hemolytic assay technique. Such couplings of laboratory and field responses are routinely employed in a weight of evidence approach to define stressor effects on aquatic systems (Brooks et al. 2004).

MATERIALS AND METHODS

Lake Possum Kingdom is a reservoir on the Brazos River, Texas, USA (Fig. 1). The dam was completed in 1941, sits within a limestone escarpment, and receives drainage from 58404 km^2. The lake has a capacity of 8.9×10^8 m^3, a surface area of 80 km^2, and a shoreline of 498 km. Depth at the dam is ~60 m (Dowell 1964). Of the many coves in this dendritic lake, we chose Echo Cove for our study area (Fig. 1). This 25 m deep cove was selected because recurrent, high *Prymnesium parvum* population densities were observed there during the early stages of bloom formation since 2001 (J Glass pers. comm.).

We performed 2 in-lake experiments in Echo Cove. The first experiment was conducted during the fall (initiated 25 October 2004), and the second experiment was conducted in the early spring (initiated 8 March 2005). We chose this time of year because *Prymnesium parvum* blooms span this period in Texas (TPWD 2003). In fact, during our experiments we observed the formation of a fish-killing *P. parvum* bloom in Echo Cove; more fish-killing blooms occurred in other coves of Lake Possum Kingdom during this period as well. In our 'Results' section, we have included data on shifts in plankton community structure and the physicochemical environment that occurred in Echo Cove during the period of our experiments, which will help place our experimental findings in the context of the natural environment.

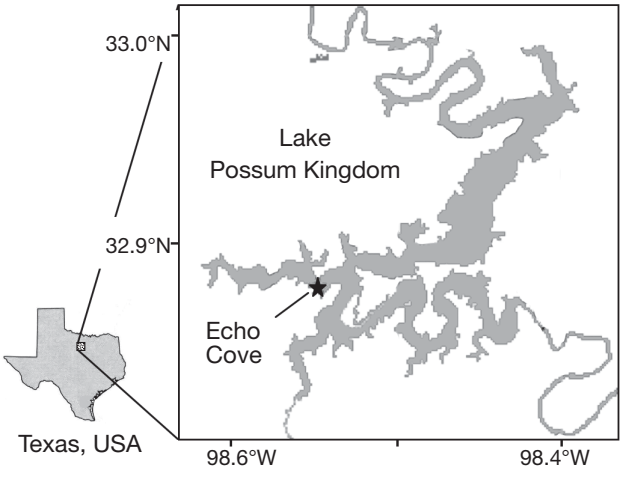

Fig. 1. Lake Possum Kingdom, Texas (USA). Our experiments were performed in Echo Cove, where high *Prymnesium parvum* population densities were observed during the early stages of bloom formation

Our 2 in-lake experiments utilized 24 translucent, closed-bottom enclosures (each 1.57 m^3) of cylindrical dimension, 1 m diameter, and 2 m depth (Aquatic Research Instruments). Each enclosure was nearly filled with water from Echo Cove (natural plankton community), vertically oriented, suspended at the surface with floatation frames, and left open to the atmosphere. The ties that fastened the enclosures to the frames were oriented to deter birds from roosting on the experimental frames. In addition, we never observed birds approaching the experiment, nor did we observe any signs of bird activity on the floatation frames.

Each experiment comprised 8 treatments, with each treatment conducted in triplicate (i.e. 24 enclosures were used). To remove the influence of 'bottom-up' effects, some of the enclosures received nutrient additions to levels of *f*/2 media (see Guillard & Ryther 1962). Initial concentrations of N and P were 800 µM N and 40 µM P, with a N:P ratio of 20. To simulate immigration, some of the enclosures received additions of *Prymnesium parvum* culture to population densities of ~5×10^5 cells l^{-1} (5% of typical bloom cell density; TPWD 2003). To test the efficacy of BSE as a mitigation tool for bloom control, extract (Ecological Laboratories) was added at 50-fold the manufacturer's recommendation (the lowest dosage that deleteriously affected *P. parvum* cultures during a pilot study). Combinations of these 3 treatments and a control completed the experimental design. Experiments lasted 28 d.

The strain of *Prymnesium parvum* used to enhance initial densities in these experiments was isolated from the Brazos River, Texas, USA (ZZ181, University of Texas Culture Collection of Algae). This strain was kept in culture using sterilized ultra-pure water (Milli-Q), enriched to *f*/2 nutrient concentrations (Guillard & Ryther 1962), then made brackish (salinity of 5) through the addition of salts (Instant Ocean). The culture was maintained in a 12:12 h light:dark cycle at 19°C under 30 W cool-white fluorescent lamps (200 µE m^{-2} s^{-1}), and transfers of culture into new media were made when the stationary growth phase was attained. Thus, our *P. parvum* culture experienced a chemical environment that oscillated between nutrient-sufficient and -deficient conditions.

Samples for characterization of the plankton assemblages were collected every 7 d for both experiments. Nutrient samples were collected at Days 0, 14, and 28

for the fall experiment and every 7 d for the early-spring experiment. Ambient toxicity to fish and cladocerans was determined from samples collected at Day 28 for the fall experiment and Days 0, 14, and 28 for the early-spring experiment. Characterizations of the plankton included estimates of total phytoplankton biomass and biomasses of higher taxonomic groups, enumerations of *Prymnesium parvum* population density, total zooplankton biomass and biomasses of higher taxonomic groups, and bacterial concentrations (early-spring experiment only). Characterizations of the chemical environment included measurements of inorganic nutrients. At the culmination of the fall experiment, only 1 enclosure from each of the treatments was sampled for toxicity. However, toxicity was assessed from each enclosure at the mid-point and finish of the early-spring experiment.

Estimates of total phytoplankton biomass and biomasses of higher taxa were achieved by measuring concentrations of phytopigments. Quantification of phytopigments followed Pinckney et al. (1998). Briefly, filters containing phytopigments were sonicated in 100% acetone (3 ml) for 30 s and then extracted in the dark for 20 to 24 h at –20°C. Extracts were filtered (0.2 µm) and injected (300 µl) into an HPLC system equipped with reverse-phase C_{18} columns in series (Rainin Microsorb-MV, 0.46 × 10 cm, 3 mm, Vydac 201TP, 0.46 × 25 cm, 5 mm). 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 (0.5 M 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 (Wright et al. 1996, Mackey et al. 1997). 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 Possum Kingdom at the time of the experiments.

Enumeration of *Prymnesium parvum* population density was performed using a settling technique (Utermöhl 1958). Typically, a 0.5 to 1 ml subsample was taken out of a well-mixed sample from the enclosures (preserved using glutaraldehyde, 3% v/v). Subsamples were settled for a 24 h period, then counted using an inverted, phase-contrast light microscope (400×, Leica Microsystem). Depending on the density of material in the samples, a range of 15 to 50 randomly selected fields of view were counted per sample, which resulted in ~200 *P. parvum* cells counted per sample.

While examining the phytoplankton samples for *Prymnesium parvum* cells, we observed other phytoplankton species present and the condition of cells. Specifically, we looked for the presence of other HAB species common to Texas lakes (e.g. multiple cyanobacteria that included *Microcystis*, *Anabaena*, and *Cylindrospermopsis*), for signs of algal pathogens (e.g. remains of lysed cells and presence of parasitic fungi), and took note of the dominant taxonomic groups present in each sample. We observed no significant accumulation of biomass of other HAB species, or signs of pathogen activity. Regarding our observations of dominant taxonomic groups present in each sample during our enumeration of *P. parvum*, these data were qualitative, because we did not measure cell dimensions and calculate biovolume for all phytoplankton types. However, our observations from microscopy agreed with the quantitative results from CHEMTAX.

Our sampling also included enumeration of zooplankton and bacteria. Zooplankton were collected using a 12 l Schindler trap, concentrated to 50 ml, and preserved in 2% buffered formalin. Subsamples, ~10 ml, were settled for a 24 h period, then counted using an inverted, phase-contrast light microscope (40× and 200×, Leica Microsystem). 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 & Likens 1991). Identification was to the taxonomic level of genus. For the purposes of this study, zooplankton were categorized into total copepod adults, copepod nauplii, total rotifers, and total protozoans. For samples collected during the fall experiment, our enumeration technique resulted in ~130 individuals counted per sample. Zooplankton densities during the early-spring experiment were extremely low, however, and the average number of individuals counted in a sample was ~3. In some cases, over half of the 50 ml subsample was counted. For bacteria, samples were preserved using para formaldehyde and prepared for enumeration by adding a fluorescent DNA stain (Rigler 1966) to 1 ml of sample, then filtered through a 25 mm diameter black filter (Hobbie et al. 1977). Counts were performed using epifluorescent microscopy, and our enumeration technique usually resulted in 100 to 300 cells counted per sample.

Samples for nutrient analyses were filtered through Whatman GF/F filters (pore size ~0.7 µm) and frozen for transport to the laboratory. Using autoanalyzer methodology (Armstrong & Sterns 1967, Harwood & Kuhn 1970), analyses included nitrate (NO_3), nitrite (NO_2), ammonia (NH_4), orthophosphate (PO_4), and silicate (SiO_3). For the purposes of this study, NO_3, NO_2, and NH_4 were summed and referred to as dissolved inorganic nitrogen (DIN).

Since toxins produced by *Prymnesium parvum* under various physiological states are not fully understood, standards for measuring concentrations of toxins are not available at this time. Toxicity can be estimated, however, using other methods. In previous studies, researchers commonly employed an *in vitro* hemolytic assay (Johannson & 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 the present study, ambient toxicity from each enclosure was evaluated rigorously using a standardized 24 h static acute-toxicity assay with the fathead minnow (*Pimephales promelas*) model and a standardized 10 d static renewal chronic-toxicity test with a cladoceran (*Daphnia magna*) model, generally following standardized aquatic toxicology methodology (US EPA 1994, 2002).

Samples collected from enclosures in Lake Possum Kingdom were transported to the laboratory, where toxicity tests were initiated within 24 h. 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 *Pimephales promelas* toxicity test sample from each experimental unit, 3 replicate chambers with 7 organisms per chamber were used to assess toxicity at each dilution level. *Daphnia 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^{-1} as $CaCO_3$) and hardness (mg l^{-1} as $CaCO_3$) of RHW were measured potentiometrically and by colorimetric titration, respectively, before initiation of acute studies (APHA 1998). Specific conductance (µS cm^{-1}), pH, and dissolved oxygen (mg l^{-1}) of RHW were also measured before toxicity testing. All toxicity tests were performed in climate-controlled chambers at 25 ± 1°C, with a 16:8 h light:dark cycle. Less than 48 h old fathead minnow larvae were fed newly hatched *Artemia* nauplii 2 h before initiation of testing (US EPA 2002). *D. magna* were fed a Cerophyll/green algal suspension daily, which was prepared according to methods reported previously (Brooks et al. 2004, Dzialowski et al. 2006). LC_{50} 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. Low values of LC_{50} imply high toxicity, while estimated $LC_{50} > 50\%$ implies that an undiluted ambient sample was toxic, but that toxicity was reduced by a relatively small dilution with RHW.

Differences in our response variables between experimental treatments were tested for significance using a repeated-measures general linear model (GLM, SPSS). For these statistics an accumulated-time approach was used. That is, the first GLM used only data from 0 and 7 d, the second GLM used data from 0, 7, and 14 d, and so forth, until the final GLM for which all time-series data were used in the analyses. In this way, the timing of differences between treatments could also be evaluated. Analysis of treatment effects on *Daphnia magna* fecundity employed an additional GLM for Day 28 of the early-spring experiment.

RESULTS

In situ lake conditions

A fish-killing *Prymnesium parvum* bloom occurred in Echo Cove during the period of our in-lake experiments. Briefly, from 3 dates that we sampled in the cove (October, January, and March; R. M. Errera et al. unpubl. data), *P. parvum* cell densities increased from non-detectable to 9.57×10^6, then to 26.6×10^6 cells l^{-1}. Throughout January and March, we observed recurrent fish kills that comprised shad *Dorosoma* sp., minnow *Pimephales* sp., buffalo *Ictiobus* sp., bullhead catfish *Ameiurus* sp., gar *Lepisosteus* sp., and crappie *Pomoxis* sp., as well as golden-colored water with surface foam that is characteristic of *P. parvum* blooms.

Concurrent with the increase in *Prymnesium parvum* population density was a >2 order of magnitude decrease in zooplankton. To quantify, biovolume of total copepod adults decreased from 29.6×10^6 to 9.28×10^6, then to 0.154×10^6 µm^3 l^{-1} during October, January and March (these biovolumes equate to ~1.7, ~0.53, and ~0.0087 adults l^{-1}). Biovolumes of copepod nauplii also decreased and were 85.8×10^6, 4.54×10^6, and 0.391×10^6 µm^3 l^{-1} (~95, ~5.0, and ~0.43 nauplii l^{-1}). Similarly, biovolumes of total rotifers decreased and were 24.9×10^6, 0.461×10^6, and 0.266×10^6 µm^3 l^{-1} (~40, ~0.73, and ~0.42 rotifers l^{-1}). Finally, the biovolume of total protozoans during October was 0.246 ×

10^6 µm^3 l^{-1} (2.8 protozoans l^{-1}). During January and March protozoans were not found in our samples.

The physicochemical environment varied in Echo Cove during these months. For example, DIN concentrations were 0.69, 6.1, and 1.6 µM N during October, January, and March, respectively, and concentrations of PO_4 were 0.13, 0.12, and 0.39 µM P. DIN:P ratios were 5.3, 51, and 4.1; Secchi depths were 1.8, 1.2, and 1.4 m; temperatures were 22, 11, and 13°C; salinities were 2.0, 1.7, and 1.8; and pH values were 8.4, 8.5, and 8.7 (taken at ~10:00 h). During each of these months, and with regards to temperature, conductivity, and pH, the upper 10 m of the water column was completely mixed. Note that surface layer mixing may have extended deeper, but we were limited by the cable length of our multi-probe.

Experimental results

Of the 3 treatments (i.e. additions of nutrients, *Prymnesium parvum* culture, and BSE), only addition of nutrients produced significant ($p < 0.05$) and lasting effects on some of our response variables. For the fall experiment, the effects of nutrient addition were apparent after 7 d for both phytoplankton biomass and *P. parvum* population density, after 14 d for rotifers and copepod nauplii, and after 28 d for copepod adults (Table 1). For the early-spring experiment, the effects of nutrient addition were apparent after 14 d for phytoplankton biomass and after 7 d for *P. parvum* population density (Table 2). On 2 occasions an interaction term was significant, but did not persist over the experiment (i.e. fall experiment from the period 0 to 7 for the *P. parvum* invasion × barley straw extract [Pp × BSE] interaction and early-spring experiment from the period 0 to 21 for the nutrients [Nuts] × Pp × BSE interaction). Zooplankton showed no significant trends during the early-spring experiment (Table 2), but again our counts were extremely low. Similarly, bacterial concentrations showed no significant trends.

Addition of nutrients resulted in rapid accumulation of phytoplankton biomass, shifts in assemblage structure, and increased *Prymnesium parvum* density. For example, during the fall experiment, phytoplankton biomass and assemblage structure (initially diatom dominated) did not change during the experiment in enclosures receiving no nutrient additions (Fig. 2A), and *P. parvum* densities slowly increased ~5-fold over a period of 28 d (Fig. 2B). With the addition of nutrients, however, phytoplankton biomass increased ~10-fold over a period of 21 d, then started to decline (Fig. 2C). In addition, several other phytoplankton taxa shared dominance with diatoms. Finally, *P. parvum* population densities increased more quickly, ~30-fold over a period of 14 d, then also declined (Fig. 2D). With the addition of nutrients *P. parvum* population densities reached a maximum of ~5×10^7 cells l^{-1}, which were 5-fold greater than typical observed cell densities during blooms under natural conditions (TPWD 2003).

In enclosures not receiving nutrient additions during the early-spring experiment, phytoplankton biomass and assemblage structure (initially dominated by prymnesiophytes) showed modest changes over the duration of the experiment, with little change in the density of *Prymnesium parvum* (Fig. 3A,B). The modest changes in phytoplankton biomass included a succession among the subdominant phytoplankton taxa that started with diatoms giving way to euglenophytes, then euglenophytes giving way to chlorophytes. As with the fall experiment, the addition of nutrients resulted in a ~10-fold increase in phytoplankton biomass, and the succession pattern involving diatoms, euglenophytes, and chlorophytes was still apparent, only now these taxa comprised the bulk of the phytoplankton biomass (Fig. 3C). Nutrient additions again stimulated *P. parvum* population growth, with cell densities increasing ~3-fold over a period of 14 d (Fig. 3D). Again, with the addition of nutrients, *P. parvum* population densities exceeded bloom proportions, with a maximum of ~10×10^7 cells l^{-1}. Note that densities for *P. parvum* at Day 0 were much greater for the early-spring experiment compared to the fall experiment. Also, both phytoplankton biomass and *P. parvum* density showed abrupt declines in the enclosures receiving nutrient additions.

In treatments receiving no addition of nutrients during the fall experiment, changes in total zooplankton biovolume were modest (Fig. 4A). The increase occurred mostly because of the rising prevalence of rotifers as the experiment progressed. The biovolume of copepod nauplii at the start of the experiment was nearly the same as the biovolume of copepod adults that occurred later.

Similar to the phytoplankton, copepod adults, copepod nauplii, and rotifers showed increased accumulated biovolume with the addition of nutrients during the fall experiment (Fig. 4). Average rotifer biovolume increased >100-fold within the first 14 d when their biovolume reached its maximum of 3023×10^6 µm^3 l^{-1} (4800 rotifers l^{-1}). Average copepod nauplii increased 1.6-fold when their biovolume reached its maximum of ~127×10^6 µm^3 l^{-1} (~140 nauplii l^{-1}) at Day 21, and average copepod adults increased 11-fold when their biovolume reached its maximum of ~325×10^6 µm^3 l^{-1} (~18 adults l^{-1}) at Day 28.

During the early-spring experiment, initial zooplankton biovolumes were >2 orders of magnitude less than the initial biovolumes for the fall experiment. As the spring experiment progressed, zooplankton popu-

lations did not accumulate, regardless of the nutrient treatment (results not shown). Concentrations of bacteria during the early-spring experiment varied and averaged 1.2 ± 0.52 × 10^8 cells l^{-1}, but no statistically significant trends were found.

In treatments receiving no addition of nutrients, DIN concentrations during the fall and early-spring experiments and PO_4 concentrations during the fall experiment showed no significant change (Figs. 5A,B & 6A). However, PO_4 concentrations during the early-spring

Table 1. Repeated-measures general linear model results for the fall 2004 in-lake experiment. Reported values are the level of significance (*F* and p) for the effect of nutrient additions (Nuts), *Prymnesium parvum* invasion (Pp), addition of barley straw extract (BSE), and combinations of these factors. An accumulated time approach was used when performing these statistics, i.e. first data from only Days 0 and 7 were used (0–7), then Days 0, 7, and 14 (0–14), and so forth, until all time-series data were included. Only nutrient additions produced a lasting effect. n/a: not applicable; *: statistically significant values ($p < 0.05$)

	0–7		0–14		0–21		0–28	
	F	p	*F*	p	*F*	p	*F*	p
Phytoplankton biomass								
Nuts	184.00	0.00*	287.00	0.00*	143.00	0.00*	119.00	0.00*
Pp	0.68	0.42	0.02	0.90	1.62	0.22	0.06	0.82
BSE	1.00	0.33	0.56	0.47	0.06	0.81	0.83	0.38
Nuts × Pp	0.87	0.37	0.31	0.59	0.04	0.84	0.96	0.35
Nuts × BSE	1.15	0.30	0.16	0.69	0.92	0.36	0.02	0.88
Pp × BSE	0.30	0.59	1.57	0.23	0.32	0.58	0.08	0.78
Nuts × Pp × BSE	0.50	0.49	0.11	0.74	0.69	0.42	0.58	0.46
***P. parvum* population**								
Nuts	83.20	0.00*	25.40	0.00*	26.30	0.00*	22.50	0.00*
Pp	0.01	0.91	1.56	0.23	2.20	0.16	1.73	0.21
BSE	0.70	0.42	0.02	0.89	0.10	0.75	0.13	0.73
Nuts × Pp	1.01	0.33	1.44	0.25	1.81	0.20	1.71	0.21
Nuts × BSE	0.19	0.67	0.02	0.90	0.15	0.70	0.13	0.73
Pp × BSE	13.80	0.00*	0.85	0.37	0.05	0.83	0.30	0.59
Nuts × Pp × BSE	4.77	0.04*	0.88	0.36	0.02	0.90	0.03	0.87
Total copepod adults								
Nuts	n/a	n/a	0.88	0.37	2.08	0.17	15.49	0.00*
Pp	n/a	n/a	0.00	0.99	0.09	0.77	0.10	0.76
BSE	n/a	n/a	3.14	0.10	3.93	0.07	3.24	0.10
Nuts × Pp	n/a	n/a	0.61	0.45	0.47	0.51	0.01	0.91
Nuts × BSE	n/a	n/a	0.19	0.67	0.05	0.83	0.17	0.69
Pp × BSE	n/a	n/a	0.11	0.74	0.55	0.47	2.62	0.13
Nuts × Pp × BSE	n/a	n/a	1.70	0.22	0.22	0.65	2.40	0.15
Copepod nauplii								
Nuts	n/a	n/a	7.49	0.02*	44.83	0.01*	65.84	0.01*
Pp	n/a	n/a	1.69	0.22	0.01	0.91	0.55	0.47
BSE	n/a	n/a	0.28	0.60	1.06	0.32	0.40	0.54
Nuts × Pp	n/a	n/a	0.94	0.35	0.29	0.60	0.02	0.89
Nuts × BSE	n/a	n/a	3.03	0.10	3.19	0.10	0.02	0.89
Pp × BSE	n/a	n/a	0.12	0.74	2.20	0.16	3.11	0.10
Nuts × Pp × BSE	n/a	n/a	0.52	0.48	1.79	0.20	2.69	0.12
Total rotifers								
Nuts	n/a	n/a	7.95	0.01*	9.71	0.01*	9.80	0.01*
Pp	n/a	n/a	0.27	0.61	0.06	0.81	0.02	0.90
BSE	n/a	n/a	0.50	0.49	1.12	0.31	2.21	0.16
Nuts × Pp	n/a	n/a	0.20	0.66	0.03	0.86	0.10	0.76
Nuts × BSE	n/a	n/a	0.39	0.54	0.81	0.38	1.16	0.30
Pp × BSE	n/a	n/a	0.58	0.46	0.25	0.63	0.12	0.73
Nuts × Pp × BSE	n/a	n/a	0.44	0.52	0.16	0.69	0.00	0.97
Total protozoans								
Nuts	n/a	n/a	3.84	0.07*	1.25	0.28	0.07	0.79
Pp	n/a	n/a	0.02	0.88	0.76	0.40	0.13	0.72
BSE	n/a	n/a	0.01	0.93	0.20	0.66	0.01	0.93
Nuts × Pp	n/a	n/a	0.02	0.88	0.21	0.65	1.74	0.21
Nuts × BSE	n/a	n/a	0.01	0.93	0.15	0.70	0.52	0.48
Pp × BSE	n/a	n/a	0.31	0.59	0.00	0.98	0.44	0.52
Nuts × Pp × BSE	n/a	n/a	0.31	0.59	0.75	0.40	0.06	0.81

Table 2. Repeated-measures general linear model results for the early-spring 2005 in-lake experiment. Reported values are the level of significance (*F* and p) for the effect of nutrient additions (Nuts), *Prymnesium parvum* invasion (Pp), addition of barley straw extract (BSE), and combinations of these factors, on phytoplankton biomass (measured as chlorophyll *a*) and *P. parvum* population density. An accumulated time approach was used when performing these statistics, i.e. first data from only Days 0 and 7 were used (0–7), then Days 0, 7, and 14 (0–14), and so forth, until all time-series data were included. Only nutrient additions produced a lasting effect. n/a: not applicable

	0–7		0–14		0–21		0–28	
	F	p	*F*	p	*F*	p	*F*	p
Phytoplankton biomass								
Nuts	1.50	0.24	440.00	0.00	603.00	0.00	609.00	0.00
Pp	2.24	0.16	1.69	0.22	0.94	0.35	0.01	0.92
BSE	0.81	0.39	0.00	0.96	0.33	0.58	0.27	0.61
Nuts × Pp	2.07	0.18	0.30	0.59	0.12	0.73	0.15	0.71
Nuts × BSE	0.63	0.44	0.03	0.86	1.61	0.23	1.05	0.33
Pp × BSE	1.13	0.31	2.27	0.16	0.00	1.00	0.31	0.59
Nuts × Pp × BSE	0.72	0.41	0.43	0.53	0.35	0.57	1.07	0.32
***P. parvum* population**								
Nuts	6.03	0.03	26.80	0.00	7.29	0.02	0.57	0.47
Pp	2.37	0.15	0.42	0.53	0.39	0.54	0.36	0.56
BSE	1.17	0.30	0.83	0.38	0.52	0.49	0.79	0.39
Nuts × Pp	2.67	0.13	0.27	0.62	0.04	0.86	0.04	0.85
Nuts × BSE	1.16	0.30	1.05	0.32	0.50	0.50	0.68	0.43
Pp × BSE	1.04	0.33	3.04	0.11	1.89	0.19	1.07	0.32
Nuts × Pp × BSE	3.58	0.08	4.19	0.06	5.20	0.04	3.79	0.08
Total copepod adults								
Nuts	0.32	0.58	0.15	0.71	0.05	0.83	0.03	0.86
Pp	1.64	0.22	1.48	0.24	1.51	0.24	1.42	0.25
BSE	0.21	0.66	0.92	0.35	1.08	0.32	1.16	0.30
Nuts × Pp	1.37	0.26	0.91	0.36	0.57	0.46	0.51	0.49
Nuts × BSE	0.21	0.66	0.19	0.67	0.25	0.63	0.21	0.65
Pp × BSE	0.32	0.58	0.02	0.88	0.00	0.96	0.01	0.93
Nuts × Pp × BSE	1.37	0.26	0.52	0.48	0.34	0.57	0.39	0.54
Copepod nauplii								
Nuts	5.38	0.04	0.23	0.64	0.48	0.50	0.20	0.66
Pp	0.38	0.55	0.11	0.74	0.01	0.92	0.13	0.73
BSE	1.24	0.28	0.13	0.72	0.01	0.94	0.01	0.91
Nuts × Pp	0.47	0.51	2.87	0.11	1.83	0.20	2.23	0.16
Nuts × BSE	0.21	0.65	0.71	0.41	0.39	0.54	0.25	0.63
Pp × BSE	1.04	0.32	1.51	0.24	1.63	0.22	1.21	0.29
Nuts × Pp × BSE	0.02	0.88	0.15	0.70	0.07	0.79	0.23	0.64
Total rotifers								
Nuts	0.35	0.56	3.18	0.09	0.55	0.47	0.01	0.91
Pp	0.40	0.54	0.11	0.75	3.04	0.10	2.93	0.11
BSE	0.35	0.56	0.95	0.35	0.64	0.44	0.00	0.99
Nuts × Pp	0.34	0.57	0.01	0.93	0.10	0.76	0.28	0.61
Nuts × BSE	0.38	0.55	1.08	0.31	0.64	0.44	0.19	0.67
Pp × BSE	0.80	0.38	1.14	0.30	0.04	0.84	0.03	0.87
Nuts × Pp × BSE	2.46	0.14	0.94	0.35	1.85	0.19	2.39	0.14
Total protozoans								
Nuts	n/a	n/a	0.79	0.39	0.71	0.41	0.71	0.41
Pp	n/a	n/a	0.98	0.34	0.71	0.41	0.71	0.41
BSE	n/a	n/a	0.98	0.34	0.85	0.37	0.85	0.37
Nuts × Pp	n/a	n/a	0.79	0.39	0.85	0.37	0.85	0.37
Nuts × BSE	n/a	n/a	0.79	0.39	0.71	0.41	0.71	0.41
Pp × BSE	n/a	n/a	0.98	0.34	0.71	0.41	0.71	0.41
Nuts × Pp × BSE	n/a	n/a	0.79	0.39	0.85	0.37	0.85	0.37
Bacteria								
Nuts	0.55	0.47	0.46	0.51	0.46	0.51	1.44	0.25
Pp	0.12	0.73	0.00	0.97	0.00	0.97	0.12	0.73
BSE	0.55	0.47	1.22	0.29	1.22	0.29	0.02	0.90
Nuts × Pp	0.69	0.42	0.62	0.44	0.62	0.44	0.53	0.48
Nuts × BSE	0.32	0.58	2.21	0.16	2.21	0.16	0.26	0.62
Pp × BSE	2.30	0.15	2.97	0.10	2.97	0.10	0.02	0.88
Nuts × Pp × BSE	0.35	0.56	0.00	0.99	0.00	0.99	0.16	0.69

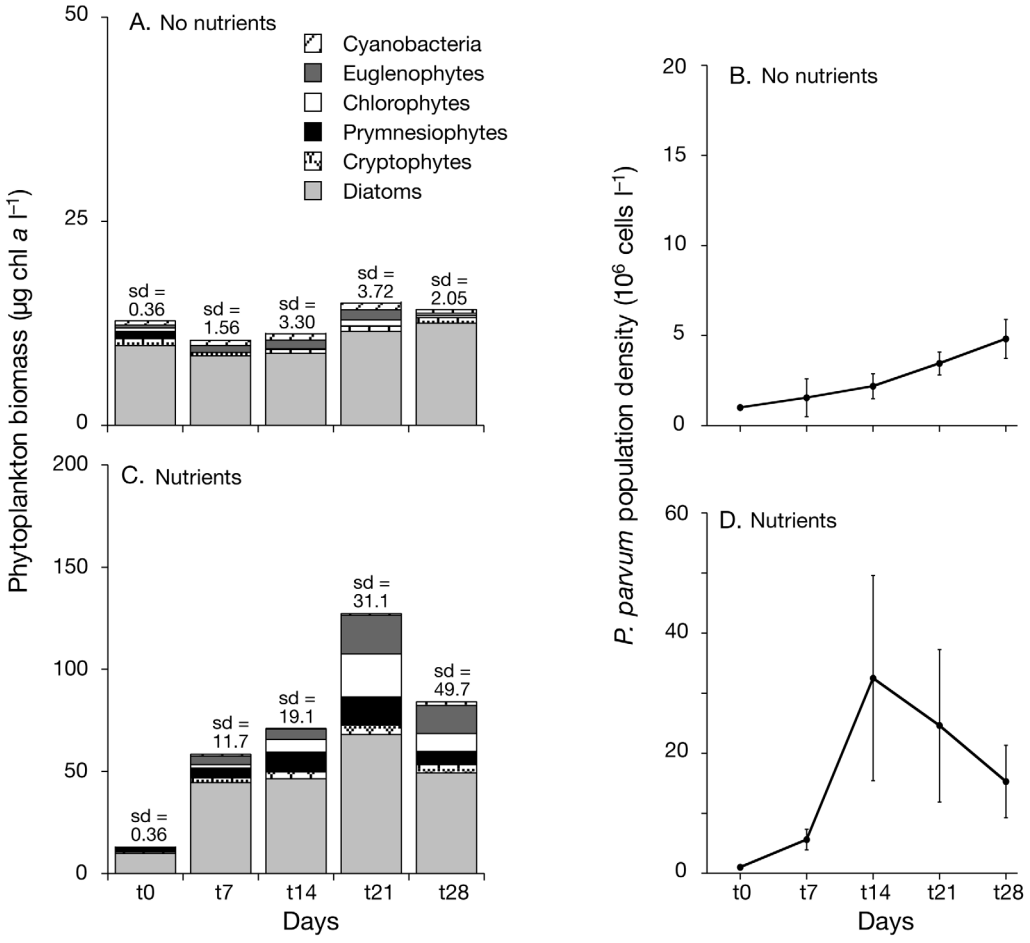

Fig. 2. Averaged phytoplankton assemblage characterizations during the fall 2004 experiment, with enclosures not receiving nutrient additions averaged (n = 12) and enclosures receiving nutrient additions averaged (n = 12). This grouping was performed because other treatments were not significantly different at the p = 0.05 level. In enclosures not receiving nutrient additions, biomass and assemblage structure (diatom dominated) remained conservative (A), while *Prymnesium parvum* population densities slowly increased ~5-fold over a period of 28 d (B). In enclosures receiving nutrient additions, biomass increased ~10-fold over a period of 21 d, with community shifts resulting in the increased prevalence of euglenophytes and chlorophytes (C); *P. parvum* population densities increased more quickly, ~30-fold over a period of 14 d (D)

experiment decreased as the experiment progressed (Fig. 6B). N:P ratios during the fall experiment remained ~8, while ratios gradually increased monotonically from ~4 to ~12 during the early-spring experiment.

In treatments receiving addition of nutrients, ~30 to 50% reduction in DIN concentrations occurred during the fall and early-spring experiments (Figs. 5C & 6C). In both experiments, the utilization of PO_4 was much more pronounced, where starting concentrations of 40 µM were depleted to 0.6 and 0.2 µM for the fall and early-spring experiments, respectively (Figs. 5D & 6D). N:P ratios, which started at 20 for both experiments, increased monotonically as PO_4 was depleted. In both experiments, and in all treatments, SiO_3 remained fairly high (frequently >100 µM, not shown) and was not likely a factor limiting growth of diatoms.

Toxicity

Nutrient additions not only affected phytoplankton biomass, *Prymnesium parvum* population density, and some zooplankton, but they profoundly reduced ambient toxicity to fish and the cladoceran *Daphnia magna*. For example, at the end of the fall experiment the estimated LC_{50} values for fathead minnows *Pimephales promelas* ranged between 21.76 and 26.18% (of sample) in representative enclosures that did not receive nutrient additions, compared to an estimated LC_{50} value of 19.39% for the untreated natural community. However, LC_{50} values ranged between 74.58% and non-toxic in representative enclosures that did receive nutrients (Fig. 7). These higher values imply that these enclosures became less or not toxic to fish.

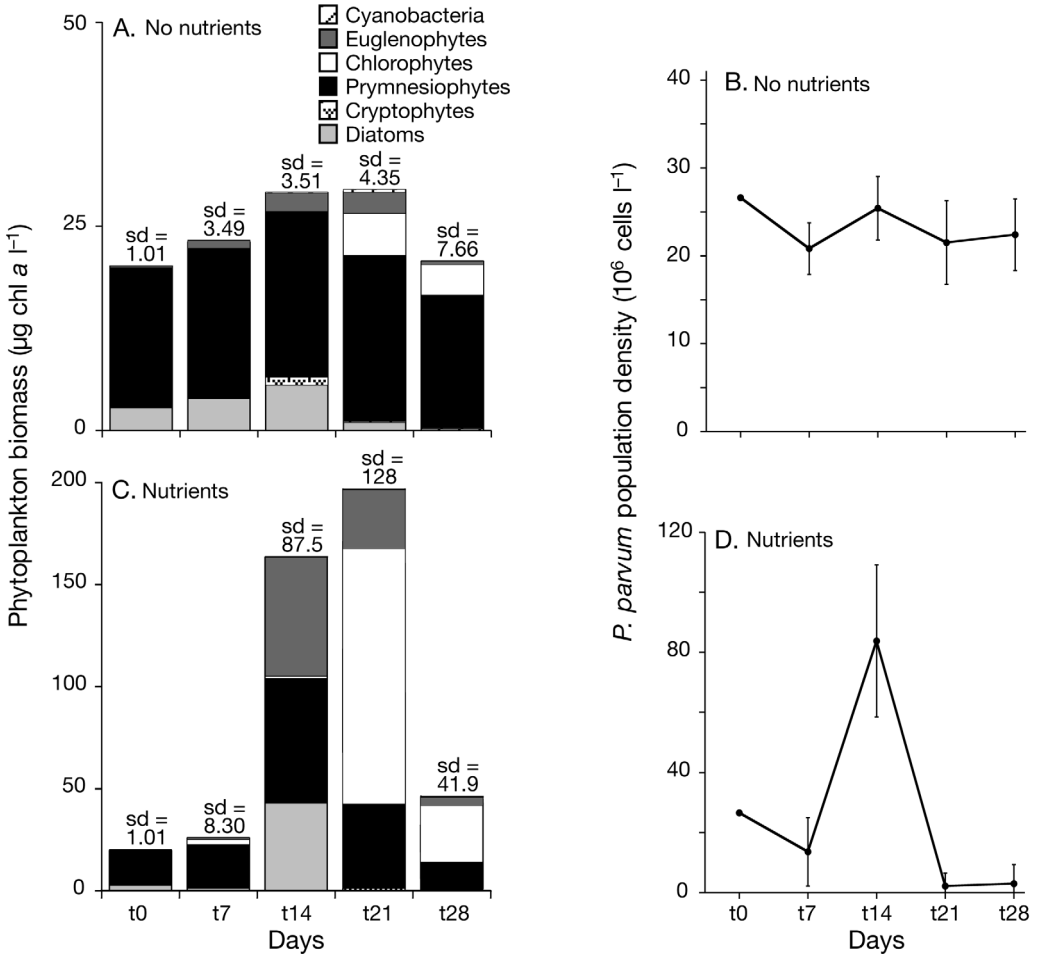

Fig. 3. Averaged phytoplankton assemblage characterizations during the early-spring 2005 experiment. In enclosures not receiving nutrient additions, changes in biomass and assemblage structure (prymnesiophyte dominated) were modest (A) and *Prymnesium parvum* population density was changed little (B). In enclosures receiving nutrient additions, biomass increased ~10-fold over a period of 21 d, with community shifts resulting in the dominance of euglenophytes and chlorophytes (C); *P. parvum* population densities increased ~3-fold over a period of 14 d (D)

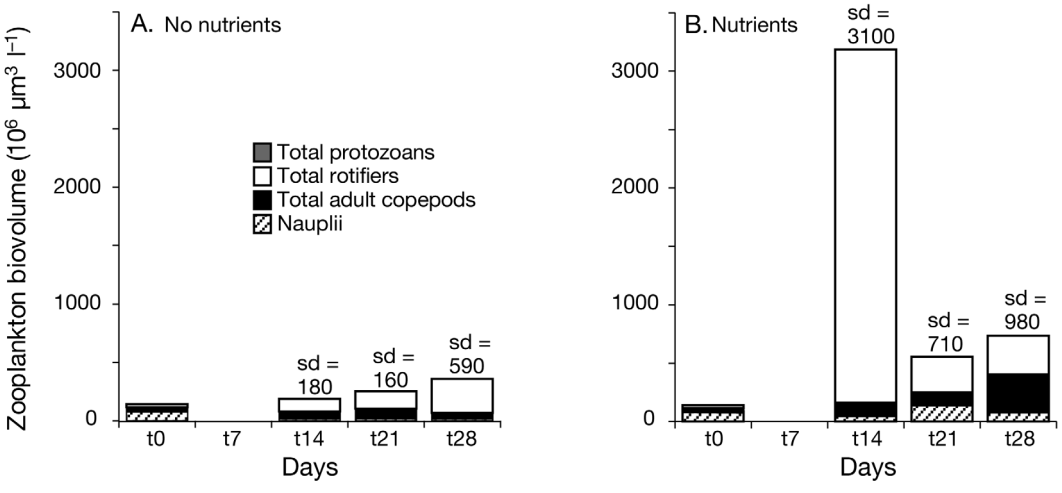

Fig. 4. Averaged zooplankton assemblage characterizations during the fall 2004 experiment. In enclosures not receiving nutrient additions, changes in biomass were modest, while an initial assemblage dominated by copepod nauplii gave way to an assemblage dominated by rotifers and adult copepods (A). In enclosures receiving nutrient additions, biomass increased ~30-fold over a period of 14 d, with rotifers making up the bulk of this increase. Copepod adults and nauplii became more prevalent as the experiment progressed (B)

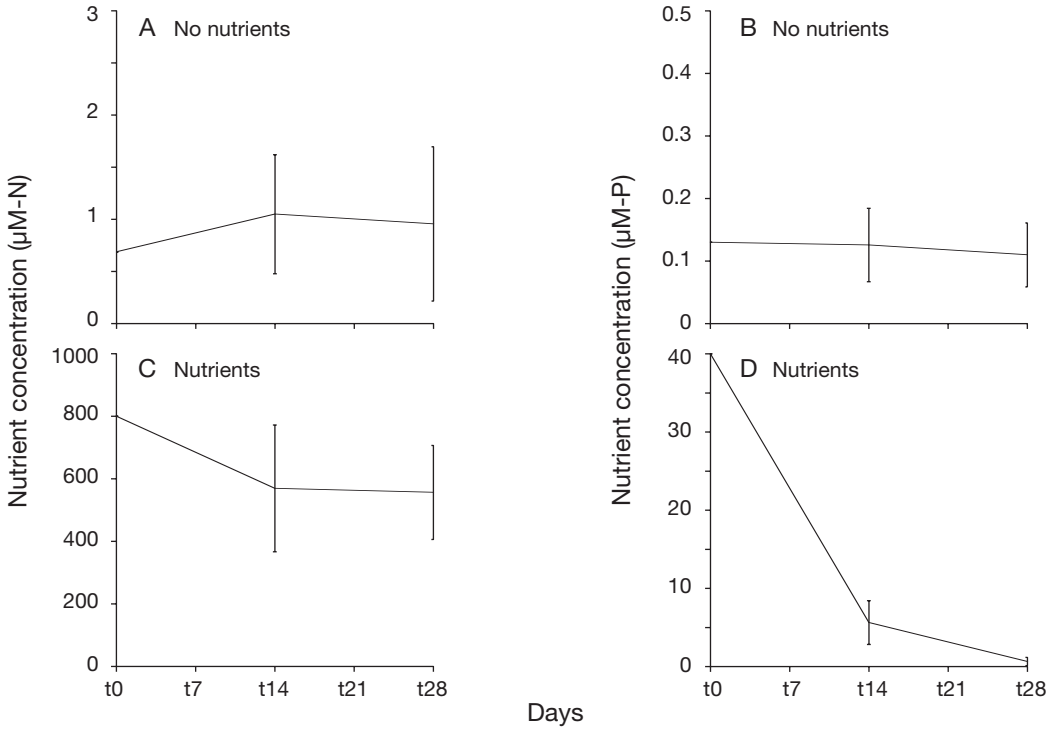

Fig. 5. Averaged dissolved inorganic nitrogen (DIN) and PO_4 concentrations during the fall 2004 experiment. In enclosures not receiving nutrient additions, DIN and PO_4 were conservative (A, B) and the N:P ratio remained ~8. In enclosures receiving nutrient additions, DIN decreased ~30% (C) and PO_4 decreased ~98% (D). The initial N:P ratio was 20, and increased as the experiment progressed

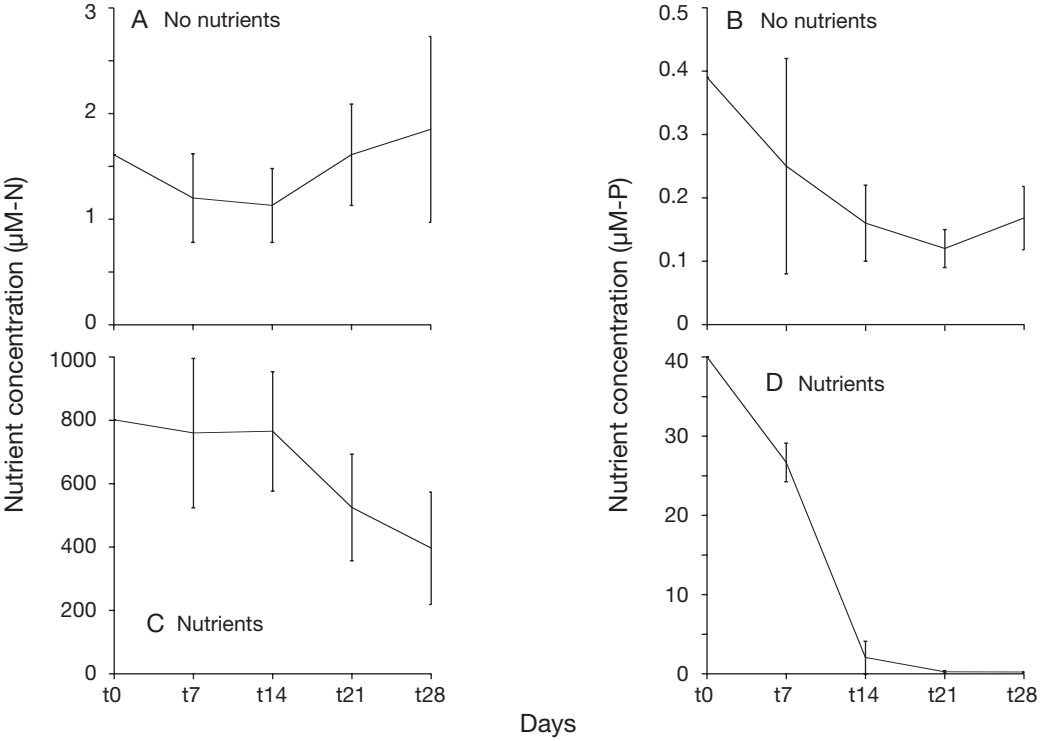

Fig. 6. Averaged DIN and PO_4 concentrations during the early-spring 2005 experiment. In enclosures not receiving nutrient additions, DIN increased slightly (A) and PO_4 decreased ~50% (B). The N:P ratio increased monotonically from ~4 to ~12 during the course of the experiment. In enclosures receiving nutrient additions, DIN decreased ~50% (C) and PO_4 decreased ~99% (D). The initial N:P ratio was 20, and increased as the experiment progressed

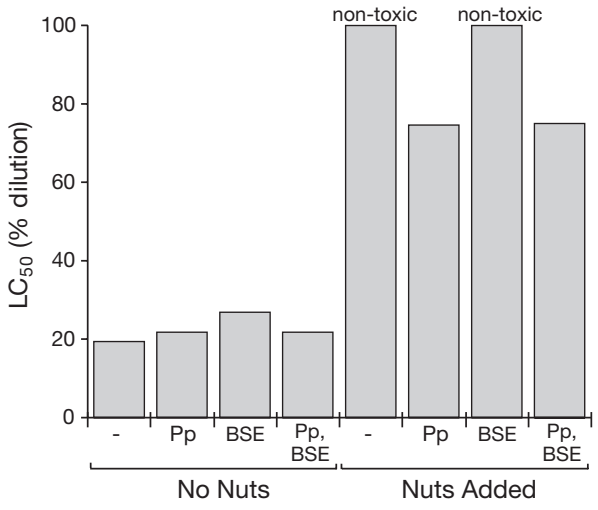

Fig. 7. Ambient toxicity to fish (fathead minnow *Pimephales promelas*) from representative enclosures during the fall experiment shown as the LC_{50} (% dilution). After 28 d, representative enclosures not receiving nutrients became more toxic, while those receiving nutrient additions became less toxic. Pp: *Prymnesium parvum* invasion; BSE: barley straw extract; Nuts: nutrients

Similarly, at the start of the early-spring experiment the fish LC_{50} was 9.8% (more toxic than the initial conditions for the fall experiment), and *Daphnia magna* could not reproduce (data not shown). After 14 d, fish LC_{50} values ranged between 3.1 and 4.4% without nutrient additions, but ranged between 3.1 and >25% with nutrient additions (Fig. 8A). At Day 14, *D. magna* reproduction remained impaired across the treatment structure (data not shown). After 28 d, fish LC_{50} values ranged between 4.8 and 8.8% without nutrients and were typically >25% with nutrients (Fig. 8B). At Day 28, a nutrient addition main effect was detected ($F = 9.174$, $p = 0.008$) for *D. magna* reproduction, indicating a significant decrease in ambient toxicity to cladocerans (Fig. 9).

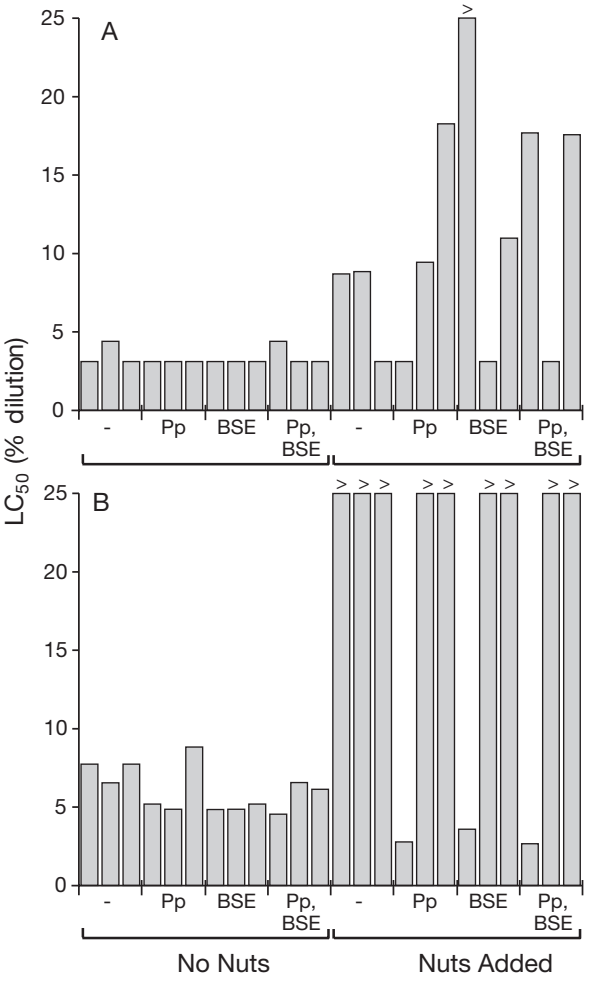

Fig. 8. Ambient toxicity to fish (fathead minnow *Pimephales promelas*) from each enclosure from the early-spring experiment shown as the LC_{50} (% dilution). After 14 d (A), all enclosures not receiving nutrients became more toxic, while some of the enclosures receiving nutrient additions became less toxic. This trend became more apparent after 28 d, at which time all but 3 of the enclosures receiving nutrients were not toxic (B). Pp: *Prymnesium parvum* invasion; BSE: barley straw extract; Nuts: nutrients

DISCUSSION

Based on findings from laboratory studies, periods of nutrient limitation appear to amplify the allelopathic, mixotrophic, and toxic properties of *Prymnesium parvum* (Nygaard & Tobiesen 1993, Barreiro et al. 2005, Uronen et al. 2005). It appears that toxin production in *P. parvum* is a result of secondary metabolite production during times of cell stress, and the competitive advantage gained through production of these toxins are what allow this otherwise moderate competitor to dominate the phytoplankton assemblage during blooms (see Johansson & Granéli 1999, Granéli & Johansson 2003a). Interestingly, and somewhat paradoxically, *P. parvum* blooms and associated fish kills typically appear in systems that are eutrophic (Krasnotshchek & Abramowitsch 1971, Holdway et al. 1978, Rijn & Shilo 1989, Kaartvedt et al. 1991, Guo et al. 1996, Amsinck et al. 2005), where the likelihood of prolonged periods of nutrient limitation may be reduced.

During the period of our in-lake experiments, fish-killing *Prymnesium parvum* blooms, with characteristic golden colored waters and fish bleeding from the gills, occurred throughout the lake and in Echo Cove. Specific to Echo Cove, no significant accumulation of harmful cyanobacteria was observed during our microscopic analyses; no excessive populations of bacteria or low dissolved oxygen were detected; nutrient avail-

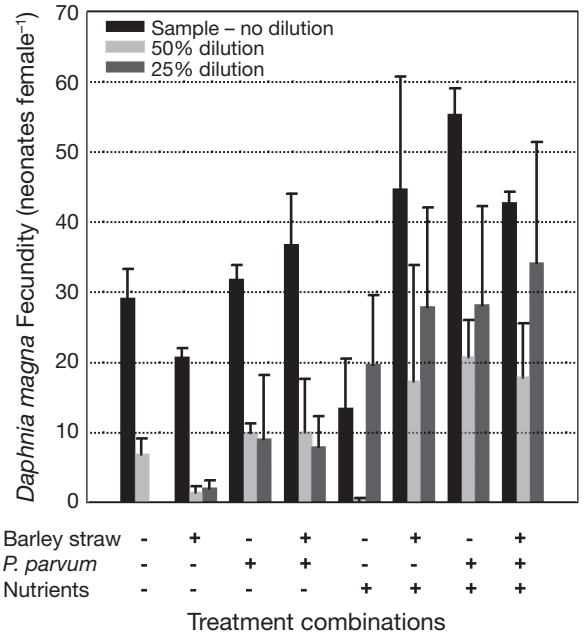

Fig. 9. *Daphnia magna* fecundity responses following a 10 d exposure to nutrient, *Prymnesium parvum* and barley straw extract treatment combinations in enclosures at Lake Possum Kingdom, Texas, USA. Samples from each experimental unit were diluted using reconstituted laboratory water. A significant fecundity increase, indicating reduced ambient toxicity, was produced by nutrients ($p = 0.008$). Average *D. magna* fecundity (±SD) in laboratory control organisms was 36.6 (±2.41)

ability was low (DIN during October and March and PO_4 during January); and competing phytoplankton and zooplankton populations declined with increases in *P. parvum* population density. All of these observations suggest that a toxin-producing *P. parvum* bloom occurred, where the toxins had a lethal effect on fish, an allelopathic influence on other phytoplankton, and a grazing-inhibitory effect (in this case through dramatically reduced grazer populations).

During our fall and early-spring experiments, enclosures that did not receive nutrient additions mirrored trends observed in Echo Cove. For example, just as the incidence of golden colored water and dying fish in the lake were greater during March compared to October, *Prymnesium parvum* population densities and toxicity in samples from our enclosures were greater in the early-spring experiment compared to the fall experiment. Evidence of pathogens influencing phytoplankton succession, significant presence of cyanobacteria known to produce toxic substances, and excessive bacterial concentrations were not observed. Nutrients were relatively low, and because of the low N:P ratio, phytoplankton growth appeared to be regulated more by DIN than PO_4. Finally, the prevalence of other phytoplankton taxa and zooplankton populations were greatly reduced in the early-spring experiment. Taken together, this evidence suggests that toxins produced by *P. parvum* were an important factor influencing plankton dynamics (through allelopathy and grazing reduction) in our enclosure experiments.

Ambient toxicity, however, was greatly reduced with the addition of nutrients, and this may have influenced plankton dynamics. For example, survival rates in our fish bioassays were much higher when using samples taken from enclosures that received nutrient additions, and sublethal reproduction effects on *Daphnia magna* were reduced in nutrient treatments (early-spring experiment). While *Prymnesium parvum* abundance was much greater with the addition of nutrients, even more so were the abundances of other algal groups. Since ambient toxicities to fish and *D. magna* are likely useful surrogates for the presence of bioavailable toxins, it might have been that *P. parvum*'s reduced levels of extracellular toxins prevented it from dominating the phytoplankton assemblage. In fact, in both experiments, there was strong indication that *P. parvum* was being excluded by euglenophytes and chlorophytes, algal groups known to compete well at higher nutrient concentrations and intermediate N:P ratios (Tilman et al. 1986, Sommer 1989, Dokulil & Padisak 1994).

These findings are consistent with laboratory studies using *Prymnesium parvum* and other cultured plankton, where the influence of allelopathy and grazer inhibition was reduced under nutrient-sufficient conditions (Johansson & Granéli 1999, Granéli & Johansson 2003a, Barreiro et al. 2005, Uronen et al. 2005). These studies suggested that secondary metabolic processes that become active when cells are nutrient limited are what lead to the production of toxins. The allelopathic and grazing inhibition effects of the toxins allow *P. parvum* to better compete for limiting resources. In this case, because toxin production is linked to secondary metabolic processes, addition of nutrients further suppresses production of toxins, and *P. parvum* must compete based on its growth rate. In another study, using the same Texas strain of *P. parvum*, maximum growth rates were determined to be 0.72 d^{-1} (Baker et al. 2007). This growth rate is intermediate compared to those of many other phytoplankton taxa (see Tilman et al. 1986, Grover 1989, Sommer 1989, Dokulil & Padisak 1994, Grover et al. 1999), so it is not surprising that *P. parvum* became less prevalent with the addition of nutrients in our experiments.

In theory, subtle differences in initial population densities of competitors can sometimes influence the dynamics of phytoplankton assemblages and lead to very different assemblage structures (Huisman & Weissing 2001, Roelke et al. 2003), so the influence of immigration can be important at times. In our study, however, immigration of *Prymnesium parvum* seemed

to have little effect on plankton dynamics. In a concurrent study where initial population densities of grazers were reduced (R. M. Errera et al. unpubl. data), immigration effects were significant. Clearly, more focused experiments are needed to better understand the relationship between *P. parvum* immigration, initial plankton community structure, and plankton dynamics.

BSE negatively affects some forms of algae (Ridge & Pillinger 1996, Everall & Lees 1997, Schrader et al. 1998), most likely through oxidation of leached tannins and lignins to form toxic polyphenolics (Gibson et al. 1990, Ridge et al. 1995, 1999). In a pilot study, an acute dosage of the same BSE used in this experiment was determined to produce a deleterious effect on *Prymnesium parvum* in culture; yet this treatment level was ineffective during our in-lake experiments. Another study investigating the efficacy of BSE in aquaculture ponds came to the same conclusion (A. Barkoh pers. comm.). It may be that in natural settings polyphenolics are degraded over periods much shorter than our 7 d sampling frequency. In any case, 1-time treatment with BSE would not appear to be an effective management strategy for *P. parvum* blooms. The efficacy of repeated barley straw treatments during bloom formation stills needs investigation.

To the best of our knowledge, our experimental findings represent the first report of nutrient influences on *Prymnesium parvum* population dynamics in natural communities coupled with toxicity estimates based on fish and cladocerans in bioassays. Our in-lake experimental findings agree with previous laboratory culture studies and suggest that nutrients strongly influence the ability of *P. parvum* to form blooms. However, this influence is complex because enrichment makes higher population densities possible, while also reducing toxicity (i.e. reducing allelopathy to competing phytoplankton and grazer inhibition). The worldwide prevalence of *P. parvum* blooms in eutrophic systems, therefore, may not be a direct function of increased nutrient availability. Instead, other processes are likely important, such as disruptions in food-web linkages that might accompany eutrophication. In the face of this complexity, it is perhaps not surprising that simple treatment with an algal suppressant, such as BSE, appears ineffective. The strong suppression of fish and cladoceran toxicity by nutrient addition, however, suggests that targeted and time-limited nutrient manipulations might be used to mitigate the effects of *P. parvum* blooms.

Acknowledgements. We are grateful to C. Miller, who assisted with the in-lake experiments, and to M. Alvarez, S. Garcia, and J. Stanley for assistance with toxicity studies. We are also grateful to the personnel associated with the Possum Kingdom State Park, especially J. Nichols for allowing use of marina facilities. This research was supported by Texas Parks and Wildlife Department, with additional support from the National Science Foundation under Grant Number DEB-0444844 to J.P.G.

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Editorial responsibility: Hugh MacIntyre, Dauphin Island, Alabama, USA

Submitted: July 20, 2006; Accepted: October 30, 2006
Proofs received from author(s): January 23, 2007