Refining a Predictive Understanding of Physical, Chemical and Biological Factors Influencing *Prymnesium parvum* Population Dynamics and Toxicity

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I. Background and Objectives

The overarching objectives of this research project were two-fold: 1. Examine the role of interactions between *Prymnesium parvum* and phytoplankton competitors through the mechanism of allelopathy – arising from substances produced both by *P. parvum* and by co-occurring algae – as it relates to bloom initiation, persistence, toxicity, and termination; and 2. Continue development of a predictive numerical model where competitive interactions, life history, the physicochemical environment, and lake dimensions are more accurately depicted. Below we summarize our observations related to accomplishing these overarching objectives. While a number of these investigations have already lead to peer-reviewed manuscripts published, in press or in review, we continue development of additional publications from studies completed over the past year. For example, Appendix A includes several publications resulting from efforts supported by this project.

II. Research Findings

1. Field Studies

1.1 Background

The field-monitoring component of this project was developed with the purpose of characterizing *P. parvum* bloom dynamics. Lake Granbury, a reservoir constructed on the main stem of the Brazos River, has experienced recent toxic blooms of *P. parvum* that have resulted in massive fish kills and concerns about general water quality. Lake Waco, a reservoir constructed on the main stem of the Bosque River, has viable *P. parvum* populations, but does not experience harmful blooms. Thus, comparisons between Lakes Granbury and Waco allow for comparisons of environmental conditions leading to bloom formation. Both Lake Granbury and Lake Waco are critical to this region as being primary water supplies, sources of revenue and recreational hotspots.

This project addressed these water quality issues by providing critical information about the relationships between *P. parvum*, salinity, nutrients and other water quality and food web parameters. In Lakes Granbury and Waco, plankton, nutrient and water quality samples were collected at fixed-location stations, and high-resolution spatial maps were generated using an on-board dataflow technology of various plankton and water quality parameters. Linkages between the toxic *P. parvum* blooms and environmental conditions were examined. Findings from the first year of monitoring in Lake Granbury were written up in manuscript form, and they are currently In-Review with a scientific journal. This manuscript and others resulting from this project are included as Appendix A. All the data from monitoring of Lakes Granbury and Waco are included in the Appendices B through I.

1.2 Project Goals and Accomplishments

Objectives
• To conduct monthly fixed-station sampling in Lakes Granbury and Waco where surface water measurements included inorganic nutrients (NO$_3^-$, NO$_2^-$, NH$_4^+$, PO$_4^{3-}$), dissolved organic carbon, phytoplankton biomass and community composition, total and fecal coliform bacteria concentration, *E. coli* concentration, and toxicity; and profile data included various water quality parameters (DO, pH, temperature, conductivity, turbidity, oxidation-reduction potential).

• To conduct monthly system-wide sampling of Lakes Granbury and Waco using a newer technology, Dataflow, where measured parameters included chlorophyll *a*, dissolved organic matter, transparency, photosynthetically active radiation, conductivity, and temperature.

• To investigate potential temporal and spatial linkages between *P. parvum*, toxicity, salinity, nutrients, and other water quality and food web parameters.

**Accomplishments**

The data records for Lakes Granbury and Waco span September 2006 through July 2009, and September 2007 through July 2009, respectively, which corresponds to the initiation of the project in 2006 and the expansion of the project in 2007. These sampling dates span a range of hydraulic conditions from high inflows into both lake systems (2007) to low inflows (2008, 2009). Inflows, estimated hydraulic residence time and our dates of sampling are shown in Appendix B.

**1.3 Sampling of Fixed Stations**

During each monitoring trip we sampled fixed-location stations (Appendix C), which enabled us to characterize the plankton seasonal succession pattern (phytoplankton and zooplankton) and determine seasonal changes in various water quality parameters (inorganic nutrients, toxicity, pH, temperature, dissolved oxygen, Secchi depth). We sampled 20 fixed-position stations in Lake Granbury and 10 stations in Lake Waco.

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 seconds and then extracted in the dark for 20-24 h at -20° C. Extracts were filtered (0.2 µm) and injected (300 ul) into an HPLC system equipped with reverse-phase C$_{18}$ columns in series (Rainin Microsorb-MV, 0.46 x 10 cm, 3mm, Vydac 201TP, 0.46 x 25cm, 5mm). A nonlinear binary gradient, adapted from Van Heukelem et al. (1994), was used for pigment separations. Solvent A consisted of 80% methanol and 20% ammonium acetate (0.5M adjusted to pH 7.2), and Solvent B was 80% methanol and 20% acetone. Absorption spectra and chromatograms were acquired using a Shimadzu SPD-M10av photodiode array detector, where pigment peaks were quantified at 440 nm.

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

Enumeration of *P. parvum* population density was performed using a settling technique (Utermöhl 1958). A 0.5-1 mL subsample was taken out of a well-mixed sample (preserved using glutaraldehyde, 3% v/v). Subsamples were settled for a 24 h period, then counted using an inverted, phase-contrast, light-microscope (400x, Leica Microsystem Inc.). Depending on the density of material in the samples, a range of 15-50 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 *P. 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 will include *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.

Our sampling also included enumeration of zooplankton and bacteria. Zooplankton were collected using a 12-liter 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 (40x and 200x, Leica Microsystem Inc.). For each individual counted, geometric shapes were determined that best corresponded to the shape of the zooplankter and dimensions were measured that enabled calculation of the individual’s biovolume (Wetzel and Likens 1991). Identification was to the taxonomic level of genus. For bacteria (Lake Granbury only), samples were preserved using paraformaldehyde 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 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 and Sterns 1967, Harwood and Kuhn, 1970), analyses included nitrate (NO₃), nitrite (NO₂), ammonia (NH₄), orthophosphate (PO₄) and silicate (SiO₃).

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

Samples were collected from Lakes Granbury and Waco and transported to the laboratory where toxicity tests were initiated within 24 hrs. 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 <i>P. promelas</i> toxicity test sample from each experimental unit, three replicate chambers with 7 organisms per chamber were used to assess toxicity at each dilution level. <i>D. magna</i> bioassays followed established US EPA protocols (US EPA 1994). RHW, was prepared according to standard methods (APHA 1998), and used as control treatment water for all toxicity assays. Alkalinity (mg/L as CaCO₃) and hardness (mg/L as CaCO₃) of RHW was measured potentiometrically and by colorimetric titration, respectively, before initiation of acute studies (APHA 1998). Specific conductance (µS/cm), pH and dissolved oxygen (mg/L) of RHW was measured before toxicity testing. All toxicity tests were performed in climate controlled chambers at 25 ±1°C with a 16:8 hour light-dark cycle. Less than 48 h old fathead minnow larvae were fed newly hatched <i>Artemia</i> nauplii two hours before initiation of testing (US EPA 2002). <i>D. magna</i> were fed a Cerophyll®/green algae suspension daily, which was prepared according to methods reported previously (Brooks et al. 2004, Dzialowski et al. 2006). LC₅₀ values for fathead minnow toxicity tests were estimated as percentage of ambient sample using Probit (Finney 1971) or Trimmed Spearman Karber (Hamilton et al. 1977) techniques, as appropriate.

1.4 Data graphs

Contour plots of fixed station data are provided in Appendices D and E for Lake Granbury. Data from the stations located in coves of Lake Granbury are provided in Appendix F. The data graphs are as follows:

Lake Granbury (deep water stations)

Figures D-1 through D-3 - Chlorophyll a, P. parvum, toxicity
Figures D-4 through D-8 – pH, temperature, salinity, Secchi depth, turbidity
Figures D-9 through D-13 – Cladoceran, copepod adult and nauplii, total rotifers, protozoan
Figures D-14 through D-15 – Phosphate, dissolved inorganic nitrogen
Figures D-16 through D-19 – Dissolved organic carbon, total bacteria, fecal coliform, E. coli
Figure D-20 - Cyanobacteria

Lake Granbury (shallow water stations)

Figures E-1 through E-3 - Chlorophyll a, P. parvum, toxicity
Figures E-4 through E-8 – pH, temperature, salinity, Secchi depth, turbidity
Figures E-9 through E-13 – Cladoceran, copepod adult and nauplii, total rotifers, protozoan
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Figures E-14 through E-15 – Phosphate, dissolved inorganic nitrogen
Figures E-16 through E-19 – Dissolved organic carbon, total bacteria, fecal coliform, E. coli
Figure E-20 - Cyanobacteria

Lake Granbury (cove stations)

F-1 - Description of cove stations
F-2, 3 - Salinity, Temperature and Secchi depth
F-4, 5 - NO3, NH4, and SRP
F-6, 7 - P. parvum, Chlorophyll a, Zooplankton
F-8, 9 - Fecal coliform, E. coli, Dissolved organic carbon
F-10, 11 - Total bacteria

Contour plots of fixed station data are provided in Appendix G Lake Waco. The contour plots are as follows:

Lake Waco

Figures G-1 through G-3 - Chlorophyll a, P. parvum, toxicity
Figures G-4 through G-8 - pH, temperature, salinity, Secchi depth, turbidity
Figures G-9 through G-13 - Cladoceran, copepod adult and nauplii, total rotifers, protozoan
Figures G-14 through G-15 – Phosphate, dissolved inorganic nitrogen
Figure G-16 - Dissolved organic carbon
Figure G-17 - Cyanobacteria

1.5 High-Resolution Spatial Mapping

We measured spatial patterns of water quality in Lakes Granbury and Waco at monthly intervals with Dataflow, a high-speed, flow-through measurement apparatus developed for mapping physicochemical parameters in shallow aquatic systems (Madden and Day 1992). We used this integrated instrument system to concurrently measure chlorophyll a, dissolved organic matter, transparency, salinity, and temperature from a boat running closely spaced transects. Measurements were taken at 4-second intervals from ~20 cm below the surface. An integrated GPS was used to simultaneously plot sample positions, allowing geo-referencing of all measurements for each variable.

GPS and dataflow information were used to create highly detailed contour maps of water quality parameters in relation to physiographic features. We also collected discrete water samples from the flow-through system during our continuous sampling for laboratory analysis of multiple parameters that were used to calibrate the dataflow unit. In addition to these samples, we measured profiles of water quality parameters that include dissolved oxygen and temperature to determine the degree of stratification of the water column.

High-resolution spatial maps are provided in Appendix D for Lake Granbury, and Appendix F for Lake Waco. The maps are as follows:
Data Maps

High-resolution data maps for Lake Granbury are provided in Appendix H, as follows:

Figures H-01 through H-26 - Chlorophyll $a$
Figures H-27 through H-53 - Salinity
Figures H-54 through H-80 - Temperature
Figures H-81 through H-104 - pH
Figures H-105 through H-131 - Turbidity
Figures H-132 through H-158 - Dissolved Organic Carbon

High-resolution data maps for Lake Waco are provided in Appendix I, as follows:

Figures I-1 through I-17 - Chlorophyll $a$
Figures I-18 through I-34 - Salinity
Figures I-35 through I-51 - Temperature
Figures I-52 through I-68 - pH
Figures I-69 through I-85 - Turbidity
Figures I-86 through I-102 - Dissolved Organic Carbon

1.6 Findings to date

Briefly, our monitoring and data analyses suggest:

Regarding the 2006/07 $P.$ parvum bloom in Lake Granbury

1. During the initiation period (September through December), lack of stress, and generally favorable conditions suggest that the $P.$ parvum bloom could have initiated from in situ growth in Lake Granbury, and that allelopathic effects were not important at this early stage. Another possibility is that conditions favorable to bloom initiation occurred in a lake up-river of Lake Granbury, i.e., Lake Possum Kingdom, and that the bloom developed due to mass effects resulting from large migrations of toxic $P.$ parvum cells into Lake Granbury.

2. During the period of bloom development and peak density (January and February 2007), environmental conditions were stressful to $P.$ parvum. While nutrient concentrations did not appear to be strongly limiting, predicted growth rates based on temperature, salinity and light were near zero, primarily due to the low winter temperatures.

3. The highest observed fish toxicities were during these winter months, the $P.$ parvum bloom was extensive throughout the entire system, and the bloom was patchy (as some areas of the lake experienced elevated phytoplankton biomass while others experienced the bloom at lower densities).

4. The $P.$ parvum bloom was near-monospecific (~92% decreasing to 87% before the first flushing event), with diatoms, green algae and cyanobacteria comprising much of the remaining biomass.
5. The presence of other algae, although not in high proportion, during this bloom might have influenced zooplankton, allowing them to persist during the bloom.

6. The first large inflow event, which occurred in April 2007, obliterated the *P. parvum* bloom. Single day estimates of hydraulic flushing for Lake Granbury at this time ranged between 2.7 and 16 d⁻¹.

7. In addition to the direct loss of cells through flushing, *P. parvum* likely ceased production of toxins with the rapid increase in nutrient concentrations. This removal of toxicity would have greatly diminished *P. parvum*’s ability to compete with other phytoplankton or inhibit zooplankton.

8. The bloom started to decline somewhat before hydraulic flushing affected the lake. It is unclear why. Pathogens might have influenced *P. parvum* population densities just prior to the impact of the first inflow event.

**Regarding the 06/09 data record for Lake Granbury (preliminary analysis)**

9. The high freshwater inflows into Lake Granbury during the winter and early spring period of 2007 lowered the salinity of the lake from ~1.5 to <0.5 (a level below which growth of *P. parvum* is severely limited). However, the lower inflows into the lake since that time have allowed the salinity of the lake to gradually increase, where salinities of ~1.0 are now observed (summer 2009).

10. Following the high inflows of 2007, *P. parvum* has not bloomed significantly in Lake Granbury. During the late winter of 2008, however, noticeable *P. parvum* populations occurred and measurable water toxicity was observed in the spring 2009. It appears that with the gradual increase in salinity, *P. parvum* populations increase in biomass during the winter period.

11. Soluble reactive phosphorus was at concentrations limiting to many phytoplankton during the fall of 2006, the months before and during the period of *P. parvum* bloom formation. After the high inflows during the spring of 2007, SRP concentrations have been at higher concentrations.

12. All zooplankton groups accumulate significantly higher population densities in years when there is no *P. parvum* bloom.

13. *P. parvum* population demographics in the coves of Lake Granbury differ from the open lake. In the coves situated towards the head of the lake, *P. parvum* occurs at high population densities in the summer and also the winter of 2008. In coves closer to the dam *P. parvum* occurred during the winter of 2008 and summer of 2009. The likelihood that coves might act as incubators for *P. parvum*, or refuges during the summer months merits further investigation.

**Regarding the 07/09 data record for Lake Waco (preliminary analysis)**

14. Significant *P. parvum* populations or water toxicity were not observed in Lake Waco during this sampling period.

15. Salinity never exceeded 0.3 in Lake Waco, which is below a level where *P. parvum* can grow effectively.
2. Laboratory Experiments

2.1 pH Experiments

We previously observed increased ambient toxicity when pH was increased in field samples from Lakes Granbury and Whitney, which were collected during P. parvum blooms in Spring 2007. In this project we performed pH-toxicity studies with P. parvum cultures in the laboratory and modeled physicochemical properties of prymnesins (see Valenti et al in press, in Appendix A).

Cultures were terminated on day 28 after reaching late stationary phase, and experiments were immediately performed to assess the toxicity of each replicate culture. Cells were enumerated at this time showing densities in high nutrient (f/2) cultures of $2.0 \times 10^5$, $1.5 \times 10^5$, and $2.1 \times 10^5$ cells mL$^{-1}$, and densities in low nutrient (f/8) cultures of $1.5 \times 10^5$, $1.3 \times 10^5$, and $1.5 \times 10^5$ cells mL$^{-1}$. Survival in the ASW and RHW controls was > 90% for all tests at all pH levels. The LC$_{50}$ values for experiments with P. promelas were consistently lower for the low nutrient (f/8) treatment compared to those for high nutrient (f/2) (Fig. 3 in Valenti et al in press). Estimated LC$_{50}$s were more variable between replicates for the f/2 treatment and increased exposure time resulted in greater toxicity, whereas temporal effects were less evident for the f/8 treatment (Fig. 3 in Valenti et al in press).

Samples of f/2 and f/8 whole cultures and cell-free filtrate were consistently more toxic to P. promelas when exposure occurred at pH 8.5 compared to pH 7.5 or 6.5 (Fig. 4). For the f/2 treatment, 50% of exposed individuals died at pH 6.5 in undiluted whole culture; however, only 15% died in the cell free filtrate. Cell free filtrates were also less potent than the whole culture at pH 7.5 and 8.5 for the f/2 treatment; however, differences in toxicity between whole cultures and cell-free filtrates were not as apparent for the f/8 treatment (Fig. 4 in Valenti et al in press). The LC$_{50}$ values were markedly lower for filtered and unfiltered cultures grown in f/8 media compared to those in f/2 media; however, endpoints were consistently lower at higher pH for all experiments (Table 3 in Valenti et al in press).

The structures of prymnesin-1 and -2 (Fig. 5 in Valenti et al in press) lead to an estimated pKa value of 8.9 for both prymnesin-1 and -2 (Table 4 in Valenti et al in press). LogD between pH 5.5 and 8.5 ranged between 2.8 and 5.2 for prymnesin-1, and 2.5 and 4.9 for prymnesin-2, respectively. At pH 6.5 approximately 16% of the prymnesins are predicted to be ionized, whereas at pH 8.5 only 0.002% are predicted to be ionized (Table 4 in Valenti et al in press).

These studies with laboratory cultures and samples from reservoirs experiencing P. parvum blooms consistently indicate that toxins released by P. parvum are more potent when exposure occurred at a higher pH of 8.5 than at lower pH. The predicted physiochemical properties of prymnesins indicate that these toxins are weak bases (pKa = 8.9) and, thus, a greater proportion of the prymnesins were likely unionized in higher pH treatment levels (e.g., 8.5). We propose that a higher proportion of prymnesins in unionized forms at pH 8.5 explains the greater toxicity observed in field and laboratory studies. This novel explanation for pH-dependent ambient toxicity associated with P. parvum suggests that variability in pH among and within aquatic systems may be an important factor governing the occurrence of fish kills.
Unionized forms of contaminants often have greater propensity to cross cellular membranes due to their lower polarity and thus are more likely to partition into organisms (Simon and Beevers 1951, Sarrikoski et al. 1986, US EPA 1986, Fisher et al. 1999, US EPA 1999, Nakamura et al. 2008, Valenti et al. In press). The importance of ionization state for ambient toxicity and environmental management is evidenced by the integration of site-specific ambient water quality criteria for contaminants such as pentachlorophenol and ammonia (US EPA 1986, 1999). Ammonia, like the prymnesins, has a pKa value of ~9. In addition, ammonia is a weak base and the ionization state of the compound changes appreciably across environmentally relevant surface water pH gradients (US EPA 1999). Consequently, acceptable ammonia loads in stream are 13-fold lower if the receiving system has a pH of 9 compared to a pH 6. Weak bases have a greater propensity to cross cellular membranes if the pH at which the exposure occurs approaches and surpasses the compound’s pKa value (US EPA 1999, US EPA 1986, Simon and Beevers 1951, Nakamura et al. 2008, Fisher et al. 1999). Greater interaction with target sites (e.g., gill membranes) would increase the likelihood of adverse effects in exposed individuals; hence, prymnesin-1 and -2 would poses greater risk to aquatic life when these toxins exist predominantly as the unionized form.

In laboratory tests examining the effectiveness of ammonium and barley straw extract to control *P. parvum*, Grover et al. (2007) only observed toxicity in samples with pH > 8. These observations were consistent with Lindholm et al. (1999) who observed fish kills attributed to *P. parvum* in a brackish-water lake when pH ranged between 8.9 and 9.4. Additional studies with *P. parvum* conducted under higher salinity conditions have reported similar pH influences during in vivo experiments. Shilo and Ashner (1953) noted that fish were 5-times more sensitive at pH 9 compared to pH 6, and demonstrated that the effects of pH manipulation were reversible during adjustments to and from pH 7 and 6. Ulitzur and Shilo (1964) investigated the toxicity of *P. parvum* toxins, along with various chemicals identified as cofactors, over a range of pH 7 to 9 and consistently noted markedly greater toxicity at higher pH. McLaughlin (1958) observed that high pH shortened the exposure time associated with onset of mortality. The pH-dependent activity of *P. parvum* toxins could also reduce internal damage to cells that are producing or storing toxins. Extracts of *P. parvum* induced “self-toxicity,” reducing growth rates and causing lysis (Olli and Trunov 2007). It is plausible that these ionizable toxins are stored inside cells of *P. parvum* at lower physiological pH, and are thus more ionized than when released outside the cell, where pH values may be higher.

Some previous results from in vitro hemolytic experiments with *P. parvum* contradict the results of the in vivo experiments reported here and elsewhere. Blood cells rupture more often when exposures are completed at pH < 6 (Igarashi et al. 1996, 1998 Kim and Padilla 1977). Prymnesin-1 and -2 have multiple ionizable groups so that changes in the protonation state could alter the configuration of the toxins. In turn, the interaction of prymnesins with specific binding sites in blood cells and fish gill membranes could depend on the structural configuration, which may be influenced by pH, and thus may be different among such in vivo and in vitro experiments. Alternatively, prymnesins might not be the only, or even the most important toxins produced by *P. parvum*, and hemolytic activity in vitro might have different determinants than lethal activity in vivo (Schug et al., In press).
Cell density alone has been long recognized as a poor predictor of toxicity for samples containing *P. parvum* (Reich and Aschner 1947, Baker et al. 2007, Grover et al. 2007), and this generalization remains apparent during monitoring in Texas reservoirs. Ionization state of the toxins may partially explain some of this variability and reduce uncertainty related to ecological risk assessments and risk management of *P. parvum* blooms. The observed pH-dependent toxicological relationships and the physiochemical properties predicted by computer modeling suggest that the toxins prymnesin-1 and -2 act as weak bases in aqueous solutions. Because their predicted pKa values are within the range of variation of pH in many surface waters, modest variations in pH could have a large influence on toxicity.

The production of ionizable toxins offers potential advantages to *P. parvum* and may be related to biochemical adaptations associated with its marine origins. The results of our studies and others suggest that the toxins released by *P. parvum* are more potent to gill-breathing organisms when exposure occurs at pH levels representative of those measured in marine systems (e.g. pH > 8). Moreover, blooms of *P. parvum* and other HABs can alter the environment and cause pH to increase through depletion of carbon dioxide during daytime photosynthesis (Pearl 1988). In fish hatchery ponds impacted by *P. parvum*, pH measurements vary by more than one unit between the daylight and evening hours (Shilo and Shilo 1953). Thus, *P. parvum* not only produces toxins during bloom formation, but could also make conditions that increase the potency of their toxins. For example, our research team recently observed high pH levels in Lake Granbury during a *P. parvum* bloom that resulted in ambient toxicity to fish, compared to lower pH levels before and after this bloom (Roelke et al. in review).

Considering site-specific pH may be especially important for environmental management and ecological risk assessments of *P. parvum* because of the inherent linkage between physiochemical properties of waters and the organisms that inhabit them. There is far greater spatiotemporal variability in the pH of inland waters compared to marine systems. Some of this variability arises from natural variations in geomorphology, geochemistry, and climate. Anthropogenic activities also influence the pH of inland waters. Inland waters where *P. parvum* blooms have occurred are often affected by altered hydrology, land use changes in the catchment, and increased nutrient loading. In the southwestern and south central U.S., *P. parvum* blooms and fish kills are often limited to waters where pH is typically high due to an arid climate, limestone bedrock, and sparse vegetation. Consequently, prospective ecological risk assessment approaches may be possible for predicting the occurrence of harmful blooms of *P. parvum* by relating watershed land-use and geography to water quality.

### 2.2 Light experiments

**Background**

Although light intensity may not strongly influence site-specific toxin production by *P. parvum* and associated ambient aquatic toxicity, relative to other physical and chemical factors, light intensity may influence the stability of toxins in aquatic ecosystems. Rahat and Jahn (1965) found that *P. parvum* growth was possible in the dark when glycerol was added to the culture medium. Results of their experiments showed that cultures grown in the dark were more toxic than those grown with alternating dark and light periods, indicating that light is not a requirement for toxin synthesis. They also concluded that any toxicity assays performed from cultures grown...
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in alternating light-dark periods only reveal the net results of production and “inactivation” of the toxin (Rahat and Jahn 1965). Another study also demonstrated that constant illumination with a fluorescent lamp reduced toxicity and “inactivated” the toxins (Reich and Parnas 1962). However, it is possible that the cultures in this study did not produce toxins while under constant illumination. Toxicity was observed when they alternated light and dark periods (Reich and Parnas 1962). While such studies manipulating the light environment of whole cultures were important for identifying factors that affect the physiological status and resulting toxicity of *P. parvum* cells, they did not allow for evaluation of which factors affect the environmental fate of toxins once released into the water column.

Only one published study performed light experiments with toxins separated from the cells (Parnas et al. 1962). Parnas et al. (1962) removed cells by centrifugation and the filtrate was exposed to artificial light and one wavelength of ultraviolet light. Toxicity tests were then performed with *Gambusia affinis* fish to evaluate the differences in toxicity of the different light treatments. They found that “photoinactivation” of the toxin occurred and that it was not affected by the presence of cells, cell pigments, oxygen, or glutathione. Whereas Parnas et al (1962) used the term “photoinactivation” to describe this observation, it is likely that photolysis of the prymnesins occurred, which reduced the concentration of bioavailable toxins, reduced exposure to fish, and thus reduced the magnitude of toxicity.

Photolysis is a chemical process by which molecules are broken down through the absorption of light (Newman and Unger 2003). Certain chemical groupings and classes, such as compounds with conjugated double bonds and aromatic rings, are more susceptible to be degraded because their internal energy states correspond to the incoming light energy (Newman and Unger 2003). There are two forms of photolysis, direct and indirect. Direct photolysis occurs when photons directly interact with and break molecular bonds. Indirect photolysis occurs when photons are absorbed by another compound, such as humic acid, which then forms a reactive species, such as a hydroxyl radical, which in turn facilitates the degradation of the contaminant (Newman and Unger 2003).

We performed two studies to examine the possible photolytic effect of natural sunlight on *P. parvum* cell-free filtrates from cultures grown under conditions comparable to when blooms occur in Texas inland water bodies. In the first study, filtrates were exposed to two different sunlight intensities (full sunlight, ~50% sunlight), or no sunlight then acute toxicity tests with *P. promelas* were performed and results compared among treatment levels. In the second study, filtrates were exposed to natural sunlight for different time periods (e.g., 0.5, 1, 2, 4, 8 hrs) then acute toxicity tests with *P. promelas* were performed. It was important to isolate excreted toxins from *P. parvum* because this eliminated any confounding results due to physiological status of the organism or toxin production when cells remain in whole culture. These studies represent the first attempt to evaluate the effect of natural light, rather than artificial laboratory light, on extracellular prymnesins.

**Materials and Methods**

*Prymnesium parvum Culture*

*P. parvum* cells originally obtained from The University of Texas at Austin Culture Collection of Algae (strain UTEX LB 2797) were cultured in an incubator (VWR Model 2015, West Chester,
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PA, USA) at 20°±1°C in 2.4 psu artificial sea water (ASW; Berges et al 2001, Appendix A) in f/8 media (Guillard 1975; Appendix B) on a 12:12 light:dark cycle. After cells reached late stationary phase they were filtered out of the media using glass fiber filters (GF/C, Whatmann), and the filtrates were collected in foil-covered Erlenmeyer flasks. These flasks were stored under refrigeration (4±1 °C) in the dark for less than 24 hours before the natural sunlight studies were initiated.

**Sunlight Exposure**

**Light Study 1:** Three light treatment levels were selected including full sunlight, partial sunlight, and no sunlight. The partial sunlight treatment was made by placing a shade cloth affixed to a frame over the top of the beakers, which were selected as experimental units for incubation of cell-free filtrate. The dark treatment level was held under a cardboard box beside the light treatments. To perform the sunlight exposures, 600-ml beakers filled with 500 mls of cell-free filtrate or ASW controls were placed in their respective light treatment level. The study was conducted at the Baylor Experimental Aquatic Research (BEAR) facility at the Lake Waco Wetlands, Waco, Texas for eight hrs beginning approximately one hour after sunrise. Light intensity was quantified approximately every 20 minutes for the full and partial sunlight treatment levels using a light meter. At the end of the eight hr period, all beakers were covered with Parafilm, placed in the dark in an ice-filled cooler, and returned to the lab where they were stored overnight under refrigeration (4±1°C) in the dark. Less than 24 hours later, toxicity assays with the model test organism *Pimephales promelas* were initiated with samples from the light exposure study. In addition, toxicity was assessed for filtrate that was stored under refrigeration in the dark after harvesting the cells, which hereafter is called “lab dark control.”

**Light Study 2:** Two light treatment levels were selected including full sunlight and no sunlight. Sunlight exposures followed those approaches described above for Light Study 1. In contrast to the first study we subsampled beakers after 0.5, 1, 2, 4 and 8 hrs of exposure to natural sunlight. At the end of the eight hr period, all beakers were covered with Parafilm, placed in the dark in an ice-filled cooler, and returned to the lab where they were stored overnight under refrigeration (4±1°C) in the dark. Less than 24 hours later, toxicity assays with juvenile *P. promelas* were initiated with samples from the light exposure study, and for lab dark controls.

**Laboratory Toxicity Bioassays**

Acute toxicity of the filtrates was assessed generally according to US EPA method 2000.0 (US EPA 2002) using *Pimephales promelas* as a model test organism. Less than 48-hour old juveniles (all hatched within 24 hours) were allowed to feed on *Artemia* nauplii at least two hours before initiation of the test. The *P. parvum* cell-free filtrate for each treatment was diluted with ASW to dilutions of 100, 50, 25, 12.5, 6.25, and 3.13% filtrate. All solutions were adjusted to pH of 8.5, including the ASW diluent, before mixing the dilutions and filling the test chambers. The test chambers consisted of three replicate 100-ml beakers filled with 80 mls of test solution at each concentration for each of the four treatments. Beakers of light-exposed ASW and reconstituted hard water (RHW; APHA et al 1995) not exposed to ambient sunlight served as controls. The beakers were loaded with five fish each and placed into an incubator (Norlake®; Hudson, Wisconsin, USA) at 25±1°C on a 16:8 L:D cycle for 48 hrs.
Modification to the EPA test method was made by using a time-to-death study design in which mortality was assessed at several time points, rather than only at 24 and 48 hrs. Time points used were 1, 2, 3, 4, 5, 6, 9, 12, 18, 21, 24, and 48 hrs. The number of dead fish was recorded at every time point, but not removed until 24 hours according to standard methods (US EPA 2002). In the second light study, aquatic bioassays were performed in the dark to avoid potential influences of incubator light on toxin stability.

**Statistical Analyses**
Median lethal concentration \( (LC_{50}) \) values were calculated for all filtrates using an appropriate method of analysis based on attributes of the data (e.g., Spearmann-Karber or Probit; US EPA 2002). Median lethal time to death \( (LT_{50}) \) values were determined using the Trimmed Spearmann-Karber method.

**Results**

**Laboratory Toxicity Bioassays**

**Study 1**
Cell-free filtrates of \( P. \) parvum exposed to either full sunlight or ~50% sunlight did not produce significant \( P. \) promelas mortality during the 48-hr acute study. In fact, there was not a single mortality observed at any dilution of these light treatments (Fig. 1); all ASW and RHW controls also had 100% survival. However, the cell-free filtrate maintained in the dark adjacent to the natural sunlight treatment levels was highly toxic to fish with a 24 and a 48-hour \( LC_{50} \) value of 19.50% filtrate (95% confidence intervals: 14.99-25.38%). Such a magnitude of toxicity to fish was similar to that of the cell–free filtrate stored in the dark under refrigeration (dark lab control) in the laboratory during the natural sunlight study period: an acute 24 and 48 \( LC_{50} \) value of 14.03% filtrate (10.29-19.13%) was determined. Results from the time-to-death study showed that the 100% treatment levels of both laboratory and field dark treatments all organisms were dead within three hrs. In the 25% filtrate treatment level for both dark treatments, time to 50% mortality \( (LT_{50}) \) was calculated at 2.3 hrs (95% confidence interval 1.37–3.84 hrs) (Fig. 2).

**Study 2**
Similar to study one, cell-free filtrates of \( P. \) parvum cultures were highly toxic to \( P. \) promelas, with a 24 and a 48-hour \( LC_{50} \) value of 1.34 (% filtrate). With increasing duration of exposure to natural sunlight, 24 hr \( LC_{50} \) values of this cell-free filtrate increased until no mortality was observed at two hrs (Fig. 3). Similarly, times to 50% mortality \( (LT_{50}) \) in the 100% filtrates also increased with duration of sunlight exposure (Fig. 3). Because both \( LC_{50} \) values and \( LT_{50} \) values were increased by sunlight treatment levels, \( P. \) parvum toxins likely were increasingly photolysized during this study. Figure 4 depicts the inverse of 24 hr \( P. \) promelas \( LC_{50} \) values (a surrogate for bioavailable toxin(s) concentrations) versus time \( (y = -0.9061x + 0.8359, R^2 = 0.8941) \).
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**Fig. 1.** *Pimephales promelas* 24 hour survivorship following exposure to *Prymnesium parvum* cell-free filtrates treated with different natural sunlight intensities and a laboratory dark control.

**Fig. 2.** *Pimephales promelas* survivorship for the first ten hours of the 48 hr acute study when exposed to 25% filtrate of the no sunlight treatment level and a laboratory dark control.
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**Fig. 3.** *Pimephales promelas* 24 hr LC$_{50}$ values (grey bars) and LT$_{50}$ values (black bars) for 100% cell free filtrate of *P. parvum* cultures that were exposed to natural sunlight for 0.5, 1 or 2 hrs.

**Fig. 4.** 1 / *Pimephales promelas* 24 hr LC$_{50}$ values for cell free filtrate of *P. parvum* cultures that were exposed to natural sunlight for 0, 0.5 or 1 hr.
Discussion
In the first experiment, the two *P. parvum* cell-free filtrates maintained in the dark demonstrated to be acutely toxic to fish while the two filtrates exposed to sunlight produced no mortality over a 48 hr period. In the second study, toxicity to fish was ameliorated after just two hrs of exposure. The complete disappearance of toxicity of filtrates after sunlight exposure in these two natural sunlight experiments provides strong evidence that photolysis of the toxins occurred. The findings in these studies are consistent with those of Parnas et al. (1962) who reported that when a *P. parvum* supernatant was exposed to light from a tungsten lamp for just 1.5 hrs toxicity to *Gambussia* sp. was removed, and such toxicity elimination was not influenced by the addition of cell pigments, oxygen, or glutathione. Parnas et al (1962) concluded that a photochemical process was involved in “inactivation” of toxins present in the *P. parvum* cell-free filtrate. However, Parnas et al (1962) could not suggest how the photons may interact with the bonds of the prymnesins because the structure was not elucidated until thirty years after the study was published (Igarashi et al. 1998).

Prymnesins are very large molecules with 90 carbon atoms and trans-1, 6-dioxadecaline units with conjugated double/triple bonds at each terminal end (Igarashi et al. 1999). These compounds are amphiphilic, with uneven distributions of sugars and hydroxyl groups, three chlorine atoms, and one nitrogen atom (Valenti et al. in press). It is not possible to identify at this time how the prymnesin molecules break apart during photolysis, especially because they are such large molecules with many bonds that could be subject to being broken by photons. However, one likely susceptible chromophore (or, a region that can absorb ultraviolet and visible light) (Connell 2005) is the series of single and double bonded carbons that is found on the hydrophilic portion of the prymnesins. It appears reasonable that this region absorbs photons and causes degradation of the parent toxins. In addition, indirect photolysis reactions may have occurred. Decaying algae, other algal exudates and bacterial activity likely increased the dissolved organic matter in the stock culture, which could not be removed when the cells were harvested and cell-free filtrate prepared for light experiments. Dissolved organic matter (DOM) is photosensitive and can release reactive species that can facilitate degradation of environmental contaminants (Newman and Unger 2003), and potentially contributed to prymnesin degradation in this study. Thus, it is not possible, based on the findings from this study, to determine whether direct and indirect photolysis was primarily responsible for photodegradation of *P. parvum* toxins. However, this question deserves additional study because the factors influencing toxin photodegradation rates, and thus the magnitude and duration of prymnesin exposures, ultimately resulting in ambient aquatic toxicity, can provide important information for environmental management of fish kills resulting from *P. parvum* bloom formation.

Not only were the dark treatments or lab dark controls in both studies highly toxic to fish, but the onset of toxicity was rapid, with complete mortality in the 100% filtrate within three hours and 50% death in the 25% filtrates at approximately 2.3 hrs in the first study. Toxins produced by *P. parvum* result in a rapid onset of toxic effects because the toxins exert toxicity at the level of the gill, which is a vulnerable surface directly exposed to bioavailable chemicals in aquatic systems (Terao et al.1996). Because fish toxicity serves as a useful surrogate for the concentration of bioavailable toxins in a sample, the magnitude of toxic response may provide a biosensor for toxin concentrations. In the first study, toxin concentrations appeared to be reduced to very low...
levels because fish toxicity was eliminated by full and ~50% sunlight exposure. In addition, LC50 values were very similar in the dark treatment in the field (LC50 = 19.5%) outside and the dark lab refrigerated control (LC50 = 14.03%), highlighted by overlapping 95% confidence intervals. Such comparable toxicity to fish suggests that temperature, which was 4°C in the lab and approximately 26°C in the field, did not influence toxin stability in this study. Further, the magnitude of survivorship responses did not continue to appreciably decrease with exposure duration in the time-to-death study after the first 3 hrs of the exposure period (Figure 1). This observation is not consistent with aquatic toxicological response thresholds, which generally decrease in concentration with increasing exposure duration (Rand 1995). Subsequently, this observation suggests that the magnitude of exposure to bioavailable toxins changed over this 3 hr period, potentially because toxins were degraded by incubator lights during the fish bioassay study.

In the second light study bioassays were performed in the dark on samples exposed to sunlight for various lengths of time. Contrary to the relationship depicted in Figure 4, 1/LC50 plots versus time should be exactly the opposite with 1/LC50 initially increasing with time if an aqueous concentration of a chemical remains stable (Rand 1995). Rather, the relationship depicted in Figure 4 may provide for initial derivation of an aggregate photolysis half-life (t1/2) for P. parvum toxins. Based on the conditions of natural sunlight exposure in this study (mean PAR = 1762), a t1/2 of 0.37 hrs is predicted for P. parvum toxins.

The observed loss in toxicity to fish in these studies when exposed to sunlight suggests that nighttime toxin release of toxins by P. parvum cells may be important for the environmental management of inland and coastal waters experiencing fish kills. Producing such large compounds is energetically costly, so it would be a more advantageous life history strategy to release the toxins when light exposure would be minimal; however, the relationship between toxin synthesis and release by P. parvum and diurnal light regimes are not understood. The information collected from this may support future fisheries management and mitigation measures for harmful P. parvum blooms. Understanding direct and indirect photolytic degradation pathways for prymnesins can also improve our understanding of fish kills. For example, if increase DOM leads to increase indirect photolysis, this relationship could advance a predictive understanding of fish kill potential in various reservoirs.

2.3 Competition experiments

We used mixed- and single-species cultures to investigate competitive relationships between P. parvum and other algae that are its potential competitors in Texas lakes. A total of five experiments were done, each consisting of a preconditioning phase and an experimental phase. The preconditioning phase consisted of growing P. parvum in single-species cultures, and an assemblage of competitors in separate cultures for three weeks in nutrient-limited media (40 μM N, 1.5 μM P) at a salinity of 3 psu. Competitor assemblages were obtained from field samples of algae collected from Lake Granbury (on 3 occasions) and Lake Waco (on 2 occasions). At the end of the preconditioning phase, half of the volume of preconditioned cultures was filtered (0.2
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μm) to remove all microorganisms. The experimental phase consisted of several mixed- and single-species cultures prepared from various mixtures of preconditioning cultures, filtrates from preconditioning cultures, and fresh sterile media of the type used in preconditioning. These treatments exposed *P. parvum* to varying abundances of competitors, concentrations of nutrients, and of potentially allelopathic substances produced by competitors (table below). All cultures were grown at the temperature of surface waters at the time of lake sampling.

<table>
<thead>
<tr>
<th>Designation</th>
<th>Replicates</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>P+C</td>
<td>4</td>
<td>50% preconditioned <em>P. parvum</em> (whole culture) + 50% preconditioned competitors (whole culture)</td>
</tr>
<tr>
<td>P+Cf</td>
<td>2</td>
<td>50% preconditioned <em>P. parvum</em> (whole culture) + 50% preconditioned competitors (filtrate)</td>
</tr>
<tr>
<td>N+P+C</td>
<td>2</td>
<td>45% fresh medium + 5% preconditioned <em>P. parvum</em> (whole culture) + 50% preconditioned competitors (whole culture)</td>
</tr>
<tr>
<td>N+P+Cf</td>
<td>2</td>
<td>45% fresh medium + 5% preconditioned <em>P. parvum</em> (whole culture) + 50% preconditioned competitors (filtrate)</td>
</tr>
<tr>
<td>C+Pf</td>
<td>2</td>
<td>50% preconditioned competitors (whole culture) + 50% preconditioned <em>P. parvum</em> (filtrate)</td>
</tr>
<tr>
<td>N+C+P</td>
<td>2</td>
<td>45% fresh medium + 5% preconditioned competitors (whole culture) + 50% preconditioned <em>P. parvum</em> (whole culture)</td>
</tr>
<tr>
<td>N+C+Pf</td>
<td>2</td>
<td>45% fresh medium + 5% preconditioned competitors (whole culture) + 50% preconditioned <em>P. parvum</em> (filtrate)</td>
</tr>
</tbody>
</table>

Dates of the experiments, incubation temperatures, and lake samples used are reported in the table below:

<table>
<thead>
<tr>
<th>Start date – preconditioning phase</th>
<th>Start date – experimental phase</th>
<th>Temperature</th>
<th>Lakes</th>
</tr>
</thead>
<tbody>
<tr>
<td>4/25/08</td>
<td>5/14/08</td>
<td>22° C</td>
<td>Granbury</td>
</tr>
<tr>
<td>10/27/08</td>
<td>11/17/08</td>
<td>21° C</td>
<td>Granbury, Waco</td>
</tr>
<tr>
<td>5/25/09</td>
<td>6/15/09</td>
<td>22° C</td>
<td>Granbury, Waco</td>
</tr>
</tbody>
</table>

During the experimental phase, samples for *P. parvum* abundance were collected on 11 days, samples for chlorophyll *a* on 3 days, and samples for acute toxicity to fish on day 21, the last day. At this time, we have analyzed data on acute toxicity for all experiments, and data on abundance of *P. parvum* and chlorophyll *a* from day 21 in the experiments initiated in May and November of 2008. Data from the experiments conducted most recently (June 2009) are still being collected and will be analyzed by the end of 2009.

Acute toxicity was measured as the percentage of diluted culture sample killing 50% of test subjects (juvenile *P. promelas*, fathead minnow) in assays following EPA-approved protocols (USEPA, 2002). For these toxicity samples, survival of fish was tested in undiluted culture samples, and a series of six dilutions into control water: 50%, 25%, 12.5%, 6.25%, 3.125%, and 1.5625%. Appropriate statistical methods were then used to estimate LC50, the percentage dilution at which 50% of subjects survive. A result of 100% indicates that the culture sample was non-toxic; lower percentage dilutions indicate increasingly toxic culture samples. A one-way
analysis of variance (ANOVA) was used to test the significance of differences in LC$_{50}$ among treatments; to conform to the assumptions of the analysis, the arcsin-square root transformation was applied to these data. One-way ANOVA was also used to test the significance of differences in $P. parvum$ abundance and chlorophyll a among treatments; to conform to the assumptions of the analysis, the natural logarithm transformation was applied to these data.

In most experiments done at different times with different lakes, two treatments differed strongly from others in being non-toxic or only weakly toxic. These were treatments C+Pf and N+C+Pf, those which did not receive an inoculum of preconditioned $P. parvum$ cells. $P. parvum$ may have been present in these cultures, since the field samples of competitor species could have contained $P. parvum$. As noted below, $P. parvum$ was either absent from these two treatments, or at lower density than in other treatments. Thus the general lack of strong toxicity to fish in the C+Pf and N+C+Pf treatments likely resulted from low abundance of $P. parvum$. The remaining treatments were usually strongly toxic, and were inoculated with $P. parvum$ from preconditioned cultures that were toxic at the time of inoculation, with LC$_{50}$ < 1.5% in bioassays with fathead minnows.

For experiments done with competitor algae sampled from Lake Granbury (Fig. 5), acute toxicity to fish (LC$_{50}$) differed significantly (one-way ANOVA, $P < 0.001$) among treatments for experiments done in May and November of 2008. For the experiment done in May 2008, post-hoc comparisons (Tukey’s HSD) show that the LC$_{50}$ of the C+Pf treatment was significantly ($\alpha = 0.05$) greater than that of all treatments where $P. parvum$ was inoculated from preconditioned cultures, and that the N+C+Pf treatment had significantly higher LC$_{50}$ than the P+C treatment. At a less stringent level of significance ($\alpha = 0.10$), post-hoc comparisons suggest that the N+C+Pf treatment had significantly higher LC$_{50}$ than the other treatments where $P. parvum$ was inoculated from preconditioned cultures. For the experiment done in November 2008, post-hoc comparisons (Tukey’s HSD) show that the LC$_{50}$ of the C+Pf treatment was significantly ($\alpha = 0.05$) greater than that of all other treatments. For experiments with competitor algae sampled from Lake Granbury, done in June 2009, LC$_{50}$ did not differ significantly among treatments (one-way ANOVA, $P = 0.08$). Like the first two experiments, the C+Pf and N+C+Pf treatments were non-toxic or weakly toxic. Unlike the first two experiments, the P+C, P+Cf, and N+P+C treatments were non-toxic or weakly toxic, and only the N+P+Cf and N+C+P treatments were strongly toxic. For this experiment, data on $P. parvum$ abundance are not yet available to help interpret this result.

For experiments done with competitor algae sampled from Lake Granbury (Fig. 5), abundance of $P. parvum$ differed significantly (one-way ANOVA, $P < 0.001$) among treatments for experiments done in May and November of 2008. For both of these experiments, post-hoc comparisons (Tukey’s HSD) show that $P. parvum$ abundances in the C+Pf and N+C+Pf treatments were significantly ($\alpha = 0.05$) lower than that of all treatments where $P. parvum$ was inoculated from preconditioned cultures. That is, those cultures that were not strongly toxic to fish also had low (or zero) abundance of $P. parvum$. Where data are now available, all cultures that were strongly toxic to fish had high $P. parvum$ abundance, with one exception (treatment N+C+Pf in November 2008). Replicate cultures for this anomalous treatment agreed well in LC$_{50}$ and all other quantities examined. It is possible that they contained another species of toxic algae, different from $P. parvum$, among the competitor assemblage.
In general, however, biomasses of competitors appeared to be low in these experiments. For experiments done with competitor algae sampled from Lake Granbury (Fig. 5), chlorophyll *a* concentration differed significantly (one-way ANOVA, *P* < 0.001) among treatments for the experiment done in May 2008. For this experiment, post-hoc comparisons (Tukey’s HSD) show that chlorophyll *a* in the C+Pf and N+C+Pf treatments was significantly (*α* = 0.05) lower than that of all treatments where *P. parvum* was inoculated from preconditioned cultures. In this experiment, much of the chlorophyll *a* was composed of *P. parvum*. Those treatments not inoculated with preconditioned cultures of *P. parvum* (C+Pf and N+C+Pf) essentially contained only competitor species, and had low biomass measured as chlorophyll *a*. For the experiment done with competitor algae sampled from Lake Granbury, done in November 2008, chlorophyll *a* did not differ significantly among treatments (one-way ANOVA, *P* = 0.073). Again, however, for treatments inoculated with preconditioned *P. parvum*, much of the chlorophyll *a* biomass was composed of this species. Those treatments not inoculated with preconditioned cultures of *P. parvum* (C+Pf and N+C+Pf) again contained only competitor species, and had low biomass measured as chlorophyll *a*. This includes the anomalous treatment N+C+Pf which had a low abundance of *P. parvum* but which was strongly toxic.
Experiments with Competitors sampled from Lake Granbury

Fig. 5. Results of competition experiments done with algal assemblages sampled from Lake Granbury. Each column of panels presents those done at the same time, as indicated. Top row of panels – LC_{50}; middle row of panels – abundance of *P. parvum*; bottom row of panels – chlorophyll *a*. Data on abundance of *P. parvum* and chlorophyll *a* have not yet been processed for the experiment done in June 2009. For each quantity, mean ± standard deviation is shown.

For experiments done with competitor algae sampled from Lake Waco (Fig. 6), acute toxicity to fish (LC_{50}) differed significantly (one-way ANOVA, *P* < 0.02) among treatments for
experiments done in November of 2008 and June of 2009. For the experiment done in November 2008, post-hoc comparisons (Tukey’s HSD) show that the LC50 of the C+Pf treatment was significantly ($\alpha = 0.05$) greater than that of the C+P treatment. At a less stringent level of significance ($\alpha = 0.10$), post-hoc comparisons suggest that the C+Pf and N+C+Pf treatments had significantly higher LC50 than the other treatments where *P. parvum* was inoculated from preconditioned cultures. For the experiment done in June 2009, post-hoc comparisons (Tukey’s HSD) show that the LC50 of the N+C+Pf treatment was significantly ($\alpha = 0.05$) greater than that of all other treatments.

For experiments done with competitor algae sampled from Lake Waco (Fig. 6), abundance of *P. parvum* differed significantly (one-way ANOVA, $P < 0.001$) among treatments for the experiment done in November 2008. Post-hoc comparisons (Tukey’s HSD) show that *P. parvum* abundances in the C+Pf and N+C+Pf treatments were significantly ($\alpha = 0.05$) lower than that of all treatments where *P. parvum* was inoculated from preconditioned cultures. That is, those cultures that were not strongly toxic to fish also had low (or zero) abundance of *P. parvum*. All cultures that were strongly toxic to fish had high *P. parvum* abundance.

For experiments done with competitor algae sampled from Lake Waco (Fig. 6), chlorophyll *a* concentration differed significantly (one-way ANOVA, $P < 0.001$) among treatments for the experiment done in May 2008. Post-hoc comparisons (Tukey’s HSD) show that chlorophyll *a* in the C+Pf and N+C+Pf treatments was significantly ($\alpha = 0.05$) lower than that of all treatments where *P. parvum* was inoculated from preconditioned cultures. Those treatments not inoculated with preconditioned cultures of *P. parvum* (C+Pf and N+C+Pf) essentially contained only competitor species, and had low to moderate biomass measured as chlorophyll *a*.
Experiments with Competitors sampled from Lake Waco

Fig. 6. Results of competition experiments done with algal assemblages sampled from Lake Granbury. Each column of panels presents those done at the same time, as indicated. Top row of panels – LC$_{50}$; middle row of panels – abundance of $P$. parvum; bottom row of panels – chlorophyll $a$. Data on abundance of $P$. parvum and chlorophyll $a$ have not yet been processed for the experiment done in June 2009. For each quantity, mean ± standard deviation is shown.

To summarize, in these laboratory culture experiments, most cultures inoculated with toxic populations of $P$. parvum from preconditioned laboratory cultures developed abundant populations of $P$. parvum and were strongly toxic to fish after three weeks of growth. The potential for $P$. parvum to develop toxic populations over this time period was not noticeably altered by the presence of competitors, the presence of filtrates from competitor cultures, the presence of filtrates from $P$. parvum cultures, or the addition of fresh medium with replenished
nutrients. These results provide no evidence that competitor algae can inhibit the formation of a toxic *P. parvum* bloom from a suitable initial population, either by competition for nutrients or production of allelopathic substances. The only exceptions to this pattern occurred in the June 2009 experiments with competitors sampled from Lake Granbury, where several treatments inoculated with toxic *P. parvum* populations were non-toxic or weakly toxic after three weeks of growth. We have not yet processed all the data on *P. parvum* abundance in these cultures to be able to say whether lack of toxicity is explained by low abundance, or whether abundant but non-toxic populations developed. It is possible that the competitor species sampled on this occasion included some that can inhibit growth or toxicity of *P. parvum*. Two cultures not inoculated with preconditioned, toxic populations of *P. parvum* also became toxic to fish despite having only very low abundance of *P. parvum*, suggesting that additional toxic species might have been present in the competitor assemblages sampled on one occasion. Otherwise all cultures not inoculated with preconditioned, toxic populations of *P. parvum* did not become strongly toxic to fish, and contained little or no *P. parvum* at the end of the growth period.

**Life history experiments**

In proposing the research described in this report we advanced a working hypothesis linking bloom development and toxicity to the life history of *P. parvum*. We suggested that when a population begins growth from low density in a nutrient-rich, favorable habitat, the population first increases and depletes nutrients. This in turn triggers toxin production, which we suspect facilitates mixotrophic nutrition and attack on microbial prey. If a dense population ages and continues to starve because prey are rare, we hypothesized that encystment eventually ensues. In mature laboratory cultures that experience stress from nutrient limitation or other suboptimal conditions, we have noted that cells of reduced motility often occur. Some of these appear to be true cysts with no flagella and thickened cell wall, while others retain flagella but do not move these rapidly enough to swim. These low motility cells resemble those recently documented in a field population of *P. parvum* (Genitsaris et al., 2009). Based on our working hypothesis that encystment, and reduced motility more generally is linked to population age and stress, we conducted three experiments exposing aging cultures of *P. parvum* to various stresses, to identify conditions under which non-motile, encysted cells would produced, and to provide further information on whether there is a relationship between toxicity and these life history shifts.

During most of our work, we have distinguished between motile and non-motile cells because this character is easy to score during routine live counts of culture samples. However, we have not consistently distinguished between non-motile but flagellated cells and those that have encysted.

The first life history experiment used batch cultures of sufficient volume (5 liters) to permit several determinations of toxicity to fish over the age of the culture, using 48-hour lethality tests with *P. promelas*. It imposed three treatments in triplicate: (1) nutrient-sufficient cultures with f/2 supplies of N and P; (2) nutrient-limited cultures with N and P supplies 40 μM and 1.5 μM respectively, which are representative of lakes in Texas impacted by *P. parvum*; and (3) nutrient-limited cultures with these N and P supplies, supplemented with 300 μM of organic C as glucose to stimulate mixotrophic nutrition. All cultures had a salinity of 3 psu and were incubated at 20° C, with an irradiance of about 150 μE m⁻² s⁻¹ on a 12:12 photoperiod. We contrasted nutrient-
sufficient with nutrient-limited cultures because nutrient limitation often enhances toxicity in *P. parvum* (Dafni et al., 1972; Johansson & Granéli, 1999). We included a treatment to encourage mixotrophy based on a hypothesis that toxicity is related to mixotrophy. In a previous laboratory experiment with glucose supplementation (Harman & Grover, 2008), we observed aggregations of cells resembling the “feeding swarms” of mixotrophic *P. parvum* observed by Tillman (2003).

During the 6-week incubation of these cultures we used a rapid, live-count protocol to determine the proportion of motile and non-motile cells at frequent intervals. We took additional samples for determinations of bacterial abundance, particulate C, N and P, and toxicity to fish (48-hour lethality tests with *P. promelas*). We also preserved samples of *P. parvum* for later determination of total population density, to check our live count protocol. Here, we present only the live count and toxicity data, because these make the major results clear.

Total densities of *P. parvum* were highest in nutrient-sufficient cultures, followed by nutrient-limited cultures, followed by nutrient-limited cultures supplemented with glucose (Fig. 7). When inoculated from stock cultures, populations in all treatments were initially dominated by non-motile cells. By day 9 the majority of cells in all cultures were motile and total population was increasing. Toxicity to fish on day 7 was low for all cultures, but was significantly greater for the nutrient-sufficient cultures than for the other treatments (one-way ANOVA, *P* = 0.016). By day 14, populations of 10^4 – 10^5 cells/ml had developed in all cultures, and toxicity to fish was high (48-hr LC50 < 10% dilution of whole culture samples). Toxicity to fish remained high (low LC50) during the rest of the experiment in nutrient-limited cultures and nutrient-limited cultures supplemented with glucose. From days 21 – 42, toxicity of the nutrient-limited and nutrient-limited, carbon-enriched cultures was significantly higher (lower LC50) than toxicity of the nutrient-sufficient cultures (one-way ANOVA, *P* < 0.01). The more toxic treatments were also consistently dominated by motile cells. In contrast to these more toxic treatments, nutrient-sufficient cultures decreased in toxicity (LC50 increased) from days 21 – 35, during which time the proportion of non-motile cells was high. These results suggest that formation of low motility cells, many of which appeared to be encysted, occurs in response to high population density, which was here associated with nutrient-sufficiency. In contrast, toxicity was highest in populations of lower density experiencing nutrient-limitation.
A. Nutrient-Sufficient Cultures

B. Nutrient-Limited Cultures

C. Nutrient-Limited Cultures with Glucose

Fig. 7. Dynamics of motile cells (solid line, solid circles), non-motile cells (dashed line, open circles), and LC50 in fish toxicity assays (large, open diamonds) during the first life history experiment. Data are means ± standard deviations for three replicate cultures. A. Nutrient-sufficient cultures; B. Nutrient-limited cultures; C. Nutrient-limited cultures supplemented with glucose.
The results of this first life history experiment suggest that stresses inducing toxicity differ from those inducing life history shifts involving reduced motility and encystment. Toxicity was observed in older cultures for all treatments in this experiment, and has been observed in response to many other stresses in earlier studies (Dafni et al., 1972; Johansson & Granéli, 1999; Baker et al., 2007). Possibly, toxicity of *P. parvum* arises in response to many forms of stress, including nutrient limitation and suboptimal physicochemical conditions. In contrast, life history shifts may be a very specific response to very high population density, perhaps mediated by an intraspecific signal, and it is likely that not all stressed populations reach a sufficient density to undergo reduced motility and encystment.

The second life history experiment was conducted using cultures similar to the nutrient-limited cultures of the first experiment. However, salinity, temperature and irradiance were adjusted to simulate winter and summer conditions with four replicates per treatment. Winter conditions were at 2 psu salinity, 14° C, and 150 μE m⁻² s⁻¹ on a 10:14 photoperiod. Summer conditions were at 4 psu salinity, 30° C, and 150 μE m⁻² s⁻¹ on a 10:14 photoperiod. For both treatment conditions, non-motile cells were relatively rare, and population densities of motile cells were somewhat higher in the summer conditions (Fig. 8). Toxicity to fish was similar for summer and winter conditions during most of the experiment, but on days 35 and 42, toxicity was significantly higher (lower LC₅₀) for the winter conditions than for the summer conditions (one-way ANOVA, *P* < 0.02).
A third life history experiment was run with nutrient-limited medium in smaller volumes (800 ml), for 28 days, at which time toxicity to fish was determined. A replicated, four-factor factorial design was used with two levels and all combinations of the following experimental factors: (1) temperature – 14° C vs. 20° C; (2) salinity – 2 psu vs. 4 psu; (3) bubbling with air – present vs. absent; and (4) silica – added at 134 μM or not added. The air-bubbling treatment was used because experiments done in another study of *P. parvum* for another project with Dr. Kevin Schug suggested that it could enhance toxicity to fish. The silica treatment was included because cysts of some flagellated algae have silicified walls. Non-motile cells were generally rare in these cultures; motile cells dominated but abundance varied among treatments (data not shown). Toxicity to fish also varied among treatments. In a full factorial ANOVA (using LC50 with the arcsin square-root transform), one three-way interaction term and two two-way interactions terms were significant (see following table), suggesting potentially complex responses of toxicity to all experimental factors.
Refining a Predictive Understanding of Physical, Chemical and Biological Factors Influencing *Prymnesium parvum* Population Dynamics and Toxicity

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Temperature (T)</td>
<td>0.507</td>
<td>1</td>
<td>0.507</td>
<td>23.394</td>
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<tr>
<td>Salinity (S)</td>
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<td>1</td>
<td>3.733</td>
<td>172.194</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Bubbling (B)</td>
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<td>1</td>
<td>0.933</td>
<td>43.052</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Silica (Si)</td>
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<td>1</td>
<td>0.099</td>
<td>4.567</td>
<td>0.048</td>
</tr>
<tr>
<td>T × S</td>
<td>1.063</td>
<td>1</td>
<td>1.063</td>
<td>49.026</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>T × B</td>
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<td>1</td>
<td>0.065</td>
<td>2.976</td>
<td>0.104</td>
</tr>
<tr>
<td>T × Si</td>
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<td>1</td>
<td>0.003</td>
<td>0.156</td>
<td>0.698</td>
</tr>
<tr>
<td>S × B</td>
<td>0.829</td>
<td>1</td>
<td>0.829</td>
<td>38.232</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>S × Si</td>
<td>0.124</td>
<td>1</td>
<td>0.124</td>
<td>5.711</td>
<td>0.03</td>
</tr>
<tr>
<td>B × Si</td>
<td>0.01</td>
<td>1</td>
<td>0.01</td>
<td>0.458</td>
<td>0.508</td>
</tr>
<tr>
<td>T × S × B</td>
<td>0.13</td>
<td>1</td>
<td>0.13</td>
<td>6.014</td>
<td>0.026</td>
</tr>
<tr>
<td>T × S × Si</td>
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<td>1</td>
<td>0.002</td>
<td>0.079</td>
<td>0.783</td>
</tr>
<tr>
<td>T × B × Si</td>
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<td>1</td>
<td>0.07</td>
<td>3.236</td>
<td>0.091</td>
</tr>
<tr>
<td>S × B × Si</td>
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<td>1</td>
<td>0.005</td>
<td>0.221</td>
<td>0.644</td>
</tr>
<tr>
<td>T × S × B × Si</td>
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<td>1</td>
<td>0.062</td>
<td>2.875</td>
<td>0.109</td>
</tr>
<tr>
<td>Error</td>
<td>0.347</td>
<td>16</td>
<td>0.022</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For most treatment combinations, cultures grown at 4 psu were strongly toxic (LC$_{50} < 20\%$). Those grown at a salinity of 2 psu were usually less toxic than those grown at 4 psu (Fig. 9). This pattern was especially strong at a temperature of 20° C, where 2 psu cultures were often non-toxic (LC$_{50} = 100\%$) or moderately toxic (LC$_{50} > 20\%$). Exceptions to these general patterns occurred when cultures were not bubbled and received no silica. Cultures under these conditions were strongly toxic when growth at a salinity of 2 psu, and when grown at 4 psu with a temperature of 14° C. However, cultures without bubbling and without silica were only moderately toxic when grown at a salinity of 4 psu and a temperature of 20° C.
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![Fig. 9. LC$_{50}$ in fish toxicity assays for the second life history experiment. Data are means ± standard deviation for duplicate cultures. Filled circles and solid line – cultures grown at 20° C; open circles and dashed line – cultures grown at 14° C.](image)

Taken together the three life history experiments confirm many previous results concerning toxicity of *P. parvum*. Many forms of stress can enhance toxicity, including nutrient limitation.
and suboptimal temperature and salinity. Notably, toxicity is higher under the simulated winter conditions of Texas reservoirs than under simulated summer conditions. Toxicity is also affected by other physicochemical factors such as mixing, aeration, and silica concentration. Finally, toxicity generally tends to increase as cultured populations age and modify their environment by consuming resources and producing metabolites. In contrast to toxicity, production of non-motile and encysted cells was only associated with mature populations of very high density (> 150,000 cells / ml), which could only be cultured under nutrient-sufficient conditions.

3. Modeling Research

During the first year of the project, modeling research focused on calibrating models for the dynamics of *P. parvum* and competing algae in a chemostat or CSTR framework. The focal site for this calibration was Lake Granbury, for which one year of monitoring data was available at the time. That work calibrated and evaluated five models that differed in whether and how competing species of algae were represented: (1) a model representing only *P. parvum* and no competitors, designated PP0; (2) a model representing four functional types of competing algae, designated PP4; (3) a model representing one functional type of competing algae (cyanobacteria), designated PP1; (4) a model representing four functional types of competing algae, and including allelopathy in the form of cyanotoxins produced by cyanobacteria, designated PP4A; and (5) a model representing one functional type of competing algae (cyanobacteria), and including allelopathy in the form of cyanotoxins, designated PP1A.

The results of this calibration and evaluation are reported in a manuscript accepted for publication in a special collection in the *Journal of the American Water Resources Association*, containing papers presented at the International Symposium on Golden Algae held in January in Fort Worth. A copy of the manuscript (Grover et al., 2010) is attached to this report. Briefly, the models we constructed did not provide accurate representations of all feature of *P. parvum* dynamics observed in Lake Granbury. Models including allelopathy in the form of cyanotoxins produced by cyanobacteria, which are assumed to inhibit the growth of *P. parvum*, did best in simulating the observed low density of *P. parvum* in summer followed by a rising density in autumn. Models without allelopathy, with calibrated parameters for the temperature response function of *P. parvum*, did better at simulating the high densities observed in winter.

During the second year of this project, our modeling efforts were focused in two directions. First, we assembled a second year of field data from Lake Granbury to extend the five models described above and in the manuscript in press. These were then re-evaluated over a two year time frame. Second, we have developed two models that provide spatially explicit representations of the dynamics of *P. parvum* or other harmful algae in the coves and main body of a reservoir. We chose this focus because field experience suggests that the most tractable mitigation options during blooms of *P. parvum* will be treatments applied to coves. Thus understanding the potential coupling of coves to the main reservoir becomes important.

*Modeling two years of P. parvum in Lake Granbury*
Continued modeling of *P. parvum* dynamics in Lake Granbury used some of the models previously constructed and calibrated as reported in Grover et al. (2010). The goal was to compare the predictions of selected, previously parameterized models to observations over a time period of more than two years. The period of record thus examined runs from 9/13/2007 to 1/17/2009, thus including the full calendar years 2008 and 2009. Only data from the 10 fixed station sites sampled on all occasions during this time period were used.

Assembly of forcing variables over this time period was a straightforward continuation of the data and calculations described in Grover et al. (2010). During the course of the study the taxonomic resolution of the zooplankton data improved. Data from more refined taxa were aggregated into the coarser taxa used in the first part of the study. However, estimates of the characteristic individual volumes of these taxa were refined in light of further information about the dominant species present. Clearance rates and algal mortality rates were then recalculated from these revised estimates. This produced negligible changes in the forcing data on grazing rates for the first part of the study as described in the submitted manuscript, since the number and magnitude of such revisions was small.

Initial values for the new simulation runs were derived from observations on 9/13/2007. For the population density of *P. parvum*, and dissolved phosphate and nitrate concentrations, the initial values were the spatial averages of the station observations. For the population density of cyanobacteria, needed for some models, the CHEMTAX abundance estimate based on pigments was converted to population density as follows. CHEMTAX estimates the abundance of cyanobacteria as the equivalent concentration of chlorophyll *a*. From a database of cell volumes measured on specimens of cyanobacteria from two Texas lakes (Grover and Chrzanowski, 2005), an average cell volume of 35 μm³ was calculated. Assuming a carbon density of 0.2 pg C / μm³ (Rocha and Duncan, 1985) gives an average cell carbon mass of 7 pg C / cell. Chlorophyll *a*: carbon ratios of algae range 10 – 100 μg Chl *a* / mg C (Chapra, 1997); the low value of 10 μg Chl *a* / mg C is assumed to apply because algae in Texas lakes are frequently nutrient-limited. This assumption leads to a conversion factor of $7 \times 10^8$ μg Chl *a* / cell, which was applied to the spatially averaged CHEMTAX estimate of cyanobacteria abundance. The initial concentration of cyanotoxin produced by cyanobacteria in allelopathy models was arbitrarily set to 1 μg / liter.

*P. parvum* observations

*P. parvum* displayed a large bloom in the winter of 2006-2007, went to low density in the summer of 2007, increased during the winter of 2007-2008 but did not form a dense bloom, fell back to low density during the summer of 2008, and then increased to a modest bloom in December of 2008, decreasing to the last observation analyzed in January 2009 (Fig. 10).
Refining a Predictive Understanding of Physical, Chemical and Biological Factors Influencing *Prymnesium parvum* Population Dynamics and Toxicity

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After the large bloom in the winter of 2006-2007, two factors made Lake Granbury less likely to develop high abundance of *P. parvum*. First, heavy rainfall beginning in late March of 2007 raised the hydraulic dilution rate considerably from its earlier, low level of about 0.001 d\(^{-1}\). From late March to late September 2007 the dilution rate varied between about 0.01 and 1.0 d\(^{-1}\). The dilution rate then fell to about 0.001 d\(^{-1}\) again from September 2007 through March 2008, rose to about 0.01 d\(^{-1}\) from March to November 2008, and then returned to lower levels around 0.001 d\(^{-1}\) through January 2009. Thus, after the bloom during the winter of 2006-2007, Lake Granbury underwent two extended periods of high flushing in the summers of 2007 and 2008. *P. parvum* increased in abundance only during winter periods of low flushing.

The second factor reducing abundance of *P. parvum* after the bloom in winter of 2006-2007 is zooplankton grazing. During the winter of 2006-2007 there was a peak of zooplankton abundance. However three larger peaks occurred at later times: February and April of 2008, and January of 2009. Rotifers were abundant during all these peaks and composed > 50% of zooplankton biovolume except during April 2008, when both cladocera and copepods were also abundant. During the winter bloom of *P. parvum* in 2006-2007 the estimated community grazing rate was about 0.6 d\(^{-1}\). During the three subsequent zooplankton peaks, community grazing rates were estimated to exceed 0.8 d\(^{-1}\). Thus *P. parvum* potentially suffered high losses from episodes of heavy grazing as well as hydraulic flushing during much of the time following the bloom in winter of 2006-2007. Estimated loss rates were relatively low, however, in November and December 2008 when the most recent population increase was observed.
Comparison of model predictions to observations

Three of the models constructed as described in Grover et al. (2010) were selected for examination here: PP0 – the single population model of *P. parvum* only; PP1 – the model with a competing population of cyanobacteria; and PP1A – the model with a competing population of cyanobacteria producing an allelopathic cyanotoxin. Models with additional competing populations were not examined, because the predictions of these models did not differ much from those of models with just one competing population, parameterized as cyanobacteria growing well in warm conditions. For each model, predictions were generated using the uncalibrated, initial parameter sets derived in Grover et al. (2010). Predictions were also generated for the calibrated models with adjusted parameter values as described in Grover et al. (2010).

Model PP0 predicted the extinction of *P. parvum* during 2008 (results not shown), due to the combination of high flushing grazing through much of this year.

Models with competitors (PP1 and PP1A) have immigration terms for both *P. parvum* and cyanobacteria to prevent competitive exclusion. These were treated as chemostat supply terms, so that total immigration increased with dilution rate. Without these immigration terms, these competition models also predicted extinction of *P. parvum* (results not shown). With immigration, *P. parvum* was predicted to persist by both model PP1 without allelopathy and model PP1A with allelopathy.

Using the uncalibrated parameter set, model PP1 without allelopathy predicted that *P. parvum* would have lower abundance in 2008 than in 2006-2007 (Fig. 11). As noted in Grover et al. (2010), predictions during the first year of observations disagreed with observations in several respects. Subsequently, however, several aspects of *P. parvum* dynamics were approximated by predictions: low population density in August 2007 followed by an increase through November 2007 to a peak in December 2007, and a decline to February 2008. The model predicted these trends only in a relative sense, as it consistently under-predicted the population density of *P. parvum* during this period. The model appeared more accurate from August through December 2008, when it predicted the development of the small bloom that was observed. However, the model wrongly predicted the demise of this bloom in January 2009, when only a modest decline occurred. The predictions of the model were somewhat sensitive to the magnitude of the immigration term. The four separate lines in Fig. 11 show predictions when the immigration density is set to values of 50, 100, 200 and 1000 cells / ml.

Using the calibrated parameter set, model PP1 was clearly less accurate in its predictions. The parameter adjustment involved sets the optimal temperature for growth of *P. parvum* to a much lower value (Grover et al., 2010). Although this adjustment improves prediction of its density during the large bloom in the winter of 2006-2007, it consistently under-predicts the abundance of *P. parvum* at all subsequent times (Fig. 11).
Using the uncalibrated parameter set, model PP1A with allelopathy also generally underpredicted the abundance of *P. parvum* during 2008 (Fig. 12). Using the calibrated parameter set, model PP1A with allelopathy predicted generally lower abundance of *P. parvum* throughout
2008 than in 2006-2007. The model predicted the development of the small bloom that was observed from August through December 2008. However, the model wrongly predicted the demise of this bloom in January 2009, when only a modest decline occurred. As with other competition models explored here, predictions were somewhat sensitive to the magnitude of the immigration term. The four separate lines in Fig. 12 show predictions when the immigration density is set to values of 50, 100, 200 and 1000 cells / ml.

The single species model (PP0) predicted extinction of *P. parvum* during the time period involved because it lacked an immigration term. The presence of immigration stabilized the persistence of *P. parvum* in the other models (PP1 and PP1A). To compare the single species model to these latter competition models, immigration term were added to model PP0. From the results (Fig. 13), the uncalibrated single species (PP0) model has similar visual fit to the uncalibrated competition model (PP1) and the calibrated allelopathy model (PP1A).

Although model predictions clearly do not reproduce the detailed dynamics of *P. parvum*, they do reproduce some of the more qualitative aspects of observations. Populations are predicted to be low in the summers of 2007 and 2008. Higher abundance is predicted during some of the cooler months in 2006-2007, 2007-2008, and 2008-2009. However, the models persistently under-predict abundance during the coldest months, especially the uncalibrated models, which use ecophysiological parameters based on lab experiments (Baker et al., 2007, 2009). This experimental work showed rather low growth rates of *P. parvum* at temperatures below about 10° C, which occur during January or February in Lake Granbury and other impacted lakes. The high abundance of *P. parvum* during these months in some years remains unexplained.
Uncalibrated Parameter Set, with Allelopathy

Calibrated Parameter Set, with Allelopathy

**Fig. 12.** Predicted abundance of *P. parvum* in Lake Granbury from the model with one competitor species having an allelopathic effect on *P. parvum* (model PP1A), shown as solid lines for different levels of upstream immigration. Markers and error bars show observed mean abundances and standard deviations from 10 fixed stations in Lake Granbury. Top panel – uncalibrated parameter set described previously (Grover et al., 2010). Bottom panel – parameter set as calibrated previously (Grover et al., 2010).
Uncalibrated Parameter Set, single species

Calibrated Parameter Set, single species

Fig. 13. Predicted abundance of *P. parvum* in Lake Granbury from a single-species model (model PP0), shown as solid lines for different levels of upstream immigration. Markers and error bars show observed mean abundances and standard deviations from 10 fixed stations in Lake Granbury. Top panel – uncalibrated parameter set described previously (Grover et al., 2010). Bottom panel – parameter set as calibrated previously (Grover et al., 2010).
Spatially explicit models – overview

We have developed two types of spatially explicit models that approach the dynamic interactions of a main lake and its coves with two different idealizations. The first type of model is an advection-dispersion-reaction system that represents the longitudinal axis ($x$) of a riverine reservoir from the headwaters ($x = 0$) to the dam ($x = L$) (Fig. 14). In the main, flowing channel of the reservoir advection occurs at a rate $\nu$ (m / d) and dispersion with coefficient $\delta$ (m$^2$ / d). The main channel is connected to an ensemble of fringing coves treated as a hydraulic storage zone (Bencala & Walters, 1983), in which there is no longitudinal transport. At any point $x$ along the length of the reservoir, there is exchange by Fickian diffusion between the main channel and storage zone at a rate $\alpha$ (d$^{-1}$). The cross-section of the channel is partitioned into area $A$ representing the main channel, and $A_S$ representing the ensemble of coves in the storage zone. Within both the main channel and the storage zone, algae grow and consume the limiting nutrient, while producing a toxin. Flow enters the headwaters at a rate $F$ (m$^3$ / d), carrying the limiting nutrient for algal growth at a concentration $R_{in}$ (μM), and a balancing flow exits at the dam, removing algae, nutrients, and algal toxin.

![Fig. 14. One-dimensional physical representation of a riverine reservoir with a hydraulic storage zone composed of fringing coves and shoreline features.](image)

The second type of spatially explicit model focuses on a single cove, rather than a distributed ensemble, which undergoes Fickian exchange with a main lake (Fig. 15). A fraction $\psi$ of the total reservoir volume $V$ occurs in the cove, and a fraction $\phi$ of the total inflow $F$ goes into the cove. The same total flow exits from the main lake, where the dam occurs. The limiting nutrient for algal growth enters the main lake at a concentration $R_{in}$ and enters the cove at a concentration $R_{in}$. 

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$R_2^{in}$. Water exchanges between the main lake and cove at a rate $E'$ (m$^3$/d) and a net flow of $\phi F$ also goes from the cove to the main lake to maintain constant volume. Flows of algae and algal toxin follow those of the nutrient.

Fig. 15. Physical representation of a coupled cove – main lake system. Zone 1 on the right is the main lake, and zone 2 on the left is the cove.

Both of these models were parameterized to represent dynamics of *P. parvum* and its toxins. The parameterization was similar to that in Grover et al. (2010), but was simplified in several respects. Temperature and salinity dependence of algal growth was neglected and only a single limiting nutrient, phosphorus was represented. Since the chemical nature of *P. parvum* toxins is still uncertain, necessary coefficients of toxin production and decay were given conjectured values analogous to those governing other types of algal toxins. The parameterized models were explored to generate predictions concerning spatial variation in algal abundance and toxin concentration, in the form of longitudinal variations and differences between coves and main lakes. Flow parameters were not designed to represent any particular lake, but to be broadly consistent with properties of Texas reservoirs, although hydrological data from Lake Granbury were used in some model simulations to generate realistic event-driven variation in flow. Nutrient loading parameters and algal mortality were set to high and low values respectively, to represent habitats prone to blooms of harmful algae.
**Governing equations for advection-dispersion-reaction system**

To simplify the hydraulic description of the system, constant reservoir volume \( V \) is assumed and the total system dilution rate is defined as \( D = \frac{F}{V} \). To maintain water balance, the advection velocity \( \nu \) must then equal \( DL \), where \( L \) is the reservoir length. Algal growth is a Monod function of the limiting nutrient

\[
\mu(R) = \frac{\mu_{max}R}{K + R}
\]

with maximal growth rate \( \mu_{max} \) (d\(^{-1}\)) and half-saturation constant \( K \) (µM). As algae grow they consume nutrient with quota \( q \) (µmol / cell). For modeling \( P. \ parvum \), toxin production is assumed to be proportion to the product of total population size (\( N \), cells / ml) and the degree to which growth is nutrient limited according to eq. (1), since nutrient limitation and other stresses enhance the toxicity of \( P. \ parvum \) in laboratory studies (Chakraborty et al., 2008; Martines et al., 2009). Toxin is also assumed to degrade with first order kinetics. Given all these assumptions, the governing partial differential equations for this model are:

\[
\begin{align*}
\frac{\partial R}{\partial t} &= -DL \frac{\partial R}{\partial x} + \delta \frac{\partial^2 R}{\partial x^2} - \left( \mu(R) - m \right) Nq + \alpha \left( R_S - R \right) \quad (2a) \\
\frac{\partial N}{\partial t} &= -DL \frac{\partial N}{\partial x} + \delta \frac{\partial^2 N}{\partial x^2} + \left( \mu(R) - m \right) N + \alpha \left( N_S - N \right) \quad (2b) \\
\frac{\partial C}{\partial t} &= -DL \frac{\partial C}{\partial x} + \delta \frac{\partial^2 C}{\partial x^2} + \varepsilon \left( \mu_{max} - \mu(R) \right) N - k_c C + \alpha \left( C_S - C \right) \quad (2c) \\
\frac{\partial R_S}{\partial t} &= -\left( \mu(R_s) - m \right) N_s q - \alpha \frac{A}{A_s} \left( R_S - R \right) \quad (2d) \\
\frac{\partial N_S}{\partial t} &= \left( \mu(R_s) - m \right) N_s - \alpha \frac{A}{A_s} \left( N_S - N \right) \quad (2e) \\
\frac{\partial C_S}{\partial t} &= \varepsilon \left( \mu_{max} - \mu(R_s) \right) N_S - k_c C_S - \alpha \frac{A}{A_s} \left( N_S - N \right) \quad (2f)
\end{align*}
\]

where \( R \) and \( R_S \) are nutrient concentrations in the main channel and storage zone, \( N \) and \( N_S \) are algal population densities in the main channel and storage zone, \( C \) and \( C_S \) are toxin concentrations in the main channel and storage zone, \( \varepsilon \) is the coefficient of toxin production, \( k_c \) the coefficient of toxin decay, and \( m \) the coefficient of algal mortality.

Boundary conditions based on the flows described above are
Governing equations for cove-lake system

For the cove-lake system, similar assumptions were made concerning algal growth and toxin production. In order to simplify the model, constant volume was assumed and the total dilution rate ($d^{-1}$) was defined as $D = F/ V$, and the total system exchange rate ($d^{-1}$) was defined as $E = E'/ V$. Governing ordinary differential equations for this model are:

\[
\frac{dR_1}{dt} = \frac{1 - \phi}{1 - \psi} R_1^m - \frac{D + E}{1 - \psi} R_1 + \frac{\phi D + E}{1 - \psi} R_2 - (\mu(R_1) - m) N_1 q \quad (4a)
\]

\[
\frac{dR_2}{dt} = \frac{\phi D}{\psi} R_2^m + \frac{E}{\psi} R_1 - \frac{\phi D + E}{\psi} R_2 - (\mu(R_2) - m) N_2 q \quad (4b)
\]

\[
\frac{dN_1}{dt} = -\frac{D + E}{1 - \psi} N_1 + \frac{\phi D + E}{1 - \psi} N_2 + (\mu(R_1) - m) N_1 \quad (4c)
\]

\[
\frac{dN_2}{dt} = \frac{E}{\psi} N_1 - \frac{\phi D + E}{\psi} N_2 - (\mu(R_2) - m) N_2 \quad (4d)
\]

\[
\frac{dC_1}{dt} = -\frac{D + E}{1 - \psi} C_1 + \frac{\phi D + E}{1 - \psi} C_2 + \varepsilon (\mu_{\text{max}} - \mu(R_1)) N_1 - k_c C_1 \quad (4e)
\]

\[
\frac{dC_2}{dt} = \frac{E}{\psi} C_1 - \frac{\phi D + E}{\psi} C_2 - \varepsilon (\mu_{\text{max}} - \mu(R_2)) N_2 \quad (4f)
\]

where $R_1$ and $R_2$ are nutrient concentrations in the cove and main lake, $N_1$ and $N_2$ are algal population densities in the cove and main lake, and $C_1$ and $C_2$ are algal toxin concentrations in the cove and main lake.
Results for advection-dispersion-reaction system

A sensitivity analysis of the steady states of equation system (2) was conducted, varying parameters through large ranges. Under nearly all conditions, steady state toxin concentration in the headwaters was at least about 50% lower than in downstream regions, and under some hydraulic conditions toxin concentration in headwaters was reduced close to 100% (e.g. Figs. 16 and 17). Algal population density at steady state was also usually lower in headwaters than downstream regions, but often not to the same extent. Under some hydraulic conditions, toxin concentration and algal population density in the headwater portion of the storage zone (coves) was notably higher than in the main channel, at steady state. Storage zones with large relative size \( A : A_S \) or sluggish exchange (low \( \alpha \)) were most likely to have elevated concentrations in headwater regions at steady state. Otherwise, the coves of the storage zone and the main channel tended to have similar concentrations at steady state. Longitudinal spatial distributions at steady state were most sensitive to the parameters governing hydraulic transport, dilution rate \( D \) and dispersion coefficient \( \delta \). The dimensionless Pe number is defined \( \text{Pe} = \frac{DL^2}{\delta} \), and is usually an important control on quantities affected by advective and dispersive transport. For Péclet numbers (Pe) in a critical range of about 10-100, algae and their toxins were partially washed out from the reservoir (Figs. 7 and 8), vanishing in the headwater regions, and for higher Pe complete washout occurred.

To examine non-steady state results with realistic hydrological events, the 40-year history of discharges at two USGS gauges on the Brazos River was used to estimate dilution rates in Lake Granbury (as in Grover et al., 2010). The annual average dilution rate was used to identify the driest year (1988), wettest year (1990), and a typical year (1975) in terms of flushing for this lake. Simulations of algal population density and toxin concentration were then made using the resulting daily dilution rates as forcing variables. The time series of dilution rates for a given year was repeated for multiple years until a stable annual cycle was observed in simulated quantities.

The results for the wettest year are the most interesting because the Pe number was often within the critical range where strong longitudinal variation occurred, due to near washout during high flow events. Longitudinally averaged population densities and toxin concentrations reveal that high flow events can produce lengthy reductions of these quantities (Fig. 18). Although the algal population is reduced to virtually zero and nearly washed out, it does persist and eventually recover.
Refining a Predictive Understanding of Physical, Chemical and Biological Factors Influencing *Prymnesium parvum* Population Dynamics and Toxicity

**Fig. 16.** Results of varying the dilution rate $D$ in the model of a riverine reservoir with a hydraulic storage zone. Top panels show predicted abundance of *P. parvum* at steady state; bottom panels show predicted toxin concentration. Left panels show distributions along the length of the main reservoir channel; right panels show predicted distributions along the length of the hydraulic storage zone.
Fig. 17. Results of varying the dispersion coefficient $\delta$ in the model of a riverine reservoir with a hydraulic storage zone. Top panels show predicted abundance of *P. parvum* at steady state; bottom panels show predicted toxin concentration. Left panels show distributions along the length of the main reservoir channel; right panels show predicted distributions along the length of the hydraulic storage zone.
Fig. 18. Results of forcing the model of a riverine reservoir with a variable dilution rate (line with markers). Predictions of spatially averaged quantities are shown for the main reservoir channel (solid line) and hydraulic storage zone (dashed line). Top panel — predicted abundance of *P. parvum*; bottom panels — predicted toxin concentration.

As events of high flow occurred, longitudinal distributions of algal population density and toxin concentration were predicted to have low values in headwater regions and higher values downstream (Figs. 19 and 20), resembling the steady state distributions found under conditions of high Pe numbers.
Refining a Predictive Understanding of Physical, Chemical and Biological Factors Influencing *Prumnesium parvum* Population Dynamics and Toxicity

**Fig. 19.** Results of forcing the model of a riverine reservoir with a variable dilution rate (line with markers, shown on the far-right panel). Predicted abundance of *P. parvum* is shown by intensity of yellow-green color as indicated by the key at the upper right. Left panel -- the predicted distribution of *P. parvum* along the length of the main reservoir channel. Middle panel -- the predicted distribution of *P. parvum* along the length of the hydraulic storage zone.
Refining a Predictive Understanding of Physical, Chemical and Biological Factors Influencing *Prymnesium parvum* Population Dynamics and Toxicity

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**Fig. 20.** Results of forcing the model of a riverine reservoir with a variable dilution rate (line with markers, shown on the far-right panel). Predicted toxin concentration is shown by intensity of red color as indicated by the key at the upper right. Left panel -- the predicted distribution of toxin along the length of the main reservoir channel. Middle panel -- the predicted distribution of toxin along the length of the hydraulic storage zone.

It is evident from these depictions that at many times, the predicted longitudinal patterns in algal population density and toxin concentration are similar in the main channel and storage zone of the reservoir. However, these displays hide larger, transient differences predicted to develop in headwater regions during high flow events. To visualize these transient differences, the ratio of toxin concentration in the storage zone ($C_s$) to that in the main channel ($C$) was calculated and then averaged for the upper kilometer of the reservoir. The resulting display shows that during brief episodes of high flow, the coves of the storage zone in the upper reaches of the reservoir
can have toxin concentrations several times those of adjacent waters in the main channel (Fig. 21). Very similar results were found for algal population densities during high flow events. In these cases, the storage zone is acting as classically expected, to retard downstream transport of dissolved or suspended constituents (Bencala & Walters, 1983). However, high flow is generally predicted to flush out the storage zone eventually and such episodes typically lasted 10 d or less. When simulations were done with dilution rates from the driest and the typical year for Lake Granbury, high flow events were too rare and too weak to have much impact on predicted spatial distributions of algal population density and toxin concentration. In these cases, predicted distributions varied little from corresponding steady state distributions (results not shown).

![Graph showing the ratio of toxin in the storage zone to the main channel](image)

**Fig. 21.** Results of forcing the model of a riverine reservoir with a variable dilution rate (line with small open markers, shown on the right scale). The ratio of toxin concentration in the main reservoir channel to that in the hydraulic storage zone is shown as a line with large solid markers (left scale).

In the simulations shown above, a constant and relatively high dispersion coefficient was used. This is probably realistic, since much of the dispersion in Texas lakes is likely wind-driven, and the climate of these lakes is generally windy. However, we conducted additional simulations where dispersion was an increasing function of flow, as would be expected if friction and shear at the channel bed were the dominant source of dispersion. Using an empirical function (Chapra,
1997, p. 246), with channel geometry appropriate for Texas reservoirs, the flow-dispersion relationship produces Pe numbers in the critical range for producing high longitudinal variation in algal population density and toxin concentration. Thus, when dilution rates from the wet year of 1988 are used, simulations with this alternative formulation for dispersion are very similar to those illustrated above. Still further exploration of the robustness of these results was examined by making the algal maximal growth rate ($\mu_{\text{max}}$) a function of temperature. The relationship used was similar to that in Grover et al. (2010) for *P. parvum* growing in low salinity waters, as was a sinusoidal function approximating seasonal temperature variations in Lake Granbury. Simulation results were again very similar to those illustrated above, except that predicted toxin concentration was higher in summer months. Higher temperature raised the potential growth rate relative to the actual, nutrient-limited rate. Because toxin production was assumed to be proportional to this difference, it was enhanced compared to simulations without temperature-dependent growth.

**Results for cove-lake system**

In the reaction-dispersion-advection system, coves are treated as an ensemble for the purpose of modeling their potential influence on longitudinal patterns in algal population density and toxin concentration. In contrast, the cove-lake system (eq. system 4) focuses on a single cove, allowing representation of such localized factors as high nutrient loading at a particular cove, or exceptional hydrological isolation.

Steady states predicted by this model were examined with a cove that has $1/100^{th}$ the volume of the main lake and receives $1/1000^{th}$ of the flow of the main lake. The nutrient loading concentration for the main lake was assumed to be 1.0 µM, and steady states were calculated for a wide range of loading concentrations ($R_{\text{in}}$) for the cove and exchange rates ($E$) between the cove and the main lake. In general, higher loading to the cove is predicted to produce higher algal population density and toxin concentration at steady state. However, the difference is very slight unless the cove receives about 10X higher loading than the lake and is strongly hydrologically isolated, with exchange rates corresponding to hydraulic residence times in the cove of at least 100 d (Fig. 22).
Refining a Predictive Understanding of Physical, Chemical and Biological Factors Influencing *Prymnesium parvum* Population Dynamics and Toxicity

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Fig. 22. Results of varying the exchange rate $E$ and the supply concentration to the cove $R_{in}$ in the model of cove coupled to a main lake. Left panels – predicted abundance of *P. parvum*; right panels -- predicted toxin concentration.

Differences between a cove and the main lake are also predicted to be modest when time-varying simulations are made using dilution rates from the wettest year found for Lake Granbury (Fig. 23). Flow events can produce somewhat higher algal population density in the cove when the cove is hydrologically isolated (low $E$), due to enhanced nutrient loading (recall that we have assumed that loading is proportional to flow). However, these events reduce toxin concentration similarly in both the cove and main lake, because production is proportional to nutrient limitation and enhanced loading from high flow is predicted to relieve this limitation.
Refining a Predictive Understanding of Physical, Chemical and Biological Factors Influencing *Przyminium parvum* Population Dynamics and Toxicity

---

**P-limited Flagellates**

*Cove with Rich Nutrient Supply (10.0 μM)*

\[ E = 0.3 \text{ d}^{-1} \]

- **Abundance (cells/ml)**
- **Toxin (μg/liter)**
- **Dilution Rate (d⁻¹)**

*Fig. 23.* Results of forcing the model of a cove coupled to a main lake with a variable dilution rate (line with markers). Predictions are shown for the main lake (solid line) and cove (dashed line). Top graph – rapid exchange between cove and main lake; bottom graph – slow exchange between cove and main lake. Within each graph: top panel – predicted abundance of *P. parvum*; bottom panel – predicted toxin concentration.

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Dissemination of Knowledge

1. Manuscripts


2. Presentations


Errera, RM, Pinckney, J, Roelke, DL. Pigment composition of the Texas strain of Prymnesium parvum during log, stationary, and senescent growth phases. 4th Annual HAB Symposium 2007


Refining a Predictive Understanding of Physical, Chemical and Biological Factors Influencing *Prymnesium parvum* Population Dynamics and Toxicity


Appendix A

Manuscript In-Review

Hydraulic flushing as a *Prymnesium parvum* bloom-terminating mechanism in a subtropical lake
Abstract: Prymnesium parvum blooms have increased in frequency and magnitude in the south-central US in recent years, resulting in large fish kills and economic losses. Here, we document seasonal and system-wide plankton dynamics of Lake Granbury over a period spanning the formation and termination of a large, highly toxic, P. parvum bloom that occurred January through March 2007. High-resolution spatial mapping showed this bloom was system-wide and patchy during its peak densities. Consistent with laboratory studies, the highest in-lake toxicity to fish occurred during peak bloom density and under the most stressful in-lake conditions (based on salinity, temperature, light and inorganic nutrients). As with other P. parvum blooms, this bloom at its peak density was near monospecific, with P. parvum accounting for ~92% of the phytoplankton biomass, and diatoms, cyanobacteria and green algae comprising most of the remaining biomass. The presence of alternative prey sources prior to and immediately after the peak bloom toxicity likely allowed selectively-feeding copepod and rotifer populations to co-occur with this bloom. A large inflow event in April obliterated this bloom, dramatically reducing population densities by 89%.
and completely removing toxicity to fish. Interestingly, the bloom had already started to decline somewhat before this hydraulic flushing event affected the lake. During this decline, in-lake conditions were not likely stressful to P. parvum, and predation did not appear to be a factor. The role of pathogens of P. parvum was not assessed during this study, however. Our findings show a strong link between hydrology and bloom-termination, which raises concerns given that in-stream flows are predicted to decline as human population increases in this region. In addition, flow reduction may be exacerbated by climate change. Increased understanding of factors that influence P. parvum blooms is paramount given the possible need to offset the effect of diminished hydraulic flushing.

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Harmful Algae Editorial Board

Uploaded is a manuscript for publication in the journal Harmful Algae titled “Hydraulic flushing as a Prymnesium parvum bloom-terminating mechanism in a subtropical lake”.

This research represents the first system-wide documentation of a P. parvum bloom from its initiation through its termination by employing both fixed station sampling and high-resolution spatial mapping. Through employing this technology, we were able to document throughout the course of a year the development and termination of this system-wide and highly toxic bloom. Our results underscore the importance of hydraulic flushing as a bloom terminating mechanism.

This finding is timely as the south-central region of the US, as well as many other areas of the world, are struggling with management issues relating to freshwater availability, where increased sequestration occurs with human population increases. In addition, these effects may be exacerbated with climate change.

In this manuscript submission, we cite works that are not yet available to reviewers (listed at the end of this letter). All five are Accepted (one with Limnology and Oceanography and four with Journal of the American Water Research Association). All of these papers are authored, in part, by individuals who were involved with this submitted manuscript. While I have not uploaded these accepted papers as supplementary materials, we can make them available to reviewers who wish to see them.

David Hambright, University of Oklahoma, was kind enough to provide a friendly review of this manuscript, whose comments strengthened this submission. We recommend the following as reviewers for this submission:

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We look forward to hearing from you.

Sincerely,
Daniel L. Roelke
Associate Professor

Accepted papers cited in this submission:


Hydraulic flushing as a Prymnesium parvum bloom-terminating mechanism in a subtropical lake

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Running head:
P. parvum bloom termination
Abstract

Prymnesium parvum blooms have increased in frequency and magnitude in the south-central US in recent years, resulting in large fish kills and economic losses. Here, we document seasonal and system-wide plankton dynamics of Lake Granbury over a period spanning the formation and termination of a large, highly toxic, *P. parvum* bloom that occurred January through March 2007. High-resolution spatial mapping showed this bloom was system-wide and patchy during its peak densities. Consistent with laboratory studies, the highest in-lake toxicity to fish occurred during peak bloom density and under the most stressful in-lake conditions (based on salinity, temperature, light and inorganic nutrients). As with other *P. parvum* blooms, this bloom at its peak density was near monospecific, with *P. parvum* accounting for ~92% of the phytoplankton biomass, and diatoms, cyanobacteria and green algae comprising most of the remaining biomass. The presence of alternative prey sources prior to and immediately after the peak bloom toxicity likely allowed selectively-feeding copepod and rotifer populations to co-occur with this bloom. A large inflow event in April obliterated this bloom, dramatically reducing population densities by 89% and completely removing toxicity to fish. Interestingly, the bloom had already started to decline somewhat before this hydraulic flushing event affected the lake. During this decline, in-lake conditions were not likely stressful to *P. parvum*, and predation did not appear to be a factor. The role of pathogens of *P. parvum* was not assessed during this study, however. Our findings show a strong link between hydrology and bloom-termination, which raises concerns given that in-stream flows are predicted to decline as human population increases in this region. In addition, flow reduction may be exacerbated by climate change. Increased understanding of factors that influence *P. parvum* blooms is paramount given the possible need to offset the effect of diminished hydraulic flushing.
1. Introduction

The haptophyte alga *Prymnesium parvum* occurs worldwide, is tolerant of large variations in temperature and salinity, and sometimes forms fish-killing blooms (Edvardsen and Paasche 1998, Lundholm and Moestrup 2006, Baker et al. 2007, Accepted). Since 2001, the incidence of *P. parvum* blooms in the south-central US has increased dramatically. In this region, fish-killing blooms are now observed in many lakes found along multiple river basins. During blooms, surface waters take on a golden color with *P. parvum* cell densities typically exceeding $10 \times 10^6$ cells L$^{-1}$ (TPWD 2003, Roelke et al. 2007, Schwierzke et al. Accepted). Many fish species ranging from herbivores to piscivores are killed during these blooms, where total mortalities number in the tens of millions (TPWD 2003, Roelke et al. 2007).

Environmental conditions conducive to blooms and the factors that lead to formation and termination of harmful algal blooms (HABs) in general are complex (Paerl 1988, Roelke and Buyukates 2001). Currently, the factors affecting the incidence of *P. parvum* blooms in the south-central US are only partly understood.

Conditions that favor *P. parvum* blooms in the south-central US might include
eutrophication and salinization. Blooms in Europe, the Middle East, and Asia have all occurred in eutrophic and brackish systems (Krasnotshchek and Abramowitsch 1971, Holdway et al. 1978, Rijn and Shilo 1989, Kaartvedt et al. 1991, Guo et al. 1996, Amsinck et al. 2005, Granéli et al. 2008). *P. parvum* blooms in the south-central US are partly consistent with these observations because they appear mostly in lakes that attain salinities of 2 to 4 practical salinity
units (psu) during low precipitation years (TPWD 2003), and these lakes may have experienced increased nutrient loading because of aging septic systems, point source discharges and expanded shoreline development. The role of nutrients is complex, however. The toxicity to fish from chemicals produced by *P. parvum*, which are believed to also act as allelochemicals and to be an important factor leading to bloom initiation, was greater when cells were nutrient-limited, based on laboratory and field experiments (Uronen et al. 2005, Roelke et al. 2007, Errera et al. 2008). In addition, *P. parvum* is sensitive to pulses of nutrients where high doses inhibited bloom formation and reduced toxicity (Barkoh et al. 2003, Grover et al. 2007, Kurten et al. 2007, Gordon and Colorni 2008). Therefore, the temporal variation in nutrient availability is likely more important than a system’s trophic state.

Factors initiating and terminating *P. parvum* blooms in the south-central US have not been determined. They may include production of chemicals toxic to grazers (Granéli and Johansson 2003, Tillmann 2003, Barreiro et al. 2005, Calliari and Tiselius 2005, Roelke et al. 2007, Sopanen et al. 2006, 2008, Michaloudi et al. 2009, Brooks et al. Accepted), use of alternative energy and nutrient sources through mixotrophy and saprophytic nourishment (Nygaard and Tobiesen 1993, Skovgaard and Hansen 2003, Burkholder et al. 2008, Lindehoff et al. 2009), suppression of competitors through allelopathy (Fistarol et al. 2003, 2005, Granéli and Johansson 2003, Roelke et al. 2007, Errera et al. 2008, Michaloudi et al. 2009), and resistance to the allelopathic effects of cyanobacteria (Suikkanen et al. 2004, Tillmann et al. 2007; but see Grover et al. Accepted, Roelke et al. Accepted). These factors are not mutually exclusive. Additional factors that seem to influence the growth of other HABs, which might also influence *P. parvum* population dynamics, include the production of beneficial or deleterious chemicals by various bacterial taxa (Kodama et al. 2006, Salomon and Imai 2006) and the pathogenic effects
of viruses (Salomon and Imai 2006, Schwierzke et al. Accepted).

In the south-central US, *P. parvum* blooms occur during the January through March months (winter through early spring). Interestingly, temperature conditions during this time of year are not optimal for *P. parvum* growth (based on observations of the Texas strain of *P. parvum*), and are in fact very near the edge of its niche (Baker et al., 2007, Accepted). This underscores the importance of other factors that might give *P. parvum* a selective advantage over its competitors, such as grazer inhibition, allelopathy and mixotrophy, thereby allowing blooms to initiate. It also raises the question of what factors terminate blooms in lakes of the south-central US, which occur at a time when temperature becomes more favorable for growth. In this manuscript we provide the first seasonal and system-wide characterization of *P. parvum* bloom formation and termination in a subtropical lake, Lake Granbury in the south-central US.

2. Methods

Lake Granbury is a reservoir on the Brazos River, Texas, US, constructed in 1969. The lake has a capacity of $167 \times 10^6$, a surface area of $34 \text{ km}^2$, and an average depth of ~5 meters. The shoreline follows the meandering river channel with an elongated, sinuous basin oriented northwest to southeast that is ~45 km long and has an average width of 0.6 km. Daily discharges from the Brazos River into the lake were measured at a location upstream from the lake (Dennis, Texas, USGS Station Number 08090800).

Monitoring in Lake Granbury encompassed monthly sampling at ten fixed stations (Fig. 1) and spanned August 2006 through August 2007. For purposes of this research, only data collected at six stations were used in the analysis (stations 1, 3, 5, 6, 8, and 10). These stations were located over the historic riverbed. The other four stations represented shallow water
environments adjacent to some of the deep-water locations and are a part of a separate study. At each station multiple parameters were measured from well-mixed surface waters (i.e., samples were taken at 0.5 meters, and vertical profiling with a multi-probe was as deep as 10 meters), including characterizations of the phytoplankton (chlorophyll \(a\), \(P. \) \(parvum\) population density, and the density of taxonomically-aggregated phytoplankton groups such as cyanobacteria, diatoms and chrysophytes), zooplankton (cladocerans, copepod adults and nauplii, rotifers, ciliated protozoa), and water quality (salinity, temperature, light, pH, inorganic nutrients, ambient water toxicity). In addition, system-wide, high-resolution spatial maps of chlorophyll \(a\) were generated during each sampling date. Throughout the study period, our vertical profiling indicated the lake was well-mixed, as all water quality profiles showed no gradients with depth.

Estimates of total phytoplankton biomass (approximated using chlorophyll \(a\)) and biomass of taxonomically-aggregated phytoplankton groups (approximated as a fraction of the total chlorophyll \(a\)) were determined from photopigment biomarker concentrations (Pinckney et al. 1998) and the use of CHEMTAX (Mackey et al. 1997, Wright et al. 1996). For the CHEMTAX model initiation, aggregated taxonomic groups were selected based on their historical prevalence in L. Granbury. For greater detail of the HPLC and CHEMTAX methods followed, see Roelke et al. (2007).

For estimation of \(P. \) \(parvum\) population density, a 100 mL phytoplankton sample was collected from each station and preserved using glutaraldehyde, 5% v/v. Enumeration of \(P. \) \(parvum\) was performed using a settling technique (Utermöhl 1958) and inverted, phase contrast light microscopy (400x, Leica Microsystems). A subsample of 1 mL was settled for 24 h. Randomly selected fields-of-view where then counted until >200 \(P. \) \(parvum\) cells were counted (5 to 40 fields-of-view).
Zooplankton samples were collected with a 12 L Schindler trap (61 µm mesh size), concentrated to 50 mL, and preserved in buffered formalin, 5% v/v. Typically, subsamples for settling were ~19 mL and were settled for 24 hours (during times of low zooplankton abundance the entire sample was settled), then counted using an inverted, phase contrast light microscope (40x and 200x, Leica Microsystems). For each individual counted, dimensions were measured corresponding to best-fit geometric shapes to estimate biovolume (Wetzel and Likens 1991). For this study zooplankton species were grouped into cladocerans, copepod adults, copepod nauplii, rotifers, and ciliated protozoa. Our enumeration technique typically resulted in ~120 individuals counted per sample.

Samples for inorganic nutrients (nitrogen and phosphorus) were filtered through pre-combusted GF/F filters, and the filtrates were frozen until analysis. Inorganic nutrient concentrations were determined using autoanalyzer methodology (Armstrong and Sterns 1967, Harwood and Kuhn 1970). For this study, nitrate (NO₃), nitrite (NO₂) and ammonium (NH₄) were summed as dissolved inorganic nitrogen (DIN), and phosphorus was soluble reactive phosphorus (SRP).

Ambient water toxicity was estimated from acute toxicity to fish. To achieve these observations, standardized 24-hour static toxicity assays with the juvenile fathead minnow (Pimephales promelas) model were employed. Toxicity assays followed standardized methods for determining aquatic toxicity of ambient surface waters (US EPA 2002). Samples were collected and transported to the laboratory where toxicity tests were initiated within 24 hours. Ambient samples were diluted using a 0.5 dilution series with reconstituted hard water, which was performed generally following US EPA recommendations (US EPA 2002). For greater
detail of the methods followed for these fish toxicity assays with *P. parvum*, refer to Brooks et al. (Accepted).

Salinity, temperature and pH in Lake Granbury were determined with a water quality multi-probe (Quanta, Hydrolab) and light penetration was determined with a Secchi disk. Estimation of average irradiance based on Secchi depth ($z_{Secchi}$) is discussed further below.

To complement our spatiotemporal characterizations of the *P. parvum* bloom, and phytoplankton as a whole (based on our fixed station data), we measured spatial patterns of chlorophyll $a$ during each sampling trip with Dataflow, a high-speed, flow-through measurement apparatus developed for mapping physicochemical parameters in shallow aquatic systems (Madden and Day 1992). We used this integrated instrument system to concurrently measure multiple water quality parameters that included chlorophyll $a$ (*in-vivo* fluorescence) from a boat following closely spaced transects (see Fig. 1). Measurements were taken at 2-second intervals from ~20 cm below the surface. An integrated GPS was used to simultaneously plot sample locations. GPS and dataflow information were then used to create detailed contour maps (Surfer v8.0).

Non-metric multidimensional scaling (NMS) was used to explore multivariate relationships within the fixed-station data (PC-ORD v.5.1; McCune and Mefford, 1999). Matrices were based on normalized physical, chemical and biological variables. We employed the Sorensen (Bray-Curtis) dissimilarity metric to determine the dimensional distances among each sampling time at each fixed-station. A final solution of two dimensions was achieved based on the lowest stress obtained using a Monte-Carlo test after 250 iterations (repeated 10 times) in a cascade procedure and using a stability criterion of 0.0001 (McCune and Grace 2002). Final stress was calculated as 12.88. Joint vectors were then used to identify significant variables that
mediate the temporal and spatial changes. Vectors were drawn as the hypotenuse of regression determination coefficients ($r^2$) between variables (see McCune and Grace 2002).

Based on our observations, hydraulic flushing played an important role as a mechanism influencing $P$. $parvum$ population dynamics. To better evaluate its relative impact, we estimated in-lake specific growth rates for $P$. $parvum$ using two mathematical models. The first model (eq. 1) was based on low-salinity laboratory experiments where salinity and temperature were varied (Baker et al. Accepted). The second model (eq. 2) was a hybrid equation (Grover et al. Accepted) that employed the first model and the effects of variable irradiance from a prior set of experiments over a wider range of salinity (Baker et al. 2007). Both sets of experiments used a strain of $P$. $parvum$ isolated from Texas waters. The models were as follows:

$$\mu = -3.531 + 0.02534(S - 1.833) - 0.06311(S - 1.833)^2 + 7.468e^{0.7((T - 20)/20)} - 3.414e^{1.4((T - 20)/20)} + 0.1697(S - 1.833)e^{0.7((T - 20)/20)} - 0.00000573(E - 222)^2$$

where $\mu$ was the specific growth rate (d$^{-1}$), $S$ was salinity (psu), $T$ was temperature ($^\circ$C) and $E$ was irradiance ($\mu$mol-m$^{-2}$-s$^{-1}$). These equations were formulated under experimental conditions where inorganic nutrients did not limit $P$. $parvum$ specific growth rate.

For purposes of this research, we used the average underwater irradiance ($I_{avg}$) as a surrogate for $E$. This was approximated using the surface irradiance, a relationship between the light extinction coefficient and Secchi depth, and the average depth of Lake Granbury. Surface irradiance (at zero depth, $I_0$) was estimated using mathematical models that accounted for time of year and latitude, and assumed cloudless conditions and a fixed water reflectance (Kirk 1994,
The light extinction coefficient \( (k) \) was estimated as a function of Secchi depth (1.7/z_{Secchi}, Wetzel 2001). The average underwater irradiance was then approximated using:

\[
I_{\text{avg}} = I_0 (1 - e^{-kz}) / (kz)
\]  

where \( z \) (meters) was the average depth of Lake Granbury.

The calculated specific growth rate was used along with flushing losses to estimate the change in population density attributable to flushing during the period when the bloom was terminated using the differential equation:

\[
\frac{d\phi}{dt} = (\mu - d)\phi
\]

where \( \phi \) was the \( P. \parvum \) population density with an initial condition equal to the March sampling date (just prior to the flushing event), \( \mu \) was the daily specific growth rate interpolated between the March and April samplings (based on equations 1 and 2), and \( d \) was the daily flushing rate for the period between March and April samplings calculated by dividing the daily inflow (USGS records) by the volume of naturally occurring well-mixed segments of the lake.

Based on the dataflow maps for chlorophyll \( a \), the length over which lake waters were well-mixed was determined to range between 1 and 6 km for the March and April samplings, respectively. By employing average lake depth and width dimensions, the volume of well-mixed segments was estimated.

3. Results

For the period of study, inflows into Lake Granbury were episodic, as is common in lakes of the south-central US. From September 2006 through March 2007 inflows were barely discernable (Fig. 2). In April 2007 the first large inflow event occurred with peak flows attaining 80 x 10^6 m^3 d^{-1}, corresponding to single-day hydraulic flushing peaks of 16 d^{-1} (if well-mixed
patches are on the scale of 1 km) and 2.7 d\(^{-1}\) (if well-mixed patches are on the scale of 6 km).

Episodic inflows of varying magnitude and duration persisted through June, where the largest inflow event reached \(~120 \times 10^6 \text{ m}^3 \text{ d}^{-1}\).

According to our CHEMTAX pigment model, phytoplankton biomass peaked in March just prior to the first large inflow event of 2007, with highest biomass occurring towards the lower end of the lake (Figure 2a). Phytoplankton composition varied somewhat during the three months leading up to the biomass peak (January through March 2007). Prymnesiophytes were prevalent from January to February, comprising \(~92\%\) of the total phytoplankton biomass.

During this same period other taxonomic groups changed little and were \(~6\%\) diatoms, \(~1\%\) green algae and \(~0.5\%\) cyanobacteria. For the period between February and March the prevalence of prymnesiophytes declined to \(~87\%\) of the total phytoplankton biomass. The prevalence of diatoms and cyanobacteria increased to \(~10\%\) and \(~2\%\) during this period.

Our direct cell counts of \(P. \text{parvum}\) were in agreement with our CHEMTAX predictions and indicated that the \(P. \text{parvum}\) bloom reached its highest population densities of \(~40 \times 10^6\) cells liter\(^{-1}\) in February with highest population densities occurring in the mid-reaches of the lake (91\% of the phytoplankton biovolume). Cell densities \(>10 \times 10^6\) cells liter\(^{-1}\) are considered bloom proportions based on historical observations in lakes of the south-central US (TPWD 2003, Roelke et al. 2007, Schwierzke et al. Accepted). Average \(P. \text{parvum}\) densities for the lake declined \(~27\%) by March (Fig. 2b), a larger proportional decrease than predicted by the CHEMTAX model. Measurements of ambient toxicity to fish were consistent with observed population densities, with LC\(_{50}\) values as low as \(~4\%\) observed in February in the mid reaches of the lake, with toxicity to fish decreasing (LC\(_{50}\) increasing) by March (Fig. 2c). \(P. \text{parvum}\) population densities for the lake were obliterated after the first large inflow event to the lake,
decreasing by 89% from the March to April sampling. In addition, waters were no longer toxic to fish.

Cyanobacteria were not abundant in Lake Granbury during the time of bloom development or termination. Cyanobacteria biomass was maximal during the months of July through September 2007 (Fig. 2d) and in the lower reaches of the lake, accounting for ~7-8% of the total phytoplankton biomass according to CHEMTAX pigment estimates.

Dataflow mapping revealed that the bloom was patchy throughout Lake Granbury with characteristic patches of ~1 km (Fig. 3a, representative map during the bloom). The location of elevated chlorophyll a patches did not appear related to morphometric attributes such as shoreline development or depth. While we have microscopic and photopigment verification of the *P. parvum* bloom only from the fixed stations, visual observations indicated ‘golden’ colored water, foam lines and dead fish throughout the lake during January to March 2007. This suggests that the in-vivo fluorescence signal attributed to chlorophyll a from the Dataflow unit indicates the *P. parvum* bloom distribution. After the first inflow event in April 2007, phytoplankton biomass was greatly diminished and patchiness decreased, where characteristic patches were ~6 km (Fig. 3b).

Zooplankton population dynamics showed varied trends for aggregated taxonomic groups. Maximum biomass of cladocera (~70 X 10^6 µm^3 liter^-1, ~12 individuals liter^-1) occurred immediately after the first large inflow event to the lake, and the large decline in *P. parvum* population density and removal of toxicity to fish (Fig. 4a). Interestingly, copepod adults were abundant just before and immediately after the *P. parvum* and toxicity maxima (~160 X 10^6 µm^3 liter^-1, ~63 individuals liter^-1), as were copepod nauplii (~80 X 10^6 µm^3 liter^-1, ~91 individuals liter^-1) to a lesser extent (Fig. 4b,c). Abundances of copepod adults and nauplii greatly reduced
after the first large inflow event in April. Rotifers, which accounted for the greatest biomass of zooplankton, accumulated during the fall months of 2006 leading to a maximum in January 2007 (~750 X 10^6 µm^3 liter^-1, ~735 individuals liter^-1), immediately prior to the P. parvum population and fish toxicity maxima (Fig. 4d). Ciliated protozoa were maximum during the summer months of 2007 (~1.4 X 10^6 µm^3 liter^-1, ~20 individuals liter^-1), but did not account for large amounts of the total zooplankton biomass at any time (Fig. 4e).

As with many of the lakes in the south-central US, Lake Granbury can sometimes be brackish. Prior to April 2007, when inflows were not significant, salinity was ~1.5 psu throughout Lake Granbury (Fig. 5a). The lake remained at salinities >1 psu until after the first large inflow event in April 2007, at which time salinity dropped to ~0.5 psu. Temperature changes in the lake were seasonal, with minima of ~6°C in February 2007 and maxima of ~32°C in August 2007 (Fig. 5b). While Secchi depth varied spatially and temporally, a prominent feature was that relatively deep Secchi depths (~1 meter) coincided with the P. parvum bloom, while a rapid decrease in light penetration (to ~0.5 meters) immediately followed the first large inflow event in April 2007 (Fig. 5c). Similarly, pH was greatest (~8.8) during the period of bloom and decreased (to ~7.8) immediately following the first large inflow event in April (Fig. 5d).

Dissolved inorganic nutrients also showed a strong relationship with inflow, and immediately following the first large inflow event in April 2007 both SRP and DIN reached their maxima of ~0.55 µM-P and ~24 µM-N (Figure 6). Highest nutrient concentrations were measured in the lower reaches of the lake at this time. During January through March 2007, when P. parvum population densities were greatest and then started to decline, the DIN:SRP was ~30 with SRP concentrations ~0.05 µM-P and DIN ~1.35 µM-N. Except for December 2006,
nutrient concentrations during the bloom were similar to the months prior to the bloom. In December, SRP concentrations were at their lowest, ~0.03 µM-P, while DIN was ~3.55 µM-N (DIN:SRP ~122).

The multivariate NMS analyses also revealed strong temporal patterns in Lake Granbury (Fig. 7). A prominent feature of the two-dimensional solution (71% and 22% of the variability in the data shown on axis 1 and 2, respectively) was the *P. parvum* bloom occurring in the winter months (January through March 2007). The maxima in copepod adults and rotifers that occurred during these months, but not coincident with the *P. parvum* maximum, was also clearly shown. Other prominent features of the analysis showed the higher nutrient concentrations following the inflow events to Lake Granbury during the spring and early summer months (April through July 2007), a period when *P. parvum* cell densities were greatly reduced. Finally, the cladocera maximum in the spring, and the cyanobacteria and ciliated protozoa maxima in the summer months, were prominent features of the analysis.

The two mathematical models used to predict the specific growth rate of *P. parvum* (equations 1 and 2) produced very similar results, indicating that the growth rate of *P. parvum* was lowest at the time when cell densities were highest, i.e., January and February 2007 (Fig. 8a). Interestingly, specific growth rate predictions increased for March 2007, at the time when *P. parvum* population densities were beginning to decline. The temperature terms in these models strongly affected predictions. As temperature increased during January to March, growth rate increased. In the late summer (August and September 2007), very high temperatures above the modeled optimum for *P. parvum* led to predictions of decreased growth rate.

The size of well-mixed surface water patches varied. During March, after an extended period of low inflows, patches were on the scale of ~1 km. During April, after a large inflow...
event, the lake was better mixed and patches were on the scale of ~6 km. Patch size influences the estimate of hydraulic flushing. Because our frequency of sampling did not match the rate of hydraulic change, we evaluated hydraulic flushing for this period bounded by lower (6 km) and upper (1 km) bounds for flushing estimates. This analysis showed that hydraulic flushing was near non-existent prior to the first large inflow event in April, and that the inflow pulses that started in April and continued sporadically through the early summer months produced flushing losses much higher than specific growth rates for *P. parvum* (Figure 8b).

Focusing on the period between March and April, when the first large inflow event occurred, average *P. parvum* population density in the lake decreased ~89%, as mentioned above. Modeled population reductions for this month-long period (based on equation 4) were 100% and 68% for the scenarios where well-mixed lake segments were 1 and 6 km (see Fig. 3). The model, however, predicts that this decrease to the *P. parvum* bloom occurred over a period of only 4 days (Fig. 9).

4. Discussion

This study is the first to document the dynamics of *P. parvum* across an entire lake and over an annual cycle, encompassing bloom formation, toxicity to fish, and bloom termination. Some of the patterns found here confirm current understanding of population dynamics and toxicity of this HAB species, while others raise questions.

Production of toxic chemicals by *P. parvum* increases when environmental conditions are stressful (Nygaard and Tobiesen 1993, Barreiro et al. 2005, Uronen et al. 2005, Roelke et al., 2007, Errera et al. 2008). Like other HAB species (Granéli and Hansen 2006, Turner 2006,
Prakash et al. 2009), production of toxic chemicals might enable *P. parvum* to initiate blooms because it imparts a competitive advantage over other phytoplankton or inhibits grazers. During the months prior to the *P. parvum* bloom in Lake Granbury (September through December 2006), it is unclear which environmental conditions might have been stressful. Salinity, temperature and light were favorable for *P. parvum* growth. In addition, nutrient concentrations during those months did not seem strongly limiting as they were never below the detection threshold of the analytical method. SRP reached its lowest concentrations in December 2006, when it was ~0.03 µM-P (DIN:SRP ~121). While this concentration might have limited growth of some phytoplankton species (Grover 1989, Grover et al. 1999, Reynolds 2006), it was not likely limiting to *P. parvum*, which has shown positive growth at SRP concentrations lower than this (Baker et al. Accepted). Lack of stress, and generally favorable conditions suggest that the *P. parvum* bloom could have initiated from in situ growth in Lake Granbury, and that allelopathic effects were not important at this early stage. Another possibility is that conditions favorable to bloom initiation occurred in a lake up-river of Lake Granbury, i.e., Lake Possum Kingdom (Roelke et al. 2007), and that the bloom developed due to mass effects (*sensu* Liebold and Miller 2004) resulting from large migrations of toxic *P. parvum* cells into Lake Granbury. In contrast to these early stages, environmental conditions were stressful to *P. parvum* during the periods of bloom development and peak population densities (January and February 2007). While nutrient concentrations did not appear to be strongly limiting, predicted growth rates based on temperature, salinity and light were near zero, primarily due to the low winter temperatures. Consistent with the notion that *P. parvum* cells are more toxic when stressed by suboptimal growth (Baker et al. 2007), the highest observed fish toxicities were during these winter months.
At this time, the *P. parvum* bloom was extensive throughout the entire system, a phenomenon observed for many other HAB species (e.g., Roelfsema et al. 2006, Chang et al. 2008, Oh et al. 2009). The bloom was also patchy, as some areas of the lake experienced elevated phytoplankton biomass while others experienced the bloom at lower densities. Blooms of *P. parvum* are typically characterized by near-complete dominance of this species, which has been observed during blooms in other lakes of the Brazos River (Roelke et al. 2007, Schwierzke et al. Accepted) and elsewhere (Sunda et al. 2006, Sopanen et al. 2008, Michaloudi et al. 2009). This *P. parvum* bloom in Lake Granbury was also near-monospecific (~92% decreasing to 87% before the first flushing event), with diatoms, green algae and cyanobacteria comprising much of the remaining biomass.

The presence of other algae, although not in high proportion, during this bloom might have influenced zooplankton. From other studies, copepods and rotifers seem sensitive to *P. parvum* toxins (Sopanen et al. 2006, 2008, Roelke et al. 2007, Brooks et al. Accepted), but here biomass peaks for copepod adults and rotifers were observed just prior to the peak of the *P. parvum* bloom and peak toxicity. Recovery of copepods immediately after the bloom peak was also observed. In general, copepods and rotifers are known to be selective grazers (Pagano et al. 1998, Zollner et al. 2003, Hambright et al. 2007). It may be that the presence of other algae during this *P. parvum* bloom allowed these zooplankton taxa to persist by providing alternative prey sources.

The first large inflow event, which occurred in April 2007, obliterated the *P. parvum* bloom. Single day estimates of hydraulic flushing for Lake Granbury at this time ranged between 2.7 and 16 d⁻¹. These estimates of flushing loss were greater than the predicted growth rate of *P. parvum* based on conditions of salinity, temperature and light. Our modeled estimate
of net population growth rate predicted population declines that agree quantitatively with the monthly observed declines, but also indicated that the actual bloom termination occurred over a much shorter period, i.e., 4 days. Hydraulic flushing as a bloom-terminating mechanism has been observed for other HABs, particularly blooms of cyanobacteria (Jacoby et al. 2000, Moustaka-Gouni et al. 2006).

In addition to the direct loss of cells through flushing, *P. parvum* likely ceased production of toxins with the rapid increase in nutrient concentrations (Roelke et al. 2007, Errera et al. 2008). Our sampling revealed the complete removal of ambient toxicity to fish with this inflow event. This removal of fish toxicity might have resulted from the cessation of toxin production coupled to degradation of the existing toxins, dilution of in-lake toxin concentrations by hydraulic flushing, or it might have resulted because the pH dropped to levels that altered the ionization state of toxins, rendering them harmless (Valenti et al. In Review). Regardless of the mechanism, the removal of toxicity would have greatly diminished *P. parvum*’s ability to compete with other phytoplankton or inhibit zooplankton.

Interestingly, the bloom started to decline (based on direct cell counts) before hydraulic flushing affected the lake. It is unclear why. The predicted growth rate of *P. parvum* based on salinity, temperature and light increased from February to March 2007, mostly because of the temperature increase. Inorganic nutrients did not appear limiting to *P. parvum*. Copepod biomass increased, suggesting increased grazing, but these biomass increases were only observed in the upper reaches of the lake, in contrast to the system-wide decrease in *P. parvum*. Factors unaccounted for in this study include pathogens of *P. parvum*. Viruses and algicidal bacteria are known to deleteriously affect other HABs (Kodama et al. 2006, Salomon and Imai 2006). Furthermore, in-situ experiments from Lake Whitney, positioned down-river from Lake...
Granbury, showed that viruses can impact population dynamics of *P. parvum* in later stages of bloom development (Schwierzke et al. Accepted). While we did not sample viruses in this study, it may be that they influenced *P. parvum* population densities just prior to the impact of the first inflow event.

After the hydraulic flushing of *P. parvum* and fish toxicity removal, cladocera became prevalent. This was not surprising in that cladoceran sensitivity to toxins produced by *P. parvum* was previously observed in laboratory and in-lake experiments (Roelke et al. 2007, Brooks et al. Accepted). In addition, cladocera are capable of parthenogenetic growth, enabling quick accumulation of biomass under more favorable conditions (Wetzel 2001).

The post-flushing distribution of phytoplankton was patchy, but at much lower densities than prior to the flushing event of April 2007. Although conditions of temperature, salinity, and light were predicted to be favorable for *P. parvum* growth, the bloom did not re-establish. This was likely due to the subsequent hydraulic flushing events that occurred in the remaining spring months of 2007. Early during the following summer, when inflows were reduced, the bloom still did not re-establish. Environmental conditions were not as stressful as spring conditions, and thus toxins were likely not being produced, leaving *P. parvum* vulnerable to zooplankton grazers, such as the rotifers that dominated the zooplankton community at this time.

Eventually, conditions did again become stressful for *P. parvum* growth as summer temperatures rose above its optimum. Yet there was no indication of toxin production by *P. parvum*, or of attendant allelopathic effects on its competitors or grazers, and *P. parvum* did not become abundant. At this time, however, cyanobacteria were present. There is indirect evidence that some cyanobacteria might have an allelopathic effect on *P. parvum* in Texas lakes (Grover...
et al. Accepted, Roelke et al. Accepted). Thus their presence during summer months of 2007 might have prevented *P. parvum* from accumulating biomass.

This study underscores the importance of hydraulic flushing as a mechanism terminating *P. parvum* blooms in lakes of the south-central US. In the decades to come, it is likely that water flow through these lakes will decrease as human populations expand and water use permits are more fully utilized. The effects of decreased through-flow might be exacerbated by climate change as well, as precipitation patterns alter. As such, reservoirs of the south-central US may represent unique ecosystems for studying such climatic effects on surface waters particularly because this region spans a broad geographical range with dramatic annual rainfall gradients. This study and other recent studies focused in the south-central US, have also produced evidence that factors other than flushing likely play important roles in *P. parvum* bloom dynamics. It may be that the effects of reduced through-flows can be offset by management efforts building on these other factors. For example, blooms might be mitigated by localized fertilization in areas where blooms develop in attempts to prevent toxin production and bloom initiation (Barkoh et al. 2003, Grover et al. 2007, Roelke et al. 2007, Errera et al. 2008), promoting growth of phytoplankton that can suppress *P. parvum* through competition (Grover et al. Accepted, Roelke et al. Accepted), manipulations of pH in hot spots of bloom initiation to negate the potency of toxins (Valenti et al. In Review), or localized introduction of natural predators and pathogens during the time of bloom initiation (Schwierzke et al. Accepted). There is much understanding still to be gained, however, before effective management of *P. parvum* in lakes of the south-central US can be implemented.
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Figure Captions

Figure 1. Lake Granbury located in the south-central US. This research coupled fixed station sampling with high-resolution spatial mapping (dark line indicates path of on-board data collection) that enabled detailed analysis of the plankton environment and system-wide characterizations of bloom dynamics.

Figure 2. Characterizations of the phytoplankton and ambient toxicity for a one-year period that spanned the formation and termination of a *P. parvum* bloom. The bloom developed during the fall months and peaked in winter, but following a high inflow event in April the bloom was greatly diminished, as evidenced by trends in chlorophyll a (a) and *P. parvum* population densities (b). Toxicity (c) was maximal during the peak of *P. parvum* population density, but ceased after this inflow event. Cyanobacteria (d), potential competitors through allelopathy, were prevalent only during the summer months.

Figure 3. System wide characterizations of phytoplankton biomass using high-resolution spatial mapping. During the winter months, biomass patterns suggest a system-wide, but patchy bloom of *P. parvum* (a). After the flushing event, phytoplankton densities were much lower and the distribution remained patchy (b).

Figure 4. Characterizations of the zooplankton for a one-year period that spanned the formation and termination of a *P. parvum* bloom. Cladocera densities (a) were only significant in the lake after the bloom was terminated. Adult copepods (b) were abundant immediately prior to the
peak in bloom density, and also immediately after the bloom peak. Copepod nauplii (c) become more abundant immediately after the bloom peak. Rotifers (d), which were the dominant grazer during this period, show a peak biomass immediately prior to the peak in bloom density. Ciliated protozoa (e) were not prevalent during the period of study, and showed maximum biomass during the summer months.

Figure 5. Characterizations of the physicochemical environment for a one-year period that spanned the formation and termination of a *P. parvum* bloom. Salinity (a) was highest in the earlier part of the study period, but decreased following the April inflow event. Temperature (b) followed a typical seasonal cycle for north-hemisphere, sub-tropical climates. Irradiance, as approximated with Secchi depth (c), showed decreased with the spring inflow events. pH (d) was highest during the peak bloom period, and decreased following the April inflow event.

Figure 6. Characterizations of inorganic nutrients for a one-year period that spanned the formation and termination of a *P. parvum* bloom. Soluble reactive phosphorus (a) and dissolved inorganic nitrogen (b) were low in the lake prior to the April inflow event, but were still at concentrations high enough to support *P. parvum* growth.

Figure 7. Non-metric multidimensional analysis (71% and 22% of the variability shown on axes 1 and 2, respectively) reinforced field observations by showing the bloom of *P. parvum* to be associated with the winter months along with adult copepods and rotifers, the nutrient loading to be associated with the spring and early-summer inflow events, cladocera abundance to be associated with the spring months, and ciliates and cyanobacteria to be associated with the
summer months. Each point in this graph represents a single sampling in space and time. That is, no averaging of data is shown.

Figure 8. Mathematical models (equations 1 and 2) that predict *P. parvum* growth rate as a function of salinity, temperature and light (a) revealed that periods of stress coincided with January and February (the period of peak bloom intensity) and late summer. Estimates of hydraulic flushing (b) show that prior to the first large inflow event in April hydraulic flushing was negligible. When inflows commenced, however, they resulted in flushing losses greater than the specific growth rate of *P. parvum*. Lower and upper estimates of flushing were calculated based on well-mixed patches of 6 and 1 km, respectively.

Figure 9. Numerical model (equation 4) simulation that reproduced our monthly observation of the *P. parvum* bloom decline (open stars), showing that the actual bloom termination occurred over an estimated period of 4 days. Lower and upper estimates of population density were from simulations that employed well-mixed patches of 6 and 1 km, respectively.
Figure 1
Deep water stations (above historic river channel)

P. parvum ($10^6$ cells liter$^{-1}$)

Phytoplankton ($\mu$g-chl a liter$^{-1}$)

River flow ($10^6$ m$^3$ d$^{-1}$)

Deep water stations (above historic river channel)

Toxicity to Fish (LC$_{50}$ as % dilution)

Cyanobacteria ($\mu$g-chl a liter$^{-1}$)

Figure 2
Deep water stations (above historic river channel)

River flow (10^6 m^3 d^-1)

- Cladocera (x10^6 µm^3 liter^-1)
- Copepod adults (x10^6 µm^3 liter^-1)
- Copepod nauplii (x10^6 µm^3 liter^-1)
- Rotifers (x10^6 µm^3 liter^-1)
- Ciliates (x10^6 µm^3 liter^-1)

Figure 4
Deep water stations (above historic river channel)

- Salinity (psu)
  - 0.5
  - 1.0
  - 1.5
- Temperature (°C)
  - 1.1
  - 19
  - 32
- Secchi depth (meters)
  - 0.3
  - 0.7
  - 1.1
- pH
  - 7.8
  - 8.3
  - 8.8

River flow ($10^6$ m$^3$ d$^{-1}$)
- 0
- 1
- 3
- 6
- 10

Figure 5
Deep water stations (above historic river channel)

River flow
(10⁶ m³ d⁻¹)

a. SRP (µM-P)

b. DIN (µM-N)

Deep water stations (above historic river channel)

2006 2007

Figure 6
Figure 7
Figure 8
Figure 9
Growth at the edge of the niche: An experimental study of the harmful alga Prymnesium parvum

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Abstract

The haptophyte Prymnesium parvum forms harmful blooms toxic to fish in coastal and inland waters. Its growth in relation to niche factors is poorly characterized for the low salinities found in the inland waters in which P. parvum blooms have occurred. The specific growth rate of P. parvum as a function of temperature and salinity was determined in nutrient-sufficient cultures with low salinity. Additionally, phosphorus-limited growth was determined at low salinity and temperatures at or below 20°C. In nutrient-sufficient cultures, decreasing salinity from 4 g L−1 to 0.5 g L−1 reduced the growth rate of P. parvum. The estimated optimal temperature for growth decreased with decreasing salinity from about 27°C at salinities above 10 g L−1, to about 24°C at 4 g L−1, to about 22°C at 0.5 g L−1. In phosphorus-limited experiments, the half-saturation concentration for growth was less than 0.02 μmol L−1 under most conditions. The phosphorus-saturated growth rate was 0.84 d−1 at 4 g L−1 salinity and 20°C, and it was reduced at lower salinities and temperatures. The salinity-temperature interaction found here weakens the negative effect of low temperature on growth at low salinity and might partially explain why blooms of P. parvum occur in the winter months in inland waters of the southwestern United States. However, the relatively slow growth of P. parvum at low temperature and salinity suggests that additional factors should be examined, such as reduced effects of competitors, pathogens, and grazers during winter.

Harmful algal blooms are among the water-quality problems receiving growing attention from aquatic scientists. There is a perception that the frequency of such blooms has increased over recent decades (Hallegraeff 1993; Sunda et al. 2006), perhaps due to ongoing eutrophication, aquaculture, and other anthropogenic changes. In some cases, the species responsible are classified as invasive because they have become abundant in locations where they were previously unnoticed. Examples include the invasion of warm, shallow lakes in temperate climate zones by the cyanobacterium Cylindrospermopsis raciborskii (Briand et al. 2004; Wiedner et al. 2007), as well as the focus of this paper: the haptophyte Prymnesium parvum, which is apparently invading brackish inland waters of the southwestern United States (James and De La Cruz 1989; Edvardsen and Paasche 1998; Roelke et al. 2007).

Studies of niche factors and resources likely to limit population growth may improve our understanding and ability to predict the spread of invasive, harmful algal species. Such approaches have long been applied in algal ecology (Rhee 1982), and quantitative information on responses to niche factors and resources is available for many species (Tilman et al. 1982; Grover 1989). However, this information is sparse for many harmful algal species of contemporary interest, including P. parvum.

Previous studies of the growth of P. parvum in relation to physical and chemical niche factors covered salinities characteristic of estuarine and coastal waters, up to about 35 g L−1, with very few observations below 4 g L−1 (Larsen et al. 1993; Larsen and Bryant 1998; Baker et al. 2007). Lower salinities of 1–3 g L−1 are common in most of the brackish inland waters of the southwestern United States where P. parvum blooms have occurred, and thus previous research provides only a limited characterization of its growth at salinities characteristic of such inland waters. Furthermore, there are no published studies that provide parameter estimates for commonly used kinetic models of nutrient-limited growth, such as the Monod model and its modifications (Istvánovics et al. 2000).

A lack of information about the specific growth rate of P. parvum at low salinities and nutrient concentrations hinders quantitative analysis of the potential for harmful blooms in many inland waters. Predictive, mechanistic models of bloom dynamics conventionally describe algal growth using a maximal rate that depends on physical factors multiplied by saturation terms that describe nutrient limitation (Chapra 1997). This study provides the necessary information for such models. Specific growth rates, abundances, and toxicity to fish under nutrient-sufficient conditions were measured in a factorial experiment at temperature and salinity levels characteristic of inland waters of the southwestern United States where harmful blooms have occurred. Additional experimental treatments compared specific growth rates at the ionic composition of seawater to specific growth rates at an ionic composition simulating inland waters. At selected combinations of temperature, salinity, and ion composition, specific growth rates were measured for a range of low-phosphorus (P) concentrations. Although the physical and
chemical niche has been studied for many algae, growth is usually characterized under conditions near optimum or across a wide range. Here, we focus on growth of *P. parvum* at the edge of the niche, in terms of salinity, an uncommon approach but one that may be valuable for understanding invasive species (Briand et al. 2004).

**Methods**

**General culture methods**—The strain of *P. parvum* culture used in this study (Culture Collection of Algae at the University of Texas at Austin, UTEX LL 2797) was isolated by J. Glass from a bloom in Texas and used in previous laboratory studies (Baker et al. 2007; Grover et al. 2007). For stock cultures, an artificial seawater (ASW) medium was prepared according to Kester et al. (1967), diluted to a working salinity of 5.8 g L⁻¹ in ultrapure water (Millipore Milli-Q, 18 MΩ cm⁻¹), and then enriched with 12 levels of nitrogen (N), P, trace metals, and vitamins (MacLachlan 1973). In the trace-metals solution, an equimolar amount of ferric chloride was substituted for ferrous ammonium sulfate due to the toxicity of ammonium to *P. parvum* (Grover et al. 2007). An additional medium simulated inland waters of western Texas affected by *P. parvum* using an ion composition similar to Lake Whitney, Texas, which was ascertained during an active bloom. This artificial lake-water (ALW) medium was modified from ASW as follows: the same molar concentration of Mg was added as MgSO₄ instead of MgCl₂ to produce higher sulfate concentrations; eightfold higher additions of CaCl₂·2H₂O were used; and eightfold higher additions of NaHCO₃ were added aseptically after autoclaving. For both ASW and ALW, full-strength preparations of the basal salts were diluted as needed with ultrapure water to achieve experimental salinities.

Stock cultures of *P. parvum* were maintained by monthly transfers in an incubator at 20°C and on a 12:12 h light:dark photoperiod with an irradiance of about 150 μmol m⁻² s⁻¹. This photoperiod was used for all stock and experimental cultures because blooms typically begin in Texas at approximately this photoperiod in autumn. Irradiance of experimental cultures was also 150 μmol m⁻² s⁻¹, a value similar to average water-column irradiances in Texas lakes (Grover and Chrzanowski 2004). Illumination and culture-vessel positions were adjusted based on irradiance values measured with a photon flux meter (LiCor model LI-1400) in several areas inside of the incubators.

**Nutrient-sufficient experiment**—This experiment determined the growth rate and toxicity of *P. parvum* in response to temperature and low levels of salinity, along with additional treatments that examined the ion composition of the medium. All nutrients were provided at the high concentrations used in stock cultures to exclude limitation. To quantify curvilinear or unimodal responses to temperature and salinity, and their interactions, a duplicated 3 × 3 factorial experimental design was used with salinities of 0.5, 1.0, and 4 g L⁻¹, and temperatures of 11°C, 20°C, and 29°C (Table 1). For this design, ASW medium was used. Additional treatments at 20°C were added to compose a duplicated 4 × 2 factorial design with four salinities (0.5, 1, 4, and 17.8 g L⁻¹) crossed with two ion compositions (ASW or ALW media). The high-salinity treatment of 17.8 g L⁻¹ was added to facilitate comparison to a similar experiment done over a higher salinity range (Baker et al. 2007).

*Additional treatments to 20°C were added to compose a duplicated 4 × 2 factorial design with four salinities (0.5, 1, 4, and 17.8 g L⁻¹) crossed with two ion compositions (ASW or ALW media). The high-salinity treatment of 17.8 g L⁻¹ was included to facilitate comparison to a similar experiment done over a higher salinity range (Baker et al. 2007).*

After diluting basal salts to the experimental salinity, NaNO₃ was added. Other nutrients (NaH₂PO₄, NaHCO₃, vitamins, trace metals) were added aseptically after autoclaving (filter-sterilization through Nalgene 0.2-μm, nylon syringe filters). The same concentration of NaHCO₃ was added to all cultures of a given medium (ASW vs. ALW), so that dissolved inorganic carbon (DIC) availability would not be affected by the experimental adjustments of salinity. All ALW cultures received 2480 μmol L⁻¹ of bicarbonate, and all ASW cultures received 379 μmol L⁻¹ of bicarbonate. One-liter borosilicate glass flasks were filled to a working volume of 800 mL, and each flask was inoculated with 100 cells mL⁻¹ of *P. parvum* from stock cultures in late exponential phase grown in the corresponding ion composition at the stock salinity of 5.8 g L⁻¹. Inoculated flasks of appropriate salinity were then distributed to incubators at different temperatures to achieve the treatment combinations in the experimental design. Flasks were mixed daily by gently swirling, and positions within incubators were rotated daily.

Samples of experimental batch cultures were taken on days 2–4, 7, 9, 11, 14, 17, and 21. Populations reached stationary phase during this time. At each sampling, aliquots of 5 mL were preserved with 0.15 mL of Lugol’s iodine (Thronsen 1978) for counts of *P. parvum*. Stock and experimental cultures were not axenic, so aliquots of 10 mL were preserved with 0.5 mL of formalin for bacterial counts. Cell concentration of *P. parvum* was obtained by direct microscopic counts, using sedimentation chambers and inverted microscopy (Margalef 1969). Cell concentrations of bacteria were obtained by direct counts of cells stained with acridine orange, collected on polycarbonate filters, and examined with epifluorescence microscopy (Hobbie et al. 1977). Maximum bacterial abundance in experimental cultures reached 2.65 × 10⁷ cells mL⁻¹, although most cultures were below this, with an average abundance of 7.84 × 10⁶ cells mL⁻¹. Bacterial data were not further analyzed. Specific growth rate (μ) of *P. parvum*...
in each culture was estimated by regressing the natural logarithm of cell concentration against time for days 2 to 9. Stationary cell concentration was taken as the average for days 17 and 21. On day 21, pH was determined with a calibrated electrode.

Acute toxicity was assessed for samples taken on day 21 by completing 48-h bioassays with <48-h-old fathead minnows (Pimephales promelas) according to U.S. Environmental Protection Agency (EPA) test method 2000.0 (USEPA 2002). The fish were fed newly hatched Artemia nauplii 2 h prior to test initiation. Their survival was assessed with whole-culture samples and at a series of six successive dilutions (0.5 at each step) from 50% to 1.56% (duplicate batches of five fish per dilution level). Reconstituted hard water (APHA 1998) was used as the dilution water and as a control, and additional controls were prepared by diluting samples of sterile culture media (ASW, ALW without algae). The percentage dilution of the whole sample causing 50% mortality of subjects (LC50) was estimated using Probit (Finney 1971) or Trimmed Spearman–Karber (Hamilton et al. 1977) methods, as appropriate. To compare toxicity among cultures with different populations of P. parvum, the percentage LC50 values were then multiplied by stationary cell concentration to estimate LC50 as the concentration of cells causing 50% mortality of subjects. For example, a culture with a stationary cell concentration of 4.9 × 10^5 cells mL^{-1} was estimated to kill 50% of subjects when diluted to 7.9% of its original concentration, giving a calculated LC50 of 3.9 × 10^4 cells mL^{-1}.

Specific growth rate (µ) was analyzed using multiple regression, where the independent variables of temperature and salinity were centered on their means to reduce collinearity. A full model was fitted to data from salinities of 0.5 to 4.0 g L^{-1} with quadratic and interaction terms for temperature and salinity, a categorical variable for ion composition, and linear and quadratic interaction terms between ion composition and salinity. Interactions between ion composition and temperature could not be estimated given the experimental design.

To allow the unimodal response to temperature to be asymmetric, temperature data were transformed using the equation (Baker et al. 2007):

\[
T_{\text{transform}} = \exp\left(\frac{\theta(T - 20)}{20}\right)
\]

where \( T \) is temperature (°C), and \( \theta \) is a transformation parameter, the value of which was chosen to maximize fit \( (R^2) \) of the full model. A best subset regression scheme was then used to find a simpler regression model with acceptable fit using Mallows' \( C_p \) statistic (Kleinbaum et al. 1998), excluding models having higher-order terms without the corresponding linear terms. Residual plots were examined for failures of the regression assumptions. Two-way factorial analysis of variance (ANOVA) was used to analyze LC50 in relation to salinity and ion composition for a subset of cultures in which acute toxicity to fish was detected.

**Nutrient-limited experiments**—These experiments used a short-term batch culture method (Tilman and Kilham 1976) to determine the responses of specific growth rate (µ) to limiting concentrations of P under selected combinations of temperature, salinity, and ion composition. Prior to inoculation of experimental cultures to determine P-limited growth kinetics, populations of P. parvum were preconditioned to reduce cellular P storage. This preconditioning was done by growth to stationary phase in media with reduced P concentration (3.6 µmol L^{-1}, 1/10 of the f/2 concentration). Preconditioned cultures for experiments at 20°C were grown for 4 weeks; those for experiments at 10°C were grown for 8 weeks due to slower growth.

With one exception, preconditioning cultures were duplicated for all experiments. Periodically, samples of 5 mL were taken from preconditioning cultures and preserved with Lugol’s iodine for counts of P. parvum. At the end of preconditioning, samples of 50 mL were filtered (0.2 µm) for soluble reactive phosphorus (SRP) analysis (Strickland and Parsons 1972). Samples of 50 mL were also filtered onto GF/F filters and stored frozen for later determination of particulate phosphorus (PP) by wet digestion with persulfate (Menzel and Corwin 1965) followed by SRP analysis. Samples of 100 mL were filtered onto precombusted GF/F filters for determinations of particulate C and N with a Perkin-Elmer CHN analyzer. Inoculation of preconditioning cultures sometimes carried non-negligible amounts of dissolved P to experimental cultures from inoculation, and such carryover was calculated from SRP measurements and added to the experimental P concentration.

Cell quota of P at the end of preconditioning was calculated as PP divided by cell concentration. Particulate composition data were not obtained for the experiment in ASW medium at 20°C and 4 g L^{-1}. A three-way factorial ANOVA was used to analyze cell (i.e., particulate) composition of preconditioned cultures in relation to temperature, salinity, and ion composition.

After dilution of basal salts to obtain the experimental salinity, vitamins, trace metals, and NaN3 were added at f/2 concentrations. After autoclaving, NaHCO3 and NaH2PO4 were added aseptically (filter-sterilization through Nalgene 0.2-µm, nylon syringe filters); the same concentrations of NaHCO3 were added to ASW and ALW media in the nutrient-sufficient experiment. For the experiment in ASW medium at 20°C and 4 g L^{-1} salinity, 24 flasks were prepared and autoclaved with 600 mL of media each. Four replicates were then prepared for each target concentration of P: 0, 0.01, 0.1, 0.5, 1, and 10 µmol L^{-1}. Cultures for the remaining experiments were prepared similarly, except that 12 flasks were used, giving duplicate cultures at each target P concentration. Each experimental culture was inoculated with 100 cells mL^{-1} of P. parvum from a preconditioning culture grown under the same temperature, salinity, and ion composition.

Experimental cultures were incubated for 4 d, mixed daily by gently swirling, and positions of flasks within the incubators were rotated daily. Samples of 100 mL were taken daily from each experimental flask and preserved with Lugol’s solution. Subsamples from preserved samples were settled in large-volume sedimentation chambers (50–100 mL) and counted with an inverted microscope to
determine cell concentration. On day 4, samples of 50 mL were filtered (0.2 μm) for SRP analysis to evaluate nutrient depletion below the target concentration during the incubations.

Samples from the first day were very sparse, with unacceptable counting errors, so μ was estimated by regressing the natural logarithm of cell concentration against time for days 2–4. Specific growth rate was regressing the natural logarithm of cell concentration on day 4. The modified Monod model (Eq. 3) was fitted preconditioning culture and the measured SRP concentration corrected for carryover from the nutrient-sufficient experiment. Symbols show growth rate under different salinities and ion composition: Triangles = 0.5 g L\(^{-1}\); circles = 1.0 g L\(^{-1}\); squares = 4 g L\(^{-1}\). Open symbols = ASW medium; closed symbols = ALW medium. Curves show the fitted regression model (Eq. 4) for different salinities: solid curve = 4 g L\(^{-1}\); long-dashed curve = 2 g L\(^{-1}\); short-dashed curve = 1 g L\(^{-1}\); dotted curve = 0.5 g L\(^{-1}\).

\[
\mu = \frac{\mu_{\text{max}} S}{K_s + S}
\]

where \(S\) is nutrient concentration, \(\mu_{\text{max}}\) is the maximal specific growth rate, and \(K_s\) is the half-saturation constant; and a modified Monod model with threshold concentration nutrient concentration \(S_T\) required for positive growth rate (Istvánovics et al. 2000):

\[
\mu = \frac{\mu_{\text{max}} (S - S_T)}{K_s + S - S_T}
\]

These kinetic models were fitted with nonlinear least-squares regression, taking as independent variable the average of the target P concentration corrected for carryover from the preconditioning culture and the measured SRP concentration on day 4. The modified Monod model (Eq. 3) was fitted for all experiments, except for experiments in which negative estimates of the threshold \(S_T\) were obtained, in which case, the conventional Monod model (Eq. 2) was fitted.

Results

Nutrient-sufficient experiment—Specific growth rate (μ) showed a unimodal function of temperature and an increasing function of salinity (Fig. 1). Asymmetry of the temperature response was represented by transforming temperature according to Eq. 1 with parameter θ = 0.7. After conducting the best subsets regression analysis, a predictive model for μ was selected with five terms, including all linear and quadratic terms for temperature and salinity, and a temperature-salinity interaction:

\[
\mu = -3.531 + 0.02534(\sigma - 1.833) - 0.06311(\sigma - 1.833)^2 + 7.468 \exp \left[ 0.7 \left( \frac{T - 20}{20} \right) \right] - 3.414 \exp \left[ 1.4 \left( \frac{T - 20}{20} \right) \right] + 0.1697(\sigma - 1.833) \exp \left[ 0.7 \left( \frac{T - 20}{20} \right) \right]
\]

where \(\sigma\) is salinity (g L\(^{-1}\)), and \(T\) is temperature (°C). The predictive model fit well (\(R^2 = 0.901\)) and was significant (\(F_{5,18} = 32.5, p < 0.001\)). Terms not included in the predictive model involved ion composition and its interaction with salinity and were not statistically significant (partial F-tests, \(df = 1, 15, p > 0.3\)). Terms included in the predictive model were statistically significant (partial F-tests, \(df = 1, 18, p < 0.01\)) with two exceptions: the linear salinity term (partial F-test, \(df = 1, 18, p = 0.71\)) was included because the marginal quadratic salinity term (partial F-test, \(df = 1, 18, p = 0.052\)) was also included. Eliminating the quadratic salinity term produced residual plots that strongly suggested curvature in the response to salinity, so the term was retained. The highest predicted μ from Eq. 4 was 0.72 d\(^{-1}\) at a temperature of 24°C and salinity of 4 g L\(^{-1}\), the upper salinity limit for applicability of this model.

Using 48-h static bioassays to assess acute toxicity to \(P.\) promelas, only samples from cultures with salinities of 4 or 17.8 g L\(^{-1}\), grown at 20°C, were toxic. Other cultures were not detectably toxic (i.e., survival of fish in 100% sample water did not differ from survival in control water).

Table 2. Acute (48-h) cellular toxicity (LC50) of \(P.\) parvum to juvenile \(Pimelphales\) promelas in relation to salinity and temperature. Each sample is a combination of salinity (g L\(^{-1}\)) and media type (ASW or ALW). All cultures were grown at 20°C.

<table>
<thead>
<tr>
<th>Salinity (g L(^{-1}))</th>
<th>Medium</th>
<th>LC50 (cells mL(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>ASW</td>
<td>39,000</td>
</tr>
<tr>
<td>4</td>
<td>ASW</td>
<td>47,000</td>
</tr>
<tr>
<td>4</td>
<td>ALW</td>
<td>32,000</td>
</tr>
<tr>
<td>4</td>
<td>ALW</td>
<td>67,000</td>
</tr>
<tr>
<td>17.8</td>
<td>ASW</td>
<td>52,000</td>
</tr>
<tr>
<td>17.8</td>
<td>ASW</td>
<td>56,000</td>
</tr>
<tr>
<td>17.8</td>
<td>ALW</td>
<td>65,000</td>
</tr>
<tr>
<td>17.8</td>
<td>ALW</td>
<td>22,000</td>
</tr>
</tbody>
</table>
concentration of *P. parvum* than those that were not detectably toxic (5.0 \( \times \) 10\(^5 \pm 1.5 \times 10^5 \) cells mL\(^{-1}\) for toxic cultures, 3.8 \( \times \) 10\(^4 \pm 9.1 \times 10^4 \) cells mL\(^{-1}\) for nontoxic cultures, mean \( \pm \) SD). For many cultures not detectably toxic, stationary cell concentrations of *P. parvum* were below the LC\(_{50}\) cell concentrations of toxic cultures, which ranged from 2.2 to 6.7 \( \times \) 10\(^4 \) cells mL\(^{-1}\) (Table 2). Toxic cultures also had significantly higher (*t*-test, *t* = 5.43, df = 26, *p* < 0.001) pH than those that were not detectably toxic (9.5 \( \pm \) 0.41 for toxic cultures, 7.5 \( \pm \) 0.97 for nontoxic cultures). For toxic cultures, LC\(_{50}\) was not significantly related to salinity, ion composition, or their interaction (ANOVA, *F*-tests, df = 1, 4, *p* > 0.5).

**Nutrient-limited experiments**—Preconditioning cultures were intended to reduce cellular P storage in *P. parvum* populations prior to inoculation into experimental cultures for determination of P-limited growth kinetics. After successful preconditioning, the cell quota of P can be interpreted as the minimal quota at which the population growth rate goes to zero (Grover 1989). Data on P quotas and cellular stoichiometry suggest that successful reduction of P storage occurred under some conditions but not others (Table 3). Cell quotas of P were less than 20 fmol cell\(^{-1}\) for three sets of preconditioning cultures (Table 3), and all of these cultures had cellular C:P and N:P ratios exceeding the Redfield ratios of 106 and 16, respectively. Cell quota was somewhat higher at 82 fmol cell\(^{-1}\) in the unreplicated preconditioning culture at 10°C, 1 g L\(^{-1}\) salinity, and ALW medium, but both cellular C:P and N:P ratios exceeded the Redfield ratios. These results suggest successful reduction of stored P by precondition in these four cases, and they suggest that the minimal P quota for *P. parvum* can approach about 4 fmol cell\(^{-1}\). For all other experimental conditions, cell quotas of P in preconditioning cultures exceeded 200 fmol cell\(^{-1}\), and cellular C:P and N:P ratios ranged from lower than the Redfield ratios to slightly higher. These results suggest that preconditioning did not successfully reduce stored P in these four cases.

For two experiments, parameter estimates for *K*\(_{\mu}\) and *S*\(_{T}\) were not biologically meaningful, i.e., estimates were negative. These cases occurred at 10°C and salinities of 1 and 3 g L\(^{-1}\) in ASW medium. In the remaining experiments, biologically meaningful parameter estimates were obtained (Table 4; Fig. 2). In three cases, estimates of *S*\(_{T}\) were positive. Otherwise, estimates of *S*\(_{T}\) were negative, so Eq. 2 was fitted.

Estimates of *μ*\(_{max}\) increased with temperature and salinity (Table 4), although they were not well predicted by regression Eq. 4 as developed from the nutrient-sufficient experiment. With one exception, estimates of *K*\(_{\mu}\) and *S*\(_{T}\) were low, indicating that P-dependent growth was saturated at concentrations lower than about 0.05 \( \mu \)mol L\(^{-1}\). In general, estimates of *K*\(_{\mu}\) were higher for growth in ASW medium than in ALW medium. For cultures grown at 20°C and 1 g L\(^{-1}\) salinity in ASW medium, the estimated *K*\(_{\mu}\) approached 0.5 \( \mu \)mol L\(^{-1}\).

**Table 3.** Cell quotas and stoichiometry from P-limited preconditioning cultures of *P. parvum*. Cultures were grown under the indicated temperature, salinity, and ion composition (ASW = artificial seawater; ALW = artificial lake water). Ranges are given for duplicate cultures, except where the asterisk (*) indicates a single culture.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Salinity (g L(^{-1}))</th>
<th>Ion composition</th>
<th>P quota (range, fmol cell(^{-1}))</th>
<th>Cellular C:P (range)</th>
<th>Cellular N:P (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1</td>
<td>ASW</td>
<td>335–341</td>
<td>69–81</td>
<td>1.3–1.4</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>ALW</td>
<td>82*</td>
<td>1001*</td>
<td>26*</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>ASW</td>
<td>447–481</td>
<td>103–121</td>
<td>5.1–5.9</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>ALW</td>
<td>246–268</td>
<td>98–121</td>
<td>6.0–6.5</td>
</tr>
<tr>
<td>20</td>
<td>1</td>
<td>ASW</td>
<td>288–298</td>
<td>71–95</td>
<td>4.5–5.2</td>
</tr>
<tr>
<td>20</td>
<td>1</td>
<td>ALW</td>
<td>15–17</td>
<td>152–168</td>
<td>17–19</td>
</tr>
<tr>
<td>20</td>
<td>3</td>
<td>ASW</td>
<td>6.7–6.8</td>
<td>457–458</td>
<td>39–40</td>
</tr>
<tr>
<td>20</td>
<td>3</td>
<td>ALW</td>
<td>3.7–4.3</td>
<td>520–588</td>
<td>44–45</td>
</tr>
</tbody>
</table>

**Table 4.** Summary of nonlinear regression fit of all nutrient kinetics experiments. Cultures were grown under the indicated temperature, salinity, and ion composition (ASW = artificial seawater; ALW = artificial lake water). Maximal growth rate (*μ*\(_{max}\)), half-saturation constant (*K*\(_{\mu}\)), and threshold (*S*\(_{T}\)) estimates are from nonlinear regressions.
Discussion

Species that become abundant where previously unnoticed, including invasive species, likely encounter conditions at the edge of their niche. Traditionally, however, ecophysiological studies have measured growth at near optimal conditions, or over broad ranges that do not focus on the edge of the niche. This study focused on the growth performance of the harmful alga *Prymnesium parvum* at the low-salinity edge of its niche. For the strain studied here, a previous study estimated an optimal salinity for specific growth rate of 22 g L\(^{-1}\) (Baker et al. 2007), and studies of other strains suggest optimal growth at salinities of 8–34 g L\(^{-1}\) (Larsen et al. 1993; Larsen and Bryant 1998). Here, specific growth rate was found to decrease strongly as salinity decreased from 4 to 0.5 g L\(^{-1}\). A lower limit of salinity for population increase appears to lie between 0.5 and 1 g L\(^{-1}\). In the range of 1–2 g L\(^{-1}\), characterizing...
many of the brackish inland waters in the southwestern United States where *P. parvum* has recently been problematic, rates of population growth reached a modest range of 0.1–0.3 d\(^{-1}\).

Previous studies of *P. parvum* at higher salinities suggest a relatively high optimum temperature for population growth, estimated to be 27°C for the strain used in this study (Baker et al. 2007) and 15–30°C for other strains (Larsen et al. 1993; Larsen and Bryant 1998). For growth of this strain at higher salinities, Baker et al. (2007) derived a regression function similar to Eq. 4. A comparison of these two regressions (Fig. 3) suggests that low salinity reduces the growth of *P. parvum* to a greater extent at high temperatures than at low temperatures. Thus, the optimum temperature for growth apparently decreases as salinity decreases, reflecting a statistically significant interaction of temperature and salinity. Optimal temperatures for growth approaching 30°C found in other studies at salinities greater than 4 g L\(^{-1}\) are hard to reconcile with the tendency for *P. parvum* to bloom during cooler weather in Texas and other parts of the southwestern United States. The salinity–temperature interaction found here shifts the optimum temperature for growth at salinities of 1–2 g L\(^{-1}\), perhaps making blooms less likely during warm weather in this region and more likely in cooler weather.

The growth performance of *P. parvum* at low-P concentration might also contribute to bloom formation in inland waters. Though extensive studies of nutrient limitation in most Texas lakes affected by *P. parvum* have not been done, nearby lakes are often but not continually P-limited (Grover et al. 1999; Grover and Chrzanowski 2004). The nutrient-saturated, maximal growth rates of *P. parvum* are modest compared to many other algal species grown at similar temperatures (Tilman et al. 1982; Grover 1989; Grover et al. 1999), but growth near maximal is maintained at low-P concentrations under some conditions (Table 4; Fig. 2). In particular, P concentrations permitting half the maximum growth rate (i.e., the sum *K_α* + *S_T*) range from 0.003 to 0.007 µmol L\(^{-1}\) when *P. parvum* is grown under low-salinity conditions in ALW medium that mimics the ion composition of inland waters. Half-saturation concentrations found here for *P. parvum* are similar to those for diatoms and green algae identified as having high competitive ability for P (Tilman et al. 1982; Grover 1989), and they are lower than those of some diatoms, green algae, and cyanobacteria found in Texas lakes (Grover et al. 1999). High competitive ability under P limitation is consistent with high expression of putative phosphate transporter genes in *P. parvum* (La Claire 2006).

High competitive ability for P by *P. parvum* appears to be enhanced by the ion composition of brackish inland waters, which are rich in divalent cations and bicarbonate compared to seawater. When *P. parvum* is grown under low salinity with the ion composition of seawater, *K_α* + *S_T* values exceed 0.01 µmol L\(^{-1}\). Thus, *P. parvum* appears to be physiologically pre-adapted to invade P-limited brackish inland waters. Few other studies have examined the influence of salinity and ion composition on nutrient-limited growth kinetics of algae. For three diatom species studied, salinity and ion composition had species-specific influences on nitrate- and ammonium-limited growth kinetics (Saros and Fritz 2000). Given the widespread occurrence of brackish and saline inland waters in arid and semiarid climates, similar information would be helpful for other algae occurring in such regions.

The nutrient-limited experiments relied on a preconditioning phase to reduce cellular P storage. Based on P quotas and stoichiometry, preconditioning was successful in four cases and unsuccessful in another four. All of the unsuccessful cases involved either low temperature or low salinity, at which growth was so slow that the anticipated P depletion did not occur. Such failure of preconditioning is an inherent risk of examining growth under suboptimal conditions. For two cases of unsuccessful preconditioning, no estimates of growth kinetic parameters were obtained due to high variability of specific growth rates. For another case of unsuccessful preconditioning (growth at 20°C, 1 g L\(^{-1}\) salinity in ASW medium), the estimated value of *K_α* + *S_T* far exceeded all other such estimates (Table 4) and probably is unreliable. For the last case of unsuccessful preconditioning (growth at 10°C, 3 g L\(^{-1}\) salinity in ALW medium), the estimated value of *K_α* + *S_T* is within the range of estimates obtained when preconditioning was successful. For one final set of conditions (growth at 20°C, 4 g L\(^{-1}\) salinity in ASW medium), data on cellular P composition were not obtained, so the success of preconditioning cannot be evaluated, but the value of *K_α* + *S_T* is within the range of estimates obtained when preconditioning was successful.

For the temperature and salinity ranges of 10–20°C and 1–4 g L\(^{-1}\), the nutrient-sufficient experiment predicts that specific growth rate increases with both of these factors. Qualitatively, estimates of the maximal growth rate *µ_\text{max}* from nutrient-limited experiments agree with this prediction. Quantitatively, however, estimates of *µ_\text{max}* consistently exceed the predictions of the regression Eq. 4 developed from the nutrient-sufficient experiment. Preconditioning may be responsible for this difference. Inocula for the nutrient-sufficient experiment were taken directly from stock...
cultures grown at 20°C and 5.8 g L\(^{-1}\) in ASW or ALW media without preconditioning, and thus they experienced shifts in temperature and salinity at the start of the experimental incubations. Inocula for the nutrient-limited experiment were preconditioned by extended growth under the experimental temperature and salinity conditions. The fact that higher nutrient-saturated growth rates were apparently produced by preconditioning suggests that \(P. \ parvum\) might acclimate to suboptimal conditions over several generations. Such acclimation deserves further study, and it could enhance the capability of this species to form blooms under suboptimal growth conditions.

Toxicity of \(P. \ parvum\) is associated with nutrient limitation (Johansson and Granéli 1999; Granéli and Johansson 2003), and for the strain studied here, growth under suboptimal temperature and salinity previously led to enhanced toxicity (Baker et al. 2007). In the current study, toxicity to fish was detected only at salinities of 4 g L\(^{-1}\) or higher. Populations cultured at lower salinities were not detectably toxic. The low abundance of most nontoxic cultures might explain such lack of toxicity. Most (75\%) of the nontoxic cultures had cell concentrations below \(2.2 \times 10^4\) cells mL\(^{-1}\), the lowest LC\(_{50}\) estimated from bioassays of acute toxicity to fish, and many (40\%) of the nontoxic cultures had much lower cell concentrations of <1000 cells mL\(^{-1}\). Even if cells of \(P. \ parvum\) were actively producing toxins, sparse populations might not accumulate enough toxins to cause mortality of fish during short-term bioassays. On the other hand, two nontoxic cultures had cell concentrations exceeding \(10^5\) cells mL\(^{-1}\). Dense populations of \(P. \ parvum\) with little or no toxicity have been observed in field experiments and lake monitoring (D. L. Roelke unpubl.; Schwierzke et al. in press). Together, these observations suggest that high abundance is a necessary but not sufficient condition for fish-kills to occur, and that regulation of toxin production also plays a role. These observations also suggest that toxin production and allelopathy are not necessary for high abundance, i.e., blooms, to occur.

The P-limited growth kinetics found here imply that only a severe depletion of P would reduce the potential for blooms to occur. Perhaps somewhat paradoxically, available evidence suggests that since specific growth of \(P. \ parvum\) becomes saturated at low-P concentrations, nutrient additions might often do little to increase its population growth, while reducing toxicity (Johansson and Granéli 1999; Granéli and Johansson 2003), and even reducing bloom initiation by stimulating competitors (Roelke et al. 2007; Errera et al. 2008).

As expected, growth performance is reduced at the low-salinity edge of the niche for \(P. \ parvum\). Nevertheless, under many low-salinity conditions, its specific growth rate is sufficient to produce blooms that are toxic to fish. Blooms of \(P. \ parvum\) in the southwestern United States occur at water temperatures of 10–20°C and salinities of 1–2 g L\(^{-1}\) (Roelke et al. 2007; Schwierzke unpubl.), where a growth rate of 0.1–0.3 d\(^{-1}\) can be expected. A bloom population of \(4 \times 10^8\) cells mL\(^{-1}\) (L. Schwierzke unpubl.) can thus be reached from a background population of \(10^2\) cells mL\(^{-1}\) in as little as 20 d, if population losses are negligible. Predictions of growth rates can be constructed by using Eq. 4 to describe the maximal growth rate (\(\mu_{\text{max}}\)) of \(P. \ parvum\) as a function of temperature and salinity, multiplied by a saturation term derived from Eq. 2 to describe phosphorus-limitation. This is a standard representation of algal growth in water-quality models (Chapra 1997). The resulting growth equations provide a reasonable model for short-term dynamics within a single bloom (D. L. Roelke et al. unpubl.). A longer-term model of annual dynamics incorporating several other processes was less successful when compared to observations in one lake (Grover et al. in press).

Ecologists often characterize growth performance under conditions close to optimal for the species they study. As a practical matter, growth rates are lower and more variable as the edge of the niche is approached, making their measurement difficult. However, there may also be an implicit view that species will most likely be abundant under conditions near the center of their niche; that is, that the fundamental and realized niches (Hutchinson 1958) do not differ much. For \(P. \ parvum\), the realized niche where it occurs in brackish inland waters appears to lie along the edge of the fundamental niche, possibly as a result of its competitors (Roelke et al. in press) or natural enemies (Schierzke et al. in press). Similarly, the tropical cyanobacterium \(Cylindrospermopsis raciborskii\) appears to be invading inland waters at midlatitudes along the low-temperature edge of its niche (Briand et al. 2004; Wiedner et al. 2007). Growth at the edge of the fundamental niche might be relevant more generally for other invasive and nuisance species, especially given global changes in climate, hydrology, and nutrient loading.

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References


Comparative Toxicity of *Prorocentrum parvum* in Inland Waters

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ABSTRACT

Numerous studies have examined the impacts of *Prymnesium parvum* to aquatic life, but the majority of information available is from experiments or field studies performed at salinities of marine and coastal ecosystems. Ambient toxicity of *P. parvum* has been characterized with *in vitro* and *in vivo* models because reliable quantitation of *P. parvum* toxins in environmental matrices is often precluded by a lack of available analytical standards. Hemolytic activity and fish mortality assays have been used most frequently to characterize toxic conditions; however, relatively few *in vivo* studies employed standardized methods. Because the relative sensitivities of different taxa to *P. parvum* toxins in inland waters were undefined, we assessed the comparative toxicity of *P. parvum* filtrate from a laboratory study (20°C, 12:12 light:dark cycle, f/8 media, 2.4 psu) to several common standardized *in vitro* and *in vivo* models. After exposure to cell-free filtrate hemolytic activity (1 hr EC$_{50}$ = 13,712 cells mL$^{-1}$) and juvenile fish (*Pimephales promelas*) survival (48 hr LC$_{50}$ = 21,754 cells mL$^{-1}$) were the most sensitive assay responses examined, followed by rotifer (*Brachionus calyciflorus*) population growth rate (48 hr NOAEL = 19,072 cells mL$^{-1}$) and cladoceran (*Daphnia magna*) reproduction (10 d NOAEL = 47,680 cells mL$^{-1}$). Green algae (*Pseudokirchneriella subcapitata*) growth (96 hr) was not adversely affected but was instead significantly stimulated by *P. parvum* toxins. We further propose an initial species sensitivity distribution approach for *P. parvum*, which may be used to support future environmental management decisions. Our findings from these laboratory studies indicate that although fish kills are increasingly associated with *P. parvum* blooms occurring in inland waters, further study is required to define the influences of toxin sensitivities of phytoplankton, zooplankton and fish communities on *P. parvum* bloom initiation and termination.
INTRODUCTION

Toxins produced by *Prymnesium parvum* Carter, a haptophyte alga, are often associated with devastating impacts to coastal and inland fisheries on the global scale (Granéli, 2006; Moestrup, 1994; Edvardsen and Paasche, 1998). Consequently, numerous studies over the past five decades employed various *in vivo* responses of aquatic organisms and *in vitro* bioassays to monitor, understand and mitigate ambient toxicity attributed to this harmful algal bloom (HAB) forming species. A literature review of those toxicity assays can be found in Table 1. Research by Igarashi et al. (1999) provided structural identification of two similar compounds named prymnesin 1 and 2 that are believed to be responsible for hemolytic activity and ambient acute toxicity to aquatic life. Prymnesins are structurally complex sterochemicals that have high molecular weights (e.g., prymnesin 1 = 2263.7) and often contain numerous polyhydroxy and polycyclic ether groups. These compounds also share some structural and toxicological characteristics with maitotoxins, which are produced by the marine dinoflagellate *Gambierdiscus toxicus* (Trevino et al., 2008). Although maitotoxins bioaccumulate and biomagnify in aquatic food chains with highest toxin levels detected in predatory fish (Trevino et al., 2008), neither bioaccumulation nor biomagnification of prymnesins have been investigated in aquatic and terrestrial organisms.

Specific toxicological mechanism(s) of action and environmental fate of prymnesins are not fully understood due to a lack of high purity compounds and analytical standards. *P. parvum*
toxins likely exert their acute toxicity to aquatic life at the level of the gill, resulting in disruption of ionoregulation (Edvardsen and Imai, 2006). Chronic responses to sublethal levels of the toxins are less understood, potentially because *P. parvum* toxins appear susceptible to “inactivation” by light (Parnas et al., 1962; Reich and Parnas, 1962; Rahat and Jahn, 1965), which may confound longer-term experimental studies of exposure and effect. Extracts of *P. parvum* were demonstrated to induce mammalian glutamate release *in vitro* through a Ca$^{2+}$ dependent mechanism similar to maitotoxin (Marriussen et al., 2005); however, it is not known whether physiochemical properties of prymnesins allow transport across the blood-brain barrier and subsequent exposure to the central nervous system; for example, previous research demonstrated effects on the frog nervous system but exposure to *P. parvum* extract was achieved by intravenous injection (Paster, 1975). Nevertheless, impacts on terrestrial vertebrates including mammals are relatively unknown compared to aquatic organisms (Table 1). Whether *P. parvum* produces toxins other than prymnesins, a trait possessed by other HAB species, has not been reported in the peer-reviewed literature (Grover et al., 2007; Schug et al., in press this issue).

In addition to lacking analytical toxin standards, numerous challenges exist to understanding and managing the aquatic impacts of *P. parvum* because the synthesis, release and resulting toxin exposures to aquatic life are responsive to physical and chemical variables such as salinity, light, temperature and nutrient availability (Padilla, 1970; Dafni et al., 1972; Shilo, 1981; Brand, 1984; Larsen et al., 1993; Larsen and Bryant, 1998; Johansson and Granéli, 1999; Granéli and Johansson, 2003; Baker et al., 2007; Kurten et al., 2007; Roelke et al., 2007).

Further complicating the characterization of toxin production are strain-specific responses to environmental variables. For example, Larsen and Bryant (1998) found a Norwegian strain of *P. parvum* to exhibit its highest growth rate at 8 psu, a Danish strain at 18 psu, and an English strain
at 30 psu. Further, of the 96 toxicity studies reviewed in Table 1 only 50.5% specifically reported salinity of the assay (10 studies reported % seawater but did not specify seawater salinity) and only 14.7% of these studies examined salinities ≤ 4 psu. Subsequently, influences of environmental factors on the aquatic toxicology of *P. parvum* strains are less understood at salinities ≤ 4 psu (Table 1). However, Baker *et al.* (2007) recently predicted 22 psu, 27°C and 275 µmol·m⁻²·s⁻¹ to be the optimal salinity, temperature and irradiance, respectively, for growth of a strain of *P. parvum* from inland waters of Texas, USA. Interestingly, non-optimal conditions for growth apparently increased toxin production and associated acute toxicity to juvenile fish by the Texas strain, as blooms are more prevalent during cooler months in inland waters with salinities ≤ 4 psu (Baker *et al.*, 2007). Such observations at non-optimal conditions are supported by previous studies, as increased ambient toxicity with *P. parvum* is often observed when cultures are grown under nutrient limitation (Dafni *et al.*, 1972; Johansson and Granéli, 1999).

Another challenge to managing *P. parvum* impacts on fisheries includes understanding differential effects to the various trophic levels of aquatic ecosystems. Differing species sensitivities to toxicants and toxins are well recognized in aquatic toxicology as organismal responses to contaminants vary due to toxicokinetic and toxicodynamic differences among taxa (Posthuma *et al.*, 2002). In fact, understanding comparative responses among components of an aquatic food web is important for the management of *P. parvum* because differential impacts on lower and higher trophic levels could influence the recovery of fisheries following termination of toxic *P. parvum* blooms. Whereas fish mortality provided the impetus for a majority of *P. parvum* studies of ambient toxicity, impacts on other aquatic organisms, including competitors and zooplankton predators, have been increasingly documented in the literature and are summarized in Table 1. However, the majority of these previous studies did not report pH of
experiments (Table 1). Comparisons of the relative toxicity of $P. \text{parvum}$ to previously studied organisms is challenging because responses to $P. \text{parvum}$ toxins are influenced by the pH of exposure media (Shilo and Aschner, 1953; McLaughlin, 1958; Yariv and Hestrin, 1961; Ultzur and Shilo, 1964; Dafni and Shilo, 1966; Padilla, 1970; Igarashi et al., 1998).

Little is understood about the relative sensitivities of aquatic organisms to $P. \text{parvum}$ in inland waters with salinities $\leq 4$ psu (Table 1). During an enclosure experiment in an impoundment (Lake Possum Kingdom, Texas, USA) experiencing a $P. \text{parvum}$ bloom, Roelke et al. (2007) identified that reproduction of the standardized cladoceran model $Daphnia \text{magna}$ was sublethally impacted. Furthermore, Schwierzke et al. (in review this issue) observed rotifer ($Notholca$ sp.) dominance of the zooplankton community in an impoundment (Lake Whitney, Texas, USA) experiencing $P. \text{parvum}$ associated fish kills, suggesting that differential sensitivity of aquatic organisms in inland waters require further study. Consequently, the objective of this study was to characterize comparative responses of several common standardized aquatic model organisms to $P. \text{parvum}$. For this study we selected conditions previously reported by our research team that may lead to bloom formation and toxin production in inland waters (Baker et al., 2007). We specifically selected growth of the green algae model $Pseudokirchneriella \text{subcapitata}$ to examine competitor responses, survival and reproduction of $D. \text{magna}$ and $Brachionus \text{calyciflorus}$ models to assess effects on predators of $P. \text{parvum}$, and $Pimephales \text{promelas}$ survival to quantify adverse impacts on a juvenile fish model. We further compared the responses of these in vivo models to in vitro hemolytic activity, which has historically been used to assess ambient toxicity associated with $P. \text{parvum}$ blooms (Table 1).
MATERIALS AND METHODS

Laboratory Culture

A strain of *Prymnesium parvum* from Texas inland waters was obtained from the University of Texas at Austin Culture Collection of Algae (UTEX LB 2797; Austin, Texas, USA). Non-axenic stock cultures were grown as previously described (Baker *et al.*, 2007) in a defined media of artificial seawater (ASW; Berges *et al.*, 2001) diluted to 2.4 psu with Nanopure® (Thermo Fisher Scientific, Waltham, Massachusetts, USA) water and enriched with f/2 levels of trace metals and vitamins. F/8 levels of nutrients, which are one quarter of the concentration of NaNO₃ and NaH₂PO₄·H₂O found in f/2 media, were added (Guillard, 1975). Briefly, cultures and experiments were performed in VWR Model 2015 incubators (West Chester, Pennsylvania, USA) at 20±1°C for a 12:12 light:dark (L:D) cycle with an irradiance of ~100 µmol photons m⁻² s⁻¹. We selected this photoperiod, temperature and salinity regime to resemble environmental conditions when *P. parvum* blooms have occurred in inland Texas water bodies (Baker *et al.*, 2007). Approximately 8 x 10³ cells mL⁻¹ of *P. parvum* was introduced from the stock cultures, which were acutely toxic to juvenile *P. promelas*, to triplicate 20L glass carboys of f/8 media. Carboys were gently swirled once per day and aliquots were collected for cell counts approximately every three days. Toxicity of cultures was preliminarily assessed using *P. promelas* on day 15 following the standard bioassay described below in order to verify that all cultures were indeed producing toxin(s). On day 36, when the cultures were in late stationary phase, cells were separated from culture media via filtration (Whatman GF/C; VWR International, West Chester, Pennsylvania, USA) in order to prepare cell-free filtrates for assessing effects of extracellular toxins (Barreiro *et al.*, 2005). Cell-free filtrate (instead of whole cultures) was used to: 1) observe effects from extracellular toxins; 2) not confound results with
toxin exposure via ingestion by grazing; and 3) prohibit the chance of further toxin production of

\textit{P. parvum} cells during exposures. Filtrates from triplicate carboys were composited to provide

sufficient volume to initiate all comparative toxicity assessments from a common culture

volume. Composited \textit{P. parvum} filtrate was collected in foil covered glass containers and stored

at 4±1°C in the dark for less than 24 hrs prior to initiation of toxicity studies; all bioassay studies

were initiated within 6 hrs on the same day. Cell-free filtrate used for these toxicity experiments

was serial diluted using 0.4 or 0.5 dilution factors to various percentages of initial \textit{P. parvum}

filtrate with 2.4 psu ASW, which was also used for controls. Media pH was adjusted to 8.5

before organisms were exposed. All incubators used in the study were supported on back-up

power supplies to ensure continuous experimental and culture conditions. Routine water quality

measurements (e.g., dissolved oxygen, temperature, salinity, pH) were performed with calibrated

multiparameter meters (YSI, Yellow Springs, Ohio, USA) throughout the studies.

\textit{Pseudokirchneriella subcapitata}

Effects of \textit{P. parvum} filtrates on \textit{P. subcapitata} growth were evaluated following general

procedures recommended by the US Environmental Protection Agency (US EPA 2002). \textit{P. subcapitata}

was obtained from UTEX, cultured for 7 d in Algal Assay Procedure (AAP) media

and harvested by centrifugation (15 minutes at 10,000 rpm at 4°C). The resulting pellet was

rinsed and then re-suspended in reconstituted hard water (RHW) and dispensed at an initial \textit{P. subcapitata}
cell density of 1 × 10^4 cells mL^{-1} to the various experimental units. Treatment levels

(N=5) of \textit{P. parvum} filtrate were added to containers following serial dilution with ASW.

Artificial seawater with 2.4 psu salinity was used as a control. To eliminate false negative results

due to low nutrient concentrations, we added each of four stock AAP nutrient solutions prior to
use in preparing the test dilutions as recommended by the US EPA (2002). Experimental units were grown at 25±1°C for 96 hours at constant illumination, and were swirled twice per day.

Growth responses were determined by \textit{in vivo} fluorescence with a Turner Design Model 10-AU-005-CE fluorometer (Sunnyvale, California, USA) at 0, 24, 48, 72, and 96 hrs (US EPA 2002).

At the conclusion of the study samples were preserved in Lugol’s solution (Wetzel and Likens, 2001) and cell densities were enumerated using a hemacytometer and a compound microscope.

\textit{Daphnia magna}

Prior to experimentation, \textit{D. magna} were cultured as previously described (Hemming \textit{et al.}, 2002) and fed a Cerophyl®-\textit{P. subcapitata} mixture daily (Knight and Waller, 1992). Effects of \textit{P. parvum} filtrates on \textit{D. magna} reproduction was assessed over a 10 d study generally according to US EPA methods (1994) with modifications previously reported by our laboratory (Dzialowski \textit{et al.}, 2006; Clubbs and Brooks, 2007; Stanley \textit{et al.}, 2007; Roelke \textit{et al.}, 2007).

Briefly, one < 24 hr old neonate was transferred to a 25 mL volume in each of ten replicate experimental units per treatment level of \textit{P. parvum} filtrate. RHW and 2.4 psu ASW served as controls. During the 10 d experiment, organisms were maintained in an incubator (Norlake®; Hudson, Wisconsin, USA) at 25±1°C with a 16:8 light:dark cycle. Neonate production was recorded when \textit{P. parvum} filtrate treatment levels were renewed every other day (US EPA 1994); filtrates were stored at 5±1°C in the dark between renewals. Experimental units were fed daily a mixture of Cerophyl®-\textit{P. subcapitata} (Knight and Waller, 1992).

\textit{Brachionus calyciflorus}
Rotifer cysts (Florida Aqua Farms, Inc., Dade City, Florida, USA) were hatched over 24 hrs in RHW (APHA et al., 1998) and fed Roti-rich™ supplemented with *P. subcapitata* prior to test initiation. Reproduction assessment was conducted according to APHA et al. (1998) generally following methods of Snell and Moffat (1992). Six neonates were introduced to each of four replicates of five *P. parvum* filtrate concentrations and a 2.4 psu ASW control. Experimental units were fed a mixture of Roti-rich™ - *P. subcapitata*. The experiment was performed in the dark at 25±1°C for 48 hrs using a Glas-Col Model 099A RD4512 rotary wheel (VWR International, West Chester, Pennsylvania, USA) to maintain the food mixture in suspension. At the end of the study period, total rotifers per tube were determined microscopically and population growth rate (*r*) calculated using Equation (1):

\[
r = (\ln N_d - \ln N_0) t^{-1}
\]

where *N*<sub>d</sub> is number of organisms at 48 hours, *N*<sub>0</sub> is number of organisms at study initiation (6), and *t* is duration of test (2 d).

**Pimephales promelas**

Acute mortality for *P. promelas* to *P. parvum* filtrates was determined generally following US EPA method 2000.0 (US EPA 2002). Following a range-finding toxicity test, serial dilutions with a 0.4 dilution factor of cell-free filtrate were prepared at seven concentrations. There were four replicates of ten individuals per treatment level. Experimental units consisted of 600 mL glass beakers filled with 250 mL of each respective concentration. Organisms were < 96 hrs old (all hatched within 24 hrs) at the initiation of the test and were allowed to feed on newly hatched *Artemia* nauplii for two hours before being loaded into experimental units. The study
was performed in an incubator (Norlake®; Hudson, Wisconsin, USA) maintained at 25±1°C with a 16:8 light:dark cycle. Mortality was assessed daily during the 48 hr study.

Hemolytic Activity

Sterile 100% packed sheep erythrocytes were obtained from Innovative Research, Inc. (Southfield, Michigan, USA) and stored at 4°C until use. Cells were diluted and re-suspended in homogenized buffer medium (HBM), which was prepared by diluting sterile RPMI 1640 culture medium (Sigma-Aldrich, St. Louis, Missouri, USA) by 10% with sterile distilled water, and adding 0.005 mg mL⁻¹ sodium heparin (Sigma-Aldrich) as an anti-coagulant. Prior to running the assay, manual cell counts for volumetric portioning (sequential dilutions in HBM) of the erythrocytes were calibrated with optical density to obtain a benchmark target absorbance corresponding to approximately 1 x 10⁷ cells mL⁻¹ for use in further preparation of red blood cell suspensions (RBCS). For analysis of hemolytic activity, 330 μL of sheep erythrocyte suspension in HBM (1 x 10⁷ cell mL⁻¹) was placed in a 1.5 mL microcentrifuge tube. A 0.5 dilution series of the algal culture medium was prepared with ASW media. 20 μL of the culture media was then added to the RBCS and the mixture was incubated for 1 hr at ambient temperature, shielded from direct light. Experiments were completed in triplicate. Following incubation, the tube was centrifuged at 6000 rpm for 5 min. 200 μL of supernatant was drawn from the top of the tube and transferred into a 96-well plate. Absorbance of the supernatant at 414 nm was read by a plate reader (Synergy 2, BioTek Inc. Winooski, Vermont, USA) and converted to percent lysis using a linear 7-point calibration curve prepared by mixing 100% lysed RBCS by sonication with unlysed RBCS to relate absorbance as a function of percent lysis. All calibrations and ASW blanks were prepared and analyzed concurrently with samples.
Statistical Analyses

Data from experimental treatment levels were converted to densities of *P. parvum* (cell mL\(^{-1}\)) from associated percentages of initial *P. parvum* filtrate to determine toxicological benchmark values, such as No Observable Adverse Effect Levels (NOAELs) and the concentrations required to cause mortality in fifty percent of organisms (LC\(_{50}\)s), on a per cell basis. This approach provides a surrogate measure of bioavailable toxin levels released per cell, which is particularly relevant for *P. parvum* because cell density need not correlate with ambient toxicity, suggesting that toxin production per unit cell varies under different environmental conditions (Baker *et al.*, 2007). To account for such influences of environmental conditions on toxin production, we performed all comparative toxicity experiments with filtrate from commonly cultured conditions. For *D. magna*, *B. calyciflorus* and *P. subcapitata* studies No Observable Adverse Effect Levels (NOAEL) and Lowest Observable Adverse Effect Levels (LOAEL) of sublethal responses to *P. parvum* filtrate were determined using a one-way ANOVA with a Dunnett’s test (SAS v.9.1., Cary, NC, USA). Statistically significant differences from controls were determined at \(\alpha \leq 0.05\). Fisher’s Exact Test was used to identify treatment level differences from control for *D. magna* survival (US EPA 2002). An LC\(_{50}\) value for *P. promelas* was estimated using Trimmed Spearman Karber (Hamilton *et al.*, 1977) and least squares regression was employed to estimate an EC\(_{50}\) of hemolytic activity (StatSoft, Inc. 2001).

Species Sensitivity Distribution

Using toxicity threshold values for the *in vitro* and *in vivo* models described above, we developed an initial species sensitivity distribution (SSD) to *P. parvum* filtrates for the
conditions of this study because SSDs are commonly used to support various environmental management decisions (Posthuma et al., 2002). Data were fitted to log-normal distributions (Burmaster and Hull, 1997; Solomon et al., 2000) by ranking the data with a Weibull formula:

\[ j = \frac{i \cdot 100}{n + 1} \]  

where \( j \) is percent rank, \( i \) is the rank assigned to toxicological benchmark value (e.g., LC\(_{50}\), NOAEL) after values were placed in increasing order (1 to \( n \)), and \( n \) is the number of toxicity model responses. A linear regression was developed for the percent rank and log10 transformed toxicity threshold data, and probabilities of percentile responses to \( P. parvum \) filtrates determined (Solomon et al., 2000, Brain et al., 2006).

RESULTS

Laboratory Cultures

Each of three \( P. parvum \) cultures experienced exponential growth through day 15 and reached late stationary growth phase by day 36 when cells were harvested (Figure 1). Cells were highly toxic to fish at the end of exponential growth (Figure 1). Cell density in composited cultures on day 36 was \( 2.98 \times 10^5 \) cells mL\(^{-1}\). The pH of \( P. parvum \) filtrates composited from these cultures was approximately 8.5, which is consistent with ambient pH levels in Texas impoundments experiencing \( P. parvum \) blooms.

Bioassay Responses

Survival of juvenile \( P. promelas \) (48 hr) and hemolytic activity (1 hr) following exposure to \( P. parvum \) filtrates indicated that laboratory cultures examined in this study were highly toxic.
*P. promelas* survival in 2.4 psu ASW was not affected (control mortality = 0). An LC50 for *P. promelas* and an EC50 value for hemolytic activity were estimated at 2.18 x 10^4 and 1.37 x 10^4 cells mL\(^{-1}\), respectively. Conversely, growth of *P. subcapitata* was not inhibited by any level of *P. parvum* filtrate; rather, growth was stimulated at the three highest treatment levels (Figure 2A). In fact, the 96 hr green algae density of 1.56 x 10^6 cells mL\(^{-1}\) (±2.3 x 10^5 cells mL\(^{-1}\)) in our highest treatment level was markedly greater than that of the 2.4 psu ASW control treatment, which had a density of 3.34 x 10^5 cells mL\(^{-1}\) (±0.85 x 10^5 cells mL\(^{-1}\); p > 0.05; Figure 2A). Accordingly, the NOAEL of *P. parvum* filtrates to *P. subcapitata* growth under these experimental conditions was at least 2.98 x 10^5 cells mL\(^{-1}\).

Survival and reproduction of *D. magna* and *B. calyciflorus* were less sensitive than hemolytic activity and juvenile fish mortality (Figure 2), but were significantly adversely affected by *P. parvum* filtrates (Figure 2B; p < 0.05). *D. magna* control survival was 100% and reproduction (mean = 41.9 ± 6.3 neonates female\(^{-1}\); N=10) was consistent with previous studies in our lab indicating that 2.4 psu ASW did not sublethally affect *D. magna* relative to RHW controls (Roelke *et al.*, 2007; Urena-Boeck, 2008). NOAEL and LOAEL values for *D. magna* survival were determined at 1.19 x 10^5 cells mL\(^{-1}\) and 2.98 x 10^5 cells mL\(^{-1}\), respectively. *D. magna* reproduction was significantly reduced at 1.19 x 10^5 cells mL\(^{-1}\) (LOAEL), but was not affected at 4.77 x 10^4 cells mL\(^{-1}\) (NOAEL). Population growth rate (r) of *B. calyciflorus* in 2.4 psu ASW controls exceeded minimum requirements for test acceptability (APHA *et al.*, 1998). Rotifer survival was adversely affected at the two highest treatment levels, resulting in a NOAEL of 4.77 x 10^4 cells mL\(^{-1}\) and a LOAEL of 1.19 x 10^5 cells mL\(^{-1}\). Not surprisingly, NOAEL and LOAEL values for *B. calyciflorus* population growth were more sensitive than survival, as determined at 1.91 x 10^4 cells mL\(^{-1}\) and 4.77 x 10^4 cells mL\(^{-1}\), respectively.
Comparative Sensitivities and Species Sensitivity Distribution

Expressed as a percent of control, relative sensitivities of bioassays evaluated under experimental conditions of this study were: sheep hemolytic activity > juvenile P. promelas survival > B. calyciforus population growth > D. magna reproduction > D. magna survival > P. subcapitata growth (Figure 3). A SSD developed for P. parvum filtrates obtained from the culture conditions of this study is presented in Figure 4 (R² = 0.93; slope = 1.587862, intercept = -7.489325). This initial distribution, which included both in vitro and in vivo models and lethal and sublethal ecotoxicological toxicological benchmark values, identified that cell densities of 8,119, 52,073 and 333,967 cells mL⁻¹ would result in 10th, 50th and 90th centile probabilities, respectively.

DISCUSSION
In this study we employed four standardized in vivo bioassays and a common in vitro model to examine the comparative toxicity of P. parvum filtrates containing excreted toxins. Standardized models are routinely used to assess wastewater effluent safety and examine the quality of ambient aquatic conditions (US EPA 2002). Specifically, standardized responses of model organisms integrate physical, biological and chemical constituents in measurable responses that are generally more repeatable and transferable among research laboratories due to higher quality control and consistency of experimental conditions (US EPA 2002). In fact, such techniques have particular relevance for understanding P. parvum impacts to aquatic life because standards
for measuring concentrations of toxins are not available at this time (Schug et al., in press this issue).

Further, these standardized laboratory responses can be used to predict field responses to contaminants and support development of predictive models for *P. parvum* (Baker et al., 2007; Baker et al., 2009; Grover et al., in press this issue). SSDs developed with aquatic toxicity data from such standardized models are used: 1) in prospective ecological risk assessments of contaminants such as safety assessments of pesticides (Solomon et al., 2000) and derivation of national ambient water quality criteria (NAWQC) for managing water quality and protecting aquatic life (Stephan, 2006); and 2) in retrospective ecological risk assessments to support environmental management decisions associated with contaminated sites or impaired water bodies (Posthuma et al., 2002). We developed an initial SSD with ecotoxicological data from the present study to demonstrate the potential utility of probabilistic ecological hazard assessment approaches in informing management decisions for HABs in general and *P. parvum* in particular. For example, the 10th centile value predicts that 90% of the responses of the *in vitro* and *in vivo* models employed here would be protected if a population of *P. parvum* did not exceed 8,119 cells mL⁻¹ under the culture conditions of this study. Much like centile values (e.g., HC₅, or 5th centile for NAWQC) are used in probabilistic assessments of other environmental contaminants, centile values presented here may serve as useful trigger values for preemptive management of *P. parvum* bloom formation, if a greater understanding of comparative aquatic toxicity under various environmental conditions known to influence toxin production is realized.

Unfortunately, most of the previous *in vivo* studies reviewed (Table 1) did not use standardized aquatic toxicological methods for inland or coastal waters. For example, those studies reported from our group represent the majority of previous experiments using clearly
defined standardized *in vivo* methodologies, particularly at salinities of inland waters (Table 1). In addition, comparisons of relative sensitivities to *P. parvum* toxins in previous published studies (Table 1) are challenging because nutrient limitation, light, temperature, salinity and pH of study conditions influences toxin production and/or potency, and corresponding toxicological responses. Thus, an understanding of the comparative sensitivities to *P. parvum* toxins remained elusive prior to this study, particularly when projecting such differential responses to inland waters with salinities ≤ 4 psu. Ongoing studies by our team, however, are combining in-lake monitoring, laboratory studies and predictive modeling to understand spatial and temporal dynamics of *P. parvum* bloom initiation and termination in Texas reservoirs.

Hemolytic activity to sheep erythrocytes and juvenile *P. promelas* survival were markedly more sensitive to *P. parvum* filtrates than the other model bioassays evaluated in this study (Figure 3). Igarashi *et al.* (1998) indicated that *P. parvum* toxins released in more saline conditions may be more potent to hemolytic activity (~3 nM) than to fish survival (~10 nM), though no quantitative data was provided to support such a relationship. Findings in this study provide the first evidence that such a relationship in potency exists under non-optimal conditions for growth representative of inland waters in Texas (Roelke *et al.*, 2007; Roelke *et al.*, in review). An important component of our study, however, was to maintain pH at approximately 8.5 during each bioassay because previous research has shown that toxicological responses to *P. parvum* toxins are influenced by the pH of the experimental conditions. *In vitro* studies assessing hemolytic activity of *P. parvum* toxins have documented heightened effects when exposure occurs at lower pH. Yariv and Hestrin (1961) commented that the toxins released by *P. parvum* rapidly lost hemolytic activity at pH > 5, which are supported by the results of Padilla (1970), who observed a decline from 90% hemolysis at pH 6 to 10% at pH 9.
Conversely, data obtained from *in vivo* experiments with fish suggest heightened toxicity at elevated pH. For example, during experiments with *Lebistes reticulates* and *Gambusia* sp., McLaughlin (1958) observed that highly toxic alkaline-grown cultures of *P. parvum* became benign when the pH was reduced to 6-6.5; however, this trend was reversible as cultures regained their toxicity when adjusted back to circumneutral pH. During these studies, time-to-death for individuals exposed to pH 7.8-8 ranged from just 3 to 40 minutes, whereas those for individuals exposed to pH 6 ranged from 18-36 hrs. Other researchers have reported similar pH-dependent toxicological relationships and that toxicity to fish could be eliminated by adjusting the pH of the exposure media to 6; however, samples again regained potency when returned to pH 7 (Shilo and Aschner, 1953). Ultzur and Shilo (1964) noted that minnows were four times more sensitive to *P. parvum* extracts when bioassays were completed at pH 9 compared to pH 8, and that no toxicity was observed for experiments completed at pH 7. Although most previous studies focused on coastal or marine environments, a similar relationship was recently reported under less saline conditions. In experiments with *P. promelas* completed at 4 psu, Grover *et al.* (2007) only observed acute toxicity in laboratory reared cultures whose pH exceeded 9, whereas those samples with pH < 8 were not toxic to fish after 48 hrs. Presence of an amine and numerous hydroxyl groups on prymnesins (Igarashi *et al.*, 1998) are characteristic of other bases and acids, respectively, and their co-occurrence suggests that prymnesins 1 and 2 may have multiple dissociation constants (pKa). Recent studies from our group suggest that ionization states of *P. parvum* toxins may account for the previous contradicting pH-dependent toxicological responses observed among *in vitro* hemolytic and *in vivo* fish responses (Valenti *et al.*, in review).
In addition to adverse affects on fish, *P. parvum* filtrate significantly impacted reproduction of *D. magna* and *B. calyciflorus* (Figure 2B, Figure 3). Previous studies demonstrated that *P. parvum* toxins adversely affect predators of *P. parvum* such as copepods, ciliates and dinoflagelates under more saline conditions than investigated in this study (Nejstgaard *et al.*, 1995; Koski *et al.*, 1999; Fistarol *et al.*, 2003; Granéli and Johansson, 2003; Rosetta and McManus; 2003; Tillmann, 2003; Barreiro *et al.*, 2005; Sopanen *et al.*, 2006). A recent experiment by Roelke *et al.* (2007) in Lake Possum Kingdom, Texas, USA demonstrated sublethal effects of *P. parvum* on *D. magna* reproduction. These observations corresponded to an absence of cladocerans in the zooplankton community and a decrease in biovolumes of copepod nauplii and total rotifers when a *P. parvum* bloom occurred (Roelke *et al.* 2007). In this laboratory study, we documented novel negative effects of *P. parvum* toxins on survival reproduction of *D. magna* and survival and population growth of the freshwater rotifer *B. calyciflorus* at salinities comparable to Lake Possum Kingdom (e.g., 2.4 psu; Figure 2B). Similarly, Barreiro *et al.* (2005) reported weak effects of *P. parvum* filtrates on population growth of *B. plicatilis* in a study performed with seawater at 6 psu, but observed more pronounced reductions in population growth when this marine *Brachionus* species ingested *P. parvum*. Interestingly, a recent experiment in Lake Whitney, Texas, USA by Schwierzke *et al.* (in press this issue) reported a marked shift in the zooplankton community during a *P. parvum* bloom that reduced *P. promelas* survival and *D. magna* reproduction to levels similar to those reported here. In the Schwierzke *et al.* (in press this issue) study the zooplankton community became dominated by a freshwater rotifer (*Notholca* sp.) under highly toxic conditions to fish and cladocerans. Lake Whitney is located downstream of Lake Possum Kingdom and Lake...
Granbury (Grover et al., in press this issue), forming a three impoundment cascade of the Brazos River in central Texas; each of these reservoirs have experienced severe fish kills associated with *P. parvum*. It is recognized that zooplankton can develop tolerance and even resistance to aquatic contaminants through various mechanisms (Clubbs and Brooks, 2007), including induction of cytochrome P450 monooxygenases, which serve as important detoxification mechanisms for toxicants and toxins. Such observations between marine and freshwater species of rotifers indicate differential sensitivities among rotifer genera and may suggest development of resistance to *P. parvum* toxins. It may be that *Notholca* sp. in these cascading reservoirs of the Brazos River are developing resistance to *P. parvum* toxins, but evidence to date of decreased susceptibility to *P. parvum* has only been observed in the most downstream impoundment. *P. parvum* has been repeatedly reported to adversely affect algal competitors (Fistarol et al., 2003; Granéli and Johansson, 2003; Legrand et al., 2003; Skovgaard et al., 2003; Uronen et al., 2005), though more recent studies suggest that allelochemicals produced by blue-green algae competitors may reduce *P. parvum* growth (Grover et al., in press this issue, Roelke et al. in press this issue). In this study growth of the model freshwater green algae *P. subcapitata* significantly increased in the presence of increasing levels of *P. parvum* filtrates (Figure 2A). This response was unexpected based on the previous literature and because all treatment levels contained the same concentrations of nutrient media to avoid differential nutrient limitation across treatment levels (US EPA, 2002). However, similar to invertebrates and vertebrates, aquatic algae and plants can develop tolerance to contaminants by inducing detoxification pathways such as glutathione (Brain and Cedergreen, 2009). Unfortunately, a mechanistic explanation of why *P. parvum* is more resistant to its toxins than competitors and predators is not
available, although even *P. parvum* may be susceptible to its allelochemicals (Olli and Trunov, 2007).

Toxic activity associated with *P. parvum* has been demonstrated to be “inactivated” by light (Parnas *et al.*, 1962; Reich and Parnas, 1962), though photolytic degradation of the molecules are a more likely explanation (Manahan, 2005), particularly when exposure to light occurs in the 400 to 510 nm and ultraviolet ranges (Rahat and Jahn, 1965). Granéli and Johansson (2003) reported that *P. parvum* cells grown under N- or P-deficient conditions produced allelopathic substances to competitors: within 0-36 hrs of their study growth rate was substantially lower for three species of marine algae exposed to *P. parvum* filtrates grown under various nutrient conditions compared to the control. However, between 36-72 hrs growth rates were occasionally higher for experiments completed with filtrates from N- and P-deficient cultures (Granéli and Johansson, 2003). In addition to *P. subcapitata* and potentially the algae studied by Granéli and Johansson (2003) developing tolerance or being less sensitive to *P. parvum* toxins, photodegradation of the toxins could have occurred during the 96 hr *P. subcapitata* growth period in this experiment, particularly since we followed US EPA (2002) protocols with continuous illumination. Subsequently, photodegradation products from the toxins and algal exudates (Vasconcelos *et al.*, 2002) could have increased dissolved organic carbon (DOC), which would have been mineralized by bacteria in these non-axenic cultures, increasing the levels of CO₂ for uptake, and potentially stimulating the growth response observed in Figure 2A. We did not, however, quantify DOC or CO₂ at each treatment level.

Here we assessed the relative sensitivities of common *in vivo* standardized bioassays and hemolytic activity to *P. parvum* filtrates following culture at experimental conditions similar to *P. parvum* bloom conditions in Texas inland waters (Roelke *et al.*, 2007; Roelke *et al.*, in press)
this issue) and those non-optimal for growth, which results in more toxin production per cell (Baker et al., 2007). Additional studies are required to understand how comparative sensitivities of inland fish, zooplankton and phytoplankton communities influence competition, predation and associated bloom dynamics of *P. parvum*. These future efforts will support predictive modeling of bloom formation and termination (Grover et al., in press this issue) and further will be supported by robust analytical methods to quantify toxins in water and tissue matrices (Schug et al., in press this issue).

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Table 1. Studies of aquatic toxicity associated with Prymnesium parvum Carter following experiments performed under various environmental conditions for samples collected in the laboratory or field.

<table>
<thead>
<tr>
<th>Author(s) and Date</th>
<th>Model System</th>
<th>P. parvum Cell Density Used for the Toxicity Test (cells ml(^{-1}))</th>
<th>Endpoint(s)</th>
<th>Lab/Field Study</th>
<th>Salinity (psu)</th>
<th>Temperature (°C)</th>
<th>pH</th>
<th>N:P NO(_3): PO(_4) (µM)</th>
<th>Light Range (µmol m(^{-2}) s(^{-1}))/Light: Dark Cycle (h)</th>
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</thead>
<tbody>
<tr>
<td>Aquatic Vertebrates</td>
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<tr>
<td>Brooks et al., this study</td>
<td>Pimephales promelas</td>
<td>1.8x10(^2) - 4.8x10(^4) Natural population, some treatments received 5x10(^{5}) additions</td>
<td>Mortality</td>
<td>Lab</td>
<td>2.4</td>
<td>20</td>
<td>6.9-9.9</td>
<td>f/8 medium</td>
<td>100/12:12</td>
</tr>
<tr>
<td>Errera et al., 2008</td>
<td>Pimephales promelas</td>
<td>Mortality</td>
<td>Field</td>
<td>1.8</td>
<td>13</td>
<td>8.7</td>
<td>1.6:0.39, 801.6:40.39, 1.6:40.39, and 801.6:0.39</td>
<td>Ambient Spring light conditions</td>
<td></td>
</tr>
<tr>
<td>Urena-Boeck, 2008</td>
<td>Pimephales promelas</td>
<td>2.3x10(^3) - 3.0x10(^4)</td>
<td>Mortality</td>
<td>Lab</td>
<td>2</td>
<td>15</td>
<td>NS(^1)</td>
<td>f/2 medium</td>
<td>140/12:12</td>
</tr>
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<td>Baker et al., 2007</td>
<td>Pimephales promelas</td>
<td>2.3x10(^3) - 1.6x10(^6)</td>
<td>Mortality</td>
<td>Lab</td>
<td>0.5, 7.5, 17.8, 28, and 35</td>
<td>5, 11, 20, 29, and 35</td>
<td>NS</td>
<td>f/2 medium + 93µM HCO(_3)</td>
<td>14, 122, 217, 336, and 420/12:12</td>
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<td>Grover et al., 2007</td>
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<td>1x10(^4) - 1.3x10(^6)</td>
<td>Mortality</td>
<td>Lab</td>
<td>4</td>
<td>10, 20, and 30</td>
<td>6-10</td>
<td>f/2 media + 93µM HCO(_3) (+ BSE(^-) or NH(_4)(^+) for some treatments)</td>
<td>150/12:12</td>
</tr>
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<td>Roelke et al., 2007</td>
<td>Pimephales promelas</td>
<td>Natural population, some treatments received 5x10(^{5}) additions</td>
<td>Mortality</td>
<td>Field</td>
<td>2</td>
<td>22</td>
<td>8.4</td>
<td>Varied</td>
<td>Ambient Fall light conditions</td>
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<tr>
<td>Author(s) and Date</td>
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<td><em>P. parvum</em> Cell Density Used for the Toxicity Test (cells ml⁻¹)</td>
<td>Endpoint(s)</td>
<td>Lab/Field Study</td>
<td>Salinity (psu)</td>
<td>Temperature (°C)</td>
<td>pH</td>
<td>N:P NO₃ : PO₄ (µM)</td>
<td>Light Range (µmol m⁻² s⁻¹)/Light: Dark Cycle (h)</td>
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<td>Lab</td>
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<td>NS, Toxin extract</td>
<td>Mortality</td>
<td>Lab</td>
<td>26-28</td>
<td>NS</td>
<td>Modified Droop medium</td>
<td>220-260 ft-candles/24:0</td>
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<td>Survival time</td>
<td>Lab</td>
<td>21-23</td>
<td>NS</td>
<td>Basal medium + various substrates</td>
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<td>Lab</td>
<td>NS</td>
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<td>Mortality and tail curvature</td>
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</table>

**Zooplankton**

| Brooks *et al.*, this study | *Daphnia magna* | 7.6x10\(^3\)-3.0x10\(^5\) Natural population, some treatments received 5x10\(^5\) additions | Reproduction | Lab | 2.4 | 20 | 6.9-9.9 | f/8 medium | 100/12:12 |
| Roelke *et al.*, 2007 | *Daphnia magna* | Reproduction | Field | 2 | 22 | 8.4 | Varied | Ambient Spring light conditions |
| Roelke *et al.*, 2007 | *Daphnia magna* | Reproduction | Field | 1.8 | 13 | 8.7 | Varied | Ambient Fall light conditions |
| Urena-Boeck, 2008 | *Daphnia magna* | 2.3x10\(^3\)-3.0x10\(^4\) Natural population, some treatments received 5x10\(^5\) additions | Reproduction | Lab | 2 | 15 | NS | f/2 medium | 140/12:12 |
| Granéli and Johansson, 2003a | *Artemia salina* | 8.0x10\(^2\)-4x10\(^4\) | Mortality | Lab | 7 | 20 | NS | 58:0.9, 14.5:3.6, and 58:3.6 | 100/16:8 |
| Granéli and Johansson, 2003b | *Artemia salina* | 4.8x10\(^3\)-7.7x10\(^4\) | Mortality | Lab | 7 | 20 | NS | 58:0.9, 14.5:3.6, and 58:3.6 | 100/16:8 |
In press, *Journal of the American Water Resources Association*

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<th>Author(s) and Date</th>
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<th>Lab/Field Study</th>
<th>Salinity (psu)</th>
<th>Temperature (°C)</th>
<th>pH</th>
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1. NS: Not Stated  
2. BSE: Barley Straw Extract  
3. DOC: Dissolved Organic Carbon
FIGURE CAPTIONS

Figure 1. *Prymnesium parvum* cell concentrations (cells mL\(^{-1}\)) in three replicate carboys with f/8 nutrients grown over a 36 study period at 2.4 psu and 20\(^\circ\)C with a 12:12 light:dark cycle. LC\(_{50}\) (±95% CI; % of culture) values were estimated for juvenile *Pimephales promelas* survival.

Figure 2. A. Mean *Pseudokirchneriella subcapitata* (cells mL\(^{-1}\); N=5; ±SD) growth following 96 hr exposure to *Prymnesium parvum* cell-free filtrate corresponding to pre-filter cell densities (cells mL\(^{-1}\)). B. *Daphnia magna* reproduction following 10 d exposure (N=10; ±SD) and *Brachionus calyciflorus* population growth rate (r; N=5; ±SD) following 48 hr exposure to *Prynmesium parvum* cell-free filtrate corresponding to pre-filter cell densities (cells mL\(^{-1}\)). * = different from control, p < 0.05.

Figure 3. Comparative responses (mean percent of control) of 1 hr hemolytic activity (N =4; ±SD), 48 hr juvenile *Pimephales promelas* survival (N=4; ±SD), *Brachionus calyciflorus* population growth rate (N=5; ±SD), and *Daphnia magna* reproduction (N=10; ±SD) models to *Prymnesium parvum* cell-free filtrate corresponding to pre-filter cell densities (cells mL\(^{-1}\)).

Figure 4. A species sensitivity distribution of toxicological benchmark values from hemolytic activity, *Pimephales promelas* survival, *Brachionus calyciflorus* survival and population growth rate, *Daphnia magna* survival and reproduction, and *Pseudokirchneriella subcapitata* growth to *Prymnesium parvum* cell-free filtrate corresponding to pre-filter cell densities (cells mL\(^{-1}\)).
Figure 1.
Figure 2. A and B.
Figure 3.
Figure 4.
Title: Current status of mathematical models for population dynamics of *Prymnesium parvum* in a Texas reservoir

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Abstract: Blooms of the harmful alga *Prymnesium parvum* have apparently increased in frequency in inland waters of the U.S., especially in western Texas. A suite of mathematical models was developed based on a chemostat (or CSTR) framework, and calibrated with data from Lake Granbury, Texas. Inputs included data on flows, salinity, irradiance, temperature, zooplankton grazing, and nutrients. Parameterization incorporated recent laboratory studies relating the specific growth rate of *P. parvum* to such factors. Models differed in the number of algal populations competing with *P. parvum*, and whether competition occurred only by consumption of shared nutrients, or additionally through production of an allelopathic chemical by one of the populations, parameterized as cyanobacteria. Uncalibrated models did not reproduce the observed seasonal dynamics of *P. parvum* in Lake Granbury, which displayed a maximum population in late February during a prolonged bloom in cooler weather, and reduced abundance in summer. Sensitivity analyses suggested two modifications leading to predictions that better resembled observations. The first modification greatly reduces the optimal temperature for growth of *P. parvum*, an approach that disagrees with laboratory experiments indicating a strong potential for growth at temperatures above 20°C. The second modification increases the growth rate of *P. parvum* at all temperatures, in models including cyanobacterial allelopathy. Despite these adjustments, calibrated models did not faithfully simulate all features of the seasonal dynamics of *P. parvum*.

Key terms: algae, aquatic ecology, harmful algal blooms, lakes, *Prymnesium parvum*, allelopathy, cyanobacteria
INTRODUCTION

Harmful algal blooms (HAB) emerged as a notable water quality issue late in the last century, and have apparently increased in frequency and intensity worldwide, in both coastal and inland waters (Hallegraeff, 1993; Van Dolah et al., 2000; Sellner et al., 2003). Although many HABs have direct implications for human health, others are harmful primarily for their disruption of aquatic ecosystems and food webs (Sunda et al., 2006). This study focuses on an example of the latter type, blooms of the haptophyte alga *Prymnesium parvum* (Edvardsen and Paasche, 1998; Edvardsen and Imai, 2006). Blooms of this species have become common in western Texas and other parts of the American Southwest, where it is referred to as golden algae. The principal impact of these blooms has been large fish kills, with an estimated $10^5$ – $10^6$ dead fish in single events.

The perceived increase in HABs has often been attributed to eutrophication and changes in nutrient supply ratios (Smayda, 1989; Paerl, 1997; Cloern et al., 2001), although many additional causes are possible (Van Dolah et al., 2000; Sellner et al., 2003; Hallegraeff and Gollasch, 2006). In water quality modeling, eutrophication is a mature subject, but to address HABs the conventional approaches provide only a starting point. Models for HABs must address the species level, rather than use an aggregated approach representing only one or a few functional types of algae. In many eutrophication models, the main processes affecting algal population dynamics are nutrient-dependent reproductive growth and competition for nutrients, sinking, grazing by zooplankton, and transport. Emerging understanding of the chemical ecology of HABs suggests considering additional biological processes, including allelopathy, here meaning
the production of toxins that impair competitors (Legrand et al., 2003; Babica, 2006; Granéli and Hansen, 2006).

This paper reports construction and calibration of models focused on *P. parvum* blooms in Texas inland waters, as part of an integrated research program addressing this problem (Baker et al., 2007; Roelke et al., 2007; Grover et al., 2007; Errera et al., 2008). Formulations of biological processes were the primary goal, based on recent experimental studies of this species (Baker, 2007; Baker et al., 2007, in press), and thus representation of hydrodynamics was intentionally highly simplified. The simplest model represented only the population of *P. parvum*, growing in relation to nutrients, other physical factors, and grazing. Models were also constructed with one and four additional populations of algae to represent algal functional types competing for nutrients with *P. parvum*. In two additional models one of the competing algal types also produced an allelopathic toxin inhibiting the growth of the focal species, *P. parvum*. These latter models were inspired by recent field experiments suggesting that cyanobacteria present in some Texas inland waters suppress the growth of *P. parvum* via an unidentified dissolved cyanotoxin (Roelke et al., this volume).

FIELD SITE

Calibration data for this study were obtained from Lake Granbury, Texas (97.8°W, 32.4°N), a reservoir on the Brazos River impounded in 1969. The lake has a volume (conservation storage) of 167.4 Mm³, an area of 3378 ha, and a mean depth of 4.98 m. The shoreline follows the meandering river channel with an elongated, sinuous basin oriented northwest to southeast, 45 km long with an average width of 0.6 km. The lake has intakes for municipal water supply and power plant cooling.
From August 2006 to August 2007 samples were taken monthly at ten fixed stations and a number of routine water quality parameters were determined, along with zooplankton and phytoplankton community composition and population densities of *P. parvum* (see digital supplemental files for this manuscript). Surface water samples were collected and processed using the methods of Roelke (2007) and Errera (2008). During the period of observations, volume varied up to 5% below conservation storage. The lake was also eutrophic during this period, with mean total phosphorus of 1.63 μmo/l, mean chlorophyll *a* of 39.8 μg/l, and mean Secchi depth of 0.65 m. Conductometric salinity during this period averaged 1.15 psu, paired deep and shallow stations differed by 0.011 psu on average, and relative longitudinal variation in salinity at different sampling times ranged 2-50% (standard deviation/mean). Similarly low spatial variation for other water quality parameters suggests strong horizontal mixing during the period of the study. Profiles of the upper 15 meters obtained during monthly sampling never revealed vertical thermal stratification. The salinity of Lake Granbury is sufficient to permit growth of *P. parvum*, and large fish-killing blooms occurred in January to June of 2001 and February to April of 2003. Since then, large populations have regularly occurred in cooler weather, with varying degrees of toxicity.

MODEL FORMULATION

A chemostat or continuously-stirred tank reactor (CSTR) was chosen as the modeling framework because the sampling program that provided calibration data revealed vigorous mixing in Lake Granbury. Model formulation thus began with conventional differential equations for coupled dynamics of algae and two dissolved nutrients (Thomann and Mueller, 1987; Chapra, 1997):
\[ \frac{dN_i}{dt} = \mu_i(R, S; \sigma, I, T)N_i - m_i N_i - v_i N_i - D(N_i - N_{in}) \]  

(1)

\[ \frac{dR}{dt} = D(R_{in} - R) - \sum_i \left[ \mu_i(R, S; \sigma, I, T)N_i / Y_{R,i} - rm_i N_i / Y_{R,j} \right] \]  

(2)

\[ \frac{dS}{dt} = D(S_{in} - S) - \sum_i \left[ \mu_i(R, S; \sigma, I, T)N_i / Y_{S,i} - rm_i N_i / Y_{S,j} \right] \]  

(3)

Here, \( N_i \) is the population density of algal type \( i \). Up to five algal types are represented in the models considered here (Table 1), indexed by subscript \( i \): \( p \) – \textit{P. parvum}; \( c \) – cyanobacteria; \( d \) – diatoms; \( f \) – flagellated algae other than \textit{P. parvum} (including chrysophytes, cryptophytes, dinophytes, and euglenophytes); and \( g \) – chlorophytes. Apart from \textit{P. parvum}, populations of other algal types are functional groups composed of many species. Three models were constructed: one with only a population of \textit{P. parvum} and no competitors, one with a single competitor type (cyanobacteria) and one with all four types of competitors (Table 1). According to Equations (1) – (3), competition occurs only through consumption of shared nutrients during growth of different populations.

Variables \( R \) and \( S \) are the concentrations of dissolved inorganic phosphorus and nitrogen, respectively. Inorganic nitrogen species (\( \text{NO}_3^- \), \( \text{NO}_2^- \), \( \text{NH}_4^+ \)) are not resolved and are assumed to affect algal growth equivalently. Although \( \text{NH}_4^+ \) at concentrations exceeding 10 \( \mu \text{mol/l} \) are toxic to \textit{P. parvum} (Barkoh et al., 2003; Grover et al., 2007), concentrations in the focal lake for this study, Lake Granbury, averaged only 2.4 \( \mu \text{mol/l} \) during the period of examined here and did not exceed 6.5 \( \mu \text{mol/l} \).

The population growth rate of algal type \( i \) is governed by the function \( \mu_i \)

\[ \mu_i(R, S; \sigma, I, T) = \mu_{\text{max},i}(\sigma, I, T) \min \left\{ \frac{R}{K_{R,i} + R}, \frac{S}{K_{S,i} + S} \right\} \]  

(4)
where $\mu_{\text{max},i}$ is a nutrient-saturated, maximal growth rate that depends on salinity ($\sigma$), irradiance ($I$), and temperature ($T$), which are time-dependent parameters based on observations described below in the section Forcing Data. The section Initial Parameterization specifies the $\mu_{\text{max},i}$ functions used for each algal type. Liebig’s law of the minimum applies to the dependence of growth on the two nutrients (Rhee, 1982), and growth rate in relation to concentration follows a rectangular hyperbola with half-saturation constants $K_{R,i}$ and $K_{S,i}$ for nitrogen and phosphorus, respectively.

The parameter $m_i$ is mortality rate due to zooplankton grazing, which is a time-dependent function developed from observational data. The parameter $v_i$ is mortality rate due to sinking, given by

$$v_i = u_i / h$$

where $u_i$ is sinking speed and $h$ is mean depth, which is taken here as a constant. Flow was parameterized by the dilution rate $D$, another time-dependent function developed from observations. The final term in Equation (1) includes an inflow of algal type $i$ at concentration $N_i$, assigned a value of 100 cells/ml to specify a small amount of algal immigration. This immigration stabilized against competitive exclusion of any algal type, and was not included in model PP0.

Equations (2) and (3) for nutrient dynamics have supply terms with input concentrations $R_{in}$ and $S_{in}$ for phosphorus and nitrogen, respectively. Remaining terms couple nutrient dynamics to algal populations. Nutrient uptake is proportional to population growth with yield coefficients $Y_{R,i}$ and $Y_{S,i}$ for phosphorus and nitrogen, respectively. Nutrient recycling is proportional to the mortality due to zooplankton
grazing, with a recycling efficiency $r$ specifying the proportion of grazed nutrient content that is recycled.

**Allelopathy**

Field experiments suggest that production of allelopathic substances by cyanobacteria might influence dynamics of *P. parvum* (Roelke et al., this volume). To explore this hypothesis, models PP4A and PP1A were constructed with an additional equation representing the dynamics of a cyanotoxin with concentration $C$. Production of the cyanotoxin is assumed to be proportional to the growth of the cyanobacteria population (Orr and Jones, 1998), with coefficient $ \varepsilon C$. Decay of cyanotoxins appears to be first order (Cousins et al., 1996; Heresztyn and Nicholson, 1997; Twist and Codd, 1997), which we assume to occur with rate constant $k C$. Thus the governing equation for cyanotoxin dynamics is

$$\frac{dC}{dt} = \mu N \varepsilon C - k C C$$

(6)

Cyanotoxins can inhibit growth of algae (Legrand et al., 2003; Granéli and Hansen, 2006), and inhibition of growth rate for *P. parvum* by cyanotoxin was suggested in field experiments (Roelke et al., this volume). Such inhibition is represented by a modified equation for population dynamics:

$$\frac{dN_p}{dt} = \mu_p N_p \left( \frac{K^{I_c}}{C + K^{I_c}} \right) - m_p N_p - v_p N_p - DN_p$$

(7)

The inhibition term in parentheses is analogous to those used in enzyme kinetics, and the parameter $K^{I_c}$ is the cyanotoxin concentration that produces a 50% reduction in growth rate. For simplicity, cyanotoxin was assumed not to affect the growth of algal types other
than *P. parvum*. Appending Equations (6) and (7) to Equations (1) – (3) adds another mechanism of competition, allelopathy, in addition to competition for nutrients.

**Forcing Data**

Eight quantities were taken as time-dependent forcing functions and developed from observational data (see digital supplemental files for this manuscript): dilution rate ($D$), salinity ($\sigma$), irradiance ($I$), temperature ($T$), supply concentrations of nitrogen and phosphorus ($R_{in}, S_{in}$), mortality due to zooplankton grazing ($m_i$), and the nutrient recycling efficiency of grazing ($r$). The time period from August 1, 2006 to August 31, 2007 was considered. Most of the necessary data was obtained from monthly sampling of Lake Granbury with daily values calculated by linear interpolation. Numerical integration of the model equations was implemented for successive days with these daily values treated as fixed; i.e. integration was restarted every day with new values. Model runs of several years duration were obtained by repeating the annual period of input data as needed.

Lake Granbury was assumed to have constant volume at conservation storage, with flow dominated by discharge of the Brazos River. Daily discharge values were obtained from two USGS gauges: 08090800, about 40 km upstream of the headwaters, and 08091000, about 60 km downstream of the dam. Daily dilution rates were calculated from discharges at each station, and these two estimates were averaged to obtain the time series used for model forcing (Fig 1A). For the annual period modeled, high dilution rates occurred from spring to summer, which was a period of unusually wet weather.

Salinity data were averaged at each monthly sampling date for the ten stations and interpolated to obtain daily values (Fig. 1B). For the annual period modeled, low salinity...
of 0.6 – 1 psu occurred during the spring-summer period of high dilution, with higher salinity of about 1.4 psu otherwise.

To estimate the average water column irradiance, exponential attenuation over the mean depth of 5.0 m was assumed. Data on Secchi depth \( h_{SD} \) were averaged at each monthly sampling date for the ten stations and interpolated to obtain daily values. Secchi depth was then converted to attenuation coefficient \( \varepsilon \) with the formula \( \varepsilon = 1.7 / h_{SD} \) (Idso and Gilbert, 1974), and interpolated to obtain daily values. Irradiance just under the water surface \( I_0 \) was calculated from solar declination and day length (Kirk, 1983), assuming a surface reflectance of 6.5% (Wetzel, 2001). This result was then averaged over the photoperiod, and expressed as the photon flux that would deliver the same irradiance over 12 hours just under the water surface. This scaling of photoperiod was used because the laboratory experiments parameterizing the dependence of \( P. parvum \) growth on irradiance used a 12-hour photoperiod (Baker et al., 2007). Finally, the depth-averaged irradiance was calculated as

\[
I = \frac{I_0}{\varepsilon h} \left(1 - e^{-\varepsilon h}\right)
\]

For the annual period modeled, high irradiances occurred in late winter and in midsummer (Fig. 1C). Irradiance was reduced in spring and early summer due to high turbidity associated with high flows.

Temperature data were averaged at each monthly sampling date for the ten stations and interpolated to obtain daily values (Fig. 1D). For the annual period modeled, temperature varied approximately sinusoidally from 7.6°C to 30.9°C.

Ideally, supply concentrations for phosphorus and nitrogen would be estimated from loading studies, but such data are not available. Theoretically, for a chemostat at
steady state, total nutrient concentration approaches the supply concentration (Grover, 1997). Therefore, total phosphorus (TP) and total nitrogen (TN) observations from the monthly monitoring program were taken as estimates of supply concentrations. These values were averaged at each monthly sampling date for the ten stations and interpolated to obtain daily values (Fig. 1E, F). For the annual period modeled, TP averaged 1.6 μmol/l (range: 1.1 – 2.7 μmol/l) and TN averaged 34 μmol/l (range: 19 – 41 μmol/l); the TN:TP molar ratio averaged 30 (range: 18 – 66).

Estimates of mortality due to zooplankton grazing were developed from data on zooplankton populations obtained by microscopy. Community grazing rates (CGR) were calculated from population densities of zooplankton taxa, using literature to estimate volumes (Wetzel and Likens, 1991) and volume-specific clearance rates (Hansen et al., 1997) of each taxon. As with other data, CGR was averaged at each monthly sampling date for the ten stations and interpolated to obtain daily values (Fig. 1G). Since not all algae are equally susceptible to the total grazing activity measured by CGR, the mortality rate for each algal type was calculated as

\[ m_i = w_i \cdot CGR \]  \hspace{1cm} (9)

where \( w_i \) is a susceptibility parameter on the interval (0,1). Although \( w_i \) could vary with time due to changes in zooplankton community composition and grazing behavior, for simplicity it was assumed to be constant for a given algal type. Over most of the annual period modeled, CGR ranged 0.02 – 0.1 1/d, but much higher grazing rates occurred during mid-winter, when rotifers (Brachionus and Keratella) were abundant. Exploratory work suggested results were very insensitive to reasonable assignments of the recycling
efficiency parameter $r$, so this quantity was taken as constant at a value of 0.5 (Sterner, 1989).

**Initial Parameterization**

For *P. parvum*, maximal growth rate as a function of salinity, irradiance and temperature was based on recent laboratory studies of a strain originating in Texas cultured under conditions representative of reservoirs where blooms have occurred (Baker, 2007; Baker et al., 2007, in press). Nutrient-saturated growth was fitted with multiple regression models as a function of temperature, salinity, and irradiance. For the modeling reported here, regression terms for temperature and salinity from low salinity experiments reported in Baker et al. (in press) were combined with regression terms for irradiance from Baker et al. (2007):

$$
\mu_{\text{max}}(\sigma, I, T) = \max \left\{ 0, \beta_0 + \beta_1 (\sigma - \sigma_{\text{ref}}) + \beta_2 e^{\frac{0.7}{\sigma - \sigma_{\text{ref}}}} + \beta_3 (I - I_{\text{ref}}) \\
+ \beta_4 (\sigma - \sigma_{\text{ref}})^2 + \beta_5 e^{\frac{1}{4} \left( \frac{T - T_{\text{ref}}}{T_{\text{ref}}} \right)} + \beta_6 (I - I_{\text{ref}})^2 \\
+ \beta_7 (\sigma - \sigma_{\text{ref}}) e^{\frac{0.7}{\sigma - \sigma_{\text{ref}}}} \right\}
$$

(10)

where terms $\beta_j$ are regression coefficients, and $\sigma_{\text{ref}}$, $I_{\text{ref}}$, and $T_{\text{ref}}$ are reference values of salinity, irradiance and temperature based on the experimental designs used (Table 2). Equation (10) describes response to salinity, irradiance, and temperature as unimodal, with an interaction such that the optimal temperature for growth declines with salinity (Fig. 2A).

For the remaining algal types, salinity-dependence of maximal growth rates was assumed negligible, since few relevant data are available and species capable of growth
under prevailing salinities are likely present in the species pools represented by the algal types modeled. Dependence of growth on temperature and irradiance was modeled as a product function

\[ \mu_{\text{max},i}(I,T) = \mu_{\text{opt},i} g_i(I) f_i(T) \]  

(11)

where \( \mu_{\text{opt},i} \) is the maximal growth rate at optimal irradiance and temperature, and functions \( f_i \) and \( g_i \) vary between 0 and 1. Values for \( \mu_{\text{opt},i} \) (Table 3) were taken as the highest nutrient-saturated growth rates observed for species of each algal type in a study of algae from Texas reservoirs (Grover et al., 1999). Temperature responses (Fig. 2B) were modeled as piecewise linear functions based on Chapra (1997). Light responses (Fig. 2C) were also modeled as unimodal, piecewise linear functions for diatoms and flagellates, approximating curves illustrated in Thomann and Mueller (1987). For chlorophytes, photoinhibition was not observed in studies of algae from Texas reservoirs (Grover et al., 1999), so a rectangular hyperbola was adopted with a half-saturation constant of 30 \( \mu \text{mol} \) photons/m²/d. For cyanobacteria, the function suggested by Litchman (2000) was used

\[ g_c(I) = \max \left\{ 0, \frac{1.683I}{33.33 + I + I^2 / 300} - 0.01 \right\} \]  

(12)

with numerical values assigned to produce a curve peaking at 1, having a shape resembling experimental data for cyanobacteria (Lee and Rhee 1999; Litchman, 2000).

The half-saturation constant for phosphorus-dependent growth of \( P. \text{parvum} \) was assigned as the average of several estimates obtained under low salinity in laboratory experiments (Baker, 2007; Baker et al., in press), and the half-saturation constant for nitrogen-dependent growth was assigned to an arbitrary low value consistent with
laboratory observations saturated growth at very low concentrations (Baker, 2007) (Table 3). Half-saturation constants for nutrient-dependent growth of other algal types were assigned based on estimates for algae from Texas reservoirs (Grover et al., 1999). Because these parameters strongly influence outcomes of competition for nutrients (Grover, 1997), further adjustments were made during exploratory simulations of model PP4 without *P. parvum*. These adjustments successfully obtained seasonal patterns of abundance resembling those documented in Texas reservoirs (Grover et al., 1999; Roelke et al., 2004; Grover and Chrzanowski, 2005).

Yield coefficients for *P. parvum* were assigned initial values within the ranges observed in the laboratory (Baker, 2007; Baker et al., 2007, in press), which were adjusted during exploratory simulations of model PP0 so that peak populations were of similar magnitude to the largest populations observed in the field. Yield coefficients for other algal types were taken from published laboratory studies of nutrient-limited representatives of each type: Hu and Zhang (1993) for cyanobacteria and diatoms; and Gotham and Rhee (1981a, b) for chlorophytes. For flagellates, the value for yield of a *Cryptomonas* sp. on phosphorus (Grover, 1989) was used to calculate yield on nitrogen from a value for the cellular N:P ratio (Sommer, 1991).

Grazing susceptibility parameters were assigned initial values based on expectations that *P. parvum*, flagellates, and diatoms would have moderate to high susceptibility while cyanobacteria and chlorophytes would have low to moderate susceptibility (Sterner, 1989). Adjustments were then made during exploratory simulations. Reservoirs in Texas are often well-mixed vertically (Grover and Chrzanowski, 2004; Roelke et al., 2004), so it was initially assumed that sinking
mortality would be negligible. During exploratory simulations, however, it was found that diatoms reached unrealistic abundances unless a modest sinking mortality was imposed, corresponding to a sinking speed of 0.9 m/d in Lake Granbury, a value near the average reported for several diatoms (Reynolds, 2006).

Parameterization of terms relating to cyanotoxin is based on considering the steady state concentration predicted by Equation (6):

\[ C^* = \frac{\mu_c N_c^* \varepsilon_c}{k_c} \]

(13)

where the asterisk denotes a steady state value. When cyanobacteria are abundant, dissolved concentrations of cyanotoxins such as nodularin and microcystin are about 1 μg/l (Heresztyn and Nicholson, 1997; Park et al., 1998; Fromme et al., 2000), which is assumed to represent a quasi-steady state. A first-order decay rate for the cyanotoxin \( k_c \) of 0.5 d\(^{-1}\) is assumed, broadly consistent with observed degradation in natural waters (Cousins et al., 1996; Heresztyn and Nicholson, 1997). Taking a cyanobacterial population density of \( 2 \times 10^4 \) cells/ml with a growth rate of 0.1 d\(^{-1}\) as representative of summertime in Texas lakes (Grover et al., 1999), the value of the production coefficient \( \varepsilon_c \) must be \( 2.5 \times 10^{-7} \) μg/cell to produce a steady state concentration of 1 μg/l. With these parameter values, simulations of model PP4A predicted cyanotoxin concentrations during summer in the expected range of about 1 μg/l. However, simulations of model PP1A led to much higher cyanotoxin concentrations. So for this model a lower value for \( \varepsilon_c \) of \( 1.25 \times 10^{-8} \) μg/cell was adopted. The value of the growth inhibition parameter for \( P. \ parvum \) \( (K_c^I) \) is assigned to be 0.1 μg/l, so that the cyanotoxin concentrations simulated should
strongly inhibit growth of *P. parvum*, consistent with field experiments (Roelke et al., this volume).

**MODEL RESULTS AND CALIBRATION**

*Overview*

Numerical integrations of models were obtained using a fourth-order Runge-Kutta algorithm with adaptive step size (Press et al., 1986). Simulations were initialized with all populations at a low density of 100 cells/ml and run for five (model PP0) or twenty simulated years (other models). All simulations became annually periodic, displaying seasonal oscillations forced by the annual time series of environmental inputs, and the same annual cycle was reached when alternative initial conditions were used. Predicted dynamics of *P. parvum* in the last simulated year were compared to observations obtained from monthly samples of Lake Granbury.

*Error Metrics*

Three error metrics were developed to screen model performance, based on a broad goal of accurately simulating dynamics of *P. parvum* during cool weather, when blooms typically occur in Texas inland waters. The first metric (*M*₁) is the relative error in predicted peak density over the year calculated as

\[
M_1 = \left| \frac{N_{\text{max},p} - \hat{N}_{\text{max},p}}{\hat{N}_{\text{max},p}} \right|
\]

(14)

where \( \hat{N}_{\text{max},p} \) is the predicted peak population density and \( N_{\text{max},p} \) is the observed peak population density. The second metric (*M*₂) is the relative error in the average natural logarithm of population density over the first 100 days of the year (January 1 to April 9)
when fish-killing blooms of *P. parvum* are most commonly observed, and when the highest abundance was observed during the year of calibration data.

\[
M_2 = \left( \frac{\langle \ln N_p \rangle - \langle \ln \hat{N}_p \rangle}{\langle \ln \hat{N}_p \rangle} \right) 
\]

(15)

where \( \hat{N}_p \) is predicted population density and \( N_p \) is observed population density, and angle brackets indicate a time-average. The third metric \( M_3 \) is the difference in timing between the predicted and observed peaks of population density, relative to the length of a year:

\[
M_3 = \min \left( \frac{t_{\text{max}} - \hat{t}_{\text{max}}}{365}, \frac{365 + t_{\text{max}} - \hat{t}_{\text{max}}}{365} \right) 
\]

(16)

where \( \hat{t}_{\text{max}} \) is the time of the predicted peak population density and \( t_{\text{max}} \) is the time of the observed peak population density.

**Model Results for Initial Parameters**

With the initial parameterization, none of predicted dynamics of *P. parvum* agreed well with observations for any of the models (Table 4, Fig. 3). Model PP0 (Fig. 3A), without competing algal populations, predicted two seasonal peaks for the *P. parvum* population, one in spring (April to June) and one in autumn (September to December). Minima were predicted in February, at the time of the observed maximum, and during summer (July to August). Model PP0 had low error in its prediction of the average natural log density of *P. parvum* in winter (metric \( M_2 \)), but this was accomplished by over-predicting the density of *P. parvum* at all other times of year.

Models PP1 and PP4 (Figs. 3B, C) predicted similar dynamics for *P. parvum*, with a seasonal maximum in late spring (early June), with a secondary peak in late
autumn (August). Predicted minima again occurred in February, at the time of the observed maximum, and in late summer (early September). In both of these models, competing populations reduced the nutrients available to P. parvum, reducing its predicted density, and to some extent correcting the problem of over-prediction for model PP0, from which competitors were absent.

Models PP1A and PP4A (Figs. 3 D, E) both predicted similar dynamics for P. parvum, with a single seasonal maximum in late autumn (early December), and a minimum in February at the time of the observed maximum. Compared to models PP1 and PP4, the inclusion of allelopathy in models PP1A and PP4A greatly reduced the predicted density of P. parvum in summer, when cyanobacteria were predicted to be abundant. The predicted density of P. parvum in winter was also low for models PP1A and PP4A, leading to under-prediction over the course of the year.

Sensitivity Analyses and Modified Parameter Sets

A sensitivity analysis was conducted for each model, varying most parameters through a range of $0.3\times - 3\times$ the initial value, except that grazing susceptibilities ($w_i$) and the recycling parameter ($r$) were truncated at 1. For the functions governing responses to temperature, light and salinity, the overall shape of the function rather than individual parameters was perturbed. The elevation of the response function was varied through a range of $0.3\times - 3\times$ by changing appropriate parameters ($\beta_0$ for P. parvum and $\mu_{opt}$ for other algae). For P. parvum, the reference levels $T_{ref}$, $I_{ref}$ and $\sigma_{ref}$ were also varied, producing translations of the response surfaces for light and salinity along the respective axis. For the other taxa, shifts in optimum values were implemented by transforming the forcing data multiplicatively through the range $0.3\times - 3\times$. In addition to truncating some
parameter variations at the biologically meaningful limit of 1, the range of 0.3× - 3× was truncated in other instances where very high values of error metrics indicated large errors.

Using the error metrics as screening tools, alternative parameter assignments were selected for further examination if they reduced at least one error metric to below 20% without large increases in other metrics (Table 5). For models PP0, PP1, and PP4, adjustment of the reference temperature ($T_{\text{ref}}$) for the growth response of *P. parvum* led to predictions that shared several features (Figs. 4A-C): a peak population occurring in late winter and early spring, shortly after the observed peak; a summer minimum population falling far below the observed minimum; and a population increase in autumn and early winter that began later and rose more rapidly than the observed increase at this time. For model PP0, the late winter-spring bloom of *P. parvum* was predicted to persist longer than was observed. Models PP1 and PP4 both predicted termination of the bloom shortly after the time that low populations began to occur in observations. There was little difference between the dynamics predicted by models PP1 and PP4, with parameter $T_{\text{ref}}$ decreased (Figs. 4B, C).

For models PP0, PP1, and PP4, reduction of $T_{\text{ref}}$ to values in a range of 6 - 15 °C led to dynamics similar to those illustrated for a value of $T_{\text{ref}} = 12.5$ °C (Fig. 4A-C). The illustrated value severely distorts of the growth response of *P. parvum* to temperature (Fig. 5) and strongly contradicts laboratory experiments with a Texas strain of *P. parvum* (Baker, 2007; Baker et al., 2007, in press; Grover et al., 2007). The very low population density of *P. parvum* predicted during summer by these models with low $T_{\text{ref}}$ results directly from lack of growth at warm temperatures.
For models PP1A and PP4A, improved agreement between predicted dynamics and observations was obtained by increasing the parameter $\beta_0$ in the growth response of *P. parvum* to abiotic conditions (Equation 10). For model PP1A, an increase of 0.161 d$^{-1}$ to $\beta_0 = -3.37$ minimized error metric $M_1$, and was close to minimal for metrics $M_2$ and $M_3$. For model PP4A, an increase of 0.131 d$^{-1}$ to $\beta_0 = -3.4$ minimized error metric $M_3$, and was close to minimal for metrics $M_1$ and $M_2$. These changes led to similar predictions of *P. parvum* dynamics (Figs. 4D and E): a peak of population density occurred in late spring – early summer, coming later than the observed peak. Although the predicted decline to a summer minimum also came later than the observed decline, the minimum population level in summer agreed with observations. A population increase was predicted through autumn and early winter, which began later than the observed increase and was faster. These models with adjusted values of $\beta_0$ also predicted a minimum population density for *P. parvum* in mid-winter, at the time of the observed peak.

For models PP1A and PP4A, increasing $\beta_0$ as indicated above increases the growth rate of *P. parvum* under all conditions of temperature, irradiance and salinity (Fig. 5). The magnitude of this increase is likely within the error arising in laboratory experiments (Baker, 2007; Baker et al., 2007, in press). For models PP1A and PP4A, improved agreement between predicted dynamics and observations could also be obtained by decreasing the parameter $T_{ref}$, as was done for other models, but only if very low and biologically implausible values were used ($< 6 \, ^\circ C$).

Predictions of all models were sensitive to parameters other than those adjusted to produce Fig. 4. Generally the most influential parameters were those of the functions describing the growth response of *P. parvum* to temperature, light and salinity (Equation
In most cases, similar improvements in model performance could be achieved by adjustments of $\beta_0$, $T_{\text{ref}}$ or $\sigma_{\text{ref}}$. For models without allelopathy (PP0, PP1, PP4) adjusting $T_{\text{ref}}$ produced the greatest agreement with observations, while for those with allelopathy (PP1A, PP4A) adjusting $\beta_0$ produced the greatest improvement. Other influential parameters included grazing susceptibilities ($w_i$) and yields on phosphorus ($Y_{R,i}$). These were left at initially assigned values, which had low values for error metrics in all cases examined. In some models, parameters describing the growth of competing algal types or production of cyanotoxin were influential, but changes from the initially assigned values were associated with tradeoffs in which one error metric declined while another increased.

**Further Modifications**

The similarity of predictions between models with one competing type of algae, parameterized as cyanobacteria, and those with four types, suggests that these particular algae could have a critical influence on the dynamics of *P. parvum*. To explore further, a modification of models PP1A was developed to use observed, monthly cyanobacterial abundances as forcing data. These observations were averaged spatially among the ten sampling stations in Lake Granbury and interpolated daily between sampling dates, as for other forcing data. Because observations estimated the biomass density of cyanobacteria as chlorophyll $a$, conversion was necessary for model parameters. The typical cell volume for cyanobacteria in Texas reservoirs was estimated as $35 \, \mu m^3$, the geometric mean from a database developed during previous studies (Grover and Chrzanowski, 2004, 2005). From this a conversion of $0.2 \, pg \, C / \mu m^3$ (Rocha and Duncan, 1985) was used to estimate $7 \, pg \, C / cell$ for cyanobacteria. Because nutrient limitation in field
populations of algae typically reduces chlorophyll-to-carbon ratios a relatively low value of 10 μg chl a / mg C was adopted (Chapra, 1997), producing a conversion of 70 fg chl a / cell.

Using initial parameter assignments, the dynamics predicted for *P. parvum* when observations of cyanobacteria are treated as forcing data (Fig. 6A) strongly resemble those predicted by models PP1 and PP4 (Figs. 3B and C). Sensitivity analysis suggested that the best improvements in model performance resulted from adjusting the reference temperature $T_{ref}$ to 12.5 °C in the growth function of *P. parvum*. After this adjustment, predicted dynamics (Fig. 6B) resembled those of models PP1 and PP4 with $T_{ref}$ adjusted (Figs. 4B and C).

DISCUSSION

Calibration of the models examined here relates to a paradox evident in studying blooms of *P. parvum* in Texas inland waters. Although some strains of this species apparently grow optimally at 15 °C, several others grow best at 25 – 30 °C (Larsen et al., 1993; Larsen and Bryant, 1998), including a strain isolated in Texas (Baker, 2007; Baker et al., 2007; in press). Based on growth potential at high temperature, blooms should occur primarily in summer. Yet, blooms in Texas have occurred primarily in winter. It is possible that Texas lakes are dominated by strains with low preferred temperatures for growth. Nevertheless, at least one well-characterized strain isolated in Texas has a high potential for growth at high temperature, so the question of why these very warm inland waters are not dominated by strains with higher preferred temperatures would still remain. Ecologists are familiar with the tendency for species to occur in the field under narrower and usually lower temperatures than those permitting growth in the laboratory.
(Magnuson et al., 1979). This phenomenon is usually attributed to lack of competition and other biological interactions for isolated populations, and the restrictive action of these processes in natural settings (Hutchinson, 1959). The fundamental niche refers to growth responses to temperature and other factors obtained in isolation, while the realized niche refers to those found in nature.

The fundamental niche of *P. parvum* is expressed graphically by Fig. 2A, and models based on this niche err in their predictions (Fig. 3), due to the much different realized niche. Constructing models PP1 and PP4 with competing populations of algae examined the classical expectation that competition for nutrient resources would suffice to alter the fundamental niche and restrict *P. parvum* to occurring under cool temperatures. For these models with resource competition only, summer populations of *P. parvum* were predicted to be high, so models PP1A and PP4A were constructed to examine whether an additional competitive mechanism, allelopathy, would restrict *P. parvum* abundance during summer. However, without additional adjustments identified during calibration, the predicted abundance of *P. parvum* during winter remained far below observations.

Calibration identified two parameter adjustments bringing predicted dynamics of *P. parvum* into closer agreement with observations: (1) modifying the temperature response of *P. parvum* by reducing the parameter $T_{\text{ref}}$, essentially aligning it with the realized niche; and (2) raising the growth rate of *P. parvum* under all conditions, which was effective only for models with allelopathy. Both of these modifications have advantages and disadvantages.
The first alternative is a strong distortion of the temperature response of *P. parvum*. Though it contradicts laboratory data, this distortion accommodates the general notion that the realized niche differs from the fundamental niche, and is a convenient way to improve model predictions when lacking specific knowledge of the ecological processes causing this niche restriction. With this adjustment, models PP1 and PP4 successfully simulate the winter bloom of *P. parvum* in the calibration data and predict its termination at about the right time. The cost of this success comes in summer, when the adjusted temperature response predicts no growth and much lower population density than observed. As a result of under-predicting the population density in summer, the predicted rise of *P. parvum* in autumn is later and faster than observed. This first alternative also violates the spirit of mechanistic modeling – it is an ad hoc adjustment that improves predictions, but whose basis and robustness are unclear.

The second alternative proposes that allelopathy from cyanobacteria negatively impacts *P. parvum*, and calibration further suggests that a modest increase in the growth response of this species improves the agreement of predictions with observations. To that extent, this exercise supports the hypothesis that allelopathy from cyanobacteria influences the population dynamics of *P. parvum* in Texas inland waters. The adjustment of the growth response during calibration is within the likely experimental error of growth response studies. Allelopathy models adjusted after calibration successfully simulate the low population density of *P. parvum* during summer and mimic the autumn rise better than models with a distorted temperature response. However, dynamics during winter are not well simulated, with a drop in population density predicted during the lowest temperatures in February, in sharp disagreement with observations. Although this
drop is followed by a recovery to near the observed population densities, the resulting spring bloom is predicted to last longer than observed.

Inclusion of allelopathy in this study was inspired by observations that growth of *P. parvum* was inhibited by mixing with waters from a source dominated by cyanobacteria, and indications that a dissolved constituent other than nutrients or salinity was responsible (Roelke et al., this volume). However, the mechanistic formulation adopted here is largely conjectural. Cyanobacteria produce many toxins (Codd et al., 2005), none of which have been tested in purified form for effects on *P. parvum* in laboratory experiments. Culture filtrates from three species of cyanobacteria did not inhibit growth of *P. parvum* (Suikkanen et al., 2004). The models constructed here assume that cyanotoxin production is proportional to the product of population density and growth rate (Orr and Jones, 1998). However, there is evidence that at least one cyanotoxin, nodularin, can be produced by non-growing populations (Stolte et al., 2002). The models also attribute toxin production to the entire assemblage of cyanobacteria, when in reality, only particular species and strains of cyanobacteria produce toxins (Kardinaal and Visser, 2005). Further information on the susceptibility of *P. parvum* to cyanotoxins, and on cyanotoxin dynamics in Texas inland waters, is required to evaluate and refine (or abandon) this process in models focusing on *P. parvum* blooms.

The study presented here examined whether a conventional approach to algal dynamics is adequate for modeling *P. parvum* blooms in a Texas reservoir. Lacking clear success in this approach, a number of model improvements could be contemplated. The representation of grazing mortality in the models presented here is highly simplified. In particular, grazing mortality rate is independent of algal population density. This is
unlikely to be true, especially for *P. parvum*, because dense, toxic populations inhibit or even kill several types of zooplankton (Tillman, 2003; Sopanen et al., 2006, 2008; Roelke et al., 2007). The use of observational inputs to drive grazing mortality could be problematic in some applications, given that detailed information on taxonomic composition is not obtained in many monitoring programs.

The models presented here did not explicitly address the toxicity of *P. parvum*, because of several current uncertainties. It is common to observe dense populations that are not toxic to fish (Shilo, 1981). One class of toxins has been characterized (Murata and Yasumoto, 2000), but there may be others, and little is known about regulation of toxin production. It is increased by stresses such as nutrient limitation (Johansson and Granéli, 1999; Granéli and Johansson, 2003) and suboptimal temperature and salinity (Baker et al., 2007), and correlates with high pH (Shilo and Aschner, 1953; Ulitzur and Shilo, 1964; Padilla 1970; Grover, 2007), but available information does not provide a strong basis for model formulation. Fortunately, knowledge of the ecological toxicology of *P. parvum* is advancing rapidly (Granéli and Flynn, 2006).

Mixotrophy on the part of *P. parvum* might also be related to its toxicity. Ingestion of bacteria has been observed under nutrient-limited conditions (Nygaard and Tobiesen, 1993; Legrand et al., 2001), and toxins produced by *P. parvum* can lyse larger microorganisms, making their cellular contents available for ingestion (Skovgaard and Hansen, 2003; Tillman, 2003). Conceivably, mixotrophy could boost the growth rate of *P. parvum* under the cool and dark conditions of winter when phototrophy is less favored. *P. parvum* also forms cysts (Carter, 1937), and like other harmful algae blooms could be
related to germination of these resting stages (Sellner et al., 2003), but no information is
currently available on factors that regulate encystment and germination in this species.

In conclusion, some first steps in model construction and calibration have been
taken to address blooms of *P. parvum* in Texas inland waters. To date, modeling
highlights a contradiction between the fundamental niche of this harmful alga, predicting
growth under warm temperatures, and the realized niche, wherein high population
densities are observed in cool weather. Competition from other algae might partly explain
this discrepancy, and the similarity between models with one and four competing
populations suggests that it is most important to represent cyanobacteria, and less
important to represent other types of algae. The models constructed and calibrated here
remain to be evaluated against further data, which is being collected from ongoing studies
of Lake Granbury and other Texas lakes. They also serve as a basis for incorporating
other processes affecting the population dynamics and toxicity of *P. parvum* as research
on this species advances.

NOTATION

\[ N_i \] Population density of algal type *i* (cells/ml)

\[ i \] Index for algal type: *p* – *P. parvum*; *c* – cyanobacteria; *d* – diatoms; *f* –
flagellates; *g* – chlorophytes

\[ R \] Concentration of dissolved inorganic phosphorus (μmol/l)

\[ S \] Concentration of dissolved inorganic nitrogen (μmol/l)

\[ C \] Concentration of cyanotoxin (μg/l)

\[ \mu(R,S;\sigma,I,T) \] Population growth rate of algal type *i* as a function of nutrients and
environmental conditions (1/d)
\( \mu_{\text{max}}(\sigma, I, T) \)  Maximal population growth rate of algal type \( i \) as a function of environmental conditions (1/d)

\( f_i(T) \)  Temperature response function for algal type \( i \) (dimensionless)

\( g_i(I) \)  Irradiance response function for algal type \( i \) (dimensionless)

\( \sigma \)  Salinity (practical salinity units, psu)

\( I \)  Average water column irradiance (\( \mu \)mol photons/m\(^2\)/s)

\( T \)  Surface water temperature (°C)

\( K_{R,i}, K_{S,i} \)  Half-saturation constants for phosphorus- and nitrogen-dependent growth of algal type \( i \), respectively (\( \mu \)mol/l)

\( Y_{R,i}, Y_{S,i} \)  Yield coefficients on phosphorus and nitrogen for algal type \( i \), respectively (cells/\( \mu \)mol)

\( v_i \)  Sinking mortality for algal type \( i \) (1/d)

\( u_i \)  Sinking speed for algal type \( i \) (m/d)

\( h \)  Mean depth (m)

\( m_i \)  Mortality due to zooplankton grazing for algal type \( i \) (1/d)

\( D \)  Dilution rate (1/d)

\( R_{\text{in}}, S_{\text{in}} \)  Supply concentrations of nitrogen and phosphorus, respectively (\( \mu \)mol/l)

\( r \)  Recycling efficiency of zooplankton grazing (dimensionless)

\( \varepsilon_C \)  Production coefficient for cyanotoxin (\( \mu \)g/cell)

\( k_C \)  Decay coefficient for cyanotoxin (1/d)

\( K_C^i \)  Inhibition parameter for effect of cyanotoxin on growth of \( P. \ parvum \) (\( \mu \)g/l)

\( h_{SD} \)  Secchi depth (m)
$\varepsilon$  Vertical attenuation coefficient for irradiance (1/m)

$I_0$  Irradiance just under the water surface ($\mu$mol photons/m$^2$/s)

$CGR$  Community grazing rate (1/d)

$w_i$  Grazing susceptibility parameter for algal type $i$ (dimensionless)

$\beta_j$  Coefficients of maximal growth function for $P.\ parvum$ (various dimensions)

$\sigma_{\text{ref}}, I_{\text{ref}}, T_{\text{ref}}$  Reference levels of salinity, irradiance and temperature in maximal growth function for $P.\ parvum$ (psu, $\mu$mol photons/m$^2$/s, °C)

$\mu_{\text{opt},i}$  Maximal growth rate of algal type $i$ under optimal conditions (1/d)

$f_i(T)$  Temperature response of growth for algal type $i$ (dimensionless)

$g_i(I)$  Irradiance response of growth for algal type $i$ (dimensionless)

**SUPPORTING INFORMATION**

Additional supporting information may be found in the online version of this article:

Table S1. Selected limnological variables measured in Lake Granbury, Texas.

Table S2. Discharges from USGS gauges 08090800 (upstream) and 08091000 (downstream) and estimated hydraulic dilution rate for Lake Granbury, Texas.

Table S3. Estimation of daily average water column irradiance for Lake Granbury, Texas, following computations outlined in the main text.

Table S4. Zooplankton abundances (as biovolume, $\mu$m$^3$/l) for Lake Granbury, Texas.
Table S5. Estimated individual volumes and volume-specific clearance rates of zooplankton taxa enumerated in Lake Granbury, Texas, based on published literature (Wetzel and Likens, 1991; Hansen et al., 1997).

Table S6. Estimated mortality increments and community grazing rates for Lake Granbury, Texas.

ACKNOWLEDGMENTS

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Berlin, Germany. Chap. 11.
Table 1. Designations and definitions of models examined for predicting *Prymnesium parvum* dynamics in a Texas reservoir.

<table>
<thead>
<tr>
<th>Designation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>PP0</td>
<td>Only the population of <em>P. parvum</em> is represented</td>
</tr>
<tr>
<td>PP1</td>
<td><em>P. parvum</em> and one competitor type, cyanobacteria, are represented</td>
</tr>
<tr>
<td>PP4</td>
<td><em>P. parvum</em> and four competitor types are represented</td>
</tr>
<tr>
<td>PP1A</td>
<td><em>P. parvum</em> and one competitor type, cyanobacteria, are represented; allelopathy is included as cyanotoxin production</td>
</tr>
<tr>
<td>PP4A</td>
<td><em>P. parvum</em> and four competitor types are represented; allelopathy is included as cyanotoxin production</td>
</tr>
</tbody>
</table>
Table 2. Parameters of the maximal growth function of *Prymnesium parvum* used in models for predicting its dynamics in a Texas reservoir.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta_0$</td>
<td>-3.531</td>
<td>$\beta_6$</td>
<td>-0.00000573</td>
</tr>
<tr>
<td>$\beta_1$</td>
<td>0.02534</td>
<td>$\beta_7$</td>
<td>0.1697</td>
</tr>
<tr>
<td>$\beta_2$</td>
<td>7.468</td>
<td>$\sigma_{\text{ref}}$</td>
<td>1.833</td>
</tr>
<tr>
<td>$\beta_3$</td>
<td>0.000611</td>
<td>$I_{\text{ref}}$</td>
<td>222</td>
</tr>
<tr>
<td>$\beta_4$</td>
<td>-0.06311</td>
<td>$T_{\text{ref}}$</td>
<td>20</td>
</tr>
<tr>
<td>$\beta_5$</td>
<td>-3.414</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Initial assignments of parameter values specific to algal types simulated in models for predicting *Prymnesium parvum* dynamics in a Texas reservoir.

<table>
<thead>
<tr>
<th>Parameter</th>
<th><em>P. parvum</em> ((p))</th>
<th>Cyanobacteria ((c))</th>
<th>Diatoms ((d))</th>
<th>Flagellates ((f))</th>
<th>Chlorophytes ((g))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\mu_{\text{opt},i})</td>
<td>NA*</td>
<td>1.52</td>
<td>1.87</td>
<td>0.79</td>
<td>1.25</td>
</tr>
<tr>
<td>(K_{R,i})</td>
<td>0.009</td>
<td>0.166</td>
<td>0.14</td>
<td>0.02</td>
<td>0.15</td>
</tr>
<tr>
<td>(K_{S,i})</td>
<td>0.01</td>
<td>3.686</td>
<td>0.04</td>
<td>3.31</td>
<td>1.15</td>
</tr>
<tr>
<td>(Y_{R,i})</td>
<td>(7.2 \times 10^8)</td>
<td>(3.5 \times 10^8)</td>
<td>(4.0 \times 10^7)</td>
<td>(8.3 \times 10^7)</td>
<td>(2.0 \times 10^8)</td>
</tr>
<tr>
<td>(Y_{S,i})</td>
<td>(3.125 \times 10^7)</td>
<td>(3.08 \times 10^7)</td>
<td>(4.4 \times 10^6)</td>
<td>(7.5 \times 10^6)</td>
<td>(7.1 \times 10^6)</td>
</tr>
<tr>
<td>(w_i)</td>
<td>0.5</td>
<td>0.49</td>
<td>0.8</td>
<td>1.0</td>
<td>0.64</td>
</tr>
<tr>
<td>(v_i)</td>
<td>0</td>
<td>0</td>
<td>0.18</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Not Applicable given definition of functions used.*
Table 4. Error metrics comparing predicted and observed dynamics of *Prymnesium parvum* in a Texas reservoir, from the initial parameterization of models predicting its dynamics.

<table>
<thead>
<tr>
<th>Model designation</th>
<th>Error in peak density, $M_1$</th>
<th>Error in average winter natural log density, $M_2$</th>
<th>Error in peak timing, $M_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP0</td>
<td>96%</td>
<td>3%</td>
<td>14%</td>
</tr>
<tr>
<td>PP1</td>
<td>94%</td>
<td>160%</td>
<td>33%</td>
</tr>
<tr>
<td>PP4</td>
<td>99%</td>
<td>227%</td>
<td>30%</td>
</tr>
<tr>
<td>PP1A</td>
<td>6425%</td>
<td>415%</td>
<td>23%</td>
</tr>
<tr>
<td>PP4A</td>
<td>2934%</td>
<td>392%</td>
<td>21%</td>
</tr>
</tbody>
</table>
Table 5. Error metrics comparing predicted and observed dynamics of *Prymnesium parvum* in a Texas reservoir, for adjusted parameters determined in a sensitivity analysis of models predicting its dynamics.

<table>
<thead>
<tr>
<th>Model designation</th>
<th>Error in peak density, $M_1$</th>
<th>Error in average winter natural log density, $M_2$</th>
<th>Error in peak timing, $M_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP0</td>
<td>95%</td>
<td>16%</td>
<td>0%</td>
</tr>
<tr>
<td>PP1</td>
<td>60%</td>
<td>5%</td>
<td>10%</td>
</tr>
<tr>
<td>PP4</td>
<td>50%</td>
<td>0%</td>
<td>10%</td>
</tr>
<tr>
<td>PP1A</td>
<td>39%</td>
<td>29%</td>
<td>10%</td>
</tr>
<tr>
<td>PP4A</td>
<td>70%</td>
<td>16%</td>
<td>10%</td>
</tr>
</tbody>
</table>
FIGURE LEGENDS

Figure 1. Forcing data for models predicting *Prymnesium parvum* dynamics in Lake Granbury, Texas: (a) dilution rate; (b) salinity; (c) irradiance; (d) temperature; (e) phosphorus supply concentration; (f) nitrogen supply concentration; (g) community grazing rate for zooplankton.

Figure 2. Functions describing maximal growth rates of algal populations used in models for predicting *Prymnesium parvum* dynamics in a Texas reservoir. (a) *P. parvum*: curves show growth rates for an irradiance of 222 μmol photons/m²/s as a function of temperature, for the salinities (psu) given in boxes. (b) Functions $f_i(T)$ governing growth responses to temperature for four types of algae represented in models (c – cyanobacteria; d – diatoms; f – flagellates; g – chlorophytes). (c) Functions $g_i(I)$ governing growth responses to light for the same four types of algae represented in models.

Figure 3. Predicted and observed population dynamics of *Prymnesium parvum* in Lake Granbury with initial parameterizations of five models. Solid line shows predicted values; symbols show observed values as the average of ten sampling stations. Whiskers show range of sampling stations; when zero observations occurred at some stations, whiskers extend to the bottom of the figure. (a) Predictions from model PP0. (b) Predictions from model PP1. (c) Predictions from model PP4. (d) Predictions from model PP1A. (e) Predictions from model PP4A.

Figure 4. Predicted and observed population dynamics of *Prymnesium parvum* in Lake Granbury with modified parameter sets selected after sensitivity analyses of five models. Solid line shows predicted values; symbols show observed values as the average of ten sampling stations. Whiskers show range of sampling stations; when zero observations
occurred at some stations, whiskers extend to the bottom of the figure. (a) Predictions from model PP0. (b) Predictions from model PP1. (c) Predictions from model PP4. (d) Predictions from model PP1A. (e) Predictions from model PP4A.

Figure 5. Maximal growth functions for *Prymnesium parvum* in relation to temperature, used in models to predict its dynamics in a Texas reservoir, and calculated for an irradiance of 222 $\mu$mol photons/m$^2$/s and a salinity of 1.2 psu: dotted line – with initial parameters for the models; dashed line – with the reference temperature parameter $T_{ref}$ reduced to 12.5 °C as suggested by sensitivity analyses of the models; solid line – with the intercept parameter $\beta_0$ increased to -3.37 1/d as suggested by sensitivity analyses of the models.

Figure 6. Predicted and observed population dynamics of *P. parvum* in Lake Granbury using observed cyanobacteria abundance as forcing data for a mathematical model that includes allelopathy in the form of cyanotoxin production (model PP1A). Solid line shows predicted values; symbols show observed values as the average of ten sampling stations. Whiskers show range of sampling stations; when zero observations occurred at some stations, whiskers extend to the bottom of the figure. (a) with initial parameters for the model. (b) with reference temperature parameter $T_{ref}$ reduced to 12.5 °C as suggested by sensitivity analysis of the model.
(a) Temperature (°C) vs. Growth Rate (1/day)

(b) Temperature Function, \( f(T) \) vs. Temperature (°C)

(c) Irradiance Function, \( g(T) \) vs. Irradiance (\( \mu \text{mol/m}^2/\text{s} \))
Population Density of *P. parvum* (cells/ml)
Day of the Year

Population Density of *P. parvum* (cells/ml)

(a) $\text{Population Density of } P.\ parvum (\text{cells/ml})$

(b) $\text{Population Density of } P.\ parvum (\text{cells/ml})$

Day of the Year
Factors influencing *Prymnesium parvum* population dynamics during bloom initiation: Results from in-lake mesocosm experiments


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Running head:
P. parvum bloom formation
Abstract

The alga *Prymnesium parvum* forms large fish-killing blooms in many Texas (USA) lakes. In some of these lakes, however, *P. parvum* occurs but does not develop blooms. In this study, we investigated factors that may influence bloom initiation by conducting a series of in-lake experiments involving mixing of waters from L. Whitney, which has a history of *P. parvum* blooms, with waters from L. Waco where no blooms have occurred. In all experiments, the addition of L. Waco waters resulted in a poorer performance of *P. parvum*. Various experimental treatments and field data show that differences in grazing, pathogens, nutrients, and salts between the two lakes were not likely factors that contributed to this observation. Industrial and agricultural contaminants, allelochemicals and algicidal chemicals were not measured as a part of this research. However, anthropogenic contaminants other than nutrients were not observed at levels exceeding water quality standards in L. Waco in recent years. On the other hand, nuisance cyanobacteria are common in L. Waco, where *Microcystis* sp. and *Anabaena* sp. were abundant during the initiation of our experiments, both taxa are known to produce chemicals with allelopathic properties. In addition, the emergent field of algal-heterotrophic bacteria interactions suggests that chemicals produced by heterotrophic bacteria should not be overlooked. Further research focusing on the chemical interactions between cyanobacteria and *P. parvum*, as well as the potential role of algicidal bacteria, in the initiation of *P. parvum* blooms is necessary, as it may be important to the management of these blooms.

Keywords: plankton, inorganic nutrients, salinity, grazing, harmful algal blooms, microcystins, prymnesins, allelopathy, cyanobacteria
Introduction

_Prymnesium parvum_ is a haptophyte alga that occurs worldwide, tolerates large variations in temperature and salinity, and sometimes forms blooms that result in large fish kills (Edvardsen and Paasche 1998, Lundholm and Moestrup 2006, Baker et al. 2007). In Texas (USA), the incidence of _P. parvum_ blooms has increased dramatically since 2001, where blooms are now observed in over 19 lakes found along five river basins. During blooms, surface waters take on a golden color with _P. parvum_ cell densities typically exceeding $10 \times 10^6$ cells L$^{-1}$. In addition, fish kills occurring with these blooms involve many species, where total mortalities number in the tens of millions (TPWD 2003, Roelke et al. 2007).

Changes in the physicochemical environment that led to the increase in Texas _P. parvum_ blooms are unknown, but might include eutrophication and salinization. For example, blooms in Europe, the Middle East, and Asia have all occurred in aquatic systems that were eutrophic and brackish (Krasnotshchek and Abramowitsch 1971, Holdway et al. 1978, Rijn and Shilo 1989, Kaartvedt et al. 1991, Guo et al. 1996, Amsinck et al. 2005). _P. parvum_ blooms in Texas are partly consistent with these observations because they appear mostly in lakes that attain salinities 2 to 4 psu (practical salinity units) during low precipitation years (TPWD 2003), and these lakes may have experienced increased non-point source nutrient loading because of aging septic systems, point source discharges and expanded shoreline development. However, the role of nutrients is complex. In laboratory and field experiments the toxicity to fish from chemicals produced by _P. parvum_, which also act as allelochemicals and are an important factor leading to bloom initiation, was greater when cells were nutrient-limited (Uronen et al. 2005, Roelke et al. 2007, Errera et al. 2008). In addition, _P. parvum_ is sensitive to pulses of nutrients where high
doses inhibited bloom formation (Barkoh et al. 2003, Grover et al. 2007, Kurten et al. 2007).

Therefore, the temporal variation in nutrient availability is likely more important than a system’s
trophic state.

In general, factors leading to the formation of harmful algal blooms (HABs) are many and
diverse (see Paerl 1988, Roelke and Buyukates 2001). In regards to P. parvum, bloom-initiating
processes may include production of chemicals toxic to grazers (Granéli and Johansson 2003,
Accepted, this issue), use of alternative energy and nutrient sources through mixotrophy (Nygaard
and Tobiesen 1993, Skovgaard and Hansen 2003), suppression of competitors through
allelopathy (Fistarol et al. 2003, 2005, Granéli and Johansson 2003, Roelke et al. 2007, Errera et
al. 2008, Grover et al. Accepted, this issue), and resistance to the allelopathic effects of other algae
(Suikkanen et al. 2004, Tillmann et al. 2007). These factors are not likely mutually exclusive.
Additional factors that seem to influence the growth of other HABs, which might also influence
P. parvum population dynamics, include the production of beneficial or deleterious chemicals by
various bacteria taxa (Kodama et al. 2006, Salomon and Imai 2006) and the pathogenic effects of

Interestingly, P. parvum occurs in some Texas lakes where blooms do not form. At
present, it is unclear why blooms do not develop in these lakes while occurring in neighboring
reservoirs. As previously mentioned, factors influencing bloom formation might involve various
aspects within the water environment ranging from zooplankton grazers to dissolved chemicals.
The overall objective of this research was to determine the relative importance of these factors, or
narrow down the number of potential influences on P. parvum bloom formation. To achieve this
goal, we conducted in-lake mesocosm experiments involving the mixing of waters from a lake frequented by *P. parvum* blooms with waters from a lake unaffected by blooms. The experimental treatments included manipulations of initial grazer and competitor population densities and community composition, along with manipulations of various ions and dissolved chemicals. Our experiments were conducted during the time of year when blooms start to develop in many Texas lakes.

**Materials and Methods**

L. Whitney is a reservoir on the Brazos River, Texas, USA, constructed in 1951. The lake receives drainage from an area of 42,107 km$^2$, has a capacity of 4.68 x 10$^8$ m$^3$, a surface area of 95 km$^2$, and a shoreline of 362 km (Bailes and Hudson 1982). River discharge in this developed watershed is flashy, with peak flows typically occurring in the late spring and early summer, lasting several days, which can result in hydraulic flushing peaks of ~0.25 d$^{-1}$. Discharge during the late summer, fall and winter months is low and sometimes undetectable. We chose an area further south in the lake for our study site (32°52.50, 97°23.10) where historically high *P. parvum* population densities have been observed during the late-fall through early-spring months, typically November through March (Glass, personal communication). L. Waco, a nearby reservoir from which waters were transported for these experiments, is located within the Bosque watershed. Relative to L. Whitney, L. Waco is smaller in area and capacity (78.7 km$^2$, 1.88 x 10$^8$ m$^3$) and drains less land area (4,325 km$^2$), but it is similar in geomorphology and hydrology, where river discharge in the late spring and early summer can result in hydraulic flushing peaks of ~0.33 d$^{-1}$ (Dowell 1972, Lind and Barcena 2003).
We performed three in-lake experiments during a 5-week period in fall 2006, each lasting 7 days. Previous experiments conducted in a neighboring system observed significant phytoplankton and zooplankton responses to treatments within a 7-day period (Roelke et al. 2007, Errera et al. 2008). In experiments employing smaller volume containers, however, experimental artifacts were observed beyond the 7-day period (Errera et al. 2008). Consequently, we selected a 7-day duration for our experiments. Over short periods, the emerging plankton composition resulting from competition and other foodweb interactions can be sensitive to the initial community composition (Roelke et al. 2003, Roelke and Eldridge In Press). Since the community composition shifts during the time of year when blooms are forming in Texas lakes, possibly influencing the outcome of our treatments, we repeated three consecutive experiments, initiated October 3rd, 17th, and 31st.

In this study, we utilized 36 transparent 25-L polycarbonate carboys during each experiment, filled to a final volume of 24.5 L. Air in the headspace of each carboy allowed neutral buoyancy. The carboys were suspended in the near-surface water from anchored floatation platforms that allowed free movement with wave turbulence, keeping the carboys well mixed. To simulate the natural light environment, the carboys were covered with a neutral density screen, reducing surface light by ~55%. Lake secchi depths are typically ~1 m at this time of year, which translates to a light extinction coefficient of ~1.7 m$^{-1}$ (see Wetzel 2001). A 55% reduction in surface light would then occur at ~0.5 m, the depth from which water was collected for experiment initiation. Measurements of pH taken at initiation and termination of the 7-day experiments suggested that CO$_2$ did not limit primary productivity in the sealed carboys.
Lake water used to initiate these experiments was collected from locations within L. Whitney and L. Waco away from the shoreline, where *P. parvum* occurred at the time. Profiles of temperature and salinity suggested that surface waters from both reservoirs were well mixed at the time of collection. Previous studies have identified that pH levels can influence the potency of prymnesins, toxins produced by *P. parvum* (Ulitzur and Shilo 1966, Shilo 1981). However, pH values between the lakes differed by only 0.25, with a mean value of 8.21. This slight variation did not cause differential potency effects between the lake waters.

Each experiment comprised 12 treatments that were conducted in triplicate. Five of the treatments represented a gradient of combined, unfiltered waters from L. Whitney and L. Waco at proportions of 100:0, 75:25, 50:50, 25:75 and 0:100 (Whitney:Waco). In these treatments, our goal was to assess the combined effect of grazers, competitors, pathogens and dissolved chemicals from L. Waco on population dynamics of *P. parvum*.

Another of our goals was to differentiate between the combined effects of grazers and competitors from L. Waco, from the combined effects of virus pathogens and dissolved chemicals from L. Waco on the population dynamics of *P. parvum*. To accomplish this, two additional treatments were performed. The first included a 50:50 mixed proportion of unfiltered L. Whitney water and 0.45 µm filtered (membrane filter) L. Waco water; the second, a 50:50 mixed proportion of unfiltered and 0.45 µm filtered L. Whitney waters. We assumed the filtered waters primarily contained dissolved constituents, viral particles, and bacteria <0.45 µm in cell size. Both treatments had the same initial population densities of phytoplankton and zooplankton, at 50% of natural abundances in L. Whitney, allowing us to account for potential effects of varied initial conditions on emergent community composition (Roelke et al. 2003, Roelke and Eldridge *In Press*).
Additional goals of our research were to investigate the roles of nutrients and salinity on *P. parvum* population dynamics when L. Waco and L. Whitney waters were mixed. To achieve this, a final five treatments were added. The first three involved mixed proportions of 100:0, 50:50 and 0:100 of unfiltered waters from L. Whitney and L. Waco with additions of inorganic nutrients, trace metals and vitamins to f/2 concentrations (Guillard and Ryther 1962). In regards to N and P, enrichment of N was to 800 µM-N and enrichment of P was to 40 µM-P (N:P ratio of 20). By enriching these treatments, the chance of growth limitation arising from the scarcity of inorganic nutrients, trace metals and vitamins was negated. The remaining two treatments included a 50:50 mixed proportion of waters with salts added, and a 50:50 mixed proportion of water from L. Whitney and 0.45 µm filtered water from L. Waco with salts added. Salinity levels were adjusted to match those found in L. Whitney. During our in-lake experiments, L. Waco salinity was ~0.14 psu, and L. Whitney salinity averaged 1.73 psu. A defined medium of artificial seawater (Kester 1967) was added to each carboy in order to reach the higher salinity characteristic of L. Whitney. Based on our observations while culturing the Texas strain of *P. parvum*, where inoculations are periodically performed into media with salinities varying by as much as 3 psu at low salinity ranges, it is not likely that an osmotic-shock due to a sudden shift of ~0.7 psu (from 1.73 to ~1 psu) influenced the outcome of our 7-day experiments.

Response variables measured in our experiments included characterizations of plankton, inorganic nutrients, and ambient toxicity (where toxicity refers to the net affect of all chemicals). Characterizations of plankton included estimates of total phytoplankton biomass and biomasses of higher taxonomic groups, enumerations of *P. parvum* population densities, and total zooplankton biomass and biomasses of higher taxonomic groups. Characterizations of the nutrients included measurements of nitrate (NO₃), nitrite (NO₂), ammonium (NH₄), and
orthophosphate (PO$_4$). Ambient toxicity was estimated using standardized fish and cladoceran bioassays previously described (Roelke et al. 2007). Initial conditions were characterized by measurements taken just prior to the initiation of each experiment from both source waters. Response variables were then sampled from each carboy at the end of each 7-day experiment. Estimates of total phytoplankton biomass and biomasses of higher taxa were determined from phytopigment concentration measurements (Pinckney et al. 1998) and the use of CHEMTAX, a matrix factorization program that enables estimates of taxonomically aggregated assemblage composition (Mackey et al. 1997, Wright et al. 1996). For the CHEMTAX model initiation, cyanobacteria, euglenophytes, chlorophytes, prymnesiophytes, cryptophytes, diatoms and chrysophytes were selected because of their historical prevalence in L. Whitney and L. Waco. For greater detail of the HPLC and CHEMTAX methods followed, see Roelke et al. (2007).

Water column chlorophyll $a$ concentrations were also determined using standard fluorometric procedures. Triplicate 50 mL samples were filtered through 47mm GF/F filters per carboy and frozen until analysis. Pigments were extracted with 90% acetone, centrifuged, and analyzed using a fluorometer (APHA 1998).

From each carboy a 100 mL phytoplankton sample was collected and preserved using glutaraldehyde, 5% v/v. Enumeration of P. parvum population density was performed using a settling technique (Utermöhl 1958). A subsample of 0.5 to 1.5 mL was settled for 24 h, then 30-40 randomly selected fields of view were counted using an inverted, phase contrast light microscope (400x, Leica Microsystems). Total cells counted per sample averaged between 50-150.
Detailed microscopy was performed on L. Whitney and L. Waco waters used to initiate the experiments, following the same methods described above. The focus of this effort was to determine the proportion of the total phytoplankton biovolume that was represented by specific cyanobacteria taxa, i.e., genera-level characterizations that are not possible using the CHEMTAX model.

Zooplankton samples were collected following two methods. For initial in-lake conditions a Schindler trap (61 µm mesh size) was used to concentrate a 12 L sample to 50 ml. To sample carboys at the termination of each experiment, 10 L were removed and filtered through the cod end portion of a Schindler trap and concentrated to 50 ml. All zooplankton samples were preserved in buffered formalin, 5% v/v. A subsample of 5 to 15 mL was settled for 24 hours, then counted using an inverted, phase contrast light microscope (40x and 200x, Leica Microsystems). For each individual counted, dimensions were measured corresponding to best-fit geometric shapes to estimate biovolume (Wetzel and Likens 1991). For this study zooplankton species were grouped into total copepod adults, copepod nauplii, total rotifers, total cladocerans and total protozoans (ciliate and amoeboid). Our enumeration technique resulted in ~100-150 total individuals counted per sample.

Samples for inorganic nutrients were filtered through GF/F filters, and the filtrates were frozen until analysis. Inorganic nutrient concentrations were determined using autoanalyzer methodology (Armstrong and Sterns 1967, Harwood and Kuhn 1970). For this study, NO$_3$, NO$_2$ and NH$_4$ were summed (DIN).

Ambient acute toxicity to fish was evaluated for initial conditions and from each experimental carboy using standardized 24-hour static toxicity assays with the juvenile fathead minnow (*Pimephales promelas*) model. Sublethal toxicity to a model cladoceran was evaluated...
with standardized 10-day static renewal chronic toxicity tests with the *Daphnia magna* model. Toxicity assays followed standardized methods for aquatic toxicology (US EPA 1994, 2002). Samples were collected and transported to the laboratory where toxicity tests were initiated within 24 hours. 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). For greater detail of the methods followed for these toxicity assays, refer to Brooks et al. (2004) and Dzialowski et al. (2006).

Using SPSS 14, comparisons of the response variables ((day 7 – day 0)/day 0) between experimental treatments involving the five mixed proportions of unfiltered waters from L. Whitney and L. Waco were tested using one-way ANOVA, followed by Bonferroni and Tukey *post hoc* tests. Independent samples t-tests were performed for all other treatment comparisons. These comparisons focused on the differences of primary interest in this study: effects of the source and proportion of waters mixed in the experimental carboys.

**Results**

*In-situ conditions for L. Whitney and L. Waco*

From the three sampling dates in L. Whitney (October 3rd, 17th and 31st), *P. parvum* cell densities were 0.72 x 10^6, 0.50 x 10^6, and 0.83 x 10^6 cells L⁻¹, well below bloom proportions in Texas waters (~10 x 10^6 cells L⁻¹), and toxicity bioassays confirmed non-toxic in-lake conditions. The state fisheries managers indicated no observations of golden-colored waters and no reports of fish kills at this time (Glass, personal communication). Foam lines were present, however, which typically correspond with the incidence of *P. parvum*. Approximately one month after our in-lake experiments golden-colored waters and fish kills were observed. Therefore, we conclude that
these experiments took place during the period of bloom initiation.

Cyanobacteria dominated the phytoplankton assemblage (50% of the biovolume, almost all Phormidium sp.; Table 1) during the October 3rd sampling of L. Whitney. During the later two October samplings, phytoplankton composition was more diverse, with co-dominance of chlorophytes, chrysophytes, cyanobacteria and prymnesiophytes, and sub-dominance of euglenophytes and cryptophytes. Diatoms were below detection based on CHEMTAX analysis of pigments, and cyanobacteria known to be harmful were not observed in microscopy (Table 1).

Total phytoplankton biomass fluctuated during the three lake samplings, with concentrations of 17.9, 25.9 and 19.8 µg-chl a L⁻¹.

Total zooplankton biovolume was similar for the first two samplings in L. Whitney, but then increased ~10-fold over the study period. On October 3rd and 17th biovolumes were 3.0 x 10⁷ and 1.3 x 10⁷ µm³ L⁻¹, but on October 31st total biovolume was 13.8 x 10⁷ µm³ L⁻¹. Rotifers accounted for 86% of the total biovolume on October 3rd (101 individuals L⁻¹), but by October 17th rotifers declined to 37% (16 individuals L⁻¹) and copepod nauplii comprised 63% (10 individuals L⁻¹) of the total biovolume. By October 31st rotifers became subdominant (15%, 70 individuals L⁻¹), and copepod nauplii (44%, 103 individuals L⁻¹) and copepod adults (34%, 7 individuals L⁻¹) were co-dominant. Cladocerans, and ciliated and amoeboid protozoa were not observed.

L. Waco was sampled on the same dates as L. Whitney, and P. parvum cell densities were very similar at 0.31 x 10⁶, 0.37 x 10⁶, and 0.62 x 10⁶ cells L⁻¹. Consistent with this lake’s history, there were no signs of P. parvum blooms during the period of our experiments. To date, there have still been no observed P. parvum blooms in L. Waco.
During October, total phytoplankton biomass in L. Waco was similar to levels found in L. Whitney: 17.4, 19.6 and 21.5 µg-chl a L⁻¹ for the three sampling dates in L. Waco. However, cyanobacteria were much more prevalent compared to L. Whitney, accounting for 77%, 69% and 51% of the total phytoplankton biovolume for the three sampling dates. In addition, potentially toxigenic species were observed in our microscopy, where *Microcystis* sp. and *Anabaena* sp. were dominant (Table 1).

Zooplankton varied in L. Waco during October, with total biovolumes of 10.7 x 10⁷, 1.3 x 10⁷ and 10.0 x 10⁷ µm³ L⁻¹ for the three sampling dates. Similar to L. Whitney, initial rotifer dominance gave way to co-dominance with copepod nauplii. On October 3rd rotifers accounted for 98% (114 individuals L⁻¹) of the total zooplankton biovolume, but on October 17th and 31st, rotifers accounted for 41% and 53% of the total biovolume (25 and 93 individuals L⁻¹). Copepod nauplii shared dominance at 57% and 37% of the total biovolume (11 and 46 individuals L⁻¹) during the latter two sampling dates. Similar to L. Whitney, cladoceran and ciliated and amoeboid protozoa population densities were very low in L. Waco during this time.

Inorganic nutrient concentrations were similar between the two lakes. In L. Whitney, average DIN and PO₄ concentrations (from the three lake samples collected at the start of each experiment) were 3.8 µM-N and 0.10 µM-P, and in L. Waco they were 2.78 µM-N and 0.22 µM-P. The ratio of N:P varied, however; L. Whitney N:P was ~38, while the L. Waco N:P was ~13.

**Experimental results**

The accumulation of *P. parvum* populations in the experimental carboys varied according to the proportions of waters mixed from L. Whitney and L. Waco. In all three experiments, *P.*
*parvum* increased 3- to 7-fold in carboys with only unfiltered L. Whitney water, while appreciable increases were not observed in the carboys with only unfiltered L. Waco water (Fig. 1). Furthermore, a dose-response was observed in all three experiments where the accumulation of *P. parvum* populations diminished with an increasing proportion of unfiltered L. Waco water. Distinct groupings along this mixing gradient were statistically significant (*p* < 0.05). Low accumulation of *P. parvum* was also observed in the carboys with filtered waters from L. Waco in all three experiments (Fig. 1). Statistically significant reductions in fish survival and cladoceran reproduction responses were never observed, including in the carboys with greater proportions of L. Whitney water where *P. parvum* population densities reached near-bloom proportions.

For phytoplankton biomass, mixing of unfiltered waters from L. Whitney and L. Waco did not produce a consistent trend as seen with *P. parvum* (Fig. 2). Reductions in chlorophyll *a* from initial conditions occurred, and the decrease was greater in carboys containing larger proportions of L. Whitney water in the first two experiments, but the opposite was observed for the third experiment.

The phytoplankton response in the carboys containing filtered L. Waco waters was consistent with the trends observed for *P. parvum*, where in all three experiments performance of the total phytoplankton assemblage was poorer in carboys with L. Waco waters, with significant differences (*p* < 0.05) for the first and third experiments (Fig. 2). Not all taxa were affected similarly. For example, cyanobacteria, euglenophytes and cryptophytes showed significantly (*p* < 0.05) poorer performance with the addition of filtered water from L. Waco. A poorer performance of chlorophytes was also observed, but only significant (*p* < 0.05) in the third experiment. Chrysophytes showed no significant difference between these treatments (Table 2).
Diatoms remained below the detection threshold for pigment analysis with the CHEMTAX model. Zooplankton biovolume increased from initial conditions in all carboys. In the first two experiments the growth of zooplankton biovolume was significantly greater in carboys with a larger proportion of L. Waco waters \((p < 0.05, \text{Fig. 3})\). Growth of copepod nauplii into adult forms was pronounced in the second and third experiments. The accumulation of zooplankton biovolume in the treatments involving filtered waters from L. Waco was not significantly different from the treatments involving filtered waters from L. Whitney \((p > 0.05, \text{Fig. 3})\). The addition of nutrients showed varying results. Enrichment did not change the deleterious effect of filtered L. Waco waters on \(P. \text{parvum}\) populations \((p < 0.05, \text{Fig. 4})\). On the other hand, nutrient additions changed the phytoplankton response to addition of filtered L. Waco waters, but the effect was not consistent between experiments. During the first and third experiments, there was no significant difference in phytoplankton growth between enriched carboys with filtered waters from L. Whitney and L. Waco. During the second experiment, however, phytoplankton growth was significantly greater in the enriched carboys with filtered L. Whitney water. Shifts in assemblage composition between the higher taxonomic groups were minor, except in carboys with 50% filtered L. Whitney waters where euglenophytes dropped below the detection level of pigment analysis based on the CHEMTAX model. Accumulation of zooplankton biovolume was much greater with nutrient additions, primarily due to the population growth of rotifers. Differences between filtered water from L. Whitney and from L. Waco, however, were not observed in any of the three experiments. Salt additions to mixtures of L. Whitney and L. Waco waters, bringing salinity levels up
to those observed in L. Whitney (from ~1 to 1.7 psu), did not alter results in any experiment. That is, no significant differences ($p < 0.05$) were found between L. Whitney and L. Waco waters (50:50) without salt added compared to salt additions for $P. parvum$, chlorophyll $a$, or zooplankton biovolume (Fig. 5). Similarly, no significant differences ($p < 0.05$) were found between L. Whitney and filtered L. Waco waters (50:50) without salt added compared to salt additions for these same parameters (Fig. 6). During the first experiment, notable increases in rotifers were observed with the salt additions, but they were highly variable between replicates.

L. Whitney and L. Waco are similar in their trophic state, and this resulted in comparable nutrient concentrations among various treatments. Excluding the carboys with enrichment, nutrients ranged from 0.9 to 1.1 $\mu$M-DIN and 0.13 to 0.15 $\mu$M-PO$_4$ at the termination of all three experiments.

**Discussion**

Waters from L. Waco, when mixed with waters from L. Whitney, had a deleterious impact on $P. parvum$ in these experiments. Zooplankton prospered in all treatments, with rapid growth of parthenogenetic rotifers and the development of copepod nauplii into adults. Undoubtedly, grazing pressure increased during the 7 days of each experiment. It was not the zooplankton from L. Waco, however, that led to the poorer performance of $P. parvum$ because both unfiltered and filtered waters from L. Waco produced the same deleterious response. In addition, accumulation of zooplankton biomass differed significantly between mixing treatments only in the first experiment, where copepod adults increased in biomass. Remaining potential causes for poor performance of $P. parvum$ in L. Waco waters then, were some small pathogen passing
through a 0.45 μm filter, possibly a virus, or dissolved chemicals, such as nutrients, salts, contaminants, allelochemicals or algicides.

The role of pathogens in phytoplankton population dynamics is becoming clearer with advances in technologies enhancing study in this area. Viruses directly influence other harmful algal species (Brussaard 2004, Salomon and Imai 2006), and there is evidence that viruses influence *P. parvum* population dynamics during later stages of blooms (Schwierzke et al. *In Review, this issue*). However, pathogens tend to be species-specific and do not affect the entire phytoplankton assemblage (Tomaru et al. 2004, Salomon and Imai 2006). In all three of our experiments, additions of filtered L. Waco waters resulted in poorer performance of cyanobacteria, euglenophytes and cryptophytes, which led to an overall poorer performance of the entire L. Whitney phytoplankton assemblage (based on chlorophyll *a*). In addition, signs of pathogens, such as lysed cells, were not observed during microscopy. These lines of evidence indicate that the causative factor in L. Waco waters reducing performance of *P. parvum* was not likely a pathogen.

A more generalized factor that could influence *P. parvum* and the phytoplankton assemblage would be nutrients. However, based on half-saturation coefficients for reproductive growth of many phytoplankton species common to lakes (Grover 1989, Grover et al. 1999), nutrient concentrations would not have been a growth-limiting factor for many species, especially at the start of these experiments. For a Texas strain of *P. parvum*, half-saturation constants for N- and P-limited reproductive growth were estimated to be 0.02 μM or lower (Baker 2007, Baker et al. *Accepted*), indicating that reproductive growth rates would be near maximum during these experiments. More conclusively, the same deleterious effect of filtered L. Waco water on *P.
parvum was observed in carboys with nutrient enrichment. Further, ammonium levels did not
approach those toxic to P. parvum or other algae in this study and did not vary appreciably
among treatments. For these reasons, it is not likely that differences in nutrient concentrations
between L. Whitney and L. Waco led to the poorer performance of P. parvum.

For bulk phytoplankton, nutrient limitation would be expected to begin when
concentrations drop below 1.0 µM-N and 0.1 µM-P (Reynolds 2006). Interestingly, in the first
and third experiments nutrient additions masked the deleterious effect of L. Waco waters when
considering the whole phytoplankton community (based on chlorophyll a). While major shifts in
the aggregated taxonomic groups were not observed, it may be that species shifts within each of
the aggregated groups occurred. For example, species resistant to the deleterious effect of L.
Waco waters, which could have higher nutrient requirements, might have prospered with the
addition of nutrients while sensitive species declined.

Another generalized factor that could influence P. parvum and the phytoplankton
assemblage is salinity. For P. parvum, relationships between growth rate and salinity are
unimodal or increasing, depending on the strain (Larsen and Bryant 1998). A strain of P. parvum
from Texas showed a unimodal relationship with an optimum at 22 psu (Baker et al. 2007). The
salinity at the time of our experiments was much lower than that, differing by ~1.3 psu between
the two reservoirs, and a 50:50 mix of these waters yielded a salinity of ~1 psu. While this
salinity is low, it is sufficient to support growth of P. parvum when other conditions are
favorable (Baker 2007, Baker et al., Accepted).

Applying the relationship between growth and salinity described by Baker (2007), the
specific growth rates of P. parvum would have been ~0.3 and ~0.15 d⁻¹ under conditions of ~1.7
and ~1 PSU. In the treatments where filtered L. Waco waters were mixed with L. Whitney waters and salts were not added (i.e., salinity was not maintained at 1.73), the difference in salinity could have contributed to the differences observed in *P. parvum* population densities. However, when salinity was maintained with the addition of filtered L. Waco waters, the same deleterious effect of filtered L. Waco waters on *P. parvum* was observed, as it was for total phytoplankton. Therefore, the difference in salt content between the two lakes did not likely cause the negative impact of L. Waco waters on *P. parvum* or the phytoplankton assemblage. Our conclusion is not that salinity has no influence on the incidence of *P. parvum* blooms, as observations suggest otherwise (Krasnotshchek and Abramowitsch 1971, Holdway et al. 1978, Rijn and Shilo 1989, Kaartvedt et al. 1991, Guo et al. 1996, Amsinck et al. 2005). Instead, this indicates that another factor, aside from salinity, played a more significant role in these experiments.

Industrial and agricultural contaminants would also act as generalized factors potentially influencing the entire phytoplankton assemblage. The recent history of L. Waco shows a wide range of contaminants being present, but of the many parameters routinely measured by the Texas Commission on Environmental Quality, only NO$_3$ was of concern. Parameters not of concern included a suite of heavy metals, polychlorinated biphenyls, organic contaminants (including herbicides), fecal coliform bacteria, suspended sediments, NH$_4$ and PO$_4$. Overall, the lake was found to be fully supporting of aquatic life, including phytoplankton (TCEQ 2004). While total phytoplankton is not threatened by present contaminants, individual species might be. Species-specific affects of contaminants have not been studied for L. Waco or L. Whitney, and are well beyond the scope of this research. However, a negative effect on *P. parvum* and major components of the phytoplankton assemblage (cyanobacteria, euglenophytes and
crypto phytes) from contaminants in L. Waco during these experiments is unlikely. On-going monitoring in L. Waco shows accumulation of phytoplankton biomass with diverse taxa and a sustained, but generally small, *P. parvum* population (Roelke, unpublished). If contaminants had an affect similar to the responses measured in our experiments, cyanobacteria, euglenophytes, cryptophytes and *P. parvum* populations would occur at much lower densities then currently observed in L. Waco.

Allelopathy is another generalized factor potentially affecting *P. parvum* and other phytoplankton. *P. parvum* is capable of producing chemicals that suppress the growth of competing phytoplankton (Fistarol et al. 2003, 2005, Granéli and Johansson 2003, Roelke et al. 2007, Errera et al. 2008), as can other harmful species, including *Microcystis* sp. and *Anabaena* sp. (Pflugmacher 2002, Legrand et al. 2003). During our experiments, both of these latter taxa were abundant in waters collected from L. Waco (possibly due to the relatively low N:P) and were absent in waters collected from L. Whitney. Furthermore, in 2006, microcystin-LR was detected by ELISA at levels between 590 and 1090 ng L\(^{-1}\) in L. Waco (Brooks, unpublished), although production of this toxin is not necessary to cause an allelopathic affect (see Sukenik et al. 2002, Beresovsky et al. 2006). Dissolved allelochemicals would have passed through filtration and been present in treatments receiving filtered L. Waco water. Thus, allelopathy could explain why performances of major taxonomic groups in L. Whitney declined with the addition of L. Waco water.

How might *P. parvum* respond to allelochemicals from cyanobacteria? Previous research using a European strain of *P. parvum* indicated a resistance to allelochemicals in studies performed at 6.8 psu (Suikkanen et al. 2004). However, the Texas and European strains have
different photopigments (Errera 2006) and may also vary in vulnerabilities to allelochemicals. The possibility of compounding chemical interactions exists as well. The higher ambient pH of Texas lakes (Roelke, unpublished), relative to the Baltic Sea, may influence the lipophilicity, bioavailability and toxicity of allelochemicals. So, it may be that *P. parvum* populations in L. Whitney are sensitive to cyanobacterial allelochemicals. In addition, a modeling study of L. Granbury, another Texas lake impacted by *P. parvum* blooms, has demonstrated that model behavior better matched in-lake observations of *P. parvum* blooms when allelochemicals from cyanobacteria were considered (Grover et al., *Accepted, this issue*). In addition to cyanobacteria, allelopathy is observed in many other phytoplankton taxa (Granéli and Hansen 2006), therefore allelochemicals from other phytoplankton species in L. Waco cannot be ruled out.

Algicidal chemicals are produced by diverse bacteria and may be another generalized factor potentially affecting *P. parvum* and other phytoplankton (Kodama et al. 2006, Salomon and Imai 2006). The impacts of algicides are also varied. Some broadly affect many phytoplankton taxa, while others are species-specific; in some cases phytoplankton growth is slowed, and in others target populations are obliterated (Doucette et al. 1998, Imai et al. 1998, Yoshinaga et al. 1998, Mayali and Azam 2004). Elucidating the possible role algicidal bacteria played in these experiments was well beyond the scope of this study, but future research should address this possible bloom-suppressing mechanism.

Allelochemicals produced by cyanobacteria, some other phytoplankton taxa, or algicidal chemicals produced by bacteria may be, in part, contributing factors leading to the suppression of *P. parvum* blooms in L. Waco, and perhaps in some other regional lakes as well. For example, in L. Somerville, *P. parvum* is present but does not form blooms. Similar to L. Waco, this lake is
frequently dominated by cyanobacteria (Roelke et al. 2004), and microcystins were detected. In addition, nutrients, dissolved organic carbon, and bacteria concentrations are high. The potential roles of allelochemical-producing phytoplankton and algicidal bacteria as ecosystem-engineering species or in suppressing *P. parvum* blooms in Texas lakes merits further investigation.

As stated previously, the environmental conditions leading to harmful algal blooms are complex and often species-specific, making it difficult to envision a universal approach to management (Roelke 2000, Roelke and Buyukates 2001, 2002). Our findings, along with those of Grover et al. (*Accepted, this issue*), suggest that allelopathic phytoplankton or algicidal bacteria might influence *P. parvum* bloom development. Should follow-on studies confirm this notion, then our findings would have important management implications for *P. parvum* in Texas lakes. For example, a management strategy could focus on the understanding of conditions where *P. parvum* blooms do not occur, e.g., in the presence of some allelopathic phytoplankton or algicidal bacteria. Manipulating an entire lake to create conditions conducive to a specific microbe may not be feasible, or even wise. However, manipulation of more restricted areas of a lake, timed during the season of bloom initiation, would likely be less detrimental to the overall health of a lake. Targeting specific coves of the dendritic lakes common in this region would be ideal. Ongoing research is investigating the role of coves as bloom initiation “hot spots”, possibly due to their longer hydrologic residence time. If true, then a focused, timely effort to promote growth of specific allelopathic phytoplankton or algicidal bacteria, if these are confirmed to suppress *P. parvum*, in these coves might help to circumvent *P. parvum* blooms. Even if blooms initiate elsewhere, manipulation in coves may still be advantageous to ward off *P. parvum* blooms, thereby creating a refuge for fish.
Acknowledgements

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Figure Captions

Fig. 1. Population density of *P. parvum* at the start (filled squares) and termination (bars) after 7 days of mixing experiments initiated on October 3rd (A), 17th (B) and 31st (C). Waters represented a gradient of mixing proportions between unfiltered waters from L. Whitney and L. Waco; 50% unfiltered L. Whitney waters added to 50% filtered L. Whitney waters; and 50% unfiltered L. Whitney waters added to 50% filtered L. Waco waters. Letter designations represent distinct groupings (*p* < 0.05).

Fig. 2. Phytoplankton biomass, as estimated by chlorophyll *a* concentration, at the start (filled squares) and termination (bars) after 7 days of mixing experiments initiated on October 3rd (A), 17th (B) and 31st (C). Waters represented a gradient of mixing proportions between unfiltered waters from L. Whitney and L. Waco; 50% unfiltered L. Whitney waters added to 50% filtered L. Whitney waters; and 50% unfiltered L. Whitney waters added to 50% filtered L. Waco waters. Letter designations represent distinct groupings (*p* < 0.05).

Fig. 3. Average zooplankton biovolume and community composition at the start (filled squares) and termination (stacked bars) after 7 days of mixing experiments initiated on October 3rd (A), 17th (B) and 31st (C). Waters represented a gradient of mixing proportions between unfiltered waters from L. Whitney and L. Waco; 50% unfiltered L. Whitney waters added to 50% filtered L. Whitney waters; and 50% unfiltered L. Whitney waters added to 50% filtered L. Waco waters. Letter designations represent distinct groupings (*p* < 0.05). No letter designations signify no significant differences were detected.
Fig. 4. Impact of L. Waco waters with the addition of nutrients to population density of *P. parvum*, phytoplankton biomass, and average zooplankton biovolume and composition at the start (filled squares) and termination (bars) after 7 days for experiments initiated on October 3rd (A), 17th (B) and 31st (C), where the treatments were 50% unfiltered L. Whitney waters added to 50% filtered L. Whitney waters; and 50% unfiltered L. Whitney waters added to 50% filtered L. Waco waters. In all experiments, L. Waco waters had a deleterious effect on *P. parvum* population growth (*p* < 0.5). Phytoplankton response was varied, however, where opposite trends in the first two experiments (A, B) were significant (*p* < 0.05), and not significant in the third experiment (C). Filtered L. Waco waters had no detectable impact on zooplankton.

Fig. 5. Impact of L. Waco waters with the addition of salts to match L. Whitney salinities to population density of *P. parvum*, phytoplankton biomass, and average zooplankton biovolume and composition at the start (filled squares) and termination (bars) after 7 days for experiments initiated on October 3rd (A), 17th (B) and 31st (C), where the treatments were 50% unfiltered L. Waco waters added to 50% L. Whitney waters; and 50% unfiltered L. Waco waters added to 50% L. Whitney waters with salt added. In all experiments, no significant difference (*p* < 0.5) was detected for *P. parvum* population growth, or accumulation of phytoplankton and zooplankton biomass.

Fig. 6. Impact of filtered L. Waco waters with the addition of salts to match L. Whitney salinities to population density of *P. parvum*, phytoplankton biomass, and average zooplankton biovolume and composition at the start (filled squares) and termination (bars) after 7 days for
experiments initiated on October 3rd (A), 17th (B) and 31st (C), where the treatments were 50% filtered L. Waco waters added to 50% L. Whitney waters; and 50% filtered L. Waco waters added to 50% L. Whitney waters with salt added. In all experiments, no significant difference ($p < 0.5$) was detected for *P. parvum* population growth, or accumulation of phytoplankton and zooplankton biomass.
Fig. 1
Fig. 2
Fig. 3
Fig. 4
Fig. 5
Fig. 6
Table 1. Contributions of cyanobacteria and Prymnesiophytes as percentages of total phytoplankton biovolume at sampling locations in Lakes Whitney and Waco at the start of each experiment.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>L. Whitney</th>
<th>L. Waco</th>
<th>L. Whitney</th>
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<td></td>
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<td>Experiment 2 initiated 10-17-06</td>
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<td>Experiment 3 initiated 10-31-06</td>
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<tr>
<td>Cyanobacteria</td>
<td>49.5</td>
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<td>68.7</td>
<td>28.2</td>
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<td>18.6</td>
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<td>-</td>
<td>-</td>
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<td><em>Calothrix</em> sp.</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.7</td>
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<td><em>Lyngbya</em> sp.</td>
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<td>1.2</td>
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<td>57.3</td>
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Table 2. Average percent change from the initial to the final taxonomic densities (n=3) as measured using CHEMTAX. In the two columns for each experiment results are shown for the treatments where either 50% filtered water from Lake Whitney or 50% filtered water from Lake Waco was added to unfiltered Lake Whitney water.

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<th>Taxa</th>
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<th></th>
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</tr>
<tr>
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<td>+ filtered L.Waco</td>
<td></td>
<td>+ filtered L.Whitney</td>
<td>+ filtered L.Waco</td>
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<td>+ filtered L.Whitney</td>
<td>+ filtered L.Waco</td>
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<td></td>
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<td>100</td>
<td>-38.2*</td>
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* significant difference ($p < 0.05$) between treatments.
Prymnesium parvum population dynamics during bloom development: a role assessment of grazers and virus

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Running head:
P. parvum field experiments
ABSTRACT

The toxic haptophyte *Prymnesium parvum* is a harmful alga known to cause fish-killing blooms that occur worldwide. In Texas (USA), *P. parvum* blooms occur in inland brackish water bodies and have increased in frequency and magnitude in recent years. In this study we conducted three consecutive field experiments (Lake Whitney) to investigate the influence of zooplankton and viruses on *P. parvum* bloom dynamics during the time of year when blooms are still typically active in Texas (early spring). A localized *P. parvum* bloom developed during our study that involved increasing levels of toxicity (based on *Pimephales promelas* and *Daphnia magna* bioassays). Only in our last experiment, during later stages of bloom development and under highly toxic conditions, did the presence of grazers show a statistically significant, negative effect on *P. parvum* population dynamics. During this experiment, a rotifer-dominated zooplankton community emerged, composed mostly of *Notholca laurentiae*, suggesting that this species was less sensitive than other grazers to toxins produced by *P. parvum*. Microzooplankton may have also been important at this time. Similarly, only our final experiment demonstrated a statistically significant, negative effect of viruses on *P. parvum*. This exploratory study, resulting in observed impacts on *P. parvum* populations by both grazers and virus, enhances our understanding of *P. parvum* ecology and highlights direction for future studies on resistance of zooplankton to prymnesin toxins and algal-virus interactions.

KEY TERMS: aquatic ecology; lakes; harmful algal blooms; toxicology; grazing; pathogens; phytoplankton; zooplankton; allelopathy; prymnesins
INTRODUCTION

Harmful algal blooms (HABs) have received the continued interest and concern of scientists, fisheries and water resource managers, and public health officials worldwide over the past two decades (Granéli and Turner 2006). Globally, aquatic ecosystems are being disrupted by the increasing incidence and severity of HABs (Smayda 1990; Hallegraeff 1993), which cause a variety of deleterious effects on aquatic systems, ranging from oxygen deficiency to human health risks (Van Dolah 2001; Granéli and Turner 2006). The haptophyte *Prymnesium parvum* is widely distributed, temperature tolerant (Baker et al. 2007), and capable of forming blooms in both coastal and brackish inland water bodies under varying salinity (Edvardsen and Paasche 1998; Lundholm and Moestrup 2006).

*P. parvum* blooms have been reported from estuarine and inland waters of the United States, Australia, China, Russia, Europe, Israel, Morocco, and other locations (Guo et al. 1996; Edvardsen and Paasche 1998). In the 1980s, the first noted blooms in brackish inland waters in the southwestern US occurred at numerous locations along the Pecos River (James and De La Cruz 1989). Since then, the state of Texas has experienced an increasing number of *P. parvum* blooms resulting in economic losses and massive fish kills. As of 2006 an estimated 31 million fish have been affected, with majority of the impact occurring in recent years (TPWD 2007). Confirmed blooms, where cell densities typically exceed $10 \times 10^6$ cells $l^{-1}$, can occur anytime throughout the year, but most of the blooms in Texas span the months between fall and spring, generally October through April (Joan Glass, TPWD, personal communication). Over 19 reservoirs along five major river systems have been affected in Texas.

Blooms of *P. parvum* persist under complex environmental conditions, and recent studies have revealed how this species might gain a selective advantage over other phytoplankton,
thereby accumulating biomass. The mixotrophic feeding strategies of *P. parvum* allow it to be photosynthetic and to feed phagotrophically, ingesting microorganisms such as bacteria and other protists (Nygaard and Tobiesen 1993; Tillmann 1998; Skovgaard and Hansen 2003). By producing allelopathic chemicals, *P. parvum* can immobilize plankton and suppress competitors, thereby fueling bloom development and persistence (Fistarol et al. 2003, 2005; Granéli and Johansson 2003; Uronen et al. 2005).

The toxicity of *P. parvum* blooms is caused by toxins that when released into the water can negatively impact some micro- and mesozooplankton. Field experiments performed by Roelke et al. (2007) revealed grazer inhibition and dramatically reduced grazer populations, with direct negative effects on cladoceran fecundity during a *P. parvum* bloom. Sopanen et al. (2006) found that exposure to *P. parvum* toxins caused inactivity and sublethal effects on the copepods *Eurytemora affinis* and *Acartia bifilosa*. Similar results were discovered in a study where the marine rotifer *Brachionus plicatilis* was negatively affected after ingesting toxic *P. parvum* cells (Barreiro et al. 2005). Furthermore, Brooks et al. (this issue) observed negative effects by *P. parvum* toxins on the freshwater rotifer *Brachionus calyciflorus*.

Factors controlling the termination of *P. parvum* blooms have not been well documented. However, various possibilities are suggested by several studies of *P. parvum* and related harmful algal species. Barkoh et al. (2003) treated samples from a fish hatchery with ammonium, reducing *P. parvum* populations to below detection with hemacytometer counts (<10^5 cells ml⁻¹) within 48 hours. More recently, ammonium doses added to laboratory batch cultures reduced toxicity and abundance of *P. parvum* (Grover et al. 2007). Similarly, field experiments indicated that nutrient additions reduced, and sometimes eliminated, *P. parvum*’s toxicity and competitive edge over other phytoplankton (Roelke et al. 2007; Errera et al. 2008). Mesocosm experiments
performed by Brussaard et al. (2005) tested the regulatory role of viruses on the prymnesiophyte
Phaeocystis globosa finding that viral infection restricted bloom formation and eventually led to
bloom termination.

This study reports findings from field experiments conducted in Lake Whitney (Texas, USA), one of the systems recently affected by P. parvum blooms. Our goal was to investigate the roles of grazers and viruses during the time of year when blooms are generally still active. This experimental approach was novel because natural plankton communities were manipulated under in-lake conditions, and the potential influence of both zooplankton and viruses on P. parvum dynamics was concurrently investigated. Response variables included phytoplankton biomass and assemblage composition, as well as zooplankton biovolume and community structure.

MATERIALS AND METHODS

Site Description

Lake Whitney is a reservoir on the Brazos River, Texas, USA, constructed in 1951. The lake receives drainage from an area of 42,107 km², has a capacity of 4.68 x 10⁸ m³, a surface area of 95 km², and a shoreline of 362 km (Bailes and Hudson 1982). We chose an area near the south shoreline of Walling Bend Island for our in-lake experiments (Figure 1). This location was selected because of the historically high P. parvum population densities typically observed there during the late-fall through early spring months (Joan Glass, TPWD, personal communication).

We performed three in-lake experiments during a 6-week period in spring 2007, each lasting 7 days. Significant phytoplankton and zooplankton responses were observed within a 7-d period during previous in-lake experiments from a nearby system (Roelke et al. 2007, Errera et al.)
2008). Although, experimental artifacts were observed beyond a 7-d period in experiments employing smaller volume containers, therefore we selected a 7-d duration (Errera et al. 2008).

Experiments were initiated February 22, and March 8 and 29. We used 24 transparent 25-l polycarbonate carboys during each study, each being filled to a final volume of 24.5 l with a small amount of air in the headspace for neutral buoyancy. Lake water was collected near our study site, away from the shoreline at a depth of 0.5 m, a location selected for the occurrence of *P. parvum* populations at the time of experiments (Joan Glass, TPWD, personal communication); surface waters were well-mixed at the time of experiments. The carboys were suspended in the near-surface water by tethering them to anchored floatation platforms that allowed free movement with wave turbulence, keeping the contents well-mixed. To simulate the natural light environment, carboys were covered with a neutral density screen, reducing surface light by ~55%. At this time of year, lake Secchi depths average ~1 m, giving a light extinction coefficient of ~1.7 m$^{-1}$ (Wetzel 2001). Therefore, a 55% reduction in surface light would occur at ~0.5 m, the collection depth for experimental initiation lake waters.

**Experimental Treatments**

Each experiment comprised three main treatments, with each using four to six carboys. Treatments included: (1) the natural plankton assemblage containing ambient populations of *P. parvum* (unmanipulated lake water), (2) the natural plankton assemblage with reduction of large grazers by filtering whole lake water through a 20-µm mesh, and (3) reduction of pathogens by passing lake water through a tangential flow filtration system to remove viral-sized particles, with original phytoplankton and zooplankton assemblages restored. Our experimental design included a fourth treatment to investigate an interaction effect of reducing both large grazers and
pathogens, but due to methodological issues, this data was compromised and is not included in
the analysis (see statistics description).

Creating the reduced pathogen treatments was a multi-step process, focused on reducing
viruses. First, lake water was collected five days prior to each experiment and transported to the
laboratory. Then tangential flow filtration, a technique used to manipulate viral particles in
previous research (see Frazatti-Gallina 2004), was employed. Lake water was passed through a
0.65-µm membrane cassette followed by a 10-kDa cassette (Millipore, Pellicon 2). Virus counts
were performed using fluorescence enumeration (described below) and confirmed this water to
be virus free. At the initiation of each in-lake experiment, the tangential flow system was again
used (0.65-µm cassette only) to separate the phytoplankton assemblage from the bulk of the
natural virus assemblage. To accomplish this, we collected the retentate from the tangential flow
system (phytoplankton-sized particles from 24.5 l of lake water concentrated to 1.0 l), which was
then added to 23.5 l of virus free water, created previously in the laboratory. Thus, particles
greater than 0.65 µm were restored to their original densities, with particles less than 0.65 µm
being diluted to approximately 4% of their initial concentration. Therefore, we refer to these
treatments as virus-reduced because the complete removal of viruses was not performed.

During tangential filtration, we discovered that the recovery efficiency of phytoplankton-
sized particles in the retentate was inconsistent, and this resulted in variable initial phytoplankton
population densities between carboys for these treatments. Tests of the potential deleterious
impacts on phytoplankton concentration within the tangential flow system showed no significant
influence. A treatment of recombined retentate and permeate from the tangential flow filtration
was compared to the grazer-reduced treatment; over 7 days, no significant different was observed
between treatments for either *P. parvum* population density or chlorophyll *a* (*p > 0.05*).
Response Variables

Response variables were sampled once from all carboys at the end of each 7-d experiment. Characterizations of plankton included estimates of biomass for total phytoplankton and higher taxonomic groups, enumerations of *P. parvum* population densities, and biovolumes of total zooplankton and taxonomic groups. Inorganic nutrients were measured, and ambient toxicity to fish and cladocerans was also determined. Initial conditions were characterized by measurements taken just prior to experimental initiation from source waters used in each treatment, and samples for these response variables were also collected from the lake at the termination of each experiment. A comparison of these in-lake samples with the control treatment at termination verified minimal bottle artifacts.

Estimates of total phytoplankton biomass and biomasses of aggregated taxa were determined from phytopigment concentration measurements following Pinckney et al. (1998), using CHEMTAX, a matrix factorization program that 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 (Mackey et al. 1997; Wright et al. 1996). Cyanobacteria, euglenophytes, chlorophytes, prymnesiophytes, cryptophytes, diatoms and chrysophytes were analyzed because of their historical prevalence in Lake Whitney. See Roelke et al. (2007) for additional detail of the HPLC and CHEMTAX methods followed.

Water column chlorophyll *a* and phaeophytin concentrations were determined using standard fluorometric procedures. A 50 ml sample was filtered through 47 mm GF/F glass microfibre filters (*n*=3 per carboy) and frozen until analysis (within 48 h of collection). Pigments were extracted with 90% acetone, centrifuged, and analyzed using a fluorometer (APHA 1998). In experiments 2 and 3, chlorophyll *a* samples were collected at initiation from
each carboy to account for differences incurred through tangential flow manipulation and grazer
reduction methods. For example, initial *P. parvum* cell density values were adjusted according
to initial chlorophyll *a* data to account for the variable initial phytoplankton population densities
in the virus-reduced carboys.

A 100 ml phytoplankton sample was collected from each well-mixed carboy and preserved
using glutaraldehyde (5% v/v). Enumeration of *P. parvum* population density was performed
using a settling technique (Utermöhl 1958). A 1.5 ml subsample was settled for a 24-h period,
then counted using an inverted, phase-contrast light microscope (400x, Leica Microsystems).
Depending of the density of material in the samples, between 5-25 randomly selected fields of
view were counted, which resulted in ~200 *P. parvum* cells counted per sample.

Zooplankton samples were collected following two methods. For in-lake conditions,
sampled at both initiation and termination of each experiment (six total data points), a Schindler
trap (61-µm mesh size) was used to concentrate a 12 l lake sample (collected at 0.5 m depth) to
50 ml. At the termination of each experiment, a 10 l sample was removed from each carboy and
filtered through the cod end portion of a Schindler trap (61 µm) and concentrated to 50 ml.
Zooplankton samples were preserved in 2% buffered formalin (10% v/v). A subsample of 15 to
18 ml was settled for 24 h, then counted using an inverted, phase-contrast light microscope (40x
and 200x, Leica Microsystems). For each individual counted, dimensions were measured
corresponding to best-fit geometric shapes to estimate biovolume (Wetzel and Likens 1991).
Zooplankton species were grouped into protozoa (mostly testate amoebae and tintinnids), total
rotifers, total copepod adults, and copepod nauplii. Zooplankton densities during these
experiments were low, and our enumeration technique resulted in ~20-50 individuals counted per
sample.
To confirm the removal of viral-sized particles, a 2 ml sample was collected at the start of each experiment from waters passed through tangential flow filtration. All virus samples were flash frozen in liquid nitrogen and stored at -86°C. Slide preparation followed methods by Noble (2001) using the nucleic acid stain SYBR Gold (Molecular Probes, Inc.), and enumeration was performed using epifluorescent microscopy (1000x).

Samples for inorganic nutrients were filtered through GF/F glass microfibre filters (0.7-µm pore size), and the filtrate was frozen until analysis. Using autoanalyzer methodology (Armstrong & Sterns 1967; Harwood & Kuhn 1970), analyses included the sum of nitrate (NO$_3$-N) and nitrite (NO$_2$-N), ammonium (NH$_4$), and orthophosphate (PO$_4$). Ambient toxicity to a cladoceran and a fish model was evaluated for initial conditions and from each experimental carboy. A standardized 24-h static acute-toxicity assay was used with the fathead minnow (*Pimephales promelas*) model (US EPA 2002), and a standardized 10-d static renewal chronic-toxicity test was used with a cladoceran (*Daphnia magna*) model (US EPA 1994) with minor modifications (Dzialowski et al. 2006). Samples were collected and transported to the laboratory where toxicity tests were initiated within 24 h. Ambient samples for both bioassays were diluted using a 0.5 dilution series with reconstituted hard water (RHW), prepared according to standardized procedures (US EPA 2002). RHW was the control treatment 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. Climate controlled chambers were utilized for all toxicity tests (25 ±1°C with a 16:8 hour light-dark cycle). Fathead minnow larvae less than 48 h old were fed newly hatched *Artemia nauplii*
two hours before initiation of testing (US EPA 2002). *D. magna* were fed a Cerophyll/green algal suspension daily, prepared according to methods reported previously (Hemming et al. 2002). 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) methods. No Observed Adverse Effect Concentrations (NOAEC) and Lowest Observed Adverse Effect Levels (LOAEL) for cladoceran reproduction responses were determined using ANOVA with Dunnett’s test (US EPA 2002)

**Data Analysis**

Relative changes in *P. parvum* densities and chlorophyll a concentrations were calculated as

\[
\frac{(Y_{7d} - Y_{\text{initial}})}{Y_{\text{initial}}},
\]

and differences between experimental treatments were tested for significance using an independent-samples t-test (SPSS). The original experimental plan comprised a 3 x 2 factorial design with the intention to analyze treatment effects with an ANOVA. However, during in-field initiation of the experiments, we discovered artifacts introduced by the tangential flow methodology, i.e., initial phytoplankton population densities were inconsistent in carboys involving the virus-reduced treatment. Initial conditions can strongly influence the outcome with this experimental design (Roelke et al. 2003; Roelke and Eldridge 2009), therefore we doubted the validity of a direct comparison between the effect of reduced grazers and reduced virus. Hence, we chose a more conservative statistical analysis with the t-test, where the effect of reduced grazers and reduced virus was only compared with the control.

**RESULTS**

*In-situ lake conditions*
A toxic *P. parvum* bloom formed during the period of our in-lake experiments, where typical *P. parvum* bloom characteristics were observed. An ongoing fish kill was recorded (composed of shad, *Dorosoma* sp.; buffalo, *Ictiobus* sp.; crappie, *Pomoxis* sp.; bullhead catfish, *Ameiurus* sp.; largemouth bass, *Micropterus salmoides*; and minnow, *Pimephales* sp.), and golden-colored waters with surface foam lines were observed. *P. parvum* population densities increased throughout the first few weeks of sampling, then leveled off at a maximum density of 37.8 x 10^6 cells l^{-1} at initiation of experiment 3. Chlorophyll *a* concentrations showed a similar trend, consistently increasing during our sampling dates. CHEMTAX data corresponded with this trend, showing that Prymnesiophytes dominated the assemblage at near monospecific levels of 96, 81, and 99% of the phytoplankton biomass during late February, and early and late March (Figure 2A). Cyanobacteria, Chlorophytes, and Chrysophytes composed the remaining small percentages of aggregated phytoplankton biomass.

Ambient toxicity bioassays confirmed the developing bloom with non-lethal conditions to *P. promelas* and moderate sublethal toxicity on *D. magna* reproduction observed at initiation of experiment 1, and then lake waters became more toxic to *D. magna* and *P. promelas* at initiation of experiment 2 and remained so through initiation of experiment 3 (Figure 3).

Concurrent with increasing *P. parvum* cell densities, the total zooplankton biovolume decreased during bloom formation. The total biovolume at the initiation of experiment 1 was 16.0 x 10^6 μm^3 l^{-1}, then a dramatic decrease from 23.1 x 10^6 to 1.86 x 10^6 μm^3 l^{-1} was observed between the last two experiments as waters became toxic (Figure 2B). A shift in the zooplankton composition also occurred. Rotifers dominated the community at the start of sampling, composed mostly of *Keratella* sp. (7.76 x 10^6 μm^3 l^{-1}, ~17 individuals l^{-1}) and *Notholca* sp. (5.50 x 10^6 μm^3 l^{-1}). Both *Notholca labis* (5.25 x 10^6 μm^3 l^{-1}, ~6 individuals l^{-1}) and *Notholca*
laurentiae \((0.25 \times 10^6 \mu m^3 l^{-1}, \sim 1 \text{ individuals } l^{-1})\) were present; this distinction between Notholca species was needed for interpretation of experimental results. At the onset of experiment 2, the zooplankton community was dominated by copepod nauplii \((11.7 \times 10^6 \mu m^3 l^{-1}, \sim 15 \text{ individuals } l^{-1})\). Adult copepods and rotifers were subdominant, with Keratella sp. and N. laurentiae representing the rotifer composition equally. At the initiation of experiment 3, copepod nauplii were still dominant \((1.33 \times 10^6 \mu m^3 l^{-1}, \sim 2 \text{ individuals } l^{-1})\), but the subdominant rotifer composition exhibited N. laurentiae dominance \((0.38 \times 10^6 \mu m^3 l^{-1})\), with Keratella sp. present as well \((0.09 \times 10^6 \mu m^3 l^{-1})\).

The physicochemical environment may have subtly influenced the bloom formation. Ammonium ranged between 1.09 and 1.28 \(\mu mol l^{-1}\), while nitrate and nitrite ranged 0.06 to 0.13 \(\mu mol l^{-1}\). Phosphate increased from 0.05 to 0.10 \(\mu mol l^{-1}\) during sampling (Figure 4A).

Nutrients were not likely growth limiting to P. parvum based on half-saturation coefficients for the Texas strain, i.e., \(K_N = 0.005\) and \(K_P = 0.0068\) (Baker 2007; Baker et al. In press).

Throughout the experiments Secchi depth was relatively deep for this region (averaging \(\sim 1 \text{ m})\), where lakes are generally turbid, suggesting that light was also not a limiting factor (Figure 4B). Temperatures steadily increased from 9 to 17ºC, and pH values remained between 8.1 and 8.6, peaking during experiment 2 (Figure 4C).

**Experimental Results**

The first treatment comparison focuses on the role of grazers, evaluating differences between reduced grazers and the control. Experiment 1 was initiated during less toxic conditions, and P. parvum population density increased in both the natural assemblage (NA) and the reduced grazer (RG) treatments; no significant difference was observed between these treatments (Figure 5A). Increases in population density ranged from 15-50%, to an average of
~32 x 10^6 cells l^{-1}. Chlorophyll a concentration decreased in both NA and RG treatments, and no significant difference between treatments was observed (Figure 5A). Decreases in concentration ranged from 5-20%, to an average of ~29 µg l^{-1}. In the NA treatment, total zooplankton biovolume increased between 50-130%, to an average of ~32 x 10^6 µm^3 l^{-1}, and no compositional shift was observed. Rotifers dominated the community, averaging ~31 x 10^6 µm^3 l^{-1}, and were composed mostly of Keratella sp., Notholca sp., and Brachionus sp. (Figure 6A). In the RG treatment, zooplankton composition showed similar rotifer dominance at the conclusion of the experiment (Figure 5A). Interestingly, by the end of the first experiment, toxicity to both D. magna and P. promelas was observed, but no differences in toxicological benchmark concentrations (e.g., LC_{50}, NOAEL values) were observed between the NA and RG treatments.

In experiment 2, initial lake waters were toxic to both D. magna and P. promelas with a fish LC_{50} value of 1.9 % of ambient sample. P. parvum population density increased in both NA and RG treatments, and no significant difference between these treatments was observed (Figure 5B). Increases in population density ranged from 2-45%, to an average of ~43 x 10^6 cells l^{-1}. Chlorophyll a concentration decreased in both NA and RG treatments, and again, the difference between treatments was not significant (Figure 5B). Concentrations decreased between 2-10%, to an average of ~34 µg l^{-1}. An overall biovolume decline of 50-75% was observed in the NA treatment for total zooplankton. Copepod nauplii declined 88-94%, but rotifers, composed mostly of Keratella sp. and N. laurentiae, remained relatively unchanged during experiment 2 (Figure 6B). Again, similar zooplankton composition was observed in the RG treatment (Figure 5B). Consistent with ambient toxicity observed in experiment 1, experimental units were toxic to P. promelas and D. magna at the termination of this study, with LC_{50} values less than 1.5 % of
ambient sample, but again there were no differences in LC$_{50}$ values between the NA and RG treatments.

The *P. parvum* bloom continued to develop, and the lake water remained toxic to *D. magna* and *P. promelas* with a fish LC$_{50}$ value of 3.86% of ambient sample at the initiation of experiment 3. A significant difference between NA and RG treatments was observed for *P. parvum* population density (Figure 5C), where greater *P. parvum* densities were measured in the RG treatment ($p = 0.038$). With grazers reduced, increases in population density ranged from 10-25%, to an average of $\sim 45 \times 10^6$ cells l$^{-1}$, while *P. parvum* population density in the NA treatment ranged from a decrease of 10% to an increase of 15% (an average of $\sim 39 \times 10^6$ cells l$^{-1}$). Chlorophyll $a$ concentration decreased in both NA and RG treatments, and the difference between treatments was not significant (Figure 5C). Overall, reductions in chlorophyll $a$ ranged from 2-14%, to an average of $\sim 38 \mu g$ l$^{-1}$. During experiment 3, total zooplankton biovolume increased 3-to 4-fold in the NA treatment. This was due in large part to the accumulation of *N. laurentiae*, which averaged $5.8 \times 10^6 \mu m^3 l^{-1}$, or $\sim 6$ individuals l$^{-1}$ (Figure 6C); copepod nauplii biovolume declined 87-100%. In the RG treatment, zooplankton composition showed similar rotifer dominance at the conclusion of the experiment (Figure 5C). Similar to experiments 1 and 2, high ambient toxicity to *P. promelas* and *D. magna* was observed in the experimental units at the end of study 3, with LC$_{50}$ values less than 1.3% of ambient sample and no differences in LC$_{50}$ values between the NA and RG treatments.

Experiment 3 differed from the other experiments in that a large cell-sized component of the phytoplankton ($\sim 11\%$ of the total chlorophyll $a$) was removed from the lake water during the grazer reduction step. This is indicated by a difference in initial chlorophyll $a$ levels between NA and RG treatments.
Our second comparison of complementary treatments focused on the role of viruses, highlighting the differences between natural assemblage (NA) and virus-reduced (VR) treatments. Due to methodological artifacts, only data from the final two experiments are shown. During experiment 2, *P. parvum* population densities and chlorophyll *a* concentrations changed little in the NA and VR treatments, and no significant difference between these treatments was observed. Rotifers dominated the zooplankton community in both treatments at the end of this experiment (Figure 7A). All carboy contents were toxic to *P. promelas* and *D. magna*, but no differences were observed between NA and VR treatments.

In experiment 3, a significant difference between NA and VR treatments was observed for *P. parvum* population density (Figure 7B), where greater changes in *P. parvum* population densities were measured in the VR treatment (*p* = 0.02). Under reduced virus conditions, increases in population density ranged from 35-65%, to an average of ~39 x 10^6 cells l⁻¹, while *P. parvum* population density in the NA treatment ranged from a decrease of 10% to an increase of 15%. Chlorophyll *a* concentration decreased in both NA and VR treatments, and the difference between treatments was not significant. The zooplankton composition was very similar in both of these treatments, showing rotifer-dominant communities (Figure 7B). Again, all contents were toxic to *P. promelas* and *D. magna* at the end of this study.

**DISCUSSION**

During the period of our experiments, fish-killing *P. parvum* blooms occurred intermittently throughout Lake Whitney, with a developing bloom recorded at our study site. Zooplankton populations dramatically declined with the onset of in-lake toxicity. Interestingly, grazers significantly impacted *P. parvum* in the third experiment, where the reduction of grazers resulted
in an increase of *P. parvum* population density. During our final experiment, the observed compositional shift to a rotifer-dominated zooplankton community, almost exclusively *N. laurentiae* (or possibly a smaller micrograzer, less than 61 µm), alongside the near complete decline of copepod nauplii, suggests that *N. laurentiae* and perhaps smaller grazers were less sensitive to toxins produced by *P. parvum* than other grazers. Conversely, a recent toxicity study by Brooks et al. (this issue) documented a negative effect on population growth of the freshwater rotifer *Brachionus calyciflorus* when exposed to *P. parvum* toxins from the TX strain. Our findings imply that rotifer sensitivities to *P. parvum* toxins may be species-specific, with *N. laurentiae* showing an ability to develop tolerance and potentially resistance to prymnesin toxins and demonstrate population growth under ambient conditions that are toxic to fish and other zooplankton.

How might these observed zooplankton community shifts compare with seasonal changes that occur dependently or independently of *P. parvum* blooms? For this study, no previous data were available for *N. laurentiae* abundances during bloom and non-bloom events. However, our on-going research currently performs monthly whole system water quality monitoring in Lake Whitney, with fixed-station sites where zooplankton communities are sampled throughout the year (Roelke, unpublished data). With this larger data set, we will better understand the seasonal shifts in zooplankton community structure, specifically of *N. laurentiae*.

While our reduced-grazer treatments improved *P. parvum* performance, this outcome did not carry over to the entire phytoplankton community. CHEMTAX estimates suggested that Prymnesiophytes dominated throughout the experiments, but we observed decreases of chlorophyll *a* in the grazer-reduced treatment concurrent with increases in *P. parvum* population densities. This could indicate that *P. parvum* had a negative effect on other algae, possibly
through competition or allelopathy (Fistarol et al. 2003, 2005; Granéli and Johansson 2003; Roelke et al. 2007; Errera et al. 2008).

The effect of viruses on phytoplankton dynamics is a developing field of study, but research has shown that viruses can negatively impact phytoplankton populations. Brussaard et al. (2005) found that viral infection can restrict the growth of the prymnesiophyte *P. globosa*, another HAB-causing species closely related to *P. parvum*, but there is no previous evidence for viral effects on *P. parvum* dynamics. Our experiments demonstrated that viral manipulation appears to affect *P. parvum* populations. During the last experiment, carboys with a reduction in virus populations resulted in an improved *P. parvum* performance. Although not a dramatic increase in population growth, this observation still suggests a negative impact of viruses on *P. parvum*.

The effect of both grazers and viruses on *P. parvum* was more pronounced, and statistically significant, in the third experiment, relative to the second, even though both experiments exhibited near identical lethal and sublethal toxicity to *P. promelas* and *D. magna*, respectively, indicating that extracellular toxin levels were likely similar during experiments 2 and 3. In these final two experiments, the total zooplankton biovolume increased only in experiment 3, most likely indicating more grazing pressure on phytoplankton communities. Under these ambient toxicity conditions, it is possible that the compositional shift among rotifers toward *N. laurentiae* dominance (or perhaps smaller micrograzers) resulted in a stronger and more significant effect of grazer reduction on *P. parvum* demographics. This potential reduced sensitivity of freshwater *N. laurentiae* to *P. parvum* toxins and the role of microzooplankton both merit further investigation.

In the case of viruses, the observed increase in *P. parvum* population densities during later stages of bloom development (under reduced viral conditions) could indicate direct interactions between viruses and *P. parvum*; conversely, the reduction of viruses may have increased
bacterial densities (Schwalbach et al. 2004), therefore causing an indirect positive facilitation of
*P. parvum*, known to utilize phagotrophic feeding strategies to prey on bacteria (Nygaard and
that phytoplankton viruses, an important factor in bloom termination, are likely to negatively
impact blooms during later stages. Over the span of our experiments, it may be that increased
viral infection and cell lysis began to affect *P. parvum* populations by the third experiment, and
perhaps both grazer and virus impacts on *P. parvum* would increase in magnitude as the bloom
continued to mature. Further research is essential to better understand the interactions of algal
virus ecology involving *P. parvum* bloom dynamics.

Based on observations in Lake Whitney, the timing of onset and termination of *P. parvum*
blooms varies locally. During the course of these experiments a localized bloom formed at our
study site, while in other areas of the lake blooms formed earlier and were more persistent
(Bryan Brooks, unpublished data; Joan Glass, TPWD, personal communication). Concentrations
of dissolved nitrogen and phosphorus during the study period remained above those limiting the
growth rate of *P. parvum* in laboratory cultures (Baker 2007; Baker et al. *In press*), which
produced toxic cells under nutrient replete conditions when the salinity, light, and temperature
environment became stressful (Baker et al. 2007). Although temperatures during these
experiments were suboptimal, based on laboratory cultures, they would support specific growth
at approximately half the optimal rate of about 0.9 d⁻¹ (Baker et al. 2007). Using equations that
estimate the average underwater irradiance (approximated with surface irradiance and a
relationship between the light extinction coefficient and Secchi depth), the average irradiance in
the lake was 183 uE m⁻² s⁻¹ (Kirk 1994; Wetzel 2001). By comparison to growth in laboratory
cultures, the in-lake irradiance was above the light conditions (140 uE m⁻² s⁻¹) used in our routine
culturing of *P. parvum* (Baker et al. 2007; Roelke et al. 2007); therefore not likely restricting the growth of *P. parvum*. Thus, it does not appear that one single factor triggered bloom development, in regards to temperature, light, pH, and nutrients, but maybe subtle changes in each of these parameters, as well as variable(s) not measured here, played a role in bloom dynamics.

*P. parvum* populations had already reached typical bloom densities at the initiation of experiment 1, yet bioassays showed non-toxic conditions to *P. promelas* and moderate effects on *D. magna* reproduction. Two weeks later, at experiment 2 initiation, lake waters were toxic to both *D. magna* and *P. promelas*. This localized development was likely triggered by a multitude of abiotic and biotic parameters, and discerning a specific factor that influenced bloom formation is difficult with the available data. If we assume the same chemicals resulting in toxicity also have an allelopathic effect (Fistarol et al. 2003, 2005; Granéli and Johansson 2003; Roelke et al. 2007; Errera et al. 2008), the lack of toxicity during the first experiment suggests that allelopathy played a minimal role in bloom development; however, these specifics involving *P. parvum* allelopathy require greater understanding. Possibly, the cooler in-lake temperatures hindered grazers preferring *P. parvum* as prey, thus enabling *P. parvum* population densities to reach bloom proportions.

The environmental conditions surrounding *P. parvum* bloom formation and termination seem complex. Our results from this exploratory research demonstrate grazer and virus impacts on *P. parvum* dynamics during toxic bloom conditions, and although follow-on research is necessary, these findings have potential implications for alga management strategies. The rise of *N. laurentiae*, apparently less sensitive to *P. parvum* toxins than other grazers, could have brought an effective grazer into the zooplankton community; this factor, in conjunction with the
proliferation of viruses affecting *P. parvum*, could possibly curb the magnitude of a bloom.

Future research initiatives could focus on continued grazer and virus affects under maturing bloom conditions, and perhaps a more detailed look at interactions between phytoplankton and virus, and their affect on *P. parvum* populations.

ACKNOWLEDGEMENTS

We are grateful to Hsiu-Ping Li for her assistance with processing samples and to the personnel at the Cliffview Resort on Lake Whitney for their hospitality. The Texas Parks and Wildlife Department supported this research, with additional support from the U.S. Environmental Protection Agency and a federal initiative through the Department of Energy.

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FIGURE CAPTIONS

FIGURE 1. Lake Whitney, Texas (USA). Experiments on *Prymnesium parvum* population dynamics were performed near Walling Bend Island, where high *P. parvum* population densities are historically observed, and a fish-killing bloom was developing during the study.

FIGURE 2. In-lake biotic parameters sampled during the six weeks of experiments in Lake Whitney. *P. parvum* population densities (dashed line), and phytoplankton biomass (solid line), estimated as chlorophyll *a* concentration, with corresponding CHEMTAX percentages showing Prymnesiophyte dominance (A). Total in-lake zooplankton biovolume decreased with the developing bloom (B). Background shading relates to in-lake toxicity conditions: solid gray = non-toxic, white = moderately toxic, and dotted gray = highly toxic.

FIGURE 3. Ambient Lake Whitney toxicity to *Pimephales promelas* 48-h survival and *Daphnia magna* 10-d reproduction (neonates female \(^{-1}\)) at the initiation of three studies performed during spring 2007. NOAEL = no observed adverse effect level.
FIGURE 4. In-lake physicochemical variables monitored to determine effects on *P. parvum* population dynamics during experiments in Lake Whitney, February 21 to March 28, 2007. Dissolved inorganic nitrogen (NO$_x$ = sum of nitrate and nitrite) and PO$_4$ concentrations suggest that nutrients where not limiting growth (A). Relatively deep readings and minor changes in Secchi depths recorded (B). Temperature steadily increased (solid line), and slight changes were observed in pH levels (dashed line, C).

FIGURE 5. Comparison of natural assemblage and reduced grazer treatments, showing initial conditions (filled squares) and data following 7-d experimental duration (bars) for experiments initiated February 22 (A), March 8 (B), and March 29 (C). Averaged *P. parvum* population densities (light gray bars); only experiment 3 demonstrated a significant difference between treatments (p = 0.038) for *P. parvum*. Averaged phytoplankton biomass, estimated as chlorophyll *a* concentration (dark gray bars). Average zooplankton biovolume and community composition (stacked bars), showing rotifer dominance in all three studies.

FIGURE 6. Comparison of rotifer zooplankton biovolumes in the natural assemblage and reduced grazer treatments for all three experiments (A, B, C). Initial conditions (filled squares) and averaged biovolumes from experimental termination (stacked bars) highlight changes in composition after 7 days. Rotifers dominated assemblages at termination of all experiments, shifting almost exclusively to *Notholca laurentiae* during the final experiment.

FIGURE 7. Comparison of natural assemblage and virus-reduced treatments for the final two experiments only (A, B), illustrating initial conditions (filled squares) and data after 7-d
experimental duration (bars). Averaged *P. parvum* population densities (light gray bars), where initial *P. parvum* conditions were adjusted, based on chlorophyll *a* concentrations, for the virus-reduced waters manipulated by tangential flow filtration. Only in experiment 3 was the difference between treatments significant (*p* = 0.02) for *P. parvum*. Averaged phytoplankton biomass, estimated as chlorophyll *a* concentration (dark gray bars). Average zooplankton biovolume and community composition (stacked bars), showing rotifer dominance in both experiments.
Lake Whitney

Study site

Fig. 1
Fig. 3

Non-toxic

Toxicological Benchmark Concentration (% ambient sample)

Study 1 | Study 2 | Study 3

D. magna NOAEL
P. promelas LC$_{50}$
Fig. 4
Fig. 5
Fig. 6

A. Zooplankton biovolume (x10^6 \mu m^3 l^{-1})

- Keratella sp.
- Notholca labis
- Notholca laurentiae
- Brachionus sp.

B. Zooplankton biovolume (x10^6 \mu m^3 l^{-1})

C. Zooplankton biovolume (x10^6 \mu m^3 l^{-1})

Natural Assemblage

Reduced Grazers

sd = 2.75

sd = 8.52

sd = 1.22

sd = 5.40

sd = 2.35

sd = 0.68

sd = 0.14

sd = 0.67

sd = 1.35

sd = 0.19

sd = 0.02

sd = 0.34

sd = 0.38

sd = 0.91
Fig. 7
A mechanistic explanation for pH-dependent ambient aquatic toxicity of Prymnesium parvum carter

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1. Introduction

Harmful algal blooms (HABs) may have devastating impacts on aquatic ecosystems, resulting in severe impacts to fisheries. Increases in the frequency and severity of HABs on the global scale has triggered scientific inquiry to define factors causing these trends (Zingone and Enevoldsen, 2000; Anderson et al., 2002; Hallegraeff, 2003); however, among the greatest challenges for managers is the spread of invasive species. Prymnesium parvum is an example of an invasive HAB species that has transitioned from marine origins to inland systems. Identified nearly a century ago as a problem in marine environments because of its toxic blooms (Liebert and Deerns, 1920), P. parvum is more recently recognized as an invasive species threatening inland systems in the arid and semiarid southwestern and south central United States (Baker et al., 2007; Roelke et al., 2007; Schwierzke et al., in press).

Anthropogenic changes to the hydrologic cycle, eutrophication, and salinization of waterways are associated...
with the spread of HABs (Anderson et al., 2002). Such changes, intertwined with climatological and geographical factors, have rendered some Texas reservoirs to be within the tolerance range of *P. parvum* (Larsen and Bryant, 1998; Baker et al., 2007; Baker et al., 2009). Specifically, the species’ euryhaline nature has apparently facilitated its transition from coastal marine and estuarine ecosystems to these weakly saline inland impoundments. Since the first harmful blooms of *P. parvum* in Texas were documented in the Pecos River (James and De La Cruz, 1989), *P. parvum* has spread to other systems in Texas resulting in toxic blooms and fish kills (Roelke et al., 2007; Schwierzke et al., in press).

*P. parvum* is a mixotrophic haptophyte that can gain energy photosynthetically as well as phagotrophically by feeding on other microorganisms (Skovgaard and Hansen, 2003). Exposure to *P. parvum* toxins can lyse cells (Yariv and Hestrin, 1961; Tillmann, 2003), disrupt cell membrane integrity (Yariv and Hestrin, 1961; Padilla, 1970; Kim and Padilla, 1977; Brooks et al., in press), and affect gill functions of aquatic organisms (Ulitzur and Shilo, 1966). Ecologists have proposed several purposes for the production and release of toxins by *P. parvum*, including acquisition of prey (Stoecker et al., 2006), elimination of algal competitors (Fistarol et al., 2003; Granéli and Hansen, 2006; Uronen et al., 2007), or reduced grazing pressure (Rosetta and McManus, 2003; Tillmann, 2003). A variety of factors, including nutrient limitation, salinity, temperature, and light are known to influence cell growth and the toxicity of laboratory cultures of *P. parvum* (Shilo and Aschner, 1953; Padilla, 1970; Dafni and Shilo, 1966; Larsen et al., 1993; Larsen and Bryant, 1998; Johansson and Granéli, 1999; Granéli and Johansson, 2003; Baker et al., 2007; Baker et al., 2009). Few studies have focused on factors governing the behavior of the toxins once they are released, or considered how bloom formation might alter the environment (e.g., light attenuation, nutrient availability, dissolved oxygen, pH) in ways that could influence the bioavailability and potency of *P. parvum* toxins.

Shilo and Aschner (1953) proposed that *P. parvum* toxins were proteins with high molecular weights. To date, the only characterized toxins are prymnesin-1 and -2, large chains of 90 carbon atoms and trans-1,6-dioxodecaline units with conjugated double/triple bonds at each terminal end; their respective chemical formulas are $C_{107}H_{154}Cl_3NO_{44}$ and $C_{96}H_{136}Cl_3NO_{35}$ (Igarashi et al., 1999). These compounds are amphiphilic, with uneven distributions of sugars and hydroxyl groups, and three chlorine atoms and one nitrogen atom. Both prymnesins are structurally similar to other HAB toxins such as maitotoxin and ciguatoxin, which are characterized by a network of hydroxylated polycyclic ether units (Murata and Yasumoto, 2000). The amine present on the prymnesins suggests that these compounds might be weak ionizable bases with pKa values >8. Prior studies suggested that some of the toxins released by *P. parvum* are ionizable, becoming more toxic to fish exposed at higher pH, with toxicity eliminated below pH 7 (Shilo and Ashner, 1953; McLaughlin, 1958; Ulitzur and Shilo, 1964). However, these experiments were completed under marine conditions, and prior to the development of standardized aquatic bioassays.

This study examines whether pH also influences the toxicity of *P. parvum* toxin in less saline waters representative of Texas reservoirs where blooms have occurred. Simultaneous bioassays were performed at three pH levels with samples obtained during *P. parvum* blooms occurring in 2007 from two reservoirs, and with samples of laboratory cultures and culture filtrates. Further, the chemical structures of prymnesin-1 and -2 were examined to estimate their physiochemical properties. We hypothesized that toxins released by *P. parvum* are ionizable weak bases.

2. Material and methods

2.1. Bioassays with samples obtained from reservoirs experiencing blooms

2.1.1. Lake Whitney

Lake Whitney is a reservoir constructed in 1951 on the Brazos River, with a capacity of $4.68 \times 10^8$ m$^3$, surface area of 95 km$^2$, and shoreline of 362 km (Bailes and Hudson, 1982). Two 4-L samples were collected in NALGENE® I-Chem Certified Series™ 300 LDPE Cubitainers™ (Fisher Scientific) from Lake Whitney during a bloom in March 2007, transported to the laboratory on ice, and stored under refrigeration at 4 °C. This lake sample contained $61.5 \times 10^6$ *P. parvum* cells mL$^{-1}$ (enumerated microscopically with a hemocytometer). Ambient pH at the site in Lake Whitney when the sample was collected was pH 8.4. Total ammonia in the samples was <1 mg/L in whole samples, which is below ambient water quality criteria for the temperature and pH at which our experiments were completed. Dilutions in our toxicity experiments at which toxicity was observed further confirmed that dose dependent responses were not due to ammonia. Toxicity tests were initiated within 96 h of sample collection following EPA recommendations for ambient toxicity studies (US EPA, 2002).

Acute bioassays with <48 h old *Pimephales promelas* were conducted in 100-mL glass beakers. Three replicates of seven individuals were prepared for each treatment level. Reconstituted hard water (RHW) prepared according to APHA et al. (1998) was used as the diluent and control (treatment consisting of 100% RHW). Treatment levels were prepared by diluting lake water with RHW to the following percentages of lake water: control (RHW), 0.01, 0.1, 1, 5, and 20%. These treatment levels were selected based upon ambient toxicity data from prior water quality monitoring efforts. A volume of 3-L was prepared for each treatment level, which was then divided into three aliquots of 1-L that were then adjusted to pH units of 6.5, 7.5, or 8.5 (-0.05) prior to dispensing experimental aliquots. The pH adjustments were achieved by slowly titrating 10% HPLC-grade nitric acid, which generally followed U.S. Environmental Protection Agency protocols for pH adjustment in Toxicity Identification Evaluations (US EPA, 1991). Test individuals were fed newly hatched brine shrimp (*Artemia sp.*) 2 h prior to the exposure, but were not fed during experiments (US EPA, 2002). Survivorship
was assessed at 24 and 48 h, and temperature, dissolved oxygen, and pH were measured at test initiation and completion. Exposures were conducted at 25 ± 1 °C under a 16:8 light:dark photoperiod.

A 10 d *Daphnia magna* reproductive study was also completed with Lake Whitney water (US EPA, 1994, modified as in Dzialowski et al., 2006). Treatment levels consisted of control (RHW), 12, 25, 50, and 100% lake sample water. Test solutions were prepared and adjusted to desired pH as previously described. Experimental units were 100-mL beakers filled with 80 mL of test solution. Organisms were fed daily with *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) augmented with filtrate from a cerophyll suspension (approximately 2 g/L RHW). The final green algae cell concentration was 30 × 10⁶ cells mL⁻¹. One *D. magna* individual <24 h old was introduced in each beaker and transferred every other day to fresh test solution. Five replicates were prepared for each treatment level. Survivorship and fecundity were monitored daily. The experiment was completed for 10 d at 25 ± 1 °C under a 16:8 light:dark photoperiod.

### 2.2. Lake Granbury

Lake Granbury is a reservoir constructed in 1969, with a capacity of 167 × 10⁶ m³, a surface area of 34 km², and an average depth of ~5 meters. Samples were collected during a *P. parvum* bloom that caused fish kills in March 2007 at three fixed monitoring stations and were handled prior to bioassay initiation as previously described. Ambient pH at the sites while the samples were collected ranged between pH 8.2 and 8.4. Total ammonia concentrations were <0.25 mg/L in the three samples, which again were lower than levels associated with ammonia toxicity (US EPA, 1999). Cell counts for samples from Sites 1–3 were 29 × 10³, 36 × 10³, and 36 × 10³ cells mL⁻¹, respectively.

Experiments were conducted with *P. promelas* similar to those previously described in order to assess acute toxicity at the three sites. Six treatments, including a control (RHW), 6, 12, 25, 50, and 100% lake water were prepared using RHW as the diluent and adjusted to pH 6.5, 7.5 and 8.5. A 96-h acute exposure experiment was initiated with <24 h old *D. magna* using a composite sample from the three stations. Treatments included a control (RHW), 6, 12, 25, 50, and 100% lake water. Ten replicates were prepared for each treatment level. Exposures were completed in 100-mL glass beakers filled with 80 mL of test solution, and water in experimental units were renewed at 48 h. Organisms were fed daily the same concentration of the mixture described for the 10 d *D. magna* experiment, at which time survivorship was assessed. Exposures were conducted at 25 ± 1 °C under a 16:8 light:dark photoperiod.

### 2.2. Laboratory culture preparation

The UTEX LL 2797 (University of Texas, Austin, Texas, USA) strain of *P. parvum* was used to initiate cultures. Cultures were grown in 20-L glass carbons filled with 14-L of an artificial seawater (ASW) prepared according to Berges et al. 2001 and then diluted to a working salinity of 5.8 g L⁻¹ with ultrapure water (18 MΩ cm⁻¹). Afterwards, nutrients (NaNO₃ and NaH₂PO₄) were added at concentrations of f/2 and f/8 media (Guillard, 1975); vitamins and trace metals were the same for both types of media. Three replicates were prepared for each treatment and all carboys were inoculated with 10³ cells mL⁻¹ of *P. parvum* from stock cultures in late exponential phase. Exposures were completed in incubators at 20 ± 1 °C for a 12:12 light:dark cycle with an irradiance of ~140 µE m⁻² d⁻¹. Carboys were repositioned and mixed daily by gently swirling.

### 2.3. Bioassays with samples obtained from laboratory cultures

Several experiments with larval *P. promelas* were completed using these *P. parvum* cultures. An initial experiment examined the toxicity of the three replicate carboys of both the f/2 and f/8 cultures. ASW was adjusted to a salinity of 5.8 g L⁻¹ used in the media served as the diluent and controls. Bioassays were completed in 100-mL beakers filled to capacity with test solution. Treatments included a control (ASW), 1, 2.5, 10, 25, and 100% culture water containing *P. parvum* cells. Four replicates of five individuals were prepared for each treatment. Experiments were conducted at 25 ± 1 °C under a 16:8 light:dark photoperiod.

For subsequent studies, we separately pooled f/2 and f/8 cultures and then filtered half of each volume through GF/C filters (Whatman GF/C; VWR International, West Chester, Pennsylvania, USA). Acute toxicity to *P. promelas* was determined for cultures (f/2, f/8) that were unfiltered and filtered (cell-free filtrate), then these samples were manipulated to pH 6.5, 7.5 or 8.5 following procedures outlined above. ASW adjusted to a salinity of 5.8 g L⁻¹ was used as the diluent and control. An additional RHW treatment for quality assurance was also prepared. Each acute toxicity study included a control (ASW), RHW, 0.1, 1, 5, 10, 25, and 100% media treatment. Four replicates of five *P. promelas* <48 h old were used for each treatment level. Experiments were conducted at 25 ± 1 °C under a 16:8 light:dark photoperiod.

### 2.4. Statistical analysis

LC₅₀ values for acute toxicity to *P. promelas* and *D. magna* were calculated by Probit analysis if data met assumptions; otherwise, the Trimmed Spearman-Karber method was applied using TOXSTAT computer software (US EPA 2002). SAS (SAS Institute, Cary, NC, USA) was used for other statistical analyses. For the 10 d experiment with *D. magna*, significant differences in survivorship between the control at each of the respective pH treatments and reservoir water dilutions were determined using Fisher’s Exact Test. Significant differences in reproduction were assessed by an ANOVA comparing the mean neonate production per female for all treatments (*α = 0.05*), followed by Dunnett’s test comparing the controls to each of the treatments at a respective pH (*α = 0.05*). In addition, we compared the mean control responses between different pH levels for the respective endpoints using ANOVAs for each series of experiments to confirm health of test organisms.
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Fig. 1. Average survivorship (±SD, n = 4) of Pimephales promelas exposed to dilutions of Lake Whitney water collected during a bloom of Prymnesium parvum in 2007. Cell density is expressed as the % lake water multiplied by the density of Prymnesium parvum cells in the undiluted sample. The error bars represent the standard deviation. Missing error bars are due to 100% survivorship in all replicates for a treatments, hence there was no variation to derive a prediction.

2.5. Estimation of prymnesin-1 and -2 physicochemical properties

Calculation of physicochemical parameters for prymnesin-1 and -2 (Fig. 1) was carried out using ACD/Labs (Advanced Chemistry Development, Inc., Toronto, Ontario, Canada) ChemSketch, pKa calculator, LogD calculator, and LogP calculator (Version 9). LogP was calculated for each whole molecule, and percent distribution of species and pKa values were calculated considering the hydrophobic component of the molecule containing the primary amine group (Murata and Yasumoto, 2000). The full molecule could not be handled by the program due to the large number of ionizable sites, especially on the hydrophilic portion of the molecule. Calculated values for logP (octanol:water partitioning coefficient when the compound is primarily unionized), logD (coefficient of octanol:water partitioning ratio of ionized to unionized over a pH range), and pKa (acid dissociation constant) are estimates based on the use of an extensive database of fragments and predicted inductive effects based on substituents near ionizable sites. LogD was calculated as the sum of logD for the hydrophobic fragment at different pHs with the logP of the hydrophilic fragment (neutral form), which remains neutral at all relevant pHs (<12).

3. Results

3.1. pH dependent toxicity in field studies: Lakes Granbury and Whitney

Ambient toxicity to juvenile P. promelas of Lake Whitney samples collected during a Spring 2007 bloom was reduced when pH was adjusted to <7.5 (Fig. 1). The 48-h LC50 value (95% confidence intervals) for experiments completed at pH 8.5 was 1.9 × 10^3 (1.6–2.7 × 10^3) cells mL^-1, whereas comparable values at pH 6.5 and 7.5 were 7.8 × 10^3 (7.1–8.8 × 10^3) and 4.1 × 10^3 (2.6–5.3 × 10^3) cells mL^-1.

Table 1

<table>
<thead>
<tr>
<th>Site</th>
<th>Cells per ml</th>
<th>pH</th>
<th>% Survivorship in undiluted sample</th>
<th>48 h LC50 (% lake water)</th>
<th>Upper and lower 95% confidence intervals</th>
<th>48 h LC50 (Prymnesium parvum cell/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.9 × 10^3</td>
<td>6.5</td>
<td>&gt;100*</td>
<td>nc</td>
<td>nc</td>
<td>nc</td>
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<tr>
<td></td>
<td></td>
<td>7.5</td>
<td>&gt;100*</td>
<td>nc</td>
<td>61–85</td>
<td>21 × 10^3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.5</td>
<td>72</td>
<td>59–100</td>
<td>28 × 10^3</td>
<td>19 × 10^3</td>
</tr>
<tr>
<td>2</td>
<td>3.6 × 10^3</td>
<td>6.5</td>
<td>80</td>
<td>41–71</td>
<td>19 × 10^3</td>
<td>15–31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.5</td>
<td>54</td>
<td>78 × 10^3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.5</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3.6 × 10^3</td>
<td>6.5</td>
<td>&gt;100*</td>
<td>nc</td>
<td>nc</td>
<td>nc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.5</td>
<td>&gt;100*</td>
<td>nc</td>
<td>nc</td>
<td>nc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.5</td>
<td>43</td>
<td>36–53</td>
<td>16 × 10^3</td>
<td></td>
</tr>
</tbody>
</table>

nc = Not calculable.

* = There was insufficient mortality to generate a point estimate.

Please cite this article in press as: Theodore W. Valenti et al., A mechanistic explanation for pH-dependent ambient aquatic toxicity of Prymnesium parvum carter, Toxicon (2009), doi:10.1016/j.toxicon.2009.09.014
There was a similar pH-dependent toxicological relationship during experiments with *D. magna*, as fecundity was significantly reduced at lower densities of *P. parvum* cells when exposure occurred at higher pH (Fig. 2). No reproduction was observed at any pH in 100% Lake Whitney water. There was no significant difference in reproduction between controls at each pH or unmodified RHW.

Susceptibility of *P. promelas* to samples from Lake Granbury during the 2007 bloom also indicated a pH-dependent toxicological relationship. Ambient toxicity to fish was ameliorated in lake samples from two stations and substantially reduced in a third by lowering pH (Table 1). There was insufficient mortality for samples collected from two sites to calculate LC₅₀ values at pH 6.5 and 7.5; thus, these values are conservatively reported as >100%. The sample from Site 2 was the only Lake Granbury sample for which LC₅₀ values could be determined for pH 6.5, 7.5, and 8.5. There was approximately a four-fold difference in LC₅₀ values between the pH 6.5 and 8.5 treatments, with higher toxicity at higher pH (Table 1). Similarly, toxicity to *D. magna* was reduced in low pH in a 96-h experiment exposing individuals to a composite sample from all three sites (Table 2). LC₅₀ values could not be calculated at pH 6.5 due to insufficient mortality, but point estimates for pH 7.5 and 8.5 differed by nearly two-fold, with higher toxicity to *D. magna* at higher pH (Table 2).

### 3.2. pH dependent toxicity in laboratory cultures

Cultures were terminated on day 28 after reaching late stationary phase, and experiments were immediately performed to assess the toxicity of each replicate culture. Cells were enumerated at this time showing densities in high nutrient (*f/2*) cultures of 2.0 × 10⁵, 1.5 × 10⁵, and 2.1 × 10⁵ cells m⁻¹, and densities in low nutrient (*f/8*) cultures of 1.5 × 10⁵, 1.3 × 10⁵, and 1.5 × 10⁵ cells m⁻¹. Survival in the ASW and RHW controls was >90% for all tests at all pH levels. The LC₅₀ values for experiments with *P. promelas* were consistently lower for the low nutrient (*f/8*) treatment compared to those for high nutrient (*f/2*) (Fig. 3). Estimated LC₅₀ values were more variable between replicates for the *f/2* treatment and increased exposure time resulted in greater toxicity, whereas temporal effects were less evident for the *f/8* treatment (Fig. 3).

Samples of *f/2* and *f/8* whole cultures and cell-free filtrate were consistently more toxic to *P. promelas* when exposure occurred at pH 8.5 compared to pH 7.5 or 6.5 (Fig. 4). For the *f/2* treatment, 50% of exposed individuals died at pH 6.5 in undiluted whole culture; however, only 15% died in the cell free filtrate. Cell free filtrates were also less potent than the whole culture at pH 7.5 and 8.5 for the *f/2* treatment; however, differences in toxicity between whole cultures and cell-free filtrates were not as apparent for the *f/8* treatment (Fig. 4). The LC₅₀ values were markedly lower for filtered and unfiltered cultures grown in *f/8* media compared to those in *f/2* media; however, endpoints were consistently lower at higher pH for all experiments (Table 3).

### 3.3. Prymnesin-1 and -2 physicochemical properties

The structures of prymnesin-1 and -2 (Fig. 5) lead to an estimated pKa value of 8.9 for both prymnesin-1 and -2 (Table 4). LogD between pH 5.5 and 8.5 ranged between 2.8 and 5.2 for prymnesin-1, and 2.5 and 4.9 for prymnesin-2, respectively. At pH 6.5 approximately 16% of the prymnesins are predicted to be ionized, whereas at pH 8.5 only 0.002% are predicted to be ionized (Table 4).

### 4. Discussion

Our studies with laboratory cultures and samples from reservoirs experiencing *P. parvum* blooms consistently indicate that toxins released by *P. parvum* are more potent...
when exposure occurred at a higher pH of 8.5 than at lower pH. The predicted physiochemical properties of prymnesins indicate that these toxins are weak bases (pKa = 8.9) and, thus, a greater proportion of the prymnesins were likely unionized in higher pH treatment levels (e.g., 8.5). We propose that a higher proportion of prymnesins in unionized forms at pH 8.5 explains the greater toxicity observed in field and laboratory studies. This novel explanation for pH-dependent ambient toxicity associated with *P. parvum* suggests that variability in pH among and within aquatic systems may be an important factor governing the occurrence of fish kills.

Unionized forms of contaminants often have greater propensity to cross cellular membranes due to their lower polarity and thus are more likely to partition into organisms (Simon and Beevers, 1951; Sarrikoski et al., 1986; US EPA, 1986; Fisher et al., 1999; US EPA, 1999; Nakamura et al., 2008; Valenti et al., in press). The importance of ionization state for ambient toxicity and environmental management is evidenced by the integration of site-specific ambient water quality criteria for contaminants such as pentachlorophenol and ammonia (US EPA, 1986, 1999). Ammonia, like the prymnesins, has a pKa value of ~9. In addition, ammonia is a weak base and the ionization state of the compound changes appreciably across environmentally relevant surface water pH gradients (US EPA, 1999).

Consequently, acceptable ammonia loads in stream are 13-fold lower if the receiving system has a pH of 9 compared to a pH 6. Weak bases have a greater propensity to cross cellular membranes if the pH at which the

Table 3
The LC50 value and respective 95% confidence intervals for experiments completed with *Pimephales promelas* and cultures of *Prymnesium parvum* grown in f/2 and f/8 media that were either unfiltered or filtered to remove cells.

<table>
<thead>
<tr>
<th>Media</th>
<th>Treatment</th>
<th>pH</th>
<th>LC50 value (% media)</th>
<th>Upper and lower 95% confidence intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>f/2</td>
<td>Unfiltered</td>
<td>6.5</td>
<td>&gt;100</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.5</td>
<td>35</td>
<td>26–46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.5</td>
<td>18</td>
<td>12–25</td>
</tr>
<tr>
<td>f/2</td>
<td>Filtered</td>
<td>6.5</td>
<td>&gt;100</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.5</td>
<td>51</td>
<td>36–73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.5</td>
<td>30</td>
<td>23–40</td>
</tr>
<tr>
<td>f/8</td>
<td>Unfiltered</td>
<td>6.5</td>
<td>7</td>
<td>5–10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.5</td>
<td>1.7</td>
<td>1.4–2.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.5</td>
<td>0.7</td>
<td>0.4–1.1</td>
</tr>
<tr>
<td>f/8</td>
<td>Filtered</td>
<td>6.5</td>
<td>4.3</td>
<td>3–6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.5</td>
<td>2.6</td>
<td>1.8–3.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.5</td>
<td>2</td>
<td>1.1–2.7</td>
</tr>
</tbody>
</table>

NC = Not calculable.
* = There was insufficient mortality to generate a point estimate.

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Fig. 5. The structures of prymnesin-1 and prymnesin-2 with the hydrophobic and hydrophilic portions of each compound differentiated. The primary amine is highlighted.

exposure occurs approaches and surpasses the compound’s pKₐ value (US EPA, 1999, 1986; Simon and Beevers, 1951; Nakamura et al., 2008; Fisher et al., 1999). Greater interaction with target sites (e.g., gill membranes) would increase the likelihood of adverse effects in exposed individuals; hence, prymnesin-1 and -2 would pose greater risk to aquatic life when these toxins exist predominantly as the unionized form.

In laboratory tests examining the effectiveness of ammonium and barley straw extract to control P. parvum, Grover et al. (2007) only observed toxicity in samples with pH > 8. These observations were consistent with Lindholm et al. (1999) who observed fish kills attributed to P. parvum in a brackish-water lake when pH ranged between 8.9 and 9.4. Additional studies with P. parvum conducted under higher salinity conditions have reported similar pH influences during in vivo experiments. Shilo and Aschner (1953) noted that fish were 5-times more sensitive at pH 9 compared to pH 6, and demonstrated that the effects of pH manipulation were reversible during adjustments to and from pH 7 and 6. Ulitzur and Shilo (1964) investigated the toxicity of P. parvum toxins, along with various chemicals identified as cofactors, over a range of pH 7–9 and consistently noted markedly greater toxicity at higher pH. McLaughlin (1958) observed that high pH shortened the exposure time associated with onset of mortality. The pH-dependent activity of P. parvum toxins could also reduce internal damage to cells that are producing or storing toxins. Extracts of P. parvum induced “self-toxicity,” reducing growth rates and causing lysis (Olli and Trunov, 2007). It is plausible that these ionizable toxins are stored as the unionized form.

Table 4
The predicted physiochemical properties of prymnesin-1 and -2 based on computer modeling and hand computation.

<table>
<thead>
<tr>
<th>Property</th>
<th>Prymnesin-1</th>
<th>Prymnesin-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log P</td>
<td>6.0 ± 1.5</td>
<td>5.6 ± 1.5</td>
</tr>
<tr>
<td>Log P (hydrophobic portion)</td>
<td>7.5 ± 0.8</td>
<td>7.5 ± 0.8</td>
</tr>
<tr>
<td>Log P (hydrophilic portion)</td>
<td>−1.7 ± 1.5</td>
<td>−2.0 ± 1.5</td>
</tr>
<tr>
<td>Log D (pH 6.5)</td>
<td>3.4 ± 1.5</td>
<td>3.1 ± 1.5</td>
</tr>
<tr>
<td>Log D (pH 7.5)</td>
<td>4.3 ± 1.5</td>
<td>4.0 ± 1.5</td>
</tr>
<tr>
<td>Log D (pH 8.5)</td>
<td>5.2 ± 1.5</td>
<td>4.9 ± 1.5</td>
</tr>
<tr>
<td>% ionized (NH₃) (pH 6.5)</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>% ionized (NH₃) (pH 7.5)</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>% ionized (NH₃) (pH 8.5)</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>pKa (1-amine)</td>
<td>8.9 ± 0.1</td>
<td>8.9 ± 0.1</td>
</tr>
<tr>
<td>pKa (hydroxyl groups)</td>
<td>13–15</td>
<td>13–15</td>
</tr>
</tbody>
</table>

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inside cells of *P. parvum* at lower physiological pH, and are thus more ionized than when released outside the cell, where pH values may be higher.

Some previous results from *in vitro* hemolytic experiments with *P. parvum* contradict the results of the *in vivo* experiments reported here and elsewhere. Blood cells rupture more often when exposures are completed at pH < 6 (Igarashi et al., 1996; 1998; Kim and Padilla, 1977). Prymnesin-1 and -2 have multiple ionizable groups so that changes in the protonation state could alter the configuration of the toxins. In turn, the interaction of prymnesins with specific binding sites in blood cells and fish gill membranes could depend on the structural configuration, which may be influenced by pH, and thus may be different among such *in vivo* and *in vitro* experiments. Alternatively, prymnesins might not be the only, or even the most important toxins produced by *P. parvum*, and hemolytic activity *in vitro* might have different determinants than lethal activity *in vivo* (Schug et al., in press).

Cell density alone has been long recognized as a poor predictor of toxicity for samples containing *P. parvum* (Reich and Aschner, 1947; Baker et al., 2007; Grover et al., 2007), and this generalization remains apparent during monitoring in Texas reservoirs. Ionization state of the toxins may partially explain some of this variability and reduce uncertainty related to ecological risk assessments and risk management of *P. parvum* blooms. The observed pH-dependent toxicological relationships and the physicochemical properties predicted by computer modeling suggest that the toxins prymnesin-1 and -2 act as weak bases in aqueous solutions. Because their predicted pKa values are within the range of variation of pH in many surface waters, modest variations in pH could have a large influence on toxicity.

The production of ionizable toxins offers potential advantages to *P. parvum* and may be related to biochemical adaptations associated with its marine origins. The results of our studies and others suggest that the toxins released by *P. parvum* are more potent to gill-breathing organisms when exposure occurs at pH levels representative of those measured in marine systems (e.g. pH > 8). Moreover, blooms of *P. parvum* and other HABs can alter the environment and cause pH to increase through depletion of carbon dioxide during daytime photosynthesis (Pearl, 1988). In fish hatchery ponds impacted by *P. parvum*, pH measurements vary by more than one unit between the daylight and evening hours (Shilo and Shilo, 1953). Thus, *P. parvum* not only produces toxins during bloom formation, but could also make conditions that increase the potency of their toxins. For example, our research team recently observed high pH levels in Lake Granbury during a *P. parvum* bloom that resulted in ambient toxicity to fish, compared to lower pH levels before and after this bloom (Roelke et al. in review).

Considering site-specific pH may be especially important for ecological risk assessments of *P. parvum* because of the inherent linkage between physiochemical properties of waters and the organisms that inhabit them. There is far greater spatiotemporal variability in the pH of inland waters compared to marine systems. Some of this variability arises from natural variations in geomorphology, geochemistry, and climate. Anthropogenic activities also influence the pH of inland waters. Inland waters where *P. parvum* blooms have occurred are often affected by altered hydrology, land use changes in the catchment, and increased nutrient loading. In the southwestern and south central U.S., *P. parvum* blooms and fish kills are often limited to waters where pH is typically high due to an arid climate, limestone bedrock, and sparse vegetation. Consequently, prospective ecological risk assessment approaches may be possible for predicting the occurrence of harmful blooms of *P. parvum* by relating watershed land-use and geography to water quality.

**Uncited references**

Meldahl et al., 1994; Pratt et al., 2008.

**Acknowledgements**

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**Conflict of interest**

The authors declare that there are no conflicts of interest.

**References**


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Prymnesium parvum

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1016

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Appendix B

Sample dates and hydrology
Figure B1. Sample dates (red dots) and inflows with resulting hydraulic flushing for Lake Granbury.
Figure B2. Sample dates (red dots) and inflows with resulting hydraulic flushing for Lake Waco.
Appendix C

Locations of fixed-stations
Figure C1. Fixed station locations for Lake Granbury
Figure C2. Fixed station locations for Lake Waco
Appendix D

Lake Granbury

Fixed station data from the head and dam of the reservoir (stations 1 and 10), and deep water locations (stations 3, 5, 6, 8), i.e., over the historic river channel.

The data collection involved monthly trips over a period from September 2006 to July 2009.

Figures D-1 through D-3 - Chlorophyll a, P. parvum, toxicity
Figures D-4 through D-8 – pH, temperature, salinity, Secchi depth, turbidity
Figures D-9 through D-13 – Cladoceran, copepod adult and nauplii, total rotifers, protozoan
Figures D-14 through D-15 – Phosphahate, dissolved inorganic nitrogen
Figures D-16 through D-19 – Dissolved organic carbon, total bacteria, fecal coliform, E. coli
Figure D-20 - Cyanobacteria
Figure D1. Phytoplankton biomass approximated using chlorophyll $a$ for a period spanning September, 2006 through July, 2009 for deep water stations, i.e., over historic river channel.
Figure D2. "Prymnesium parvum" population density for a period spanning September, 2006 through July, 2009 for deep water stations, i.e., over historic river channel.
Figure D3. Ambient toxicity estimated using fish bioassays for a period spanning September, 2006 through July, 2009 for deep water stations, i.e., over historic river channel.
Figure D4. Surface water pH for a period spanning September, 2006 through July, 2009 for deep water stations, i.e., over historic river channel.
Figure D5. Surface water temperature for a period spanning September, 2006 through July, 2009 for deep water stations, i.e., over historic river channel.
Figure D6. Surface water salinity for a period spanning September, 2006 through July, 2009 for deep water stations, i.e., over historic river channel.
Figure D7. Secchi depth for a period spanning September, 2006 through July, 2009 for deep water stations, i.e., over historic river channel.
Figure D8. Surface water turbidity for a period spanning September, 2006 through July, 2009 for deep water stations, i.e., over historic river channel.
Figure D9. Total cladoceran biovolume for a period spanning September, 2006 through July, 2009 for deep water stations, i.e., over historic river channel.
Figure D10. Total copepod biovolume for a period spanning September, 2006 through July, 2009 for deep water stations, i.e., over historic river channel.
Figure D11. Total copepod nauplii biovolume for a period spanning September, 2006 through July, 2009 for deep water stations, i.e., over historic river channel.
Figure D12. Total rotifer biovolume for a period spanning September, 2006 through July, 2009 for deep water stations, i.e., over historic river channel.
Figure D13. Total protozoan biovolume for a period spanning September, 2006 through July, 2009 for deep water stations, i.e., over historic river channel.
Figure D14. Phosphorus concentration for a period spanning September, 2006 through July, 2009 for deep water stations, i.e., over historic river channel.
Figure D15. Dissolved inorganic nitrogen concentration for a period spanning September, 2006 through July, 2009 for deep water stations, i.e., over historic river channel.
Figure D16. Dissolved organic carbon concentration for a period spanning September, 2006 through July, 2009 for deep water stations, i.e., over historic river channel.
Figure D17. Total bacteria cell concentration for a period spanning September, 2006 through July, 2009 for deep water stations, i.e., over historic river channel.
Figure D18. Fecal bacteria concentration for a period spanning September, 2006 through July, 2009 for deep water stations, i.e., over historic river channel.
Figure D19. *E. coli* concentration for a period spanning September, 2006 through July, 2009 for deep water stations, i.e., over historic river channel.
Figure D20. Cyanobacteria for a period spanning September, 2006 through July, 2009 for deep water stations, i.e., over historic river channel.
Appendix E

Lake Granbury

Fixed station data from the head and dam of the reservoir (stations 1 and 10), and shallow water locations (stations 2, 4, 7, 9), i.e., submerged banks adjacent to the historic river channel.

The data collection involved monthly trips over a period from September 2006 to July 2009.

Figures E-1 through E-3 - Chlorophyll a, P. parvum, toxicity
Figures E-4 through E-8 – pH, temperature, salinity, Secchi depth, turbidity
Figures E-9 through E-13 – Cladoceran, copepod adult and nauplii, total rotifers, protozoan
Figures E-14 through E-15 – Phosphahate, dissolved inorganic nitrogen
Figures E-16 through E-19 – Dissolved organic carbon, total bacteria, fecal coliform, E. coli
Figure E-20 - Cyanobacteria
Figure E1. Phytoplankton biomass approximated using chlorophyll $a$ for a period spanning September, 2006 through July, 2009 for shallow water stations, i.e., opposite bank from historic river channel.
Figure E2. *Prymnesium parvum* population density for a period spanning September, 2006 through July, 2009 for shallow water stations, i.e., opposite bank from historic river channel.
Figure E3. Ambient toxicity estimated using fish bioassays for a period spanning September, 2006 through July, 2009 for shallow water stations, i.e., opposite bank from historic river channel.
Figure E4. Surface water pH for a period spanning September, 2006 through July, 2009 for shallow water stations, i.e., opposite bank from historic river channel.
Figure E5. Surface water temperature for a period spanning September, 2006 through July, 2009 for shallow water stations, i.e., opposite bank from historic river channel.
Figure E6. Surface water salinity for a period spanning September, 2006 through July, 2009 for shallow water stations, i.e., opposite bank from historic river channel.
Figure E7. Secchi depth for a period spanning September, 2006 through July, 2009 for shallow water stations, i.e., opposite bank from historic river channel.
Figure E8. Surface water turbidity for a period spanning September, 2006 through July, 2009 for shallow water stations, i.e., opposite bank from historic river channel.
Figure E9. Total cladoceran biovolume for a period spanning September, 2006 through July, 2009 for shallow water stations, i.e., opposite bank from historic river channel.
Figure E10. Total copepod biovolume for a period spanning September, 2006 through July, 2009 for shallow water stations, i.e., opposite bank from historic river channel.
Figure E11. Total copepod nauplii biovolume for a period spanning September, 2006 through July, 2009 for shallow water stations, i.e., opposite bank from historic river channel.
Figure E12. Total rotifer biovolume for a period spanning September, 2006 through July, 2009 for shallow water stations, i.e., opposite bank from historic river channel.
Figure E13. Total protozoan biovolume for a period spanning September, 2006 through July, 2009 for shallow water stations, i.e., opposite bank from historic river channel.
Figure E14. Phosphorus concentration for a period spanning September, 2006 through July, 2009 for shallow water stations, i.e., opposite bank from historic river channel.
Figure E15. Dissolved inorganic nitrogen concentration for a period spanning September, 2006 through July, 2009 for shallow water stations, i.e., opposite bank from historic river channel.
Figure E16. Dissolved organic carbon concentration for a period spanning September, 2006 through July, 2009 for shallow water stations, i.e., opposite bank from historic river channel.
Figure E17. Total bacteria cell concentration for a period spanning September, 2006 through July, 2009 for shallow water stations, i.e., opposite bank from historic river channel.
Figure E18. Fecal bacteria concentration for a period spanning September, 2006 through July, 2009 for shallow water stations, i.e., opposite bank from historic river channel.
Figure E19. *E. coli* concentration for a period spanning September, 2006 through July, 2009 for shallow water stations, i.e., opposite bank from historic river channel.
Figure E20. Cyanobacteria concentration for a period spanning September, 2006 through July, 2009 for shallow water stations, i.e., opposite bank from historic river channel.
Appendix F

Lake Granbury

Cove station data for Lake Granbury.

The data collection involved monthly trips over a period from November 2007 to July 2009.

F-1  - Description of cove stations
F-2, 3 -  Salinity, Temperature and Secchi depth
F-4, 5 -  NO3, NH4, and SRP
F-6, 7 -  P. parvum, Chlorophyll a, Zooplankton
F-8, 9 -  Fecal coliform, E. coli, Dissolved organic carbon
F-10, 11 -  Total bacteria
Lake Granbury Cove Stations

St. A
- Description: Dredged
- Formed Sides
- Developed
- Moderate Size

St. B
- Description: Shallow
- Rock Sides
- Limited Development
- Small Size

St. C
- Description: Shallow
- Rock Sides
- Limited Development
- Small Size

St. D
- Description: Shallow
- Rock Sides
- Limited Development
- Small Size

St. E
- Description: Shallow
- Formed Sides
- Developed
- Moderate Size
- Flooded Timber

St. F
- Description: Moderate Depth
- Developed
- Large Tributary
- Flooded Timber

St. G
- Description: Moderate Depth
- Formed Sides
- Developed
- Moderate Size

St. H
- Description: NOT A COVE
- Dredged
- Power Plant Discharge
- Deep

St. I
- Description: Shallow
- Anthropogenic Inputs
- Developed
- Moderate Size

St. J
- Description: Shallow
- Anthropogenic Inputs
- Agriculture
- Moderate Size
Lake Granbury Cove Stations

Salinity

Temperature (°C)

Secchi (m)
Lake Granbury Cove Stations

\[ \text{NO}_x (\mu \text{M}) \]

\[ \text{NH}_4^+ (\mu \text{M}) \]

\[ \text{SRP (} \mu \text{M)} \]
Lake Granbury Cove Stations

**P. Parvum (cells L⁻¹)**

**Chlorophyll-a (µg L⁻¹)**

**Zooplankton Biovolume (×10⁶ µM³ L⁻¹)**
Lake Granbury Cove Stations

*P. Parvum* (cells L\(^{-1}\))

Chlorophyll-\(a\) (µg L\(^{-1}\))

Zooplankton Biovolume (\(\times 10^6\) µM\(^3\) L\(^{-1}\))
Lake Granbury Cove Stations

Fecal Coliform (cfu 100 mL⁻¹)  

E. coli (cfu 100 mL⁻¹)  

DOC (ppm)
Lake Granbury Cove Stations

Total Bacteria (*10^6 cells mL^-1)
Lake Granbury Cove Stations

Total Bacteria (*10^6 cells mL^-1)
Appendix G

Lake Waco

Fixed station data from both heads of the reservoir, and the location of the dam (stations 1 (head), 2, 3, 4 (dam), 5, 6, 7 (head)).

The data collection involved monthly trips over a period from November 2007 to July 2009.

Figures G-1 through G-3 - Chlorophyll a, P. parvum, toxicity
Figures G-4 through G-8 – pH, temperature, salinity, Secchi depth, turbidity
Figures G-9 through G-13 – Cladoceran, copepod adult and nauplii, total rotifers, protozoan
Figures G-14 through G-15 – Phosphate, dissolved inorganic nitrogen
Figure G-16 – Dissolved organic carbon
Figure G-17 – Cyanobacteria
Figure G1. Phytoplankton biomass approximated using chlorophyll $a$ for a period spanning November, 2007 through July, 2009
Figure G2. *Prymnesium parvum* population density for a period spanning November, 2007 through July, 2009.
Figure G3. Ambient toxicity estimated using fish bioassays for a period spanning November, 2007 through July, 2009.
Figure G4. Surface water pH for a period spanning November, 2007 through July, 2009
Figure G5. Surface water temperature for a period spanning November, 2007 through July, 2009.
Figure G6. Surface water salinity for a period spanning November, 2007 through July, 2009.
Figure G7. Secchi depth for a period spanning November, 2007 through July, 2009
Figure G8. Surface water turbidity for a period spanning November, 2007 through July, 2009
Figure G9. Total cladoceran biovolume for a period spanning November, 2007 through July, 2009.
Figure G10. Total copepod biovolume for a period spanning November, 2007 through July, 2009.
Figure G11. Total copepod nauplii biovolume for a period spanning November, 2007 through July, 2009.
Figure G12. Total rotifer biovolume for a period spanning November, 2007 through July, 2009.
Figure G13. Total protozoan biovolume for a period spanning November, 2007 through July, 2009.
Figure G14. Phosphorus concentration for a period spanning November, 2007 through July, 2009.
Figure G15. Dissolved inorganic nitrogen concentration for a period spanning November, 2007 through July, 2009.
Figure G16. Dissolved organic carbon concentration for a period spanning November, 2007 through July, 2009.
Figure G17. Cyanobacteria concentration for a period spanning November, 2007 through July, 2009.
Appendix H

Dataflow maps for Lake Granbury

Monthly sampling from September 2006 through July, 2009. Note, early in the data record transects were not system wide due to troubles encountered while sampling. Months were no data flow maps are presented indicate that on-board instrument troubles prevented collection of flow-through data, or that some of the sensors in the dataflow were malfunctioning.

Figures H-01 through H-26 - Chlorophyll a
Figures H-27 through H-53 - Salinity
Figures H-54 through H-80 - Temperature
Figures H-81 through H-104 - pH
Figures H-105 through H-131 - Turbidity
Figures H-132 through H-158 - Dissolved Organic Carbon
Figure H-1. Chlorophyll $a$ dataflow map for Lake Granbury
Lake Granbury, Texas
October 18, 2006

Chl-a (ug L$^{-1}$)

Figure H-2. Chlorophyll a dataflow map for Lake Granbury
Lake Granbury, Texas
November 11, 2006

Chlorophyll - a (μg L⁻¹)

Figure H-3. Chlorophyll a dataflow map for Lake Granbury
Figure H-4. Chlorophyll $a$ dataflow map for Lake Granbury
Lake Granbury, Texas
June 2, 2007

Chl-a (ug L⁻¹)

Figure H-5. Chlorophyll a dataflow map for Lake Granbury
Lake Granbury, Texas
August 4, 2007

Chl-a (ug L⁻¹)

Figure H-6. Chlorophyll a dataflow map for Lake Granbury
Lake Granbury, Texas
September 8, 2007

Chl-a (ug L$^{-1}$)

Figure H-7. Chlorophyll $a$ dataflow map for Lake Granbury
Figure H-8. Chlorophyll $a$ dataflow map for Lake Granbury
Figure H-9. Chlorophyll $a$ dataflow map for Lake Granbury
Figure H-10. Chlorophyll $a$ dataflow map for Lake Granbury
Lake Granbury, Texas
January 15, 2008

Chl-a (ug L$^{-1}$)

Figure H-11. Chlorophyll a dataflow map for Lake Granbury
Figure H-12. Chlorophyll $a$ dataflow map for Lake Granbury
Figure H-13. Chlorophyll $a$ dataflow map for Lake Granbury
Lake Granbury, Texas
May 15, 2008

Chl-a (ug L$^{-1}$)

Figure H-14. Chlorophyll $a$ dataflow map for Lake Granbury
Lake Granbury, Texas
June 17, 2008

Chl-a (ug L$^{-1}$)

Figure H-15. Chlorophyll $a$ dataflow map for Lake Granbury
Figure H-16. Chlorophyll α dataflow map for Lake Granbury
Lake Granbury, Texas
August 16, 2008

Figure H-17. Chlorophyll $a$ dataflow map for Lake Granbury
Figure H-18. Chlorophyll a dataflow map for Lake Granbury
Lake Granbury, Texas
October 24, 2008

Chl-a (ug L $^{-1}$)

Figure H-19. Chlorophyll a dataflow map for Lake Granbury
Lake Granbury, Texas
January 17, 2009

Chl-a (ug L $^{-1}$)

Figure H-20. Chlorophyll a dataflow map for Lake Granbury
Figure H-21. Chlorophyll a dataflow map for Lake Granbury
Lake Granbury, Texas  
March 17, 2009  

Chl-a (ug L$^{-1}$)

Figure H-22. Chlorophyll a dataflow map for Lake Granbury
Figure H-23. Chlorophyll a dataflow map for Lake Granbury
Lake Granbury, Texas
May 20, 2009

Chl-a (ug L$^{-1}$)

Figure H-24. Chlorophyll $a$ dataflow map for Lake Granbury
Figure H-25. Chlorophyll a dataflow map for Lake Granbury
Lake Granbury, Texas
July 18, 2009

Chl-a (ug L$^{-1}$)

Figure H-26. Chlorophyll a dataflow map for Lake Granbury
Figure H-27. Salinity dataflow map for Lake Granbury
Lake Granbury, Texas
October 18, 2006

Salinity

Figure H-28. Salinity dataflow map for Lake Granbury
Figure H-29. Salinity dataflow map for Lake Granbury
Figure H-30. Salinity dataflow map for Lake Granbury
Lake Granbury, Texas
March 24, 2007

Salinity

Figure H-31. Salinity dataflow map for Lake Granbury
Lake Granbury, Texas
June 2, 2007

Salinity

Figure H-32. Salinity dataflow map for Lake Granbury
Lake Granbury, Texas
August 4, 2007

Salinity

Figure H-33. Salinity dataflow map for Lake Granbury
Figure H-34. Salinity dataflow map for Lake Granbury
Lake Granbury, Texas
October 20, 2007

Salinity

Figure H-35. Salinity dataflow map for Lake Granbury
Lake Granbury, Texas
November 13, 2007

Salinity

Figure H-36. Salinity dataflow map for Lake Granbury
Lake Granbury, Texas
December 11, 2007

Salinity

Figure H-37. Salinity dataflow map for Lake Granbury
Lake Granbury, Texas
January 15, 2008

Salinity

Figure H-38. Salinity dataflow map for Lake Granbury
Lake Granbury, Texas
February 12, 2008

Salinity

Figure H-39. Salinity dataflow map for Lake Granbury
Figure H-40. Salinity dataflow map for Lake Granbury
Lake Granbury, Texas
May 15, 2008

Figure H-41. Salinity dataflow map for Lake Granbury
Lake Granbury, Texas
June 17, 2008

Salinity

Figure H-42. Salinity dataflow map for Lake Granbury
Figure H-43. Salinity dataflow map for Lake Granbury
Figure H-44. Salinity dataflow map for Lake Granbury
Lake Granbury, Texas
September 19, 2008

Salinity

Figure H-45. Salinity dataflow map for Lake Granbury
Lake Granbury, Texas
October 24, 2008

Salinity

Figure H-46. Salinity dataflow map for Lake Granbury
Lake Granbury, Texas
January 17, 2009

Salinity

Figure H-47. Salinity dataflow map for Lake Granbury
Figure H-48. Salinity dataflow map for Lake Granbury
Figure H-49. Salinity dataflow map for Lake Granbury
Figure H-50. Salinity dataflow map for Lake Granbury
Figure H-51. Salinity dataflow map for Lake Granbury
Lake Granbury, Texas
June 13, 2009

Salinity

Figure H-52. Salinity dataflow map for Lake Granbury
Lake Granbury, Texas
July 18, 2009

Salinity

Figure H-53. Salinity dataflow map for Lake Granbury
Figure H-54. Temperature dataflow map for Lake Granbury
Lake Granbury, Texas
October 18, 2006

Temperature (°C)

Figure H-55. Temperature dataflow map for Lake Granbury
Lake Granbury, Texas
November 11, 2006

Temperature (°C)

Figure H-56. Temperature dataflow map for Lake Granbury
Lake Granbury, Texas
February 21, 2007

Temperature (°C)

Figure H-57. Temperature dataflow map for Lake Granbury
Lake Granbury, Texas
March 24, 2007

Temperature (°C)

Figure H-58. Temperature dataflow map for Lake Granbury
Lake Granbury, Texas
June 2, 2007

Temperature (°C)

Figure H-59. Temperature dataflow map for Lake Granbury
Lake Granbury, Texas
August 4, 2007

Temperature (°C)

Figure H-60. Temperature dataflow map for Lake Granbury
Lake Granbury, Texas
September 8, 2007

Temperature (°C)

Figure H-61. Temperature dataflow map for Lake Granbury
Lake Granbury, Texas
October 8, 2007

Temperature (°C)

Figure H-62. Temperature dataflow map for Lake Granbury
Lake Granbury, Texas
November 13, 2007

Temperature (°C)

Figure H-63. Temperature dataflow map for Lake Granbury
Lake Granbury, Texas
December 11, 2007

Temperature (°C)

Figure H-64. Temperature dataflow map for Lake Granbury
Lake Granbury, Texas
January 15, 2008

Temperature (°C)

Figure H-65. Temperature dataflow map for Lake Granbury
Lake Granbury, Texas
February 12, 2008

Temperature (°C)

Figure H-66. Temperature dataflow map for Lake Granbury
Lake Granbury, Texas
April 24, 2008

Temperature (°C)

Figure H-67. Temperature dataflow map for Lake Granbury
Lake Granbury, Texas
May 15, 2008

Temperature (°C)

Figure H-68. Temperature dataflow map for Lake Granbury
Lake Granbury, Texas  
June 17, 2008  

Temperature (°C)  

Figure H-69. Temperature dataflow map for Lake Granbury.
Lake Granbury, Texas
July 18, 2008

Temperature (°C)

Figure H-70. Temperature dataflow map for Lake Granbury
Figure H-71. Temperature dataflow map for Lake Granbury
Lake Granbury, Texas
September 19, 2008

Temperature (°C)

Figure H-72. Temperature dataflow map for Lake Granbury
Lake Granbury, Texas
October 24, 2008

Temperature (°C)

Figure H-73. Temperature dataflow map for Lake Granbury
Lake Granbury, Texas
January 17, 2009

Temperature (°C)

Figure H-74. Temperature dataflow map for Lake Granbury
Lake Granbury, Texas
February 14, 2009

Temperature (°C)

Figure H-75. Temperature dataflow map for Lake Granbury
Figure H-76. Temperature dataflow map for Lake Granbury
Figure H-77. Temperature dataflow map for Lake Granbury
Lake Granbury, Texas
May 20, 2009

Temperature (°C)

Figure H-78. Temperature dataflow map for Lake Granbury
Lake Granbury, Texas
June 13, 2009

Temperature (°C)

Figure H-79. Temperature dataflow map for Lake Granbury
Figure H-80. Temperature dataflow map for Lake Granbury
Lake Granbury, Texas
February 21, 2007

Figure H-81. pH dataflow map for Lake Granbury
Lake Granbury, Texas
March 24, 2007

Figure H-82. pH dataflow map for Lake Granbury
Lake Granbury, Texas
June 2, 2007

Figure H-83. pH dataflow map for Lake Granbury
Lake Granbury, Texas
August 4, 2007

Figure H-84. pH dataflow map for Lake Granbury
Figure H-85. pH dataflow map for Lake Granbury
Lake Granbury, Texas
October 20, 2007

pH

Figure H-86. pH dataflow map for Lake Granbury
Figure H-87. pH dataflow map for Lake Granbury
Lake Granbury, Texas
December 11, 2007

Figure H-88. pH dataflow map for Lake Granbury
Figure H-89. pH dataflow map for Lake Granbury
Lake Granbury, Texas
February 12, 2008

Figure H-90. pH dataflow map for Lake Granbury
Figure H-91. pH dataflow map for Lake Granbury
Lake Granbury, Texas
May 15, 2008

Figure H-92. pH dataflow map for Lake Granbury
Figure H-93. pH dataflow map for Lake Granbury
Figure H-94. pH dataflow map for Lake Granbury
Lake Granbury, Texas
August 16, 2008

Figure H-95. pH dataflow map for Lake Granbury
Lake Granbury, Texas
September 19, 2008

Figure H-96. pH dataflow map for Lake Granbury
Figure H-97. pH dataflow map for Lake Granbury
Lake Granbury, Texas
January 17, 2009

pH

Figure H-98. pH dataflow map for Lake Granbury
Figure H-99. pH dataflow map for Lake Granbury
Lake Granbury, Texas
March 17, 2009

Figure H-100. pH dataflow map for Lake Granbury
Lake Granbury, Texas
April 25, 2009

Figure H-101. pH dataflow map for Lake Granbury
Lake Granbury, Texas
May 20, 2009

Figure H-102. pH dataflow map for Lake Granbury
Figure H-103. pH dataflow map for Lake Granbury
Figure H-104. pH dataflow map for Lake Granbury
Lake Granbury, Texas
February 21, 2007

Figure H-81. pH dataflow map for Lake Granbury
Lake Granbury, Texas
March 24, 2007

Figure H-82. pH dataflow map for Lake Granbury
Lake Granbury, Texas
June 2, 2007

Figure H-83. pH dataflow map for Lake Granbury
Figure H-84. pH dataflow map for Lake Granbury.
Lake Granbury, Texas
September 8, 2007

pH

Figure H-85. pH dataflow map for Lake Granbury
Lake Granbury, Texas
October 20, 2007

Figure H-86. pH dataflow map for Lake Granbury
Figure H-87. pH dataflow map for Lake Granbury
Lake Granbury, Texas
December 11, 2007

Figure H-88. pH dataflow map for Lake Granbury
Lake Granbury, Texas
January 15, 2008

pH

Figure H-89. pH dataflow map for Lake Granbury
Figure H-90. pH dataflow map for Lake Granbury
Figure H-91. pH dataflow map for Lake Granbury
Figure H-92. pH dataflow map for Lake Granbury
Lake Granbury, Texas
June 17, 2008

Figure H-93. pH dataflow map for Lake Granbury
Lake Granbury, Texas
July 18, 2008

pH

Figure H-94. pH dataflow map for Lake Granbury
Figure H-95. pH dataflow map for Lake Granbury
Figure H-96. pH dataflow map for Lake Granbury
Figure H-97. pH dataflow map for Lake Granbury
Figure H-98. pH dataflow map for Lake Granbury
Figure H-99. pH dataflow map for Lake Granbury.
Lake Granbury, Texas
March 17, 2009

Figure H-100. pH dataflow map for Lake Granbury
Lake Granbury, Texas
April 25, 2009

Figure H-101. pH dataflow map for Lake Granbury
Lake Granbury, Texas
May 20, 2009

Figure H-102. pH dataflow map for Lake Granbury
Lake Granbury, Texas
June 13, 2009

Figure H-103. pH dataflow map for Lake Granbury
Lake Granbury, Texas
July 18, 2009

pH

Figure H-104. pH dataflow map for Lake Granbury
Figure H-132. Dissolved organic matter dataflow map for Lake Granbury
Figure H-133. Dissolved organic matter dataflow map for Lake Granbury
Figure H-134. Dissolved organic matter dataflow map for Lake Granbury

Lake Granbury, Texas
November 11, 2006
Lake Granbury, Texas
February 21, 2007

FDOM (ppb)

Figure H-135. Dissolved organic matter dataflow map for Lake Granbury
Figure H-136. Dissolved organic matter dataflow map for Lake Granbury
Figure H-137. Dissolved organic matter dataflow map for Lake Granbury.
Lake Granbury, Texas
August 4, 2007

Figure H-138. Dissolved organic matter dataflow map for Lake Granbury
Lake Granbury, Texas
September 19, 2008

Figure H-139. Dissolved organic matter dataflow map for Lake Granbury
Lake Granbury, Texas
October 20, 2007

Figure H-140. Dissolved organic matter dataflow map for Lake Granbury
Lake Granbury, Texas
November 13, 2007

Figure H-141. Dissolved organic matter dataflow map for Lake Granbury
Figure H-142. Dissolved organic matter dataflow map for Lake Granbury
Lake Granbury, Texas
January 15, 2008

Figure H-143. Dissolved organic matter dataflow map for Lake Granbury
Figure H-144. Dissolved organic matter dataflow map for Lake Granbury
Figure H-145. Dissolved organic matter dataflow map for Lake Granbury
Lake Granbury, Texas
May 15, 2008

FDOM (ppb)

Figure H-146. Dissolved organic matter dataflow map for Lake Granbury
Figure H-147. Dissolved organic matter dataflow map for Lake Granbury
Figure H-148. Dissolved organic matter dataflow map for Lake Granbury
Figure H-149. Dissolved organic matter dataflow map for Lake Granbury
Figure H-150. Dissolved organic matter dataflow map for Lake Granbury
Figure H-151. Dissolved organic matter dataflow map for Lake Granbury
Figure H-152. Dissolved organic matter dataflow map for Lake Granbury
Figure H-153. Dissolved organic matter dataflow map for Lake Granbury
Figure H-154. Dissolved organic matter dataflow map for Lake Granbury
Figure H-155. Dissolved organic matter dataflow map for Lake Granbury
Lake Granbury, Texas
May 20, 2009

FDOM (ppb)

Figure H-156. Dissolved organic matter dataflow map for Lake Granbury
Lake Granbury, Texas
June 13, 2009

Figure H-157. Dissolved organic matter dataflow map for Lake Granbury
Figure H-158. Dissolved organic matter dataflow map for Lake Granbury
Appendix I

Dataflow maps for Lake Waco


Figures I-1 through I-17 - Chlorophyll $a$
Figures I-18 through I-34 - Salinity
Figures I-35 through I-51 - Temperature
Figures I-52 through I-68 - pH
Figures I-69 through I-85 - Turbidity
Figures I-86 through I-102 - Dissolved Organic Carbon
Figure I-1. Chlorophyll a dataflow map for Lake Waco
Lake Waco, Texas
December 12, 2007

Chl-a (ug L$^{-1}$ )

Figure I-2. Chlorophyll a dataflow map for Lake Waco
Figure I-3. Chlorophyll a dataflow map for Lake Waco

Lake Waco, Texas
January 16, 2008

Chl-a (ug L$^{-1}$)
Figure I-4. Chlorophyll $a$ dataflow map for Lake Waco
Figure I-5. Chlorophyll a dataflow map for Lake Waco
Figure I-6. Chlorophyll a dataflow map for Lake Waco
Figure I-7. Chlorophyll $a$ dataflow map for Lake Waco
Lake Waco, Texas
July 16, 2008

Chl-a (ug L$^{-1}$)

Figure I-8. Chlorophyll $a$ dataflow map for Lake Waco
Figure I-9. Chlorophyll a dataflow map for Lake Waco
Figure I-10. Chlorophyll $a$ dataflow map for Lake Waco
Lake Waco, Texas
January 18, 2009

Chl-a (μg L\(^{-1}\))

Figure I-11. Chlorophyll \(a\) dataflow map for Lake Waco
Lake Waco, Texas
February 15, 2009

Chl-a (ug L$^{-1}$)

Figure I-12. Chlorophyll a dataflow map for Lake Waco
Figure I-13. Chlorophyll a dataflow map for Lake Waco
Lake Waco, Texas
April 26, 2009

Chl-a (ug L$^{-1}$)

Figure I-14. Chlorophyll a dataflow map for Lake Waco
Lake Waco, Texas
May 21, 2009

Chl-a (ug L$^{-1}$)

Figure I-15. Chlorophyll $a$ dataflow map for Lake Waco
Figure I-16. Chlorophyll $a$ dataflow map for Lake Waco
Lake Waco, Texas
July 19, 2009

Chl-a (ug L⁻¹)

Figure I-17. Chlorophyll a dataflow map for Lake Waco
Lake Waco, Texas
November 14, 2007

Salinity

Figure I-18. Salinity dataflow map for Lake Waco
Lake Waco, Texas
December 12, 2007

Salinity

Figure I-19. Salinity dataflow map for Lake Waco
Lake Waco, Texas
January 16, 2008

Salinity

Figure I-20. Salinity dataflow map for Lake Waco
Figure I-21. Salinity dataflow map for Lake Waco
Lake Waco, Texas
April 23, 2008

Salinity

Figure I-22. Salinity dataflow map for Lake Waco
Figure I-23. Salinity dataflow map for Lake Waco
Figure I-24. Salinity dataflow map for Lake Waco
Figure I-25. Salinity dataflow map for Lake Waco
Figure I-26. Salinity dataflow map for Lake Waco

Lake Waco, Texas
August 17, 2008

Salinity
Figure I-27. Salinity dataflow map for Lake Waco

Lake Waco, Texas
September 20, 2008

Salinity

Longitude

Latitude

0.15
0.1
Lake Waco, Texas
January 18, 2009

Salinity

Figure I-28. Salinity dataflow map for Lake Waco
Figure I-29. Salinity dataflow map for Lake Waco
Figure I-30. Salinity dataflow map for Lake Waco
Figure I-31. Salinity dataflow map for Lake Waco
Figure I-32. Salinity dataflow map for Lake Waco

Lake Waco, Texas  
May 21, 2009
Figure I-33. Salinity dataflow map for Lake Waco
Figure I-34. Salinity dataflow map for Lake Waco
Lake Waco, Texas
November 14, 2007

Temperature (°C)

Figure I-35. Temperature dataflow map for Lake Waco
Lake Waco, Texas
December 12, 2007

Temperature (°C)

Figure I-36. Temperature dataflow map for Lake Waco
Lake Waco, Texas
January 16, 2008

Temperature (°C)

Figure I-37. Temperature dataflow map for Lake Waco
Figure I-38. Temperature dataflow map for Lake Waco
Figure I-39. Temperature dataflow map for Lake Waco
Figure I-40. Temperature dataflow map for Lake Waco
Lake Waco, Texas
June 16, 2008

Temperature (°C)

Figure I-41. Temperature dataflow map for Lake Waco
Lake Waco, Texas
July 18, 2008

Temperature (°C)

Figure I-42. Temperature dataflow map for Lake Waco
Figure I-43. Temperature dataflow map for Lake Waco
Figure I-44. Temperature dataflow map for Lake Waco
Figure I-45. Temperature dataflow map for Lake Waco
Lake Waco, Texas
February 15, 2009

Temperature (°C)

Figure I-46. Temperature dataflow map for Lake Waco
Figure I-47. Temperature dataflow map for Lake Waco
Figure I-48. Temperature dataflow map for Lake Waco
Figure I-49. Temperature dataflow map for Lake Waco
Figure I-50. Temperature dataflow map for Lake Waco
Lake Waco, Texas
July 19, 2009

Temperature (°C)

Figure I-51. Temperature dataflow map for Lake Waco
Figure I-52. pH dataflow map for Lake Waco
Figure I-53. pH dataflow map for Lake Waco
Figure I-54. pH dataflow map for Lake Waco
Figure I-55. pH dataflow map for Lake Waco
Lake Waco, Texas
April 23, 2008

pH

Figure I-56. pH dataflow map for Lake Waco
Figure I-57. pH dataflow map for Lake Waco
Figure I-58. pH dataflow map for Lake Waco
Figure I-59. pH dataflow map for Lake Waco
Figure I-60. pH dataflow map for Lake Waco
Lake Waco, Texas
September 20, 2008

pH

Figure I-61. pH dataflow map for Lake Waco
Figure I-62. pH dataflow map for Lake Waco
Figure I-63. pH dataflow map for Lake Waco
Figure I-64. pH dataflow map for Lake Waco
Lake Waco, Texas
April 26, 2009

pH

Figure I-65. pH dataflow map for Lake Waco
Figure I-66. pH dataflow map for Lake Waco
Figure I-67. pH dataflow map for Lake Waco
Figure I-68. pH dataflow map for Lake Waco

Lake Waco, Texas
July 19, 2009

pH

Latitude

Longitude

Figure I-68. pH dataflow map for Lake Waco
Lake Waco, Texas
November 14, 2007

Turbidity (% Transmittance)

Figure I-69. Turbidity dataflow map for Lake Waco
Lake Waco, Texas
December 12, 2007

Turbidity (% Transmittance)

Figure I-70. Turbidity dataflow map for Lake Waco
Lake Waco, Texas
January 16, 2008

Turbidity (% Transmittance)

Figure I-71. Turbidity dataflow map for Lake Waco
Lake Waco, Texas
February 15, 2009

Turbidity (% Transmittance)

Figure I-72. Turbidity dataflow map for Lake Waco
Figure I-73. Turbidity dataflow map for Lake Waco
Lake Waco, Texas
May 13, 2008

Turbidity (% Transmittance)

Figure I-74. Turbidity dataflow map for Lake Waco
Figure I-75. Turbidity dataflow map for Lake Waco
Figure I-76. Turbidity dataflow map for Lake Waco
Figure I-77. Turbidity dataflow map for Lake Waco
Lake Waco, Texas
September 20, 2008

Turbidity (% Transmittance)

Figure I-78. Turbidity dataflow map for Lake Waco
Lake Waco, Texas
January 18, 2009

Turbidity (% Transmittance)

Figure I-79. Turbidity dataflow map for Lake Waco
Lake Waco, Texas
February 15, 2009

Turbidity (% Transmittance)

Figure I-80. Turbidity dataflow map for Lake Waco
Lake Waco, Texas
March 18, 2009

Turbidity (% Transmittance)

Figure I-81. Turbidity dataflow map for Lake Waco
Lake Waco, Texas
April 26, 2009

Turbidity (% Transmittance)

Figure I-82. Turbidity dataflow map for Lake Waco
Lake Waco, Texas
May 21, 2009

Turbidity (% Transmittance)

Figure I-83. Turbidity dataflow map for Lake Waco
Figure I-84. Turbidity dataflow map for Lake Waco
Lake Waco, Texas
July 19, 2009

Turbidity (% Transmittance)

Figure I-85. Turbidity dataflow map for Lake Waco
Figure I-86. Dissolved organic matter dataflow map for Lake Waco
Figure I-87. Dissolved organic matter dataflow map for Lake Waco.
Figure I-88. Dissolved organic matter dataflow map for Lake Waco
Lake Waco, Texas
February 13, 2008

FDOM (ppb)

Figure I-89. Dissolved organic matter dataflow map for Lake Waco
Lake Waco, Texas
April 23, 2008

FDOM (ppb)

Figure I-90. Dissolved organic matter dataflow map for Lake Waco
Lake Waco, Texas
May 13, 2008

FDOM (ppb)

Figure I-91. Dissolved organic matter dataflow map for Lake Waco
Lake Waco, Texas
June 16, 2008

FDOM (ppb)

Figure I-92. Dissolved organic matter dataflow map for Lake Waco
Figure I-93. Dissolved organic matter dataflow map for Lake Waco
Figure I-94. Dissolved organic matter dataflow map for Lake Waco
Figure I-95. Dissolved organic matter dataflow map for Lake Waco
Figure I-96. Dissolved organic matter dataflow map for Lake Waco
Figure I-97. Dissolved organic matter dataflow map for Lake Waco
Lake Waco, Texas
March 18, 2009

FDOM (ppb)

Figure I-98. Dissolved organic matter dataflow map for Lake Waco
Figure I-99. Dissolved organic matter dataflow map for Lake Waco
Lake Waco, Texas
May 21, 2009

FDOM (ppb)

Figure I-100. Dissolved organic matter dataflow map for Lake Waco
Figure I-101. Dissolved organic matter dataflow map for Lake Waco
Figure I-102. Dissolved organic matter dataflow map for Lake Waco