Vol. 52: 33–44, 2008
doi: 10.3354/ame01199

AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol

Printed July 2008
Published online June 26, 2008

Effect of imbalanced nutrients and immigration on *Prymnesium parvum* community dominance and toxicity: results from in-lake microcosm experiments

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ABSTRACT: *Prymnesium parvum*, a haptophyte species, forms harmful blooms, including those that have caused severe fish kills in Texas, USA, over the past 6 yr. We studied *P. parvum* dynamics using *in situ* microcosm experiments at Lake Possum Kingdom, Texas, during 3 seasons (fall 2004, winter and spring 2005). Experimental treatments included full and partial nutrient enrichment (encompassing nitrogen [N] and phosphorus [P] deficient treatments), *P. parvum* immigration and combinations of these factors. In the control and N and P deficient treatments, *P. parvum* populations dominated the community, but only in the N deficient treatments did *P. parvum* experience a significant growth in the population. In contrast, when nutrients were not limiting, *P. parvum* tended to lose its competitive edge to other taxa such as chlorophytes, euglenophytes and diatoms, which then dominated the community. Population growth of *P. parvum* was also stimulated through immigration, but only during the winter experiment, a period of the year when bloom initiation is common. This finding suggests that movement into the water column may be an important process leading to *P. parvum* bloom initiation. Toxicity of *P. parvum* to fish was also affected by the nutrient changes: during conditions of no nutrient addition *P. parvum* was most toxic; intermediate toxicity was observed under N and P deficient conditions, and full nutrient enrichments resulted in nearly non-toxic conditions.

KEY WORDS: Allelopathy · Phytoplankton · Competition · CHEMTAX · Nutrient limitation · HAB · Toxins

INTRODUCTION

Prymnesium parvum, a harmful algal species, occurs worldwide and is responsible for large fish kills in coastal and inland water bodies (Edvardsen & Paasche 1992). In Texas, USA, blooms of *P. parvum* have affected 21 lakes and 5 river basins (TPWD 2003). Recent estimates suggest that over 17.5 million fish, valued at over $6.5 million, have died as a result of *P. parvum* blooms in Texas (TPWD 2003).

The mechanisms controlling *Prymnesium parvum* blooms in Texas lakes are poorly understood. However, the availability of nutrients probably plays a role. In field enclosures under nutrient sufficient conditions

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dominance of *P. parvum* in phytoplankton assemblages and ambient toxicity were greatly reduced, but under nutrient deficient conditions *P. parvum* became dominant and toxicity increased (Roelke et al. 2007). These findings are consistent with laboratory experiments using the Texas (Barkoh et al. 2003, Grover et al. 2007) and European (Granéli & Johansson 2003a, Uronen et al. 2005) strains of *P. parvum* where toxicity was linked to nutrient availability.

Some studies suggest that production of toxins may be a key mechanism by which *Prymnesium parvum* gains a selective advantage over other phytoplankton, thereby allowing bloom formation (Fistarol et al. 2003, Granéli & Johansson 2003a). *P. parvum* is known to produce several toxins that when released into the water column affect fish, heterotrophic dinoflagellates, bacteria, phytoplankton and ciliates (Nygaard & Tobiesen 1993, Fistarol et al. 2003, Granéli & Johansson 2003a,b, Barreiro et al. 2005, Sopanen et al. 2006, Uronen et al. 2007). Effects of the toxins include ichthyotoxic, cytotoxic, and hemolytic activity, while some act as allelopathic substances that inhibit the growth of competing phytoplankton and as grazer inhibitors (Fistarol et al. 2003, Granéli & Johansson 2003a,b, Uronen et al. 2005, 2007).

Another possible bloom initiating mechanism is immigration. This may be especially relevant to species with benthic resting stages (Beltrami et al. 2007). Previous theoretical studies have demonstrated that subtle differences in the initial composition of phytoplankton assemblages could have a profound effect on plankton dynamics (Huisman & Weissing 2001, Roelke et al. 2003). Immigration, which can change the initial phytoplankton assemblage composition, can alter plankton dynamics at rates as low as 0.1% of the total phytoplankton biomass per day (Roelke & Eldridge 2008).

Few studies have investigated the role of nutrient limitation and immigration as it influences *Prymnesium parvum* population dynamics using natural plankton assemblages. Here we report findings from in-lake experiments conducted in Lake Possum Kingdom, a system affected by *P. parvum* blooms in the last 6 yr. Our microcosm experiments were conducted concurrently with larger volume experiments reported in Roelke et al. (2007). Because our microcosm experiments were more easily controlled, we were able to expand the number of treatments beyond the scope of our large volume study. In this paper, we present findings from these additional treatments, i.e. nutrient manipulations that comprised full (f/2 nutrient concentrations) and partial (nitrogen [N] and phosphorus [P] deficient) additions and immigration as they relate to *P. parvum* bloom dynamics, plankton community shifts and potential management strategies.

MATERIALS AND METHODS

Study site. Lake Possum Kingdom is a reservoir on the Brazos River, Texas (Fig. 1), with maximum and mean depths of 60 and 11 m, respectively, and a surface area of 80 km^2. The drainage area is a limestone escarpment 730 times greater than the lake's surface area (Dowell 1964). The reservoir is periodically brackish depending on annual rainfall. We conducted our research at Echo Cove, a site where high *Prymnesium parvum* population densities were historically observed during early stages of seasonal bloom formation (see Roelke et al. 2007 for further information). During the course of our experiments a *P. parvum* bloom occurred in Echo Cove. The description of this bloom in regards to enumerations of phytoplankton and zooplankton, changes in the physicochemical environment and observations of dead fish are provided in Roelke et al. (2007).

In-lake experiments. As part of a larger project involving lake monitoring, in-lake large volume enclosure experiments (Roelke et al. 2007), laboratory experiments (Baker et al. 2007, Grover et al. 2007) and numerical modeling, we conducted in-lake bottle experiments performed alongside the enclosure experiments. Three 28 d experiments were performed in Echo Cove during the fall (initiated on October 25, 2004), winter (initiated on January 11, 2005), and early spring (initiated on March 8, 2005). We used 36 transparent 2 l polycarbonate bottles, and experimental water was collected in the vicinity of Echo Cove at a depth of 0.5 m. To exclude large zooplankton, which may cause bias in small bottle experiments (Sommer 1985, Roelke et al. 2003), lake water was filtered

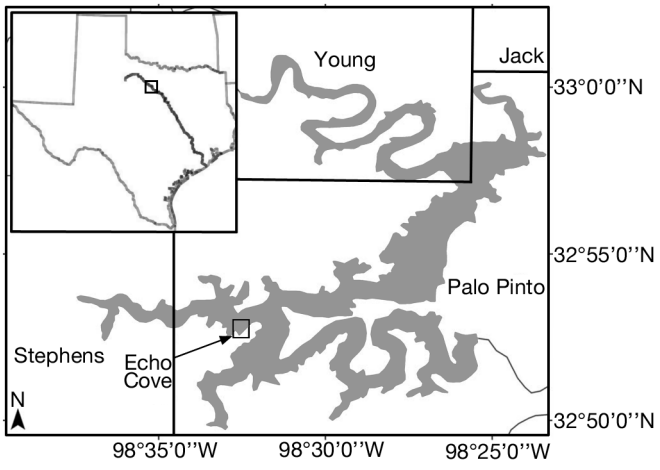

Fig. 1. Lake Possum Kingdom, Texas. Experiments were conducted in Echo Cove, an area where *Prymnesium parvum* blooms have formed in recent years. Inset shows general location of Lake Possum Kingdom (□) in the state of Texas

through a 153 µm mesh. Bottles were capped leaving enough air in the headspace to allow neutral buoyancy. Protective frames housed bottles in the lake and frame covers provided shade through neutral density screening (~55% reduction in light). The 2 l polycarbonate bottles further reduced light by ~10%. Each frame was tethered within the cove, which allowed bottles to experience turbulence, temperature and light variations similar to natural conditions.

Our treatments included (1) full nutrient additions (to concentrations of f/2 media; 800 µM N, 40 µM P, N:P ratio = 20:1; see Guillard & Ryther 1962), (2) addition of barley straw extract (Ecological Laboratories), (3) addition of *Prymnesium parvum* culture that increased initial population density by ~5×10^5 cells l^{-1} (5% of typical bloom cell density, TPWD 2003), and (4) combinations of these 3 factors (Table 1). These treatments represented a full 2^3 factorial design with 3 replicates. Bottles receiving nutrient additions had nitrogen added in the form of nitrate, not ammonium, because the latter form appears to be toxic to *P. parvum* (Barkoh et al. 2003, Grover et al. 2007). Because deleterious effects on phytoplankton in similar reservoirs have not been reported for the nutrient levels added in our experiments (Grover et al. 1999), we assumed that the only potential effect of added nutrients was the relief of potential growth limitation.

Table 1. Treatments (all contain the natural phytoplankton assemblage) performed in the experiment. The gray shaded area represents treatments that were also tested in Roelke et al. (2007) and used to quantify the bottle effect. These data are not represented in the manuscript

Treatment	Full nutrients	Barley straw extract	*Prymnesium parvum*	N deficient	P deficient
1 (control)					
2		x			
3			x		
4		x	x		
5	x				
6	x	x			
7	x		x		
8	x	x	x		
9				x	
10			x	x	
11					x
12			x		x

Additional treatments involving partial nutrient enrichment were also conducted. These were an f/2 medium lacking $NaNO_3$ (N) and an f/2 medium without NaH_2PO_4 (P). These treatments were conducted in triplicate with and without additions of *Prymnesium parvum*. Together with unenriched controls, these treatments represented a 4 × 2 factorial design, again with 3 replicates. Our nutrient analyses verified that initial nutrient concentrations were non-limiting in bottles receiving full nutrient additions; initial nutrients were N deficient in bottles receiving nutrient additions without N, and initial nutrients were P deficient in bottles receiving nutrient additions without P.

The strain of *Prymnesium parvum* used to enhance initial population densities in these experiments was isolated from the Colorado River, Texas (strain ZZ181, University of Texas Culture Collection of Algae). This strain was cultured in the laboratory as described in Roelke et al. (2007).

Samples of 210 ml for characterization of phytoplankton assemblages were collected every 7th day for each of the 3 experiments and preserved in glutaraldehyde, 5% v/v. Because zooplankton samples require large volumes, collection was taken only at termination of each experiment. Samples were concentrated by passing the remaining water in each experimental bottle (~1400 ml) through a 63 µm mesh net, collecting the retained particles, and then preserving them in 5% buffered formalin. Nutrient samples of approximately 100 ml were collected at Days 0, 14 and 28 for the fall experiment and every 7th day for the winter and spring experiments. Nutrient samples were filtered through 47 mm GF/F filters, and the filtrate was frozen until analysis. Ambient toxicity to fish was determined at Day 28 for the spring experiment only.

Estimates of total phytoplankton biomass and biomasses of higher taxonomic groups were made from measurements of photopigments following Pinckney et al. (1998). Phytoplankton were collected on filters (25 mm GF/F) and 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 Nucleopore) 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 photopigments concentrations, biomasses of higher phytoplankton taxa were estimated with CHEMTAX (see Pinckney et al. 1998). The higher taxa targeted in the analyses were based on their prevalence in Lake Possum Kingdom; these included cyanobacteria, euglenophytes, chlorophytes, prymnesiophytes, cryptophytes and diatoms.

Enumeration of *Prymnesium parvum* population densities were performed using a Utermöhl settling technique. A 500 to 1000 µl subsample was settled for 24 h and then the number of cells were 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.

For zooplankton enumeration, subsamples were settled for a 24 h period and counted using an inverted, phase contrast, light microscope (40× and 200×, Leica Microsystem). Dimensions corresponding to best-fit geometric shapes were measured for each zooplankter and biovolume calculated (Wetzel & Likens 1991). We then aggregated the zooplankton into the following groups: total adult copepods, copepod nauplii, total rotifers and total protozoa.

Nutrient concentrations were determined using autoanalyzer methods (Armstrong & Sterns 1967, Harwood & Kuhn 1970) and included nitrate (NO_3), nitrite (NO_2), ammonia (NH_4), orthophosphate (PO_4) and silicate (SiO_3). Concentrations of NO_3, NO_2 and NH_4 were summed and are reported as dissolved inorganic nitrogen (DIN). Initial nutrient conditions within each treatment were determined based on the ambient lake conditions plus the enrichment concentrations.

We measured ambient toxicity to fish at the completion of the spring experiment following methods described in Roelke et al. (2007). Briefly, using the filtrate of ~1200 ml after zooplankton were sampled (which included particles < 63 µm), toxicity was evaluated by means of 24 h static acute toxicity assays with fathead minnows *Pimephales promelas*. Samples collected from experimental units were diluted using a 0.5 dilution series with reconstituted hard water (RHW); RHW 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. Specific conductance (µS cm^{-1}), pH and dissolved oxygen (mg l^{-1}) of RHW were also measured before toxicity testing. All culturing and toxicity tests were performed at 25 ± 1°C with a 16 h light : 8 h dark cycle. Fathead minnow larvae <48 h old were fed newly hatched *Artemia* nauplii 2 h before initiation of testing. LC_{50} values for fathead minnow toxicity tests were estimated as percentage of ambient sample using Probit or Trimmed Spearman Karber techniques, depending on data properties.

Differences between experimental treatments in total phytoplankton biomass (estimated using chlorophyll *a* [chl *a*]), *Prymnesium parvum* population density and biomasses of zooplankton taxa were tested for statistical significance using a repeated measures general linear model (GLM, using SPSS Professional Statistics 16.0 for Windows XP). We used an accumulated time approach: the first GLM used only data from time 0 and 7 d, the second GLM used data from 0, 7 and 14 d, and so forth, until the final GLM where all time series data were used in the analysis. This procedure permitted evaluation of the timing of significant differences between treatments.

Two separate statistical analyses were conducted. The first analysis tested for treatment effects in the 2^3 factorial design with 3 factors (enrichment with full nutrients, simulated immigration of *Prymnesium parvum* and addition of barley straw extract). These results were very similar to findings reported in Roelke et al. (2007). Therefore, we present them here primarily to evaluate potential bottle effects. The second analysis tested for treatment effects in the factorial design where 3 nutrient treatments (full enrichment, P deficient and N deficient) and a control were crossed with presence or absence of *P. parvum* inoculation. Post hoc tests were performed for the second analysis. If the data contained equal variances, then Bonferroni tests were conducted. If variances were not homogeneous, then Dunnett's *T3*-tests were conducted due to the test's conservative nature (Scheiner & Gurevitch 2001).

Characterization of potential bottle effects. The large volume enclosure experiments (Roelke et al. 2007), which ran concurrently during our fall and spring bottle experiments, used twenty-four 1.57 m^3 translucent, closed bottom enclosures of cylindrical dimension, 1 m diameter and 2 m depth (Aquatic Research Instruments). These enclosure experiments had 8 treatments: full nutrient additions (to f/2, Guillard & Ryther 1962), addition of *Prymnesium parvum* culture that increased initial population density by ~5×10^5 cells l^{-1} (5% of typical bloom cell density, TPWD 2003), addition of barley straw extract (50-fold the manufacturer's recommendation), and combinations of these. The same treatments were also used in our bottle experiments (Table 1), which enabled comparisons of results between the 2 experiments.

To evaluate bottle effects we tested for statistical differences between the control treatments for the large enclosure and bottle experiments using a repeated measures GLM (SPSS). We compared the phytoplankton biomass and assemblage composition between the 2 designs, as well as the likelihood of nutrient recycling based on the relative concentrations of NH_4 to total dissolved inorganic nitrogen (DIN).

For the fall and spring experiments, *Prymnesium parvum* population dynamics were not significantly different ($p > 0.05$) between the experimental designs. In addition, the difference between *P. parvum* and chl *a* in the 2 experiments was minimal for the barley

straw treatments. Even though the bottles were of smaller volume than the enclosures and the initial conditions for the bottles had large grazers removed, accumulation of phytoplankton biomass and shifts in assemblage composition were remarkably similar between the 2 experiments.

The proportion of NH_4 in total DIN was greater in the bottle experiments than in large volume enclosures, but only during the fall experiment. In the bottles, ~78 and ~71% of the total DIN was NH_4 at Days 14 and 28, respectively, while ~41 and ~25% of the total DIN was NH_4 in the large volume enclosures.

From these observations, 'small-bottle' effects did influence results between the 2 experiments. But, such effects occurred only during the fall experiment and affected only recycling of nutrients. Plankton dynamics seemed unaffected.

As in the large volume experiments, addition of barley straw extract to bottles had no significant effect on *Prymnesium parvum* population dynamics. Consequently, we do not further present these results here.

RESULTS

The effects of partial and full nutrient additions on phytoplankton biomass and *Prymnesium parvum* population density were apparent at 7 d in all 3 experiments (Tables 2 to 4). Following full nutrient enrichment, the highest phytoplankton biomass occurred in the treatments receiving nutrient additions that were N deficient, followed by nutrient additions that were P deficient, with controls having the lowest biomass (Fig. 2).

Shifts in community composition varied between nutrient treatments. For example, composition varied little between P deficient nutrient additions and controls (Fig. 2), while treatments receiving additions of P (i.e. full and N deficient nutrient additions) showed greater prevalence of diatoms, chlorophytes and euglenophytes. This trend was most obvious during the spring experiment where *Prymnesium parvum* was the dominant species in the water column at the start of the experiment, but throughout the experiment the community composition shifted away from *P. parvum* dominance (Fig. 2B,C, spring).

Prymnesium parvum population density was greatest in treatments receiving N deficient nutrient additions (Fig. 3). Early in all 3 experiments, full nutrient additions generally produced *P. parvum* population densities comparable with the N deficient nutrient additions, but *P. parvum* populations dramatically declined in full nutrient addition treatments midway through the experiments (Fig. 3B), with the exception of the spring experiment when *P. parvum* populations declined after 7 d. Although *P. parvum* populations in

Table 2. Repeated measures general linear model results for the fall 2004 partial nutrients experiment; results are reported for nutrient class, *Prymnesium parvum* (*Pp*) additions and interactions. During sampling periods when the interaction between treatments was not significant, post hoc Dunnett's *T3*-test was used to determine significance between nutrient classes. Nutrient treatments connected by under-bars were not statistically different. None = no nutrient manipulation, Nutrients = full f/2 nutrient additions, N = f/2 nutrients without nitrogen additions, P = f/2 nutrients without phosphorus additions, *statistically significant values ($p < 0.05$)

	0–7 d		0–14 d		0–21 d		0–28 d	
	F	p	*F*	p	*F*	p	*F*	p
Phytoplankton biomass								
Nutrient class	235.28	<0.01*	203.82	<0.01*	113.83	<0.01*	67.53	<0.01*
Pp	0.04	0.85	0.04	0.84	0.04	0.85	0.02	0.88
Nutrient class × *Pp*	1.08	0.39	1.16	0.36	1.09	0.38	1.57	0.24
	Nutrients N None P		Nutrients N None P		Nutrients N None P		Nutrients N None P	
***P. parvum* population**								
Nutrient classs	120.00	<0.01*	88.75	<0.01*	97.24	<0.01*	147.47	<0.01*
Pp	0.57	0.46	0.01	0.91	1.41	0.25	3.60	0.08
Nutrient class × *Pp*	2.20	0.13	0.82	0.50	0.21	0.89	1.02	0.41
	Nutrients N None P		Nutrients N None P		N Nutrients None P		N Nutrients None P	
	Copepod adults 0–28 d		Copepod nauplii 0–28 d		Total rotifers 0–28 d		Total protozoa 0–28 d	
Zooplankton								
Nutrient class	3.85	0.30	2.19	0.13	5.45	<0.01*	0.91	0.46
Pp	0.23	0.64	1.87	0.19	0.06	0.81	0.30	0.59
Nutrient class × *Pp*	0.33	0.80	3.64	0.04	0.02	1.00	1.48	0.26
					None P Nutrients N			

Table 3. Repeated measures general linear model results for the winter 2005 partial nutrients experiment; results are reported for nutrient class, *Prymnesium parvum* (*Pp*) additions and interactions. During sampling periods when the interaction between treatments was not significant, post hoc Dunnett's *T3*-test was used to determine significance between nutrient classes. Nutrient treatments connected by under-bars were not statistically different. For *P. parvum* population density Days 0 to 7, Bonferroni's multi-comparison test was used. None = no nutrient manipulation, Nutrients = full f/2 nutrient additions, N = f/2 nutrients without nitrogen additions, P = f/2 nutrients without phosphorus additions, na = not applicable, *statistically significant values ($p < 0.05$)

	0–7 d		0–14 d		0–21 d		0–28 d	
	F	p	*F*	p	*F*	p	*F*	p
Phytoplankton biomass								
Nutrient class	68.39	<0.01*	350.19	<0.01*	401.14	<0.01*	613.42	<0.01*
Pp	7.83	0.01*	14.08	<0.01*	2.85	0.11	4.04	0.06
Nutrient class × *Pp*	1.65	0.22	3.83	0.03*	0.70	0.57	1.45	0.27
	Nutrients N None P				Nutrients N None P		Nutrients N None P	
***P. parvum* population**								
Nutrient class	23.92	<0.01*	49.48	<0.01*	77.88	<0.01*	104.61	<0.01*
Pp	13.79	<0.01*	6.62	0.02*	9.48	0.01*	14.86	<0.01*
Nutrient class × *Pp*	2.42	0.10	1.75	0.20	3.95	0.03*	9.66	<0.01*
	N Nutrients None P		Nutrients N None P					
	Copepod adults (0–28 d)		Copepod nauplii (0–28 d)		Total rotifers (0–28 d)		Total protozoa (0–28 d)	
Zooplankton								
Nutrient class	0.66	0.59	0.31	0.82	1.78	0.19	na	na
Pp	0.43	0.52	1.93	0.18	1.13	0.30	na	na
Nutrient class × *Pp*	0.72	0.56	0.55	0.66	1.27	0.32	na	na

Table 4. Repeated measures general linear model results for the spring 2005 partial nutrients experiment; results are reported for nutrient class, *Prymnesium parvum* (*Pp*) additions and interactions. During sampling periods when the interaction between treatments was not significant, post hoc Dunnett's *T3*-test was used to determine significance between nutrient classes. Nutrient treatments connected by under-bars were not statistically different. None = no nutrient manipulation, Nutrients = full f/2 nutrient additions, N = f/2 nutrients without nitrogen additions, P = f/2 nutrients without phosphorus additions, na = not applicable, *statistically significant values ($p < 0.05$)

	0–7 d		0–14 d		0–21 d		0–28 d	
	F	p	*F*	p	*F*	p	*F*	p
Phytoplankton biomass								
Nutrient classs	21.95	<0.01*	250.02	<0.01*	133.10	<0.01*	105.72	<0.01*
Pp	2.62	0.13	1.36	0.26	1.04	0.32	0.61	0.45
Nutrient class × *Pp*	4.49	0.02*	2.27	0.12	0.59	0.63	0.17	0.92
			Nutrients N None P		Nutrients N None P		Nutrients N None P	
***P. parvum* population**								
Nutrient classs	15.20	<0.01*	32.37	<0.01*	56.78	<0.01*	12.10	<0.01*
Pp	1.38	0.26	1.21	0.29	0.32	0.58	0.81	0.38
Nutrient class × *Pp*	1.06	0.40	0.39	0.76	0.13	0.94	0.62	0.61
	N Nutrients None P		N None Nutrients P		N None Nutrients P		N None P Nutrients	
	Copepod adults (0–28 d)		Copepod nauplii (0–28 d)		Total rotifers (0–28 d)		Total protozoa (0–28 d)	
Zooplankton								
Nutrient class	1.01	0.42	1.91	0.17	0.79	0.52	na	na
Pp	0.44	0.52	0.04	0.85	0.88	0.36	na	na
Nutrient class × *Pp*	1.08	0.39	0.64	0.60	0.79	0.52	na	na

the control and P deficient treatments remained lower than in the other treatments, they represented a significant portion of the phytoplankton community, especially during the spring experiment.

The addition of *Prymnesium parvum* cells, our simulated immigration treatment, produced significant differences only during the winter experiment (Table 3). This treatment effect was significant for the duration of the experiment for *P. parvum* population density and through Day 14 for total phytoplankton biomass. The interaction between nutrient treatments and *P. parvum* addition was significant for Days 21 and 28 for the

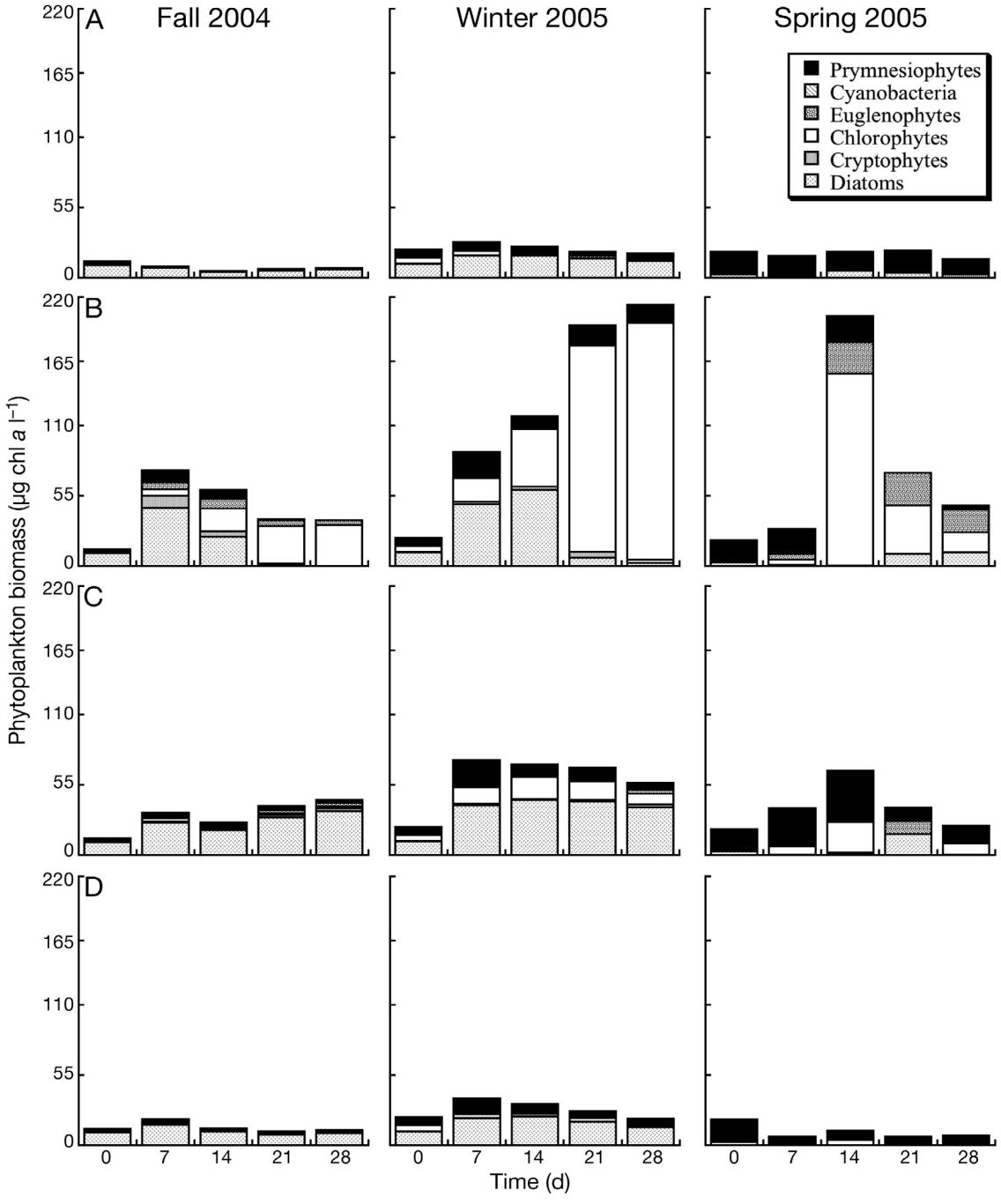

Fig. 2. Average phytoplankton biomass and assemblage composition for the fall, winter and spring in-lake bottle experiments. (A) No nutrient additions, (B) full nutrient enrichment, (C) N deficient nutrient enrichment, (D) P deficient nutrient enrichment. Bottles with enhanced initial *Prymnesium parvum* populations were included in the averages, but bottles receiving additions of barley straw extract were not included in the averages because there were no N and P deficient treatments where barley straw extract was added

winter experiment (Table 3), in which *P. parvum* population densities were highest in the treatments receiving both N deficient nutrient additions and inoculations of *P. parvum* (Fig. 3B).

Significant treatment effects on zooplankton were detected only during the fall experiment when the *P. parvum* concentration was the lowest in Lake Possum Kingdom, and an effect of nutrients was only observed for rotifers (Table 2).

Changes in nutrient concentrations were similar in all 3 experiments. When nitrogen was added to a bottle, DIN concentration remained high (~600 µM) for

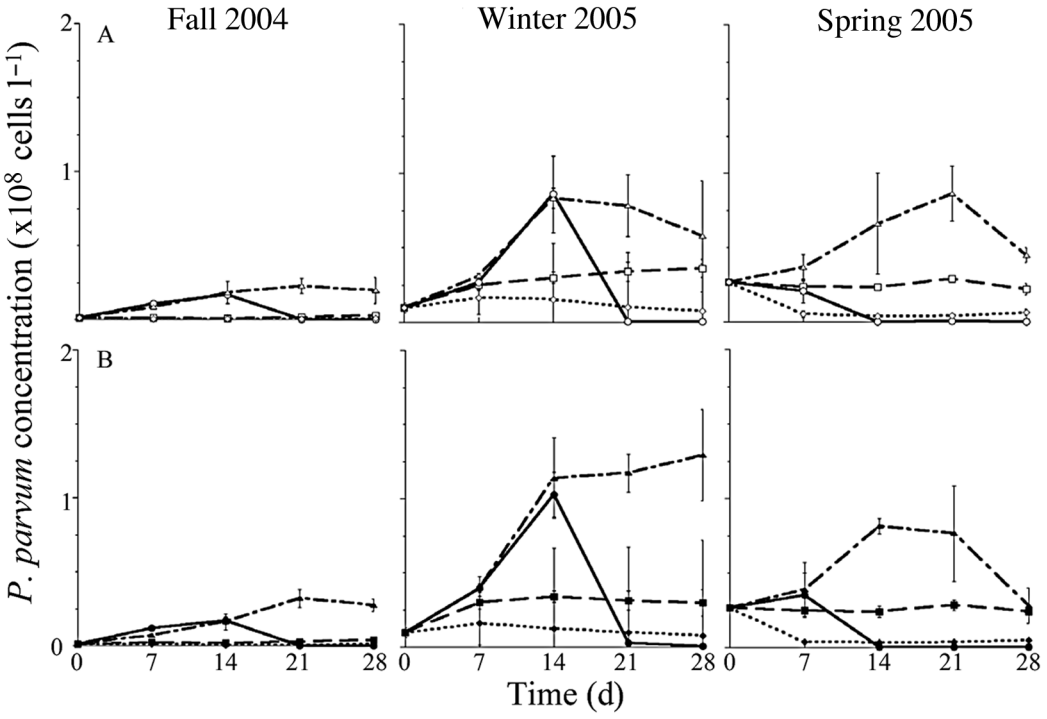

Fig. 3. *Prymnesium parvum*. Mean (±SD) population density representing (A) no *P. parvum* additions (open symbols) and (B) enhanced initial *P. parvum* populations (closed symbols) for the fall, winter and spring experiments. Dashed lines represent treatments with no nutrient additions; solid lines represent treatments that received full nutrient additions; broken lines represent N deficient nutrient additions; dotted lines represent P deficient nutrient additions

the duration of the experiment (Table 5). When phosphorus was added to a bottle, however, PO_4 concentration showed rapid utilization generally decreasing from ~20 µM to <1 µM in full nutrient addition treatments, and decreasing to ~5 µM in N deficient nutrient addition treatments (Table 5). Similarly, utilization of SiO_3 was apparent in treatments receiving full nutrient additions (Table 5). During the fall and spring experiments, SiO_3 dropped to concentrations that probably limited reproductive growth of diatoms.

Table 5. Concentrations of DIN, PO_4 and SiO_3 for the fall, winter and spring experiments. We present the initial (Day 0) concentration and final (Day 28) mean (±SD) concentration for each treatment type to describe potential limitations over the course of the experiment

Treatment	Initial			Final (mean ± SD)		
	µM N	µM P	µM Si	µM N	µM P	µM Si
Fall						
No nutrients	0.68	0.14	101.56	0.53 ± 0.36	0.09 ± 0.10	102.63 ± 9.16
Full nutrients	800.68	40.14	201.56	716.48 ± 72.24	0.09 ± 0.04	11.17 ± 3.25
N deficient nutrients	0.68	40.14	201.56	1.10 ± 0.31	6.21 ± 2.34	147.32 ± 23.96
P deficient nutrients	800.68	0.14	201.56	692.14 ± 79.52	0.14 ± 0.01	156.03 ± 19.15
Winter						
No nutrients	6.14	0.12	88.02	1.79 ± 0.27	0.08 ± 0.07	103.55 ± 4.85
Full nutrients	806.14	40.12	188.02	634.50 ± 17.44	0.34 ± 0.10	55.92 ± 9.45
N deficient nutrients	6.14	40.12	188.02	2.64 ± 0.69	4.82 ± 0.38	150.30 ± 5.65
P deficient nutrients	806.14	0.12	188.02	745.79 ± 23.34	0.11 ± 0.10	150.17 ± 6.24
Spring						
No nutrients	1.60	0.39	105.64	1.07 ± 0.19	0.18 ± 0.01	82.76 ± 8.56
Full nutrients	801.60	40.39	205.64	604.25 ± 34.97	0.39 ± 0.08	12.42 ± 5.56
N deficient nutrients	1.60	40.39	205.64	2.37 ± 0.42	2.29 ± 0.32	100.17 ± 9.11
P deficient nutrients	801.60	0.39	205.64	639.75 ± 40.64	0.19 ± 0.02	89.66 ± 18.51

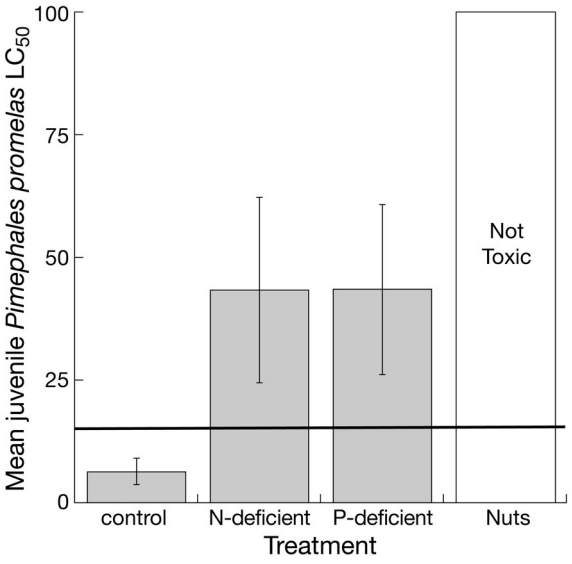

Fig. 4. *Pimephales promelas*. Mean (±SD) LC_{50} values during the spring experiment. Bottles with full nutrient additions (Nuts) were not toxic and had 100% survival of *P. promelas*. —: LC_{50} at the start of the experiment

Nutrient additions reduced ambient toxicity to fish. During the spring experiment, estimated LC_{50} values for fathead minnows ranged between 2.68 and 10.90% (dilution of sample) in control bottles (Fig. 4). But, in full nutrient addition treatments, toxicity was greatly reduced and many samples were non-toxic (calculation of an LC_{50} was not possible in such cases, but these results are presented as 100% LC_{50} in Fig. 4). Samples deficient in N and P were less toxic than the controls, but more toxic than the full nutrient addition samples.

DISCUSSION

The population dynamics and dominance of *Prymnesium parvum* varied as a function of nutrient availability in these experiments and we interpret this as indication of its potential for competitive success. Although *P. parvum* populations were stimulated with full nutrient additions, other phytoplankton were also stimulated, and chlorophytes eventually dominated. When P deficient nutrients were added, *P. parvum* population dynamics were not much different from that seen in the controls, where *P. parvum* dominated the community. But, when initial nutrients were N deficient, *P. parvum* populations performed best.

Allelopathic substances produced by *Prymnesium parvum* are known to influence competing algae (Johansson & Granéli 1999, Fistarol et al. 2003, 2005, Granéli & Johansson 2003a,b, Barreiro et al. 2005, Uronen et al. 2005). In addition, nutrient stress is known to increase toxicity (Granéli & Johansson 2003a, Uronen et al. 2005). Our results suggest that allelopathy was probably not a prominent factor influencing the biomass and composition of the phytoplankton when nutrients were not limiting (i.e. samples were not toxic and *P. parvum* dominance did not occur). In treatments receiving N and P deficient nutrient additions, however, allelopathy could have played an essential role.

Physiological differences in growth kinetics between competitors may also be a likely factor involved in the competitive success of *Prymnesium parvum*. N:P ratios were high in the full nutrient addition and P deficient nutrient addition treatments, and low in the N deficient nutrient addition treatment. Using classic resource competition theory (see Grover 1997) superior competitors can be identified based on their half-saturation coefficient for reproductive growth (k_S) and their maximum specific growth rate (μ_{max}). Species with the highest value for the growth affinity μ_{max}/k_S are superior competitors when nutrients become limiting, and species with the highest value for μ_{max} are superior competitors when nutrients are non-limiting (Healey 1980).

In recent laboratory experiments using the Texas strain of *Prymnesium parvum* P limited growth kinetics were examined through ranges of temperatures and salinities relevant to conditions during the seasons when this experiment was conduced, 10 to 20°C and 1 to 4 psu (Baker 2007). The half-saturation coefficient for reproductive growth varies from 0.0026 to 0.47 µM, depending on temperature and salinity, but is usually below 0.01 µM. The maximum specific growth rate ranges from 0.11 to 0.84 d^{-1}. For chlorophytes, k_P values are typically 0.01 µM or higher, and μ_{max} values vary between 0.5 d^{-1} and greater than 2 d^{-1} (Tilman et al. 1986, Grover 1989, Sommer 1989, Grover et al. 1999). For diatoms, k_P values vary between less than 0.003 µM and 1.9 µM, and μ_{max} values vary between 0.3 to 2.1 d^{-1} (Tilman et al. 1986, Grover 1989, Sommer 1989, Grover et al. 1999). Given these parameters, some chlorophytes would likely have a significant competitive advantage over *P. parvum* in the full nutrient addition and P deficient treatments because of higher μ_{max}, but did not dominate in the latter, possibly due to the allelopathic effect of the toxins production by *P. parvum* (Fig. 4). Thus, *P. parvum* was able to dominate the community. Diatoms probably did not co-dominate with chlorophytes in the full nutrient addition treatments because SiO_3 reached concentrations known to be limiting for many diatoms (Tilman et al. 1982, Grover 1997). In the P deficient treatments, at least for the fall and winter experiments, SiO_3 was not depleted and some diatoms would probably have had a competitive advantage over *P. parvum* because of their higher μ_{max}. In summary, available evidence on P

dependent growth kinetics suggests that many algal species present in these experiments were probably better competitors than *P. parvum* under P deficient conditions. An estimate of k_N for *P. parvum* is ~0.005 µM (J. Baker unpubl.). In comparison, k_N values for chlorophytes studied in Texas reservoirs exceed about ~0.7 µM, while values for diatoms, cryptophytes and cyanobacteria exceed about 0.1 µM (Grover et al. 1999). This comparison suggests that *P. parvum* is a better competitor than co-occurring species under N deficient conditions, and probably contributed to the higher population densities of *P. parvum* under these conditions.

With the addition of full nutrients, ambient toxicity of *Prymnesium parvum* to fish was greatly reduced, becoming non-toxic in most samples, which mirrored observations described in Roelke et al. (2007) and is consistent with previous work showing elevated toxicity of *P. parvum* under nutrient limitation (Johansson & Granéli 1999, Fistarol et al. 2003, Granéli & Johansson 2003a,b, Barreiro et al. 2005). However, nutrient enrichments that were either N or P deficient only moderately reduced toxicity compared with controls receiving no nutrient additions. Our findings with *Pimephales promelas*, and those of others, suggest that toxic effects of *P. parvum* might decrease with eutrophication because the incidence of prolonged periods of nutrient limitation might decrease. Worldwide locations of *P. parvum* blooms and associated fish kills, however, indicate otherwise (van Rijn & Shilo 1989, Kaartvedt et al. 1991, Guo et al. 1996, Amsinck et al. 2005), which suggests that toxic bloom formation is influenced by more than just availability of nutrients. For example, *P. parvum* is known to display mixotrophic behaviors (Granéli 2006); therefore, its dominance may be enhanced both by its abilities to produce toxin and to consume competing algae and bacteria.

The natural *Prymnesium parvum* bloom that occurred in the waters of Echo Cove during our experiments disrupted grazing by dramatically reducing populations of zooplankton (see Roelke et al. 2007). In turn, this impacted our experimental findings. During our winter and spring experiments, treatments produced no significant effects on zooplankton because their populations were so low at the start of these experiments. On the other hand, the plankton community for our fall experiment had higher zooplankton populations (not yet affected by the *P. parvum* bloom). In this experiment, nutrient additions significantly increased rotifer populations and decreased adult copepod populations. *Prymnesium parvum* populations initially increased then declined rapidly in the full nutrient addition treatments during this experiment. *Prymnesium parvum* cells were apparently non-toxic and rotifer populations increased dramatically in this treatment. While not measured directly, this observation suggests that grazing by rotifers might have been an important loss factor for *P. parvum* during the fall.

Regarding immigration, in theory, initial population densities of competitors can influence the dynamics of phytoplankton assemblages (Huisman & Weissing 2001, Roelke et al. 2003). Research focused on spatial ecology in terrestrial systems suggests a complex unimodal relationship between immigration rate and community structure (Loreau & Mouquet 1999, Liebold & Miller 2004). At some immigration rates, local interactions are much stronger than the destabilizing effect of immigration, and immigration at these rates has little influence on community dynamics. At other rates of invasion, however, local interactions are not strong enough to mask the effect of immigration. A recent theoretical study that focused on aquatic systems demonstrated that immigration rates as low as 0.1% of the total phytoplankton biomass per day could dramatically alter plankton succession dynamics, in some cases leading to single species dominance (Roelke & Eldridge 2008).

In our bottle experiments, enhancement of initial *Prymnesium parvum* populations produced no significant effects during our fall and spring experiments. During the winter experiment, however, our simulated immigration event significantly enhanced *P. parvum* population dynamics. It appears that there are unidentified local interactions whose resistance to immigration varies in time, and which were strong enough during the fall and spring to prevent immigration effects. However, during the winter, these local interactions were apparently not as strong, and the community was vulnerable to immigration effects.

CONCLUSIONS

The role of nutrients in the formation of toxic *Prymnesium parvum* blooms seems complex in that enrichment with full nutrients allowed higher population densities, but reduced toxicity. This enrichment allowed other algae to accumulate biomass as well, so that unlike natural blooms, *P. parvum* did not dominate the assemblage despite its increased cell densities. Furthermore, when zooplankton were present at greater population densities during the start of an experiment (i.e. fall), treatments receiving nutrient enrichment showed increased rotifers with eventual decreases in *P. parvum*, suggesting that grazing losses were significant. Apparently, *P. parvum* only gained a competitive advantage under N deficiency when the N:P ratio was low. The apparent inability of *P. parvum*

to compete with other phytoplankton during P deficient, high N:P conditions was probably linked to its low maximal growth rate and intermediate affinity for P compared with other algae. However, *P. parvum* bloom formation probably involves more than resource availability. The timing of immigration events might also contribute to bloom development. If *P. parvum* bloom initiation is a function of immigration, e.g. from excystment and movement into the water column, then localized fertilization over 'seed beds' might prevent *P. parvum* cells from becoming toxic and circumvent bloom development. From a management perspective, this merits further study.

Acknowledgements. We are grateful to C. Miller, C. Robertson and H. P. Li, who assisted with the in-lake experiments, and M. Alvarez, S. Garcia and J. Stanley for assistance with toxicity studies. We are also grateful to personnel associated with Possum Kingdom State Park, especially Mr. J. Nichols for allowing us to use the 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: Frede Thingstad, Bergen, Norway

Submitted: May 9, 2007; Accepted: March 4, 2008
Proofs received from author(s): June 18, 2008