# INHIBITION AND SUCCESS OF *PRYMNESIUM PARVUM* INVASION ON PLANKTON COMMUNITIES IN TEXAS, USA

# AND

# *PRYMNESIUM PARVUM* PIGMENT DYNAMICS

A Thesis

by

REAGAN MICHELLE ERRERA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2006

Major Subject: Wildlife and Fisheries Sciences

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## ABSTRACT

Inhibition and Success of *Prymnesium parvum* Invasion on Plankton Communities in Texas, USA and *Prymnesium parvum* Pigment Dynamics.

(May 2006)

Reagan Michelle Errera, B.S., Trinity University

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*Prymnesium parvum* Carter, a haptophyte species capable of forming harmful algal blooms (HABs), has been identified in fresh and brackish water habitats worldwide. In Texas, *P. parvum* blooms have diminished local community revenues from losses to tourism, fishing, and hatchery production. In this thesis, *P. parvum* dynamics were studied using *in-situ* microcosm experiments at Lake Possum Kingdom, Texas during three seasons (fall, winter, spring) in 2004-2005. Specifically, nutrient additions were used to test the hypothesis that increased nutrient levels would not enhance *P. parvum*'s ability to invade phytoplankton communities. In addition to full nutrient additions to levels of f/2 media, other treatments included nutrient additions deficient in either nitrogen (N) or phosphorus (P). Additionally, barley straw extract was tested as a growth inhibitor to prevent *P. parvum* blooms. Furthermore, *P. parvum* initial population density was examined to test the hypothesis that increased initial populations could promote an increase in *P. parvum* population densities.

Findings indicated that *P. parvum* populations in Lake Possum Kingdom would not likely gain a selective advantage over other species when inorganic nutrients (nitrogen and phosphorus) were not limiting. *P. parvum* did, however, gain an advantage during both N- and P-limited conditions as indicated by toxicity, cell concentrations, and bulk phytoplankton community shifts. Furthermore, *P. parvum* blooms in Lake Possum Kingdom would likely not be inhibited by barley straw extract application. Initial population densities affected the final population density, but only when initial populations were low.

A method to quickly and accurately detect the presence of *P. parvum* is needed due to *P. parvum*'s potential to cause toxic and lethal blooms. This thesis tested whether *P. parvum* photopigments are conservative regardless of growth conditions and could be used to quantify the relative abundance of *P. parvum* in mixed community samples. If biomarker pigments are conservative, then an optimized version of CHEMTAX could be employed as an alternative diagnostic tool to microscopy for enumeration of *P. parvum*. However, *P. parvum* pigments in the Texas strain were not conservative throughout the growth cycle and therefore may not be a reliable indicator of cell abundance.

## ACKNOWLEDGEMENTS

To Dan Roelke, from the start you were willing to take a risk on my abilities and achievements, for that I am truly grateful. Your guidance and support has been key in launching my career.

To Richard Kiesling, thank you for the support in the field (especially with equipment) and out, you have helped launch my insight into aquatic systems. Jay Pinckney, thank you for the countless hours of guidance on the photopigments, HPLC, and statistics. Kirk Winemiller, thank you for understanding during my times in the field and for your support. It was an honor to work and learn from my entire committee.

Leslie Schwierzke, Carrie Miller, Jose Montoya, Elizabeth Herbert, Erin Li, and George Gable; the most supportive lab in Old Heep! Thank you for your support both in the field and in the lab. Leslie, without your assistance and encouragement in the field and your efficiency and dedication in the lab I would still be processing samples today. To all my friends, thank you for your support and countless hours of listening, reading, and knowing when it was time to leave the lab and go out for a drink.

To Fabiola Ureña-Montoya and Bryan Brooks for performing the toxicity testing. Marcus Gary, thank you for developing a new way to fill up the limnocorrals and for your help in the field. Thank you, Jeff Nichols, the marina owner at the Possum Kingdom State Park, for the use of the boat slip.

To my family, each member new and old has always supported me in my silly fascination with the oceanic and aquatic ecosystems. I still remember my first times fishing off Mather's Bridge with my brothers and father; I had to play with each shrimp

properly before it could be placed on a hook. Without these types of memories and experience from my childhood, the appreciation of coastal and aquatic systems I gained growing up in Florida, and the support of my family I would have not pursued this course of education.

This research was funded and supported by Texas Parks and Wildlife.

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# CHAPTER I

# INTRODUCTION

## 1. Introduction

Incidences of harmful algal blooms (HABs) have increased in frequency, duration, and severity worldwide (Smayda, 1990; Hallegraeff, 1993). Blooms of harmful and nuisance species have been reported in coastal and inland waters, and observed in fresh and saltwater environments. Formation of HABs is a result of select biological, physical, and chemical conditions, which are achieved through a number of interacting phenomena (Roelke and Buyukates, 2001). These conditions can vary greatly for different HAB species. Consequently, predicting the initiation of blooms is difficult. The causes for this apparent global expansion of the frequency and duration of bloom outbreaks are unknown; however, human alteration of water quality is believed to be a contributing factor (Hallegraeff, 1993).

HABs have a direct impact on surrounding ecosystems. One impact is the production of toxins by HAB species (Hallegraeff, 1993; Harvell et al., 1999, Van Dolah, 2000; Van Dolah et al., 2001). In many cases, these toxins accumulate in the food web and affect human health when contaminated commercial species are involved. For example, brevitoxins produced by the dinoflagellate *Karenia brevis* can accumulate in the tissues of shellfish. When contaminated shellfish are consumed by humans, the toxins can cause neurotoxic shellfish poisoning (NSP) (Steidinger et al., 1998, p. 133).

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This thesis follows the journal style of Harmful Algae.

HABs can directly affect natural resources and local economies. For instance, an outbreak of *Pfiesteria piscicida* in Maryland caused an estimated $43 million loss in seafood sales for the region (Lipton, 1998).

A newly investigated HAB species *Prymnesium parvum* Carter, a haptophyte, occurs worldwide. This species was first identified by scientists in the late 1930s in Denmark and Holland where it was responsible for numerous fish kills in coastal waters (Shilo & Aschner, 1953; McLaughlin, 1958). Today *P. parvum* blooms have been identified in fresh and brackish-water environments from New South Wales, Australia to Texas, USA (Edvardsen and Paasche, 1998). The occurrences of the blooms have increased the need for water treatment plants to treat water from reservoirs, and have diminished local community revenues from lost tourism, fishing, and hatchery production.

*P. parvum* is able to produce an array of toxins (ichthyotoxic, neurotoxic, cytotoxic, hepatoxic, and hemolytic compounds) (Igarashi et al., 1996, 1999). The toxins are released into the water column yielding an assortment of impairments (Ulitzer and Shilo, 1966; Edvardsen and Paasche, 1998, Igarashi et al; 1996, 1999) that affect fish, heterotrophic dinoflagellates, bacteria, phytoplankton and ciliates (Nygaard and Tobiesen, 1993; Tillmann, 2003; Fistarol et al., 2003; Granéli & Johansson, 2003a, b; Rosetta and McManus, 2003; Barreiro et al., 2005; Uronen et al., 2005). The toxins are especially devastating to fish because the hemolytic effects cause the cells located in the

gills to lyse, followed by mortality due to blood loss. To date there have been no reports of negative health effects of *P. parvum* toxins to humans.

In 1985, the state of Texas officially confirmed a *P. parvum* bloom along the Pecos River (TPWD, 2003). Since the initial identification, *P. parvum* blooms have affected 19 reservoirs along five river basins in Texas (TPWD, 2003) with bloom occurrences appearing to be associated with brackish water (1 – 5 mS/C). Due to *P. parvum*'s ability to produce dense blooms in a wide range of environmental conditions, it has caused extensive damage to local economies. The Lake Possum Kingdom Chamber of Commerce estimated economic losses of $18 to $20 million due to *P. parvum* blooms from 1998 to 2001 (TPWD, 2002). Additionally, *P. parvum* blooms have resulted in fish kills exceeding 17.5 million fish in Texas since 1985 (TPWD, 2003).

Eutrophication of local water bodies may explain, in part, the increase in frequency and intensity of *P. parvum* blooms in Texas reservoirs. Texas continues to experience high levels of urban and suburban development, resulting in increased clearing of trees, and the application of fertilizers to the landscape. Cultural eutrophication of the surrounding water systems occurs with runoff and can stimulate harmful algal blooms (Hallegraeff, 1993). An increase in nutrient loading can lead to a shift in species compositions and in doing so; destabilize plankton communities (Riegman, 1998). These events may be beneficial to *P. parvum* growth and promote bloom initiation.

Another theory suggests that during times of nutrient limitation *P. parvum* is able to dominate phytoplankton communities (Granéli and Johnasson, 2003a). Several studies have shown that limiting nitrogen or phosphorus enhances the toxic effects of *P. parvum*, thereby providing *P. parvum* with the ability to compete for limited nutrients. The toxins (alleopathy) could shift the phytoplankton community composition to one in which *P. parvum* is the superior competitor (Johnasson and Granéli, 1999). Granéli and Johnasson (2003a) demonstrated this phenomenon by introducing cell-free filtrates of *P. parvum* culture grown under nitrogen and phosphorus deficient conditions to cultures of *Thalassiosira weissflogii*, *Procentrum minimum* and *Rhodomonas cf. baltica*. In these experiments, the three phytoplankton species experienced a rapid decline in cell abundances.

More specifically, some of the toxins have the capability to act as allelopathic substances that inhibit growth of competing phytoplankton or deter potential predators. In a study preformed by Fistarol et al. (2003), cell-free filtrate of *P. parvum* grown under nutrient sufficient conditions was added to cultures of cyanobacteria, dinoflagellates, nanoflagellates, and diatoms isolated from the Baltic Sea. This resulted in a decrease in cell numbers of the phytoplankton groups. The effect of toxins produced by *P. parvum* is not limited to competitors. They also negatively affect potential predators. For instance, feeding by *Euplotes affinis*, a ciliate, ceased after it was exposed to *P. parvum* (Granéli and Johansson, 2003b). Furthermore, when offered an alternative prey, *E. affinis* consumption of the prey decreased when *P. parvum* was present. In the same study, they also demonstrated that survival of *E. affinis* decreased

when exposed to *P. parvum* limited by either N- or P-, suggesting that toxin production was enhanced when *P. parvum* became nutrient limited.

The devastation caused by *P. parvum* to Texas' rivers, reservoirs, and fish hatcheries has resulted in the need to develop strategies for mitigating the effects of blooms. A potential mitigation strategy involves the use of barley straw extract (BSE). Field trials and laboratory experiments suggested that BSE was effective in suppressing some phytoplankton taxa, including cyanobacteria, diatoms and chlorophytes (Gibson et al, 1990; Newman and Barrett, 1993; Everall and Lees, 1996; Ridge et al., 1999; Barrett et al., 1999). However, algal species exhibited a range of sensitivities to BSE. For example, Martin and Ridge (1999) demonstrated that the cyanobacterium *Anabaena flos-aquae* experienced inhibition by 50% when treated with 371 g $m^{-3}$ of barley straw, but another cyanobacterium, *Anabaena cylindrical,* was resistant to the putative inhibitors.

The mechanism by which BSE affected phytoplankton involved leaching of growth inhibitors (oxidized polyphenolics) (Gibson et al., 1990; Ridge et al., 1995, 1999). It was essential that the BSE experienced oxidation to effectively inhibit algae growth (Gibson et al., 1990, Martin and Ridge, 1999). The advantage of using BSE as a control substance is that there was no apparent effect on macrophytes and other aquatic life (Everall and Lees, 1996; Ridge et al., 1999); Everall and Lees (1996) reported that barley straw had no effect on living invertebrates, rotifer communities, or nesting waterfowl. Furthermore, in preliminary studies of artificial ponds, barley straw did not affect trout fisheries (Ridge et al., 1999).

Based on the conflicting theories regarding the mechanisms that enhance *P. parvum* competitive abilities, I investigated *P. parvum* demographics. Specifically, I examined the role of nutrient availability in *P. parvum* population dynamics, and the use of BSE as a potential growth inhibitor. I tested whether different nutrient conditions and BSE would select against *P. parvum*. I also manipulated the initial population density of *P. parvum* to test its potential effect on population dynamics.

Research and monitoring related to *P. parvum* has increased dramatically in Texas over the past five years. A method to quickly and accurately detect the presence of *P. parvum* is needed. Enumeration by microscopy is accurate (Jordon et al., 1994), but also time-consuming (Millie et al., 1993). Consequently, the number of samples that can be counted using a microscope is limited (Wilhelm and Manns, 1991). In addition, personnel must also be trained by a taxonomist to identify *P. parvum* cells within mixed assemblages of phytoplankton. Microscopic identification of *P. parvum* is difficult due to its similarity with other haptophytes and chrysophytes, and *P. parvum* cell size and shape vary.

An alternative to microscopy is the use of photosynthetic pigment profiles as chemotaxonomic markers of phytoplankton groups (Jeffery et al., 1997; Zapata et al., 2004). Individual phytoplankton groups have characteristic pigments that may be used as chemotaxonomic biomarkers (Gieskes and Kraay, 1983; Bidgare et al., 1990; Althuis et al.; 1994, Schübert et al., 2000). CHEMTAX (CHEMical TAXonomy) is a matrix factorization program that enables the user to estimate the abundances of major algal groups using photopigment biomarkers (Mackey et al., 1996; 1997; Wright et al., 1996,

Wright and van den Enden, 2000). Briefly, the program uses an initial estimate of pigment ratios for targeted algal classes and a steepest descent algorithm that determines the "best fit" of the unknown sample to known pigment ratios of algal groups. Input for the program consists of the photopigment concentrations obtained by high performance liquid chromatography HPLC analyses.

CHEMTAX can be optimized for better performance in targeted systems by adjusting the initial estimates of pigment ratios to algal groups that are more taxonomically defined. Therefore, I also studied *P. parvum* photopigment dynamics in an attempt to optimize CHEMTAX for estimates of the relative abundance of *P. parvum*. I assumed that if pigment ratios were conservative throughout different stages of growth, an optimized version of CHEMTAX could be employed as an alternative diagnostic tool to microscopy.

## 2. Objectives

In this research the *overarching objective* is to further the understanding of *P. parvum* bloom dynamics and toxicity in Lake Possum Kingdom, Texas. Specifically, this research focused on the relative roles of inorganic nutrients, the efficacy of barley straw extract as a mitigation tool, and initial population densities in formation bloom densities. In addition, this research investigated if CHEMTAX could be optimized for estimation of *P. parvum* relative abundance in terms of total phytoplankton biomass (Chl *a*).

## 3. Hypotheses

The primary hypotheses guiding the research were:

1. *P. parvum* populations will not gain a selective advantage over other species in natural assemblages in Lake Possum Kingdom when nutrient concentrations are high.
2. *P. parvum* blooms in Lake Possum Kingdom will be inhibited by barley extract.
3. *P. parvum* initial population density will affect population dynamics.
4. *P. parvum* pigments will be conservative throughout changing growth stages.

# CHAPTER II

# INHIBITION OF *PRYMNESIUM PARVUM* INVASION ON NATIVE PLANKTON COMMUNITIES WITH THE USE OF BARLEY STRAW EXTRACT IN TEXAS, USA

Refer to Chapter I for main introduction; additional introduction material pertaining to the objectives of the experiment conducted in Lake Possum Kingdom is described below.

## 1. Purpose

In this chapter, my objective is to investigate changes in phytoplankton biomass and *P. parvum* population dynamics in response to addition of nutrients and barley straw extract (BSE), and inoculations of *P. parvum.* Specifically, nutrient additions were used to test the hypothesis that elevated levels of inorganic nutrients would not enhance *P. parvum*'s ability to invade phytoplankton communities. Additions of BSE tested the hypothesis that known phytoplankton growth inhibitors would prevent *P. parvum* blooms. *P. parvum* initial population density was manipulated to test the hypothesis that inoculations would promote increased *P. parvum* population densities. In-lake experimental incubations were conducted over three seasons (fall, winter, and spring) to test these hypotheses.

## 2. Methods

### *2.1. Site description*

Lake Possum Kingdom (32° 52' N, 98° 26'W) is located approximately 103 km northwest of Fort Worth, Texas in Palo Pinto, Young, and Stephens counties (Fig. 2.1). The reservoir contains relatively high content of salt (2.93 – 3.98 mS/C). Morris Sheppard Dam construction was completed by the Brazos River Conservation and Reclamation District (currently known as Brazos River Authority) in 1941, and is the first dam constructed on the mainstem of the Brazos River system. According to Texas Parks and Wildlife (TPWD) (2005), Lake Possum Kingdom has been severely impacted by *P. parvum* blooms since 1997, with the most devastating blooms occurring since 2000. This study was performed in Echo Cove, near the grounds of the Possum Kingdom State Park. Historically, the cove is located in an area where blooms have initiated (Joan Glass, pers. comm.) making it an ideal location for this study.

### *2.2. Culturing*

A strain of *P. parvum* isolated from west Texas, USA (ZZ181, University of Texas- Culture Collection of Algae) was used for this study. This strain was kept in culture using sterilized ultra-pure water enriched to f/2 nutrient concentrations (800 μM-N, 40 μM-P, N:P=20; Guillard and Ryther, 1962) and autoclaved. The culture was maintained under a 12h light: 12h dark cycle at 19°C under 30W cool-white fluorescent lamps (200 $\mu E\ m^{-2}\ s^{1}$) until travel to the field site. The culture was transported to the Possum Kingdom reservoir in an 18.9-L blue tinted polycarbonate carboy and shaded from direct sunlight.

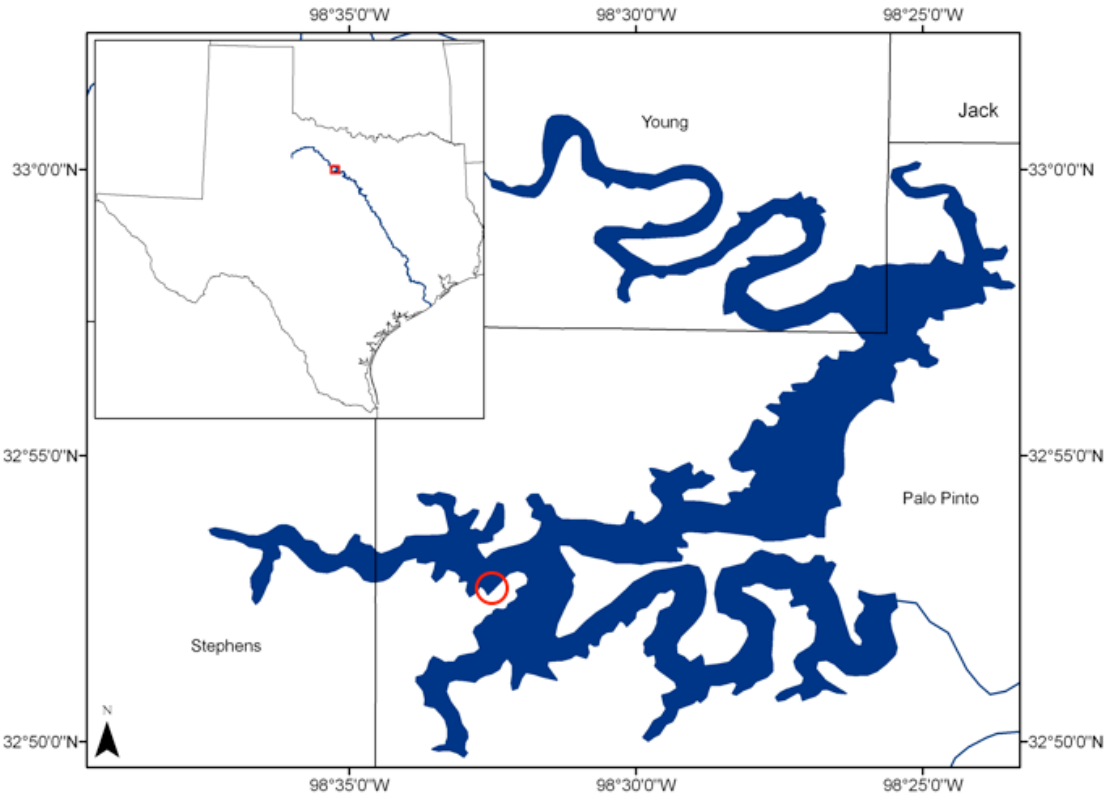


Fig. 2.1. Study site location, Lake Possum Kingdom (reservoir). Lake Possum Kingdom is the first reservoir built on the Brazos River in Young, Stephens and Palo Pinto counties, Texas, USA. The circled area represents the location of Echo cove.

*2.3. Field procedures*

Experiments were conducted during three seasons: fall (October through November, 2004), winter (January through February, 2005), and spring (March through April, 2005). The timing of the experiments was based on the time of year when historical *P. parvum* blooms occurred in Lake Possum Kingdom. Each experimental incubation ran for 28 days.

Experiments included eight treatments, and each treatment was tested in triplicate (Table 2.1). Water contained the natural plankton assemblage was collected in the vicinity of Echo Cove, Possum Kingdom reservoir (32.87391°N, -98.54570°W) at a depth of 0.5 m. To exclude large zooplankton, which may cause bias in small bottles (Sommer, 1985; Roelke et al., 2003), lake water was filtered through a 153 μm mesh. Manipulations were conducted using twenty-four transparent 2-L polycarbonate bottles which reduced light by 10% ($\lambda$ = 379-979). To some bottles, nutrients were added to concentrations of f/2 media (Guillard and Ryther, 1962). A pilot study determined that concentrated BSE (Microb-Lift CBSE, Ecological Laboratories Inc.) at 50-fold greater (1.56 mL of extract) than the manufacture's recommendation negatively affected *P. parvum* cultures. Therefore, BSE was added to some experimental bottles at this higher dosage. Some bottles received inoculations from a *P. parvum* culture to yield approximately 300 cells $mL^{-1}$ in the bottle (3% of bloom level) (Table 2.1). After capping, the bottles contained enough air in the headspace to allow them to float. The bottles were then placed within protective frames that were shaded by neutral density irradiance filters (55.25% reduction in light). In total, the bottles received an

Table 2.1. Microcosm experiment design. The experiments were initiated using 2 L of filtered (153 µm) water from Lake Possum Kingdom. Treatments included nutrient additions to levels of f/2 media, BSE, additions of *P. parvum*, and a combination of these factors.

| Microcosm treatment additions (triplicate) | f/2 nutrients | BSE | *P. parvum* |
|---|---|---|---|
| 1 | | | |
| 2 | | + | |
| 3 | | | + |
| 4 | | + | + |
| 5 | + | | |
| 6 | + | + | |
| 7 | + | | + |
| 8 | + | + | + |

approximate 65% reduction in light transmission. Each frame was tethered within the cove, which allowed the bottles to experience turbulence, temperature and light similar to natural conditions.

Samples were collected weekly for analyses of *P. parvum* population densities and photopigments concentrations. For enumeration of *P. parvum* population densities, 10 mL of water was collected from each bottle and preserved using glutaraldehyde solution (5% v/v). For estimation of photopigment concentrations, water samples ranging between of 65 mL to 150 mL were collected from each bottle. These samples were filtered under gentle vacuum (5< kPa, Whatman GF/F) and under minimal light, then stored at -70° C. Water temperature and salinity readings for Echo Cove were recorded using a HydroLab Quanta multi-probe. The Quanta multi-probe was calibrated for salinity using a freshwater standard (0.781 mS/cm).

*2.4. Laboratory procedures*

Measurement of photopigment concentrations was done using HPLC following the procedure described in Pinckney et al. (1996; 2000, 2002). Briefly, filters containing the pigments were placed into 100% acetone (1 ml) and sonicated, then extracted for 20-24 h at -20° C. Filtered extracts (300 µL) were then injected into the HPLC system, which was equipped with two reverse-phase $C_{18}$ columns placed in series (Rainin Microsorb-MV, 0.46 x 10 cm, 3mm, Vydac 201TP, 0.46 x 25cm, 5mm). A nonlinear binary gradient 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 and pigment peaks were quantified at 440 nm. Authentic photopigment standards (DHI, Denmark) were used for instrument calibration.

Cell counts were performed using the Utermöhl (1958) settling technique for only *P. parvum* cells. Typically, 500-1000 μL were settled for a 24-h period, then counted using an inverted microscope (400x, Leica Microsystem Inc.). A range of 15-50 randomly selected fields of view were counted per sample, which resulted in ~200 *P. parvum* cells counted per sample, with a 5% error.

The pigment concentrations acquired through HPLC and an initial pigment ratio file were used to determine major algal groups (cyanobacteria, euglenophytes, chlorophytes, prymnesiophytes cryptophytes, and diatoms) using CHEMTAX (Mackey et al., 1996, 1997; Schübert et al., 2000; Pinckney et al., 1996, Pinckney et al., 2001, Pinckney et al., 2002) .

At the completion of the third experiment, ambient acute toxicity of samples was determined using an assay based on survivability of *Pimephales promelas* over 24 h (US EPA methods, 2002). These procedures were carried out at Baylor University. Samples were diluted using a 0.5 dilution series with reconstituted hard water (RHW), which was prepared according to US EPA recommendations (US EPA, 2002). RHW was used as control treatment water for all toxicity assays. All culturing and toxicity tests were performed at 25 ± 1°C with a 16:8 hour light-dark cycle. *P. promelas* larvae were fed newly hatched *Artemia* nauplii two hours before initiation of testing (US EPA, 2002).

LC 50 values for *P. promelas* toxicity tests were estimated using Probit (Finney, 1971) or Trimmed Spearman Karber (Hamilton et al., 1977) techniques as appropriate.

*2.5. Statistical analyses*

Differences in total chlorophyll a (Chl *a*) and *P. parvum* cell concentrations between treatments were evaluated using a general linear model (GLM) repeated measures analysis of variance (ANOVA) (SPSS Inc. Chicago, Illinois). The factors included filtered assemblage (control) and filtered assemblages with additions of nutrients, BSE and *P. parvum*. A cumulative repeated measures approach was used with the GLM to assess when factors became statistically significant ($p<0.05$), i.e., the model was run starting at the experiment's initiation through each sampling date (days 0-7, days 0-14, days 0-21, and days 0-28). In this way, the timing of bloom and senescence events could be accounted for when assessing the significant of treatments effects. For the winter and spring experiments, the Chl *a* data were log transformed to achieve normality.

**3. Results**

There was a seasonal difference in water temperature and salinity. Temperature varied between the three seasons (Table 2.2), with the fall representing the warmest mean water temperature and the winter having the coldest mean temperature. Salinity ranged between 2.0 and 1.6 during the three experiments (Table 2.2).

*3.1. Fall experiments*

During the fall experiment, biomass was affected by the addition of nutrients (Fig 2.2A, appendix A). Only moderate changes were observed in bottles receiving no

Table 2.2. Summary of lake temperature (mean over 28 days) and salinity at the start of each season. Seasonal temperature varied by approximately 10 ºC. Salinity has a moderate variance.

| Sampling periods | Mean temp(°C) | Salinity at T0 (PSU) |
|---|---|---|
| Oct - Nov '04 (Fall) | 19.28 | 2.00 |
| Jan - Feb '05 (Winter) | 9.26 | 1.62 |
| March - April '05 (Spring) | 12.82 | 1.87 |

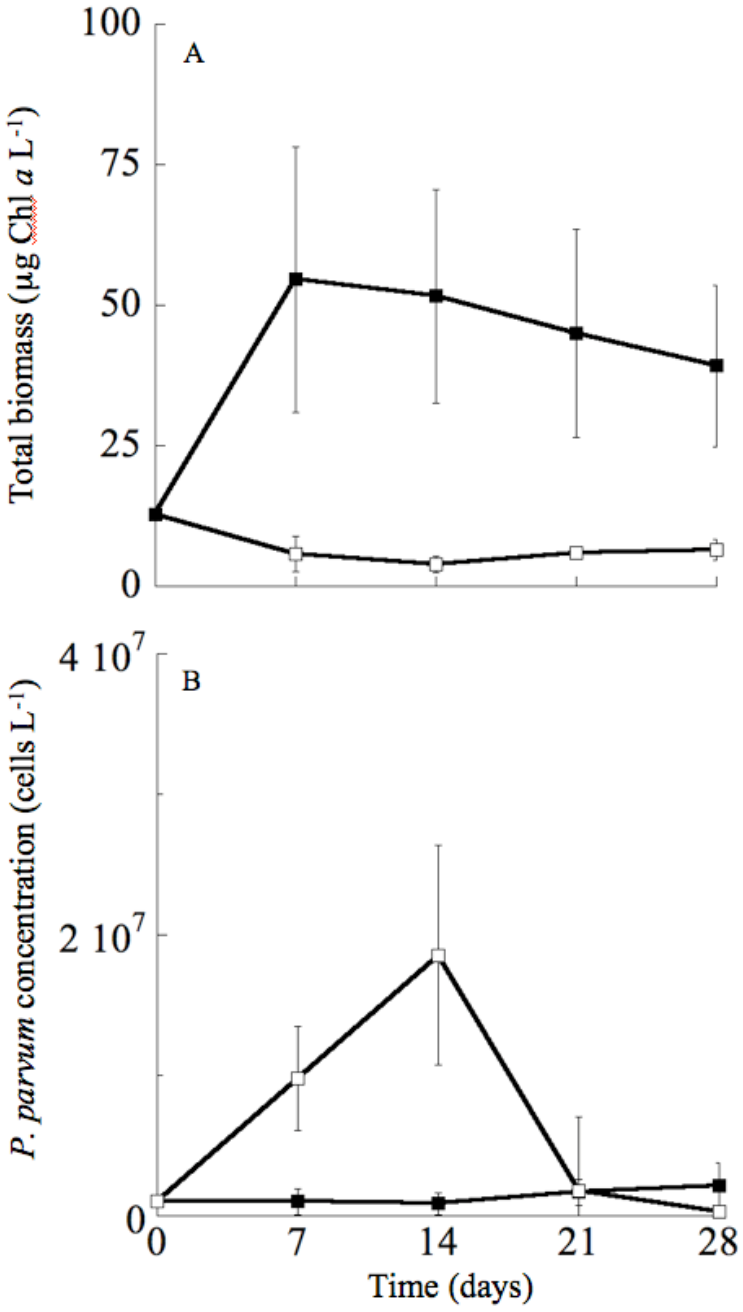


Fig. 2.2. Fall season experiment. Divided into (A) Total biomass, and (B) *P. parvum* concentration. The open boxes (□) represent treatment averages that did not receive nutrient additions, closed boxes (■) represent treatments averages that did receive f/2 nutrient additions. Total biomass increased within the first 7 days then gradually declined. *P. parvum* cell concentration increased through day 14 then noticeably decreased to levels below the control. Standard errors are indicated.

nutrients (Table 2.3A, appendix C). After 7 days, biomass increased approximately 4-fold in the bottles receiving nutrients then gradually declined (Fig. 2.2A). Statistically, the difference between bottles receiving no nutrients and bottles receiving nutrients was significant for the duration of the experiment (Table 2.3A, appendix C).

The other factors had varying impacts on the experiment. BSE had a negative impact on biomass through day 14, but after that time its affect was not significant ($p>0.05$). The interaction between BSE and nutrients were significant during the first 14 days if the experiment. This interaction was not significant from day 14 onward. *P. parvum* additions were not significant during this experiment.

*P. parvum* cell concentrations were also influenced by the addition of nutrients (Fig. 2.2B, appendix A). In bottles that did not receive nutrient additions, *P. parvum* growth was moderate. In bottles receiving nutrient additions, however, *P. parvum* concentration increased 15-fold through day 14, but then decreased dramatically, to levels below bottles that did not receive nutrient additions. Statistically, the influence of nutrient additions was significant ($p<0.05$) for the entire duration of the experiment (Table 2.3B, appendix C). BSE additions, *P. parvum* inoculations, and the other interaction terms were not significant during this experiment.

Quantitatively, the initial phytoplankton community was dominated by diatoms, and this dominance persisted throughout the experiment in bottles that did not receive nutrient additions (Fig. 2.3, appendix A). However, when nutrients were added a community shift occurred. In these bottles, diatoms dominated until day 14 when the community shifted to dominance by chlorophytes and euglenophytes (Fig. 2.3B).

Table 2.3. Fall experiment GLM repeated measures ANOVA. (A) Total biomass, and (B) *P. parvum* concentration. Notation Nut. represents treatments which received nutrient additions and BSE represents treatments that received barley straw extract. For total biomass, several levels and interactions were significant for a short period of time, however, nutrient additions was the only significant level throughout the experiment. Nutrient additions again were significant for the duration of the experiment for P. parvum concentration. Degrees of freedom for the models was 11. Note: shaded areas represent values where $p<0.05$.

**A.**

| Total Chl*a* | 0-7 | 0-14 | 0-21 | 0-28 |
|---|---|---|---|---|
| | F (*p*) | F (*p*) | F (*p*) | F (*p*) |
| Nut. | 189.297 (< 0.001) | 127.391 (< 0.001) | 106.342 (< 0.001) | 107.974 (< 0.001) |
| *P. parvum* | 0.075 (0.788) | 0.040 (0.843) | 0.139 (0.714) | 0.458 (0.508) |
| BSE | 42.130 (< 0.001) | 14.217 (0.002) | 3.471 (0.081) | 1.916 (0.185) |
| Nut. x BSE | 23.223 (< 0.001) | 7.628 (0.014) | 1.215 (0.287) | 0.367 (0.553) |
| Nut. x *P. parvum* | 0.044 (0.837) | 0.045 (0.835) | 0.124 (0.729) | 0.458 (0.508) |
| BSE x *P. parvum* | 0.557 (< 0.001) | 0.550 (0.469) | 0.401 (0.535) | 0.565 (0.463) |
| Nut. x BSE x *P. parvum* | 0.487 (0.495) | 0.469 (0.503) | 0.374 (0.549) | 0.526 (0.479) |

**B.**

| | 0-7 | 0-14 | 0-21 | 0-28 |
|---|---|---|---|---|
| Cells L-1 | F (*p*) | F (*p*) | F (*p*) | F (*p*) |
| Nut. | 63.718 (< 0.001) | 73.528 (< 0.001) | 57.672 (< 0.001) | 48.303 (< 0.001) |
| BSE | 4.181 (0.058) | 0.127 (0.726) | 0.002 (0.963) | 0.035 (0.854) |
| *P. parvum* | 0.000 (0.996) | 0.225 (0.642) | 0.567 (0.462) | 0.399 (0.537) |
| Nut. x BSE | 0.588 (0.454) | 0.175 (0.681) | 0.996 (0.333) | 1.827 (0.195) |
| Nut. x *P. parvum* | 0.368 (0.552) | 0.877 (0.363) | 1.820 (0.196) | 1.948 (0.182) |
| BSE x *P. parvum* | 1.063 (0.318) | 1.136 (0.302) | 2.059 (0.171) | 2.094 (0.167) |
| Nut. x BSE x *P. parvum* | 0.299 (0.639) | 0.552 (0.468) | 1.042 (0.323) | 0.875 (0.364) |

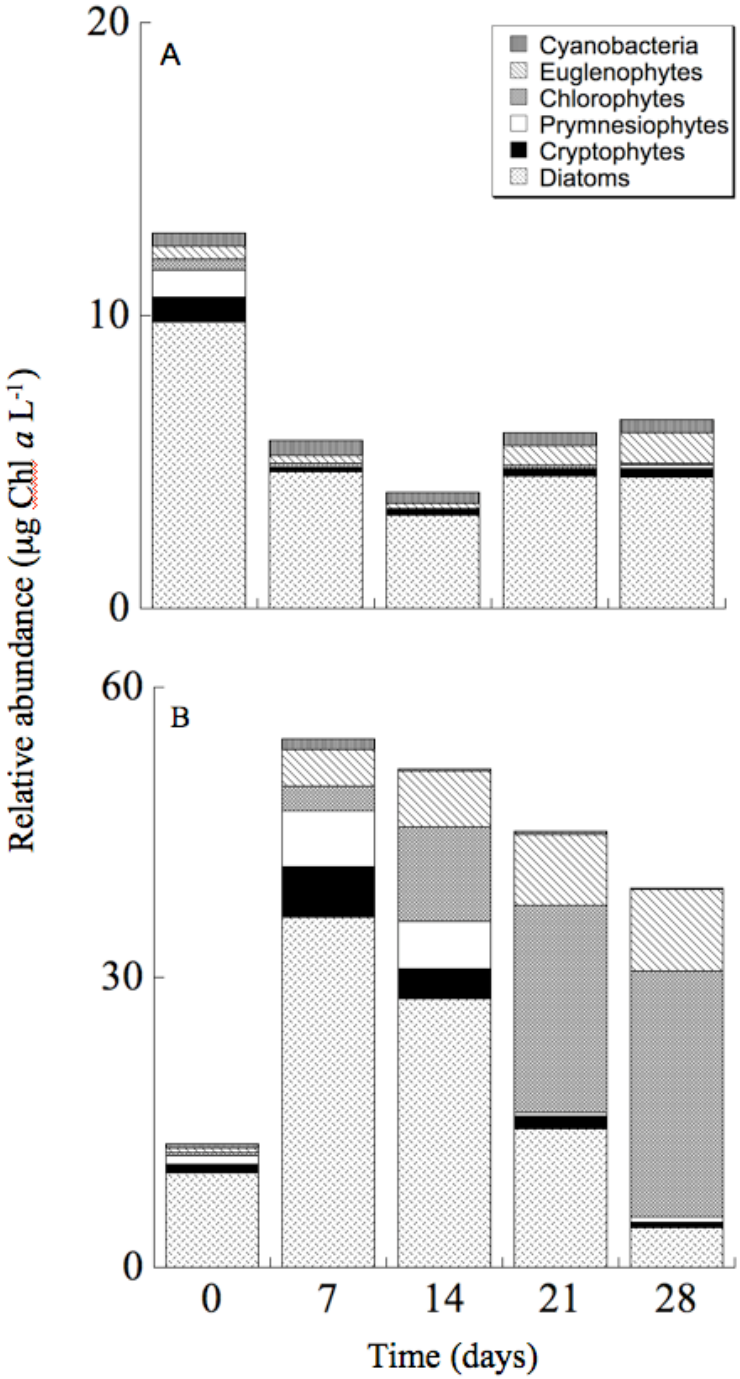


Fig. 2.3. Average fall phytoplankton bulk community composition. Divided into (A) bottles receiving no nutrient additions, and (B) bottles receiving nutrient additions. The community was dominated by diatoms over the 28 day experiment in bottles receiving no nutrients. A community shift from dominance by diatoms to dominance by euglenophytes and chlorophytes occurred in bottles receiving nutrient additions.

*3.2. Winter experiments*

During the winter experiment, biomass was again affected by the addition of nutrients (Fig. 2.4A, appendix A). Similar to the fall, moderate changes were observed in bottles receiving no nutrient additions, and a 10-fold increase was observed in bottles receiving nutrients. In this experiment, however, biomass accumulated gradually throughout the duration of the experiment, instead of decreasing as observed during the fall experiment. Again, there was a significant difference between the bottles that received no nutrients and the nutrient treatments for the entire experimental period (Table 2.4A, appendix C).

With the additional nutrients, changes in *P. parvum* cell concentrations during the winter experiment were similar to the fall experiment (Fig. 2.4B, appendix A). Bottles that did not receive nutrient additions showed an increase in population densities, and bottles receiving nutrients showed an 11-fold increase by day 14. Population density then declined to levels below bottles that did not receive nutrient additions. Differences between bottles resulting from the addition of nutrients were significant throughout the duration of the experiment (Table 2.4B, appendix C). Several interaction terms were significant (Table 2.4B, appendix C); however, these interactions were not significant after day 7.

At the start of the winter experiments the phytoplankton community was dominated by prymnesiophytes and diatoms, with chlorophytes present (Fig. 2.5, appendix A). This community composition trend changed little in bottles that did not receive nutrient additions (Fig. 2.5A). When nutrients were added, however, community

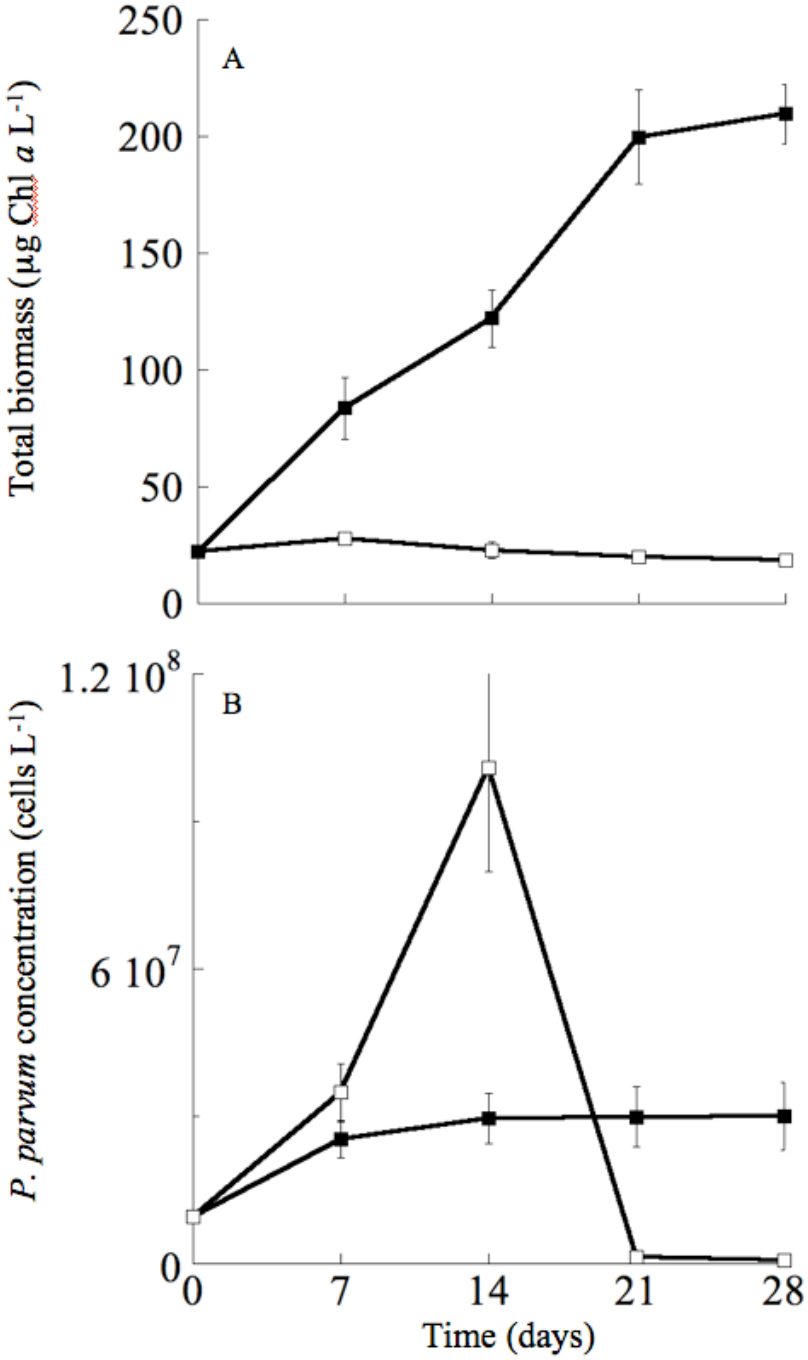


Fig. 2.4. Winter season experiment. Divided into (A) Total biomass, and (B) *P. parvum* concentration. The open boxes (□) represent treatment averages that did not receive nutrient additions, closed boxes (■) represent treatments averages that did receive f/2 nutrient additions. Total biomass for bottles that received nutrients gradually increased for the duration of the experiment. *P. parvum* cell concentration increased through day 14 then noticeably decreased to levels below the control. Standard errors are indicated.

Table 2.4. Winter experiment GLM repeated measures ANOVA. (A) Natural log of the total biomass, and (B) *P. parvum* concentration. Notation Nut. represents treatments which received nutrient additions and BSE represents treatments that received barley straw extract. The natural log of the total biomass consistently indicated a significant difference between bottles which did not receive nutrient additions and bottles which received nutrient additions. While, *P. parvum* concentration indicated that several interactions were significant, for short periods during the duration of the experiment. But nutrient additions were again consistently different throughout the experiment. Degrees of freedom for the models was 11. Note: shaded areas represent values where $p<0.05$.

| **A.** | 0-7 | 0-14 | 0-21 | 0-28 |
|---|---|---|---|---|
| LN Total Chl *a* | F (*p*) | F (*p*) | F (*p*) | F (*p*) |
| Nut. | 455.87 (< 0.001) | 1442.194 (< 0.001) | 2523.870 (< 0.001) | 3936.069 (< 0.001) |
| BSE | 1.905 (0.186) | 1.516 (0.236) | 0.665 (0.427) | 1.305 (0.27) |
| *P. parvum* | 6.36 (0.023) | 3.644 (0.074) | 1.496 (0.239) | 0.767 (0.394) |
| Nut. x BSE | 1.667 (0.215) | 0.248 (0.625) | 0.920 (0.352) | 0.855 (0.369) |
| Nut. x *P. parvum* | 0.035 (0.854) | 2.864 (0.11) | 1.860 (0.192) | 1.835 (0.194) |
| BSE x *P. parvum* | 0.059 (0.811) | 0.132 (0.721) | 0.023 (0.882) | 0.002 (0.966) |
| Nut. x BSE x *P. parvum* | 0.018 (0.894) | 0.108 (0.747) | 0.002 (0.962) | 0.013 (0.911) |

| **B.** | 0-7 | 0-14 | 0-21 | 0-28 |
|---|---|---|---|---|
| Cells L-1 | F (*p*) | F (*p*) | F (*p*) | F (*p*) |
| Nut. | 51.845 (< 0.001) | 142.960 (< 0.001) | 53.597 (< 0.001) | 8.651 (0.01) |
| BSE | 0.055 (0.818) | 0.384 (0.544) | 0.030 (0.864) | 0.031 (0.863) |
| *P. parvum* | 11.398 (0.004) | 2.578 (0.128) | 1.873 (0.19) | 1.043 (0.322) |
| Nut. x BSE | 7.466 (0.015) | 3.412 (0.083) | 4.403 (0.052) | 5.389 (0.034) |
| Nut. x *P. parvum* | 4.103 (0.06) | 1.271 (0.276) | 1.964 (0.18) | 2.344 (0.145) |
| BSE x *P. parvum* | 11.484 (0.004) | 1.608 (0.223) | 1.697 (0.211) | 1.096 (0.311) |
| Nut. x BSE x *P. parvum* | 0.769 (0.393) | 0.160 (0.694) | 0.150 (0.704) | 0.303 (0.59) |

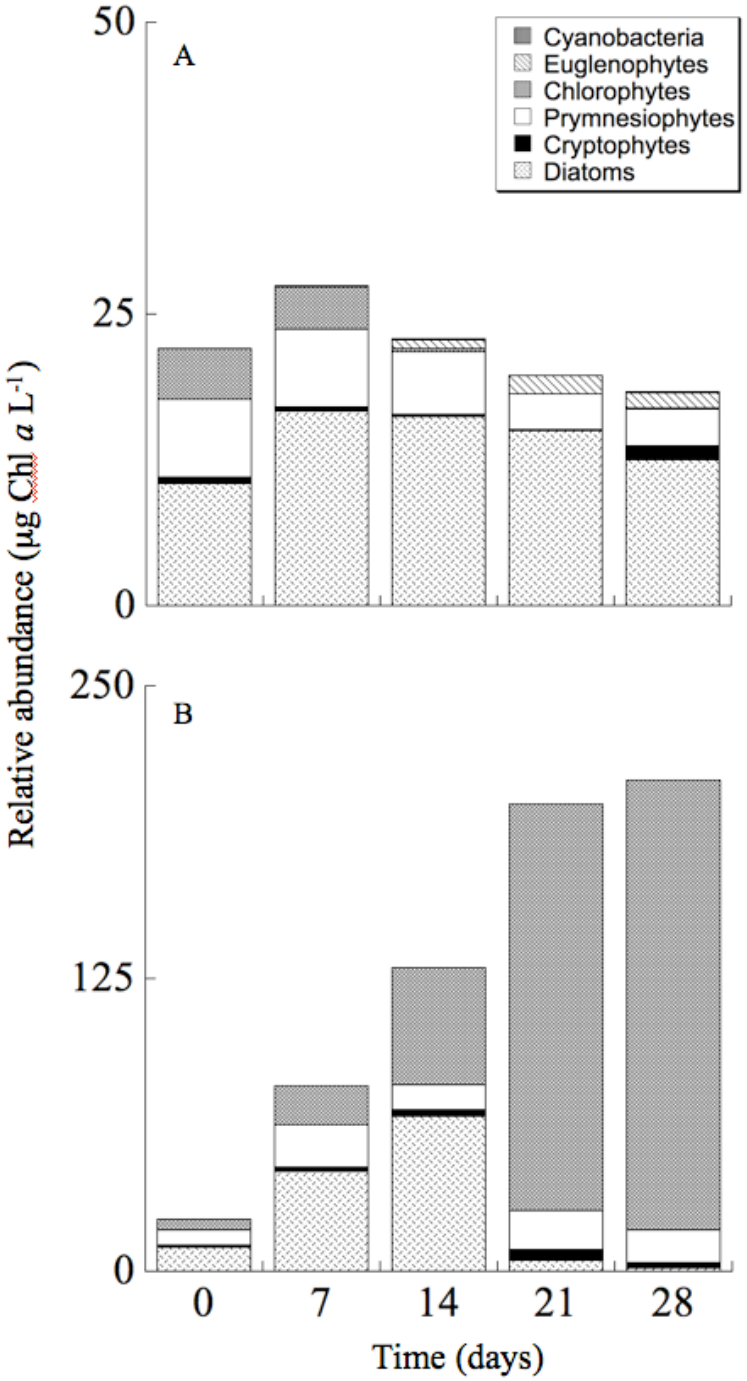


Fig. 2.5. Average winter phytoplankton bulk community composition. Divided into (A) bottles receiving no nutrient additions, and (B) bottles receiving nutrient additions. The community was dominated by diatoms over the 28 day experiment in bottles receiving no nutrients. A community shift from dominance by diatoms to dominance by euglenophytes and chlorophytes occurred in bottles receiving nutrient additions.

composition shifts were pronounced. In these bottles, diatoms and prymnesiophytes were replaced by chlorophytes after day 14 (Fig. 2.5B).

*3.3. Spring experiments*

During the spring, again total biomass differed between bottles that received no nutrients and those that did (Fig. 2.6A, appendix A). When nutrients were added biomass increased. Biomass increased approximately 10-fold 7 and 14 (Fig. 2.6A, appendix A). Significant differences were detected between no nutrients additions and nutrient additions for the duration of the experiment (Table 2.5A, appendix C).

Significant ($p<0.05$) differences were apparent for the additions of BSE and *P. parvum*, however, these were not detected until after day 14 (Table 2.5A, appendix C). Significant interactions effects occurred after day 21.

Changes in *P. parvum* cell concentrations during the spring experiments showed a pattern different from the other two experiments (Fig. 2.6B, appendix A). In bottles that did not experience nutrient additions population growth was again modest (Fig. 2.6B). When nutrients were added, however, *P. parvum* cell concentrations, showed an initial increase (small) but then experienced a large decrease of approximately 110-fold (Fig. 2.6B). This affect of nutrient additions was significant ($p<0.05$) after day 7 (Table 2.5B, appendix C). No other treatments were significant during this experiment.

The initial community composition for the spring experiment consisted largely of prymnesiophytes (Fig. 2.7A). This composition changed little in bottles that did not receive nutrient additions. When nutrients were added, the community composition shifted, and became dominated by chlorophytes and euglenophytes (Fig. 2.7B).

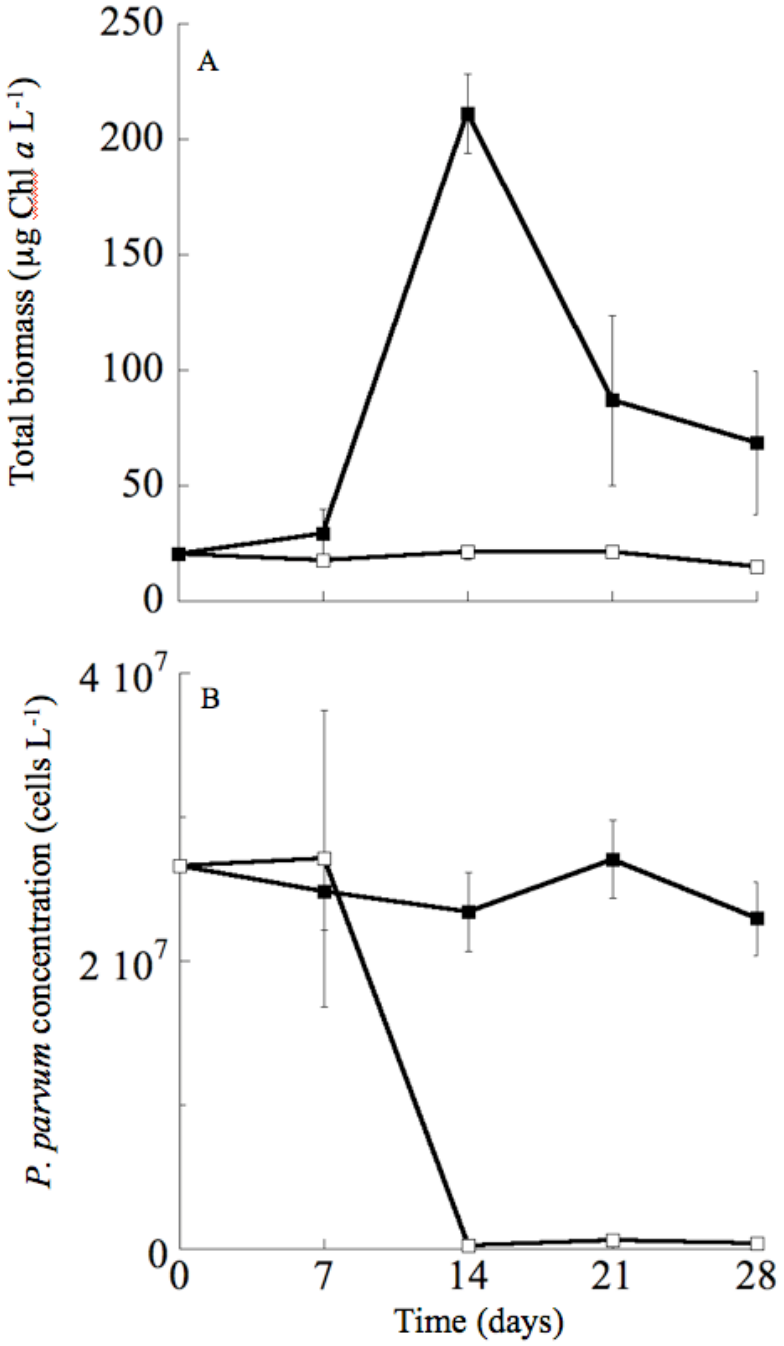


Fig. 2.6. Spring season experiment. Divided into (A) Total biomass, and (B) *P. parvum* concentration. The open boxes (□) represent treatment averages that did not receive nutrient additions, closed boxes (■) represent treatments averages that did receive f/2 nutrient additions. Total biomass increased for 14 days then rapidly declined. While *P. parvum* cell concentration had a slight initial increase but then a sharp decreased to levels below the control. Standard errors are indicated.

Table 2.5. Spring experiment GLM repeated measures ANOVA. (A) Natural log of the total biomass, and (B) *P. parvum* concentration. Notation Nut. represents treatments which received nutrient additions and BSE represents treatments that received barley straw extract. The natural log of the total biomass consistently indicated a significant difference between bottles which did not receive nutrient additions and bottles which received nutrient additions. However, after day 14, other levels and interactions became significant, therefore, interpretation is difficult. After day 7, *P. parvum* concentration were significantly impacted by nutrient additions for the duration of the experiment. But nutrient additions were again consistently different throughout the experiment. Degrees of freedom for the models was 11. Note: shaded areas represent values where $p<0.05$.

| **A.** | 0-7 | 0-14 | 0-21 | 0-28 |
|---|---|---|---|---|
| LN Total Chl *a* | F (*p*) | F (*p*) | F (*p*) | F (*p*) |
| Nut. | 7.722 (0.013) | 167.118 (< 0.001) | 145.215 (< 0.001) | 219.639 (< 0.001) |
| BSE | 0.614 (0.445) | 0.982 (0.336) | 3.755 (0.071) | 7.265 (0.016) |
| *P. parvum* | 2.690 (0.120) | 0.053 (0.821) | 7.323 (0.016) | 4.860 (0.042) |
| Nut. x BSE | 0.311 (0.585 | 0.041 (0.843) | 2.147 (0.162) | 5.384 (0.034) |
| Nut. x *P. parvum* | 5.263 (0.036) | 0.061 (0.808) | 7.919 (0.012) | 6.324 (0.023) |
| BSE x *P. parvum* | 1.876 (0.190) | 1.844 (0.193) | 0.417 (0.527) | 2.407 (0.140) |
| Nut. x BSE x *P. parvum* | 1.815 (0.197) | 1.603 (0.224) | 0.314 (0.583) | 5.362 (0.034) |

| **B.** | 0-7 | 0-14 | 0-21 | 0-28 |
|---|---|---|---|---|
| Cells L-1 | F (*p*) | F (*p*) | F (*p*) | F (*p*) |
| Nut. | 0.558 (0.466) | 39.844 (< 0.001) | 181.873 (< 0.001) | 354.086 (< 0.001) |
| BSE | 0.017 (0.898) | 0.041 (0.842) | 0.384 (0.544) | 0.353 (0.561) |
| *P. parvum* | 1.225 (0.285) | 2.058 (0.171) | 2.045 (0.172) | 3.227 (0.091) |
| Nut. x BSE | 0.230 (0.638) | 0.118 (0.735) | 0.021 (0.887) | 0.042 (0.841) |
| Nut. x *P. parvum* | 0.809 (0.382) | 0.209 (0.654) | 0.176 (0.680) | 0.000 (0.983) |
| BSE x *P. parvum* | 2.265 (0.152) | 1.228 (0.284) | 0.824 (0.378) | 0.536 (0.475) |
| Nut. x BSE x *P. parvum* | 1.656 (0.216) | 2.193 (0.158) | 2.166 (0.160) | 2.260 (0.152) |

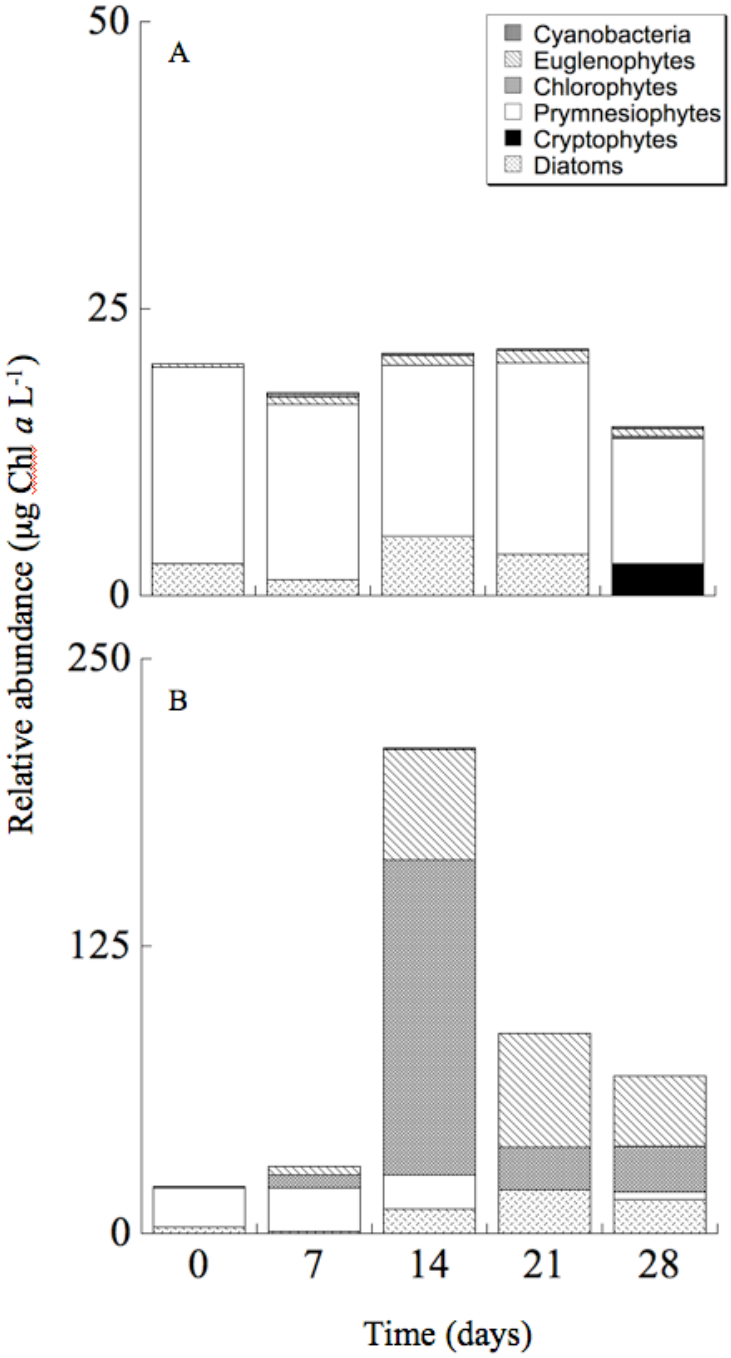


Fig. 2.7. Average spring phytoplankton bulk community composition. Divided into (A) bottles receiving no nutrient additions, and (B) bottles receiving nutrient additions. The community was dominated by prymnesiophytes over the 28 day experiment in bottles receiving no nutrients. A community shift from dominance by prymnesiophytes to dominance by euglenophytes and chlorophytes occurred in bottles receiving nutrient additions.

Nutrient additions had a large effect on ambient toxicity. After 24 hours, the LC 50 of *Pimephales promelas* exposed to free-cell filtrate from no nutrient addition bottles ranged between 4-6% of the original sample. Filtrates from bottles receiving nutrient additions were not toxic (Fig. 2.8).

**4. Discussion**

*4.1. Inorganic nutrients*

In my study, inorganic nutrients impacted total phytoplankton biomass and *P. parvum* concentrations throughout the duration of the experiments in all three seasons. The increase in total biomass in these bottles occurred because of the nutritional enrichment of the system. The inconsistency of the total biomass growth patterns, i.e. a decrease in biomass for the fall and spring experiments following the initial increase, and a gradual increase during the winter experiment, maybe due to food-web destabilization, or the paradox of enrichment (Rosenzweig ,1971). The paradox of enrichment suggests that steady state predator/prey populations begin to oscillate when prey populations are stimulated because predators respond slower and prey accumulate biomass. Predators eventually "catch up" to a point were overgrazing occurs. At this point, prey biomass becomes greatly diminished, followed by a decline in predators. The cycle might then repeat. Because samples from zooplankton were not collected in this study, further research is needed to verify this conclusion.

During the fall and winter experiments, *P. parvum* population growth occurred until day 14 (Fig. 2.2B and 2.4B, appendix A), then decreased. During the spring experiment, the population declined after day 7 (Fig. 2.6B, appendix A). Interestingly,

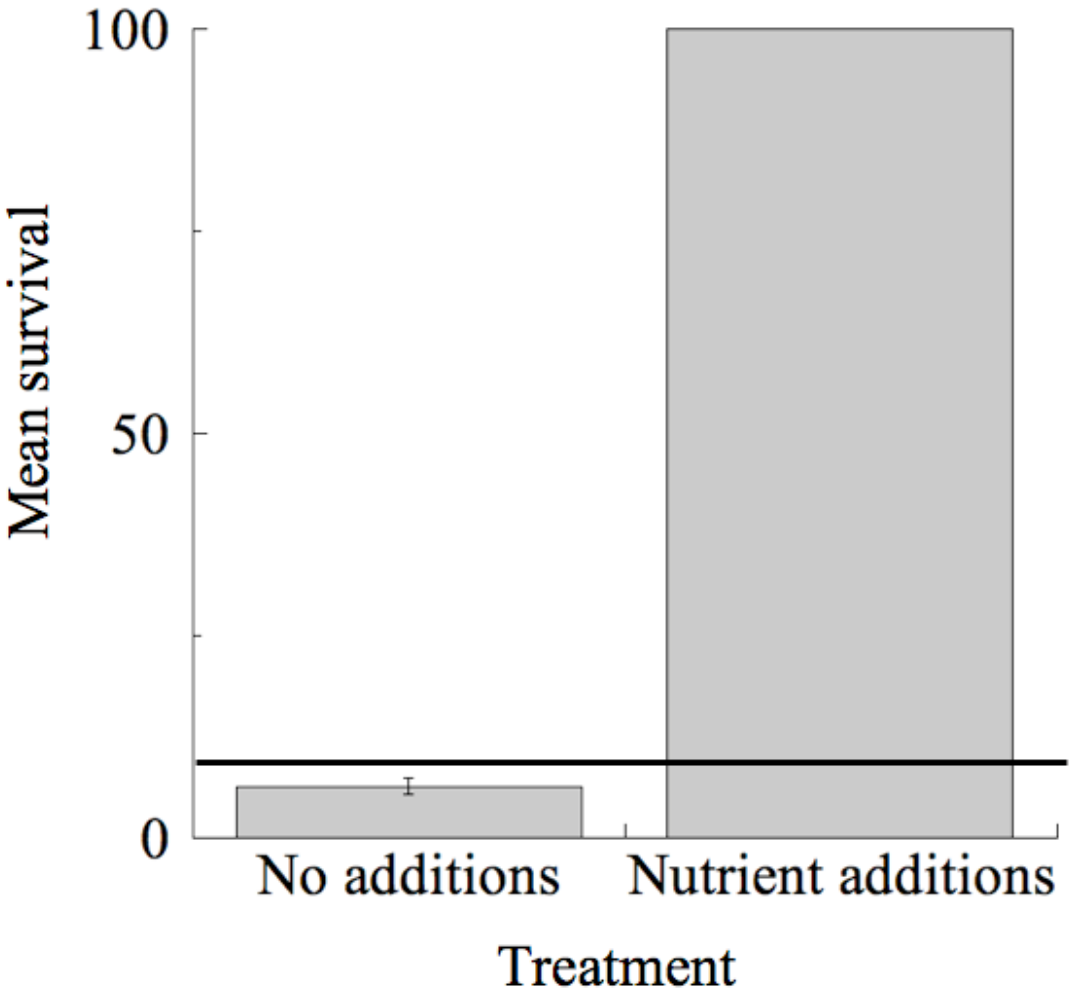


Fig. 2.8. Mean survival during a LC 50 for *Pimephales promelas*. No nutrient addition bottles were near the initial toxic levels. While bottles with nutrient additions reported a 100 percent survival of *P. promelas,* which indicated the water was not toxic. Note: the heavy line represents toxicity at the initiation of the spring experiment.

the decline in *P. parvum* cell density occurred when chlorophytes started to dominate the phytoplankton community (Figs. 2.3B, 2.5B,and 2.7B). Chlorophytes are good competitors under conditions of high N:P ratio (Tilman, 1977, Tilman et al, 1982, Tilman et al, 1986). The N: P of f/2 media is approximately 24:1 ratio (Guillard and Ryther, 1962). Chlorophytes have higher affinity for phosphorus than *P. parvum*, and is therefore able to exclude *P. parvum* during prolonged conditions of high N:P. If *P. parvum* is a stronger competitor under conditions of low N:P, the species may invade phytoplankton communities when N is limiting. This concept will be discussed further in chapter III.

*P. parvum* toxicity results indicated that bottles that received no nutrient additions were highly toxic. However, when nutrients were added the water was not toxic (Fig. 2.4). If *P. parvum*'s toxicity is a mechanism influencing competition through the mechanism of allelopathy, one would expect the full nutrient treatments would have been highly toxic, since *P. parvum* would have to compete with emerging chlorophytes. However, the toxicity dramatically decreases as nutrients are added. Thus *P. parvum* lost its competitive advantage and other species become superior competitors.

*4.2. Barley straw*

Barley straw extract did not inhibit *P. parvum* growth during these experiments. A limitation of BSE is that leached tannins must be oxidized before they inhibit alga growth, inhibition then continues after the initial oxidization because of on-going leaching from the barley straw (Gibson et al., 1990; Martin and Ridge, 1999). A

laboratory experiment conducted prior to the field experiments tested the effect of BSE on *P. parvum*. After a 24-h exposure, BSE did inhibit cultured *P. parvum* growth. Thus, it can be concluded that the chemical composition of the commercial BSE was able to inhibit *P. parvum* growth. In the bioassay experiments, inhibition may have occurred in the early days of the experiments, but by day 7 BSE lost its potency and *P. parvum* recovered. If this is the case, then approaches to management involving acute exposures to BSE may not be effective. BSE occasionally affected biomass, but when it did, the interaction term between nutrients and BSE was significant. An explanation of those findings would be complex, involve additional research, and is beyond the scope of my research.

*4.3. Mitigation*

This research revealed a potential strategy for mitigating *P. parvum* blooms. Based on the findings of the study, nutrient additions with high N:P, or non-limiting conditions, appear to inhibit *P. parvum* population growth, as well as toxin production. Application of nutrients in enclosed areas, such as coves in lake systems where *P. parvum* blooms often initiate, might prevent bloom formation. Localized nutrient additions, however, would need to be closely monitored so that accumulation of phytoplankton biomass would not be excessive. Large accumulation of phytoplankton biomass could result in a variety of other negative impacts to the environment, such as hypoxia/anoxia, the emergence of other nuisance/toxic species, and alteration of the food web.

# CHAPTER III

# THE ROLE OF INORGANIC NUTRIENTS ON THE INVASION SUCCESS OF *PRYMNESIUM PARVUM* ON NATIVE PLANKTON COMMUNITIES IN TEXAS, USA

Refer to Chapter I for main introduction; additional introduction material pertaining to the objectives of the experiment conducted in Lake Possum Kingdom is described below.

## 1. Purpose

In this chapter, my objective is to investigate changes in phytoplankton biomass and *P. parvum* population dynamics in response to full and partial nutrient additions. In these experiments, the role of nitrogen- and phosphorus-limitation as well as, the influence of *P. parvum* initial population density on community dynamics was examined. Nutrient additions were used to test the hypothesis that increased nutrients will not enhance *P. parvum*'s ability to invade phytoplankton communities and the inoculation densities of *P. parvum* were manipulated to test the hypothesis that variation in initial population densities of *P. parvum* affects population demographics.

## 2. Methods

Refer to Chapter II for details regarding site description, culturing, basic field procedures, and laboratory procedures. New field procedures are described below.

*2.1. Field procedures*

In addition to the treatments described in Chapter II, i.e. natural assemblage and full nutrient additions with and without *P. parvum* additions, four additional treatments were performed during each of the three experiments (Table 3.1). These included the addition of f/2 media deplete in $NaNO_3$ (N-deplete) with and without *P. parvum*, and $NaH_2PO_4$ (P-deplete) with and without additions of *P. parvum*. Again, *P. parvum* was added so that initial population densities were approximately 300 *P. parvum* cells $mL^{-1}$ (3% problematic level). The additional four treatments were also run in triplicate.

*2.2. Statistical analyses*

Differences in *P. parvum* cell concentrations and total chlorophyll a (Chl *a*) between treatments were evaluated using a general linear model repeated measures analysis of variance (ANOVA) (SPSS Inc., Chicago, Illinois). The two factors tested were *P. parvum* inoculations and nutrient class. The nutrient addition factor had four levels: control, full nutrient additions, N-deplete nutrients additions, and P-deplete nutrient additions. A GLM repeated measures ANOVA was conducted to determine statistical significance ($p<0.05$) for each sampling period.

**3. Results**

Biomass was affected by nutrient treatment in all three seasonal experiments (Fig. 3.1A, appendix A). In each season, bottles that received full nutrient additions had a higher biomass than the other treatments, with the exception of day 28 during the fall (Fig. 3.1A, appendix A). Bottles from the N-deplete treatments had the second highest accumulation of biomass, again except during day 28 of the fall experiment. Bottles

Table 3.1. Microcosm experiment design. These experiments were initiated using 2 L of filtered (153 µm) water from Lake Possum Kingdom. Treatments included nutrient additions to levels of f/2 media, N-deplete nutrient additions, P-deplete nutrient additions, and additions of *P. parvum*, and a combination of these factors.

| Microcosm treatment additions (triplicate) | f/2 nutrients | N-deplete f/2 nutrients | P-deplete f/2 nutrients | *P. parvum* |
|---|---|---|---|---|
| 1 | | | | |
| 2 | | | | + |
| 3 | + | | | |
| 4 | + | | | + |
| 5 | | + | | |
| 6 | | + | | + |
| 7 | | | + | |
| 8 | | | + | + |

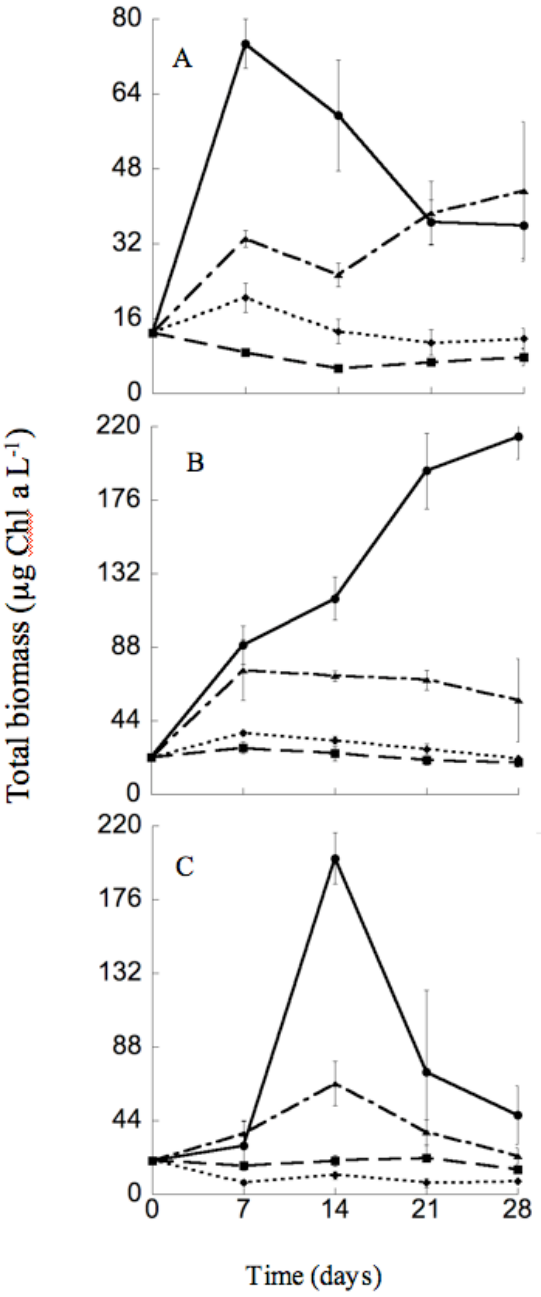


Fig. 3.1. Seasonal total biomass. Divided into (A) Fall (B) Winter, and (C) Spring. A dashed line (--■--) represents no nutrient additions, a solid line ( ● ) represents treatments that received full nutrient additions, a broken line (—— - ▲) represents N-deplete nutrient additions, and dotted line (•• ••) represents P-deplete nutrient additions. Biomass was affected by nutrient classes. Specifically, bottles that received full nutrient additions usually had the highest biomass, followed by N-deplete bottles, with P-deplete and control bottles having the lowest biomass accumulation.

from the P-deplete treatment and the control additions showed low accumulation of biomass, with P-limited conditions having slightly higher biomass than the controls during the fall and winter experiments. Statistical analyses indicated that inoculation of *P. parvum* was usually not significant (Table 3.2, 3.3, 3.4, appendix C). Therefore, biomass results were presented as the averages of the different nutrient treatments.

In the fall experiments, bottles that received full nutrient additions showed a rapid increase in biomass through day 7, but then declined (Fig. 3.1A, appendix A). Bottles receiving N-deplete nutrients also showed growth, but it occurred gradually over the course of 28 days. In this experiment, the nutrient treatment was significant ($p<0.05$) and all nutrient treatments were different (Table 3.2, appendix C).

Changes in biomass during the winter experiments responded differently to the addition of nutrients, where accumulation of biomass occurred with no decline over the course of the experiments (Fig 3.1B, appendix A). N-deplete bottles showed a buildup of biomass until day 7, but then declined. The control and P-deplete bottles showed modest growth. Nutrient treatments were again significantly ($p<0.05$) different throughout the experiment and all treatments were different (Table 3.3A, appendix A). The addition of *P. parvum* was significant ($p<0.05$), but only through day 14.

During the spring experiment biomass in bottles receiving full nutrient additions differed from the winter and fall. Although, similar pattern occurs the duration is different, where a rapid increase occurred through day 14, then rapidly declined (Fig. 3.1C, appendix A). N-deplete bottles followed a similar pattern as the full nutrient bottles, but the maximum accumulation of biomass was much less. P-deplete bottles had

Table 3.2. Fall experiment GLM repeated measures ANOVA. (A) Total biomass, and (B) *P. parvum* concentration. Nut_class represents treatments which received combinations of f/2 nutrients. For total biomass, nut_class was the significant factor for the duration of the experiment and all nutrient levels were significantly different. Nut_class again were significant for the duration of the experiment for *P. parvum* concentration. The control and P-deplete bottles had similar population growth throughout the experiment. Dunnent's T3 post hoc test was performed. Degrees of freedom for the models was 23. Highlighted areas represent significance at $p < 0.05$.

| A. | 0-7 | 0-14 | 0-21 | 0-28 |
|---|---|---|---|---|
| Total Chl *a* | F (*p*) | F (*p*) | F (*p*) | F (*p*) |
| Nut_class | 235.278 (< 0.001) | 203.815 (< 0.001) | 113.828 (< 0.001) | 67.527 (< 0.001) |
| *P. parvum* | 0.036 (0.852) | 0.043 (0.838) | 0.037 (0.85) | 0.022 (0.883) |
| Nut _ class x *P. parvum* | 1.075 (0.389) | 1.164 (0.356) | 1.092 (0.383) | 1.566 (0.239) |
| | Nuts N None P | Nuts N None P | Nuts N None P | Nuts N None P |

| B. | 0-7 | 0-14 | 0-21 | 0-28 |
|---|---|---|---|---|
| *P. parvum* cells $L^{-1}$ | F (*p*) | F (*p*) | F (*p*) | F (*p*) |
| Nut_class | 120.000 (< 0.001) | 88.754 (< 0.001) | 97.240 (< 0.001) | 147.473 (< 0.001) |
| *P. parvum* | 0.574 (0.462) | 0.014 (0.909) | 1.413 (0.252) | 3.599 (0.076) |
| Nut _ class x *P. parvum* | 2.202 (0.128) | 0.820 (0.502) | 0.207 (0.89) | 1.024 (0.408) |
| | Nuts N None P | Nuts N None P | N Nuts None P | N Nuts None P |

Table 3.3. Winter experiment GLM repeated measures ANOVA. (A) Total biomass, and (B) *P. parvum* concentration. Nut_class represents treatments which received combinations of f/2 nutrients. For total biomass, nut_class was the significant factor for the duration of the experiment. *P. parvum* inoculations were also significant through day 14. *P. parvum* concentration were significantly affected by both nut_classes and *P. parvum* additions throughout the experiment. Dunnent's T3 post hoc test was performed, a bonferroni's post hoc test is indicated by ♠. Degrees of freedom for the models was 23. Highlighted areas represent significance at $p < 0.05$.

| A. | 0-7 | 0-14 | 0-21 | 0-28 |
|---|---|---|---|---|
| Total Chl a | F (*p*) | F (*p*) | F (*p*) | F (*p*) |
| Nut_class | 68.386 (< 0.001) | 350.191 (< 0.001) | 401.144 (< 0.001) | 613.423 (< 0.001) |
| *P. parvum* | 7.827 (0.014) | 14.076 (0.002) | 2.853 (0.112) | 4.036 (0.064) |
| Nut _ class x *P. parvum* | 1.646 (0.221) | 3.831 (0.032) | 0.702 (0.565) | 1.445 (0.272) |
| | Nuts N P None | | Nuts N P None | Nuts N P None |

| B. | 0-7 ♠ | 0-14 | 0-21 | 0-28 |
|---|---|---|---|---|
| *P. parvum* cells $L^{-1}$ | F (*p*) | F (*p*) | F (*p*) | F (*p*) |
| Nut_class | 23.923 (< 0.001) | 49.475 (< 0.001) | 77.876 (< 0.001) | 104.608 (< 0.001) |
| *P. parvum* | 13.793 (0.002) | 6.618 (0.02) | 9.479 (0.007) | 14.858 (0.001) |
| Nut _ class x *P. parvum* | 2.415 (0.104) | 1.747 (0.198) | 3.954 (0.028) | 9.661 (0.001) |
| | N Nuts None P | Nuts N None P | | |

Table 3.4. Spring GLM repeated measures ANOVA. (A) Total biomass, and (B) *P. parvum* concentration. Nut_class represents treatments which received combinations of f/2 nutrients. For total biomass, nut_class was the significant factor for the duration of the experiment. All treatment levels were significantly different. *P. parvum* concentration were significantly affected by nut_classes. Dunnent's T3 post hoc test was performed. Degrees of freedom for the models was 23. Highlighted areas represent significance at $p < 0.05$.

| A. | 0-7 | 0-14 | 0-21 | 0-28 |
|---|---|---|---|---|
| Total Chl a | F (*p*) | F (*p*) | F (*p*) | F (*p*) |
| Nut_class | 21.951 (< 0.001) | 250.021 (< 0.001) | 133.100 (< 0.001) | 105.718 (< 0.001) |
| *P. parvum* | 2.619 (0.126) | 1.355 (0.263) | 1.043 (0.323) | 0.612 (0.446) |
| *P. parvum* x Nut _ class | 4.493 (0.019) | 2.270 (0.122) | 0.585 (0.634) | 0.170 (0.915) |
| | | Nuts N None P | Nuts N None P | Nuts N None P |

| B. | 0-7 | 0-14 | 0-21 | 0-28 |
|---|---|---|---|---|
| *P. parvum* cells $L^{-1}$ | F (*p*) | F (*p*) | F (*p*) | F (*p*) |
| Nut_class | 15.204 (< 0.001) | 32.370 (< 0.001) | 56.783 (< 0.001) | 12.102 (< 0.001) |
| *P. parvum* | 1.379 (0.259) | 1.212 (0.288) | 0.317 (0.582) | 0.805 (0.384) |
| *P. parvum* x Nut _ class | 1.062 (0.395) | 0.393 (0.76) | 0.130 (0.941) | 0.618 (0.614) |
| | N Nuts None P | N None Nuts P | N None Nuts P | N None P Nuts |

the lowest accumulation of biomass. Significant differences ($p<0.05$) was determined between the nutrient classes (Table 3.4A, appendix C), and the nutrient treatments were all different.

*P. parvum* accumulation was also affected by the addition of nutrients, although the response was varied across classes. Unlike biomass dynamics, *P. parvum* growth responded to the addition of *P. parvum* (Fig. 3.2, appendix A).

Focusing on the fall experiments, *P. parvum* in bottles receiving full nutrient additions grew until day 14, then abruptly dropped to levels below the control (Fig. 3.2A, appendix A). Bottles receiving *P. parvum* and N-deplete nutrients appeared to have high population growth than N-deplete bottles that did not receive *P. parvum* (Fig. 3.2A, appendix A), and both treatment types had a population decline after day 21. *P. parvum* population growth in the control and P-deplete bottles were not statistically different (Dunnett's T3, $p>0.05$, Table 3.2B, appendix C).

During the winter, *P. parvum* displayed similar trends to the fall experiments (Fig 3.2B, appendix A). Bottles that received full nutrient additions showed a rapid increase in *P. parvum* concentrations, and then rapidly decreased to levels below the control. Again, bottles receiving *P. parvum* and N-deplete nutrients showed a different population growth trend than N-limited bottles that did not receive *P. parvum*. N-deplete bottles that received *P. parvum* additions underwent high population growth that continued throughout the experiment, whereas populations in N-deplete bottles that did not receive *P. parvum* additions declined after day 14. The control and P-deplete bottles had moderate growth. Bottles receiving *P. parvum* showed significantly different

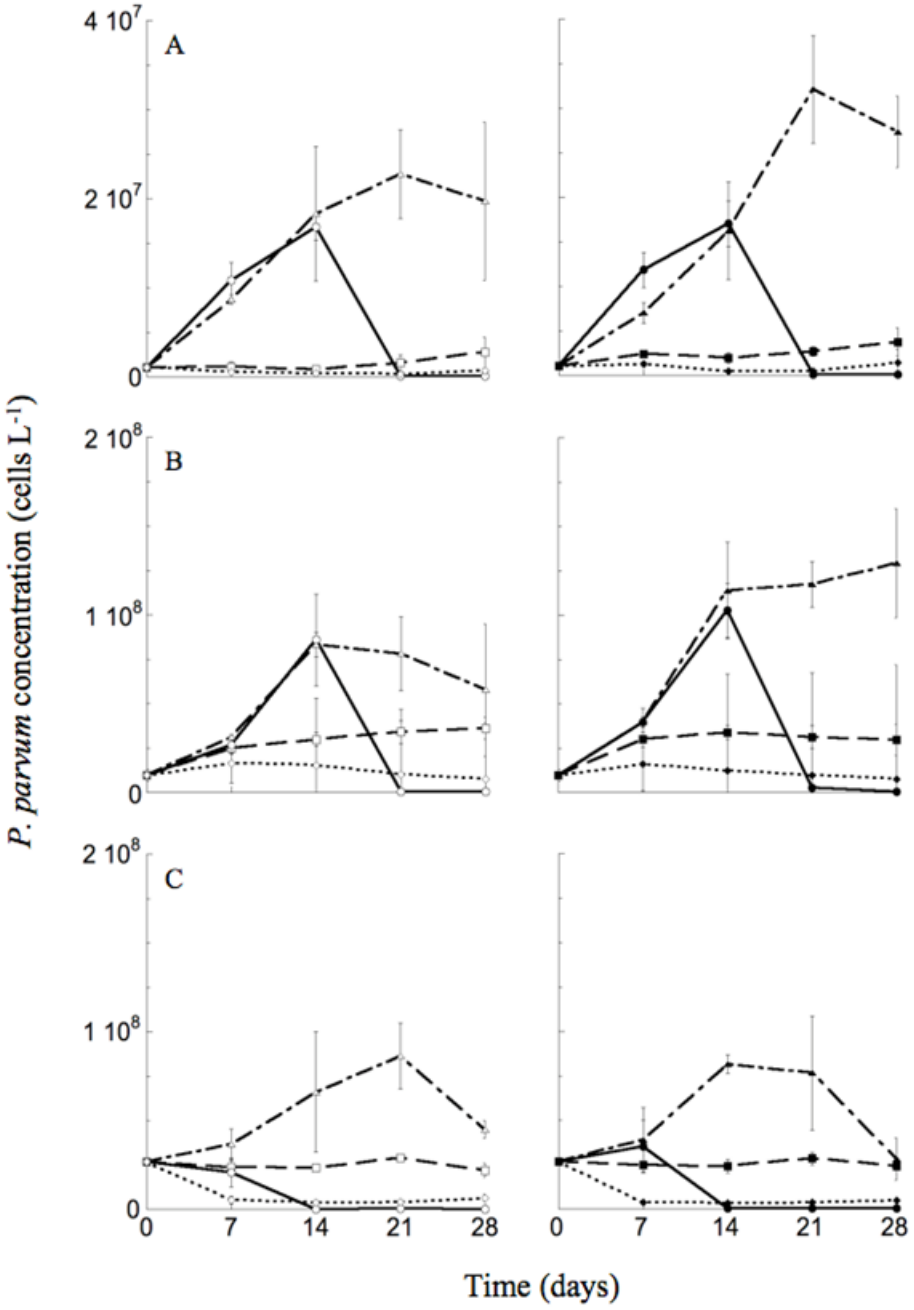


Fig. 3.2. Seasonal *P. parvum* concentration. Divided into (A) Fall (B) Winter, and (C) Spring. A dashed line (--■--) represents the control, a solid line ( ● ) represents treatments that received full nutrient additions, a broken line (—— - ▲) represents N-deplete nutrient additions, and an dotted line (••□••) represents P-deplete nutrient additions. The open symbols represent treatments that did not receive *P. parvum* additions, closed symbols received *P. parvum* additions. *P. parvum* accumulation was affected by nutrient classes and *P. parvum* inoculations (depending on the season).

population densities from (Table 3.3B), but the interaction term was also significant, so the interpretation is complex (Table 3.3B).

When nutrients were added during the spring experiment, *P. parvum* experienced initial population growth, but then declined to concentrations similar to P-deplete treatments (Fig. 3.3C, appendix A). The N-deplete bottles showed higher growth through about day 21, then declined. Control bottles showed a little population growth during the spring experiments. Statistically, full nutrient additions were similar to bottles receiving P-limited nutrient additions (Dunnett's T3, $p>0.05$, Table 3.4B, appendix C). The P-depleted bottles were also comparable to the control. *P. parvum* inoculations did not have a significant effect (Table 3.4, appendix C) during the spring experiments. However, the in-field *P. parvum* densities were already high at the start of the spring experiment.

During the fall and winter experiments, diatoms dominated the community composition in the control bottles and bottles receiving nutrient additions deplete in N and P (Fig. 3.3A and B, appendix A). A community shift occurred in bottles receiving full nutrient additions. For example, after day 14, diatoms were replaced by chlorophytes and euglenophytes as the dominate algal groups (Fig. 3.3A and B, appendix A). Fall and winter experiments differed in that the overall biomass was higher for all phytoplankton groups during the winter.

Spring community composition was dominated by prymnesiophytes in the control bottles, and bottles receiving nutrient additions deplete in N- and P- (Fig. 3.3C,

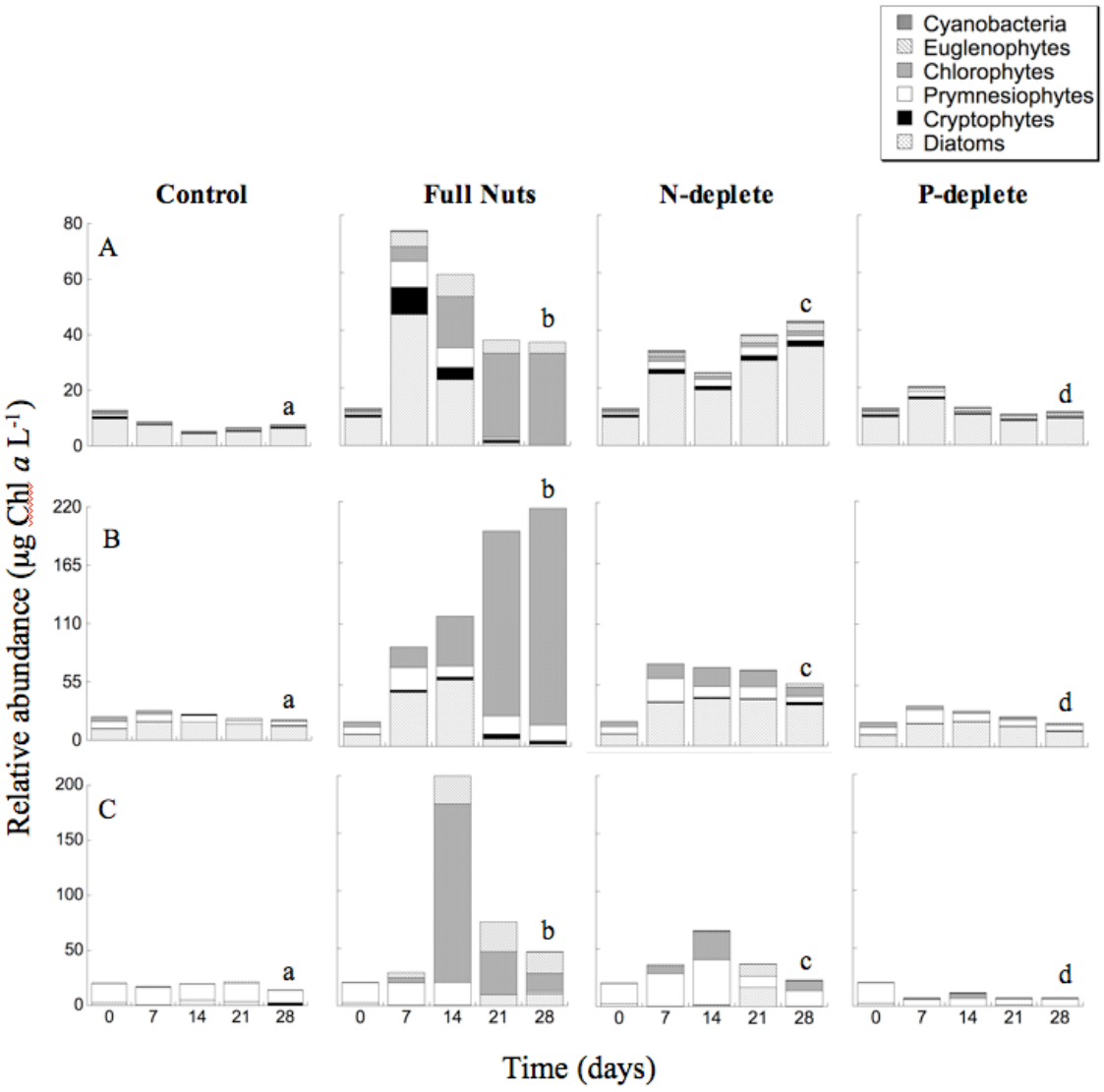


Fig. 3.3. Phytoplankton bulk community compositions. Divided into (A) Fall (B) Winter (C) Spring. During the fall experiments addition of full nutrients resulted in a community shift from diatoms, cryptophytes and prymnesiophytes to euglenophytes and chlorophytes. During the winter experiments initial concentrations of prymnesiophytes was higher compared to the fall. Again, bottles that received full nutrients resulted in a community shift from diatoms to chlorophytes. During the spring initial community structure was dominated by prymnesiophytes. A community shift again occurred in bottles receiving full nutrients from a dominance of prymnesiophytes to chlorophytes, euglenophytes and diatoms. Final Chl *a* values for each nutrient treatment where determined to be significantly different ($p < 0.05$) as indicated (a, b, c, d).

appendix A). In the bottles receiving full nutrients bottles, prymnesiophytes dominated through day 7, then the dominance shifted to chlorophytes and euglenophytes.

Toxicity differed between the nutrient treatments (Fig. 3.4). The LC 50 for *Pimephales promelas* at the start of the spring experiment was 9.81 % of the ambient water. In the control bottles toxicity at this level persisted, when full nutrients were added to the bottles toxicity was not detectable. Toxicity was lessened in N- and P-deplete bottles as compared to the control. The bottles receiving nutrient additions deplete in N- and P- were toxic, but less so than the controls.

**4. Discussion**

The experiments presented in Chapter II suggested that *P. parvum* was an inferior competitor when N:P ratios were high. This finding was supported with the data presented in this chapter as well. During the fall and winter experiments performance of *P. parvum* was best in bottles receiving nutrients deplete in N, i.e. low N:P. When N:P was high, e.g. in bottles receiving full nutrients and P-limited nutrients, *P. parvum* growth was decreased. This trend was also apparent during the spring experiment. However, it was not as pronounced due to the decline of *P. parvum* concentrations in the N-limited treatment following day 21 (Fig 3.2C). In bottles where treatments resulted in high N:P, *P. parvum* concentration declines corresponded to the emergence of chlorophytes. For example, after day 14 during the fall and winter experiments and after day 7 during the spring experiments, chlorophytes began to dominate the community structure just as *P. parvum* population densities began to decline.

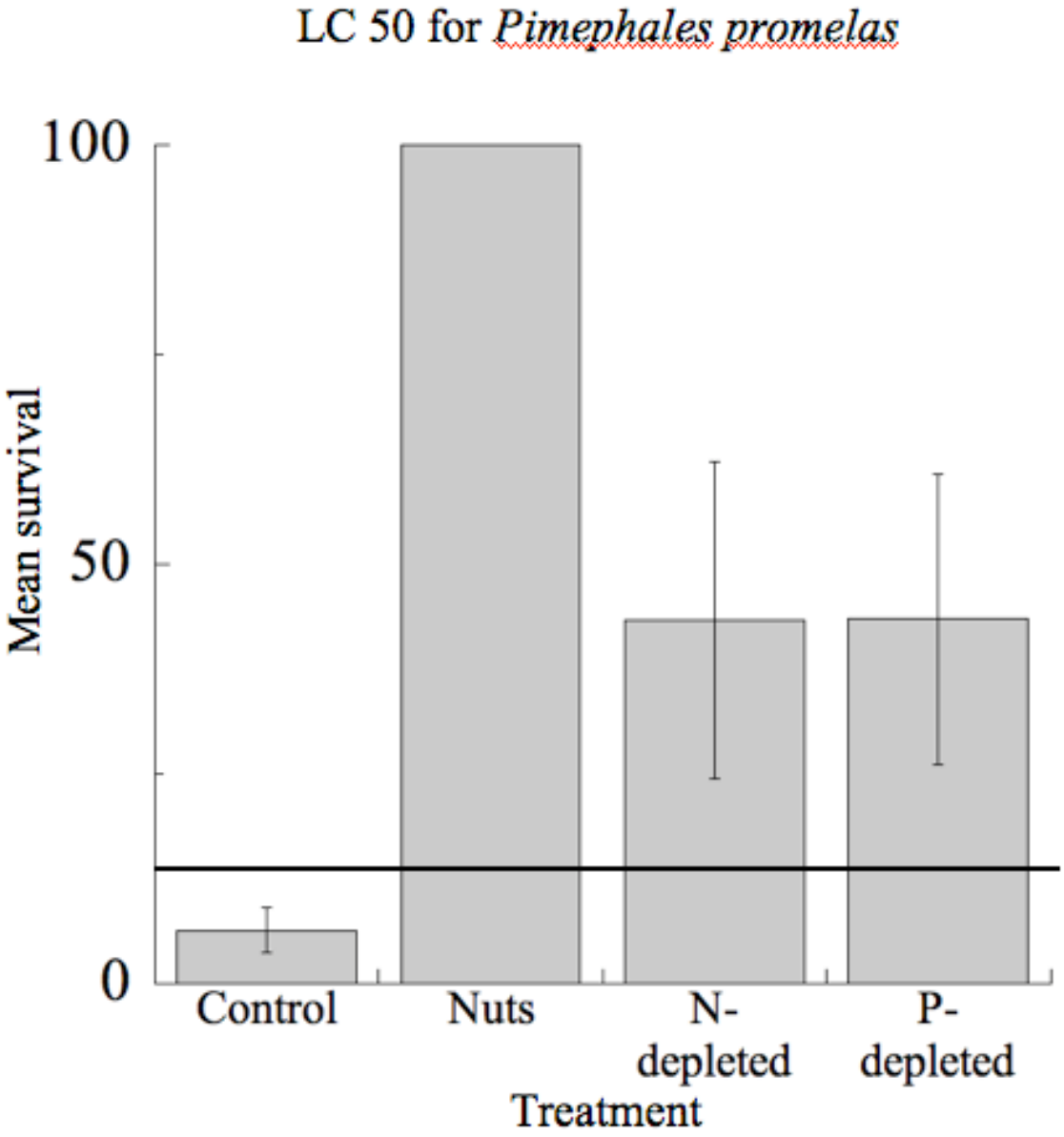


Fig. 3.4. Mean survival during a LC 50 for *Pimephales promelas*. No nutrient addition bottles were near the initial toxic levels. While bottles with full nutrient additions reported a 100 percent survival of *P. promelas,* which indicated the water was not toxic. Bottles receiving nutrients deplete in N- or P- were toxic but toxicity was less than the control bottles and initial conditions. Note: the heavy line represents toxicity at the initiation of the spring experiment.

The Monod equation (Monod, 1950) can interpret this shift through consideration of growth rate and the concentration of the limiting nutrient, P. Typically, chlorophytes utilize P efficiently (Kp ≈ 0.02 μM) and gain a competitive advantage over other algal groups when N:P ratios are high or intermediate (Tilman et al 1986, Sommer, 1989), due to their maximum reproductive rate (μmax ≈ 2 $d^{-1}$). *P. parvum*'s affinity for P is 0.006 μM (Grover, in prep), making it a more effective user of P, then chlorophytes, however, under high nutrient conditions, *P. parvum* cannot compete, regardless of toxicity, due its maximum growth rate (μmax = 0.72 $d^{-1}$). Furthermore, my research suggests that *P. parvum* could also be a superior competitor for N, i.e. during N-limited conditions *P. parvum* accumulated high concentrations (Fig. 3.1, 3.2, 3.3, appendix A).

*P. parvum* toxicity was similar to results discussed in Chapter II, in which that bottles that received no nutrient additions were highly toxic. However, when nutrients were added the bottles were not toxic (Fig. 3.4). In addition, when the bottles are N-or P-limited toxicity increases compared to full nutrient additions. Again, if *P. parvum*'s toxicity was a mechanism influencing competition, through the use of allelopathy, the full nutrient treatments and P-limited nutrient additions should have been highly toxic due to competition with chlorophytes. In addition, N-limited nutrient additions bottles would not have been toxic; however, *P. parvum* it exhibited toxicity levels comparable to P-limited nutrient addition bottles. These results support previous work showing that toxicity increases during N- or P-limitation (Johansson and Granéli, 1999; Granéli and Johansson, 2003a, b; and Fistarol et al., 2003). My experiment suggests that toxicity is

more complicated then a simple competitive response and perhaps linked to nutrient limitation at the cellular level.

Additional ideas materialized based on the findings in this experiment. For example, *P. parvum* additions influenced the population dynamics of *P. parvum* during the fall and winter experiment although during the fall this difference was not significant at levels of $p<0.05$. However, during the spring experiments, additions of *P. parvum* had no apparent effect on *P. parvum* population dynamics. The major difference between the fall and winter compared to the spring experiments was the in-field concentrations of *P. parvum* at the initiation of the experiments. During the fall and winter *P. parvum* populations were approximately three times lower than the spring. Spring *P. parvum* population densities may have been sufficient and the addition of more cells had no effect on the population. Therefore, the influence of invading cells is likely a function (in part) of the resident *P. parvum* population density.

My findings did support my hypothesis that nutrient loading into Lake Possum Kingdom does not contribute to an increase in *P. parvum* blooms. What maybe the underlying mechanism is Lake Possum Kingdom is still a young reservoir (~65 years old). Therefore, the lake may still be experiencing a maturing process and possibly has yet to reach a state where the physiochemical environment and the ecology are in "balance". Based on the findings of this experiment, I suspect that nitrogen concentrations may now become limiting during the winter, thereby providing an environment where *P. parvum* blooms are favored. In the past N was not limiting in the winter. Further investigations are needed to support this hypothesis.

# CHAPTER IV

# *PRYMNESIUM PARVUM* PIGMENT DYNAMICS

## 1. Purpose

In this chapter my objectives were to determine if *P. parvum* photopigments were conservative, and if so, could an optimized version of CHEMTAX be employed as an alternative diagnostic tool to microscopy for enumeration of *P. parvum*. These objectives were based on uncertainties regarding Texas *P. parvum* photopigments. Most of the research on *P. parvum* regarding photopigments was has focused on strains isolated from Europe, Australia or New Zealand (Fawley, 1989 and Zapata et al., 2004), and one study suggested that photopigments relative to Chl *a* were conservative (Wilhelm and Manns, 1991). However, the cellular content of carotenoids and Chl *a* are influenced by variations in irradiance and nutrient availability (Latasa, 1995 and Goericke and Montoya, 1998).

## 2. Methods

Refer to Chapter II for methods on *P. parvum* culturing.

A laboratory experiment was designed to investigate potential variation in pigment ratios as a function of the physiological growth stage of cultures. Stock culture was injected into triplicate 4 L flasks containing 2L of f/2 media. The size of the inoculations was such that the initial concentration of *P. parvum* cells were $9.6*10^3$ cells $L^{-1}$. The flasks were then incubated for 33 days under the same conditions as described in Chapter II, 2.2. At three-day intervals, samples were collected for analyses

of photopigments using HPLC. Refer to Chapter II for methodology regarding HPLC. Samples were also taken for microscopic cell counts.

Cell counts followed procedures described in Chapter II, 2.4. Minor modifications to the method included counting a range of 20-80 randomly selected fields of view for counting which resulted in ~2877 *P. parvum* cells counted in a typical 250 μL settled sample. Cell biovolume was approximated by measuring cell dimensions corresponding to geometric shapes that best fit cell morphology (Wetzel and Likens, 1981).

The coefficient of variation was calculated for each pigment ratio to determine if pigment ratios were conservative (discussed later). Changes in *P. parvum* population density growth phases were determined through observation. That is, the transition from log growth to stationary phase was determined at the point were population increases were notably slower. The coefficient of variation was determined for pigment ratios (pigment concentration/Chl *a* concentration) over the period of the growth cycle.

**3. Results**

A number of studies have shown that *P. parvum* has a moderate growth rate under a wide range of physicochemical conditions (Holdway et al, 1978; Brand, 1984; and Larsen and Bryant, 1998). Under our experimental conditions, the *P. parvum* culture had a mean growth rate of 0.069 $d^{-1}$ (during log-growth phase), which is slightly lower relative to growth rates reported previously (Jim Grover, pers. comm.).

We determined that our *P. parvum* culture had eight characteristic pigments that were consistently present throughout its growth cycle. They were chlorophyll $c_1c_2$,

chlorophyll c3, fucoxanthin, violaxanthin, diadinoxanthin, diatoxanthin, zeaxanthin, and β-cartone (Fig. 4.1). The number of pigments detected might be lower. Diatoxanthin maybe converted to diadinoxanthin (xanthophylls cycle) during filtration, and violaxanthin, a derivative of zeaxanthin, may also be produced during filtration.

Pigment ratios were not conservative, i.e. constant, over the course of growth. For example, during early log-growth phase, while turbidity remained low in the flasks, pigments ratios experienced a period of relative stability. From day 12 to day 21, pigment ratios remained moderately constant (see Fig. 4.2 for representative examples, appendix B). As cell densities and turbidity increased, after day 21, the pigment ratios (per unit Chl *a*) were dynamic. Some ratios increased, while others decreased (see Table 4.1, appendix C). The coefficient of variation typically had values above 10% (Table 4.2).

## 4. Discussion

### *4.1. Pigment variation*

Cellular Chl *a* concentrations are variable in order to facilitate adaptation in a fluctuating environment (Jeffery et al., 1997). In particular, algae shift Chl *a* concentrations based on shifts in light availability (Jeffery et al., 1997; Goericke and Montoya, 1998; Mackey et al., 1998; Schlüter et al., 2000). For example, when exposed to low light conditions Chl *a* concentrations increase, but during high light exposure Chl *a* will decrease (Sakshaug et al., 1987; Goericke and Montoya, 1998; Descy et al., 2000). In this study, the culture incubation irradiance was held constant.

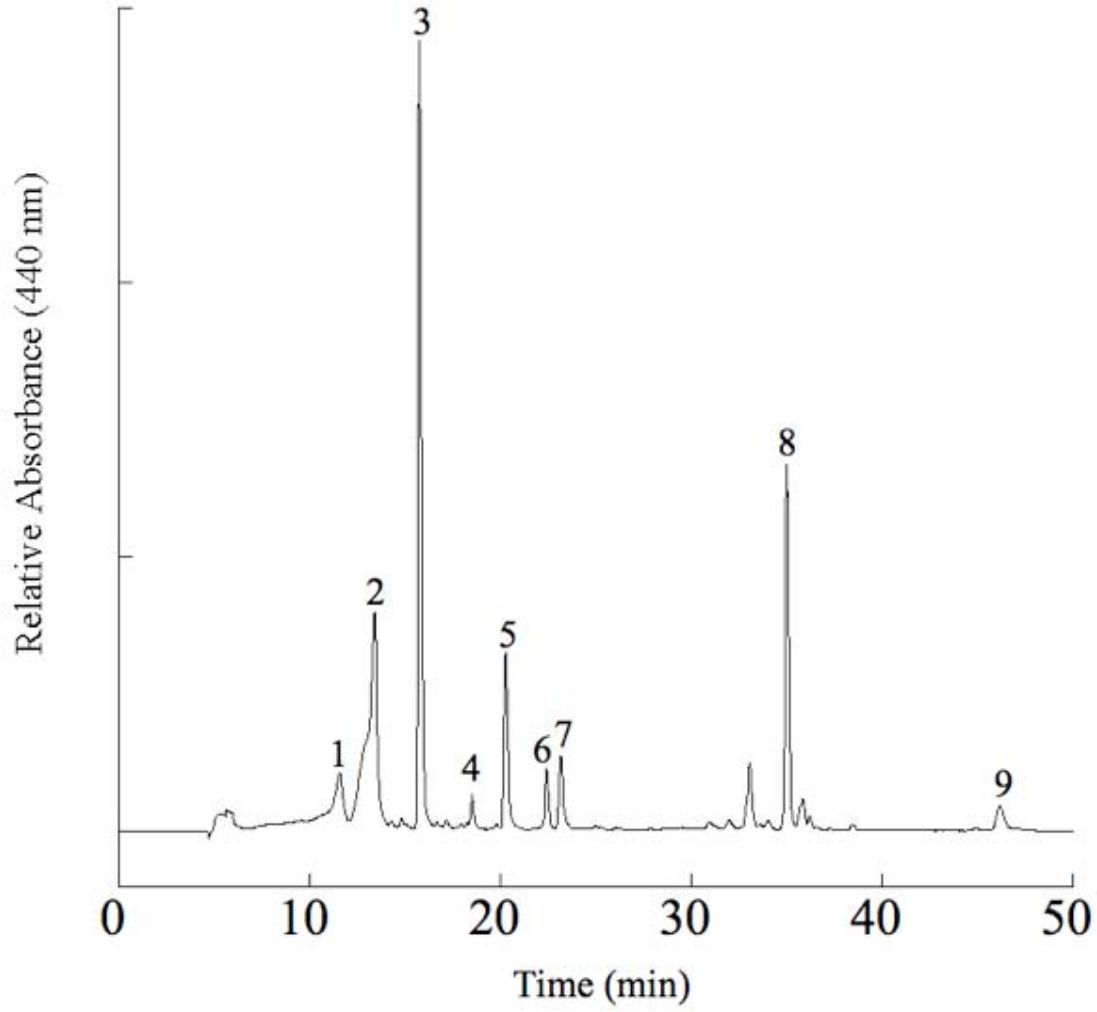


Fig. 4.1. Representative chromatogram for *P. parvum*. Nine pigments were consistently present during early and late log growth phase, which were (1) chlorophyll c3, (2) chlorophyll c1c2, (3) fucoxanthin, (4) violaxanthin, (5) diadinoxanthin,(6) diatoxanthin, (7) zeaxanthin, (8) chlorophyll a, and (9) β-cartone phytopigments.

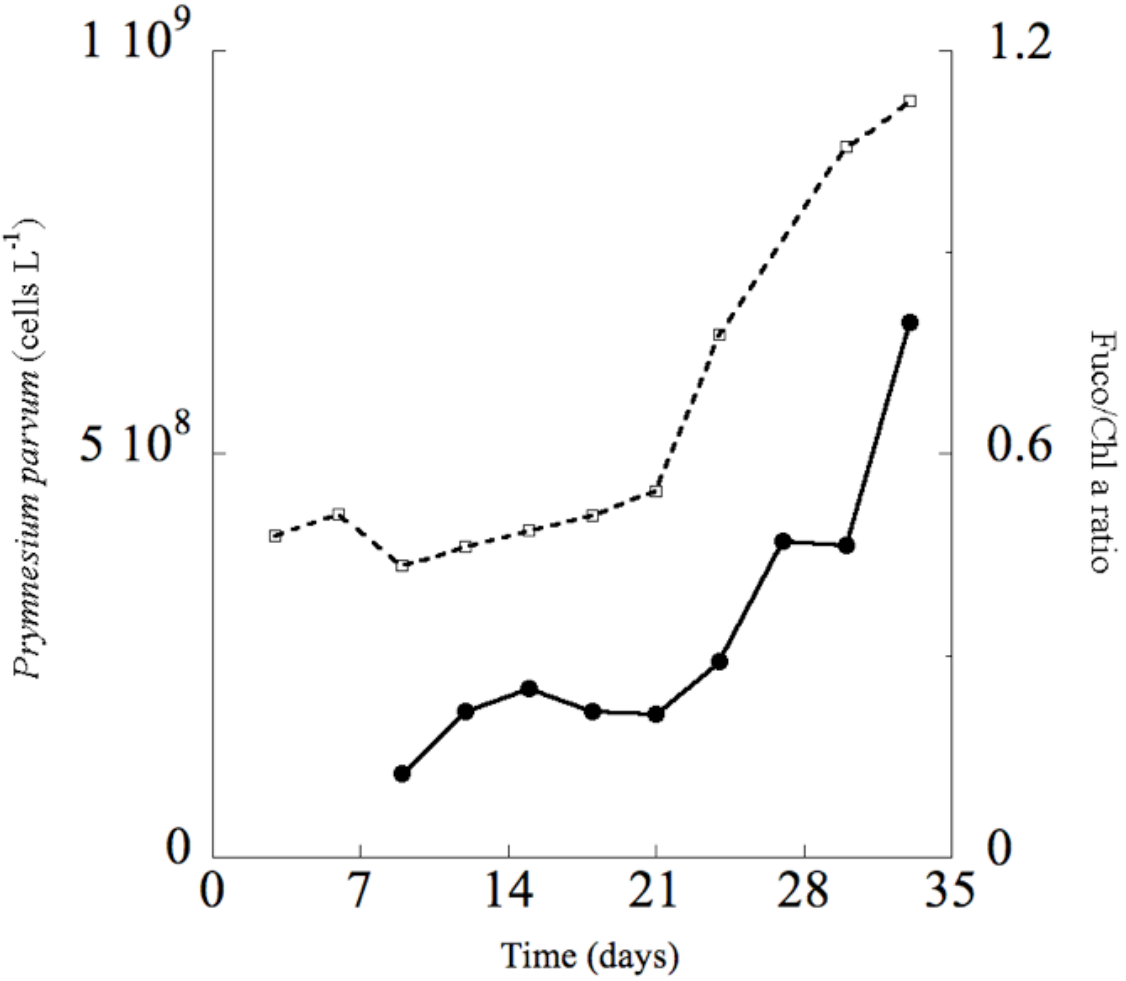


Fig. 4.2. Example of *P. parvum* cell concentration and pigment/Chl *a* over the course of log growth. A solid line ( ● ) represents cells $L^{-1}$, a dashed line (--□--) represents pigment ratio. Fucoxanthin per chlorophyll a ratio showed a noticeable increase after day 21, this corresponded to an increase in *P. parvum* cell concentrations. Results shown are typical across triplicate flasks and pigments.

Table 4.1. Minimum, maximum and mean pigment/chlorophyll *a* ratio for *Prymnesium parvum*. Gradient has two states of low turbidity and high turbidity, corresponding ratios are presented. Ratios (mean) increase as they progress from low to high turbidity.

| | | Chl $c_3$/Chl *a* | Chl $c_1c_2$/Chl *a* | Fuco/Chl *a* | Viola/Chl *a* | Diad/Chl *a* | Diat/Chl *a* | Zea/Chl *a* | β-Car/Chl *a* |
|---|---|---|---|---|---|---|---|---|---|
| **Low Turbidity** | minima | 0.353 | 0.162 | 0.408 | 0.000 | 0.070 | 0.016 | 0.023 | 0.017 |
| | maximum | 0.450 | 0.690 | 0.492 | 0.003 | 0.105 | 0.186 | 0.039 | 0.031 |
| | mean | 0.389 | 0.272 | 0.459 | 0.001 | 0.088 | 0.051 | 0.030 | 0.023 |
| **High Turbidity** | minima | 0.367 | 0.239 | 0.518 | 0.000 | 0.043 | 0.038 | 0.034 | 0.001 |
| | maximum | 2.398 | 0.600 | 1.139 | 0.017 | 0.559 | 0.142 | 0.300 | 0.010 |
| | mean | 1.474 | 0.424 | 0.825 | 0.006 | 0.263 | 0.086 | 0.132 | 0.005 |

Table 4.2. Coefficient of variation for each pigment. Total includes the COV for the duration of the experiment. Flask 3 high turbidity represents ratios with the least variability.

| Concentrations | Pigments | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Chl $c_3$ | Chl $c_1c_2$ | Fuco | 19'HFuco | 9'cis-Neo | Viola | Diad | Diat | Zeax | β-Car |
| **Flask 1** | | | | | | | | | | |
| Total | 90.50 | 77.85 | 45.25 | 60.12 | 214.69 | 206.82 | 108.73 | 113.01 | 111.29 | 99.90 |
| Low turbidity | 25.90 | 115.57 | 6.04 | 49.97 | 17.13 | 92.18 | 27.22 | 164.27 | 25.86 | 35.05 |
| High turbidity | 53.50 | 36.73 | 31.25 | 41.53 | 160.02 | 223.61 | 96.50 | 51.53 | 83.78 | 49.20 |
| **Flask 2** | | | | | | | | | | |
| Total | 91.72 | 53.31 | 41.00 | 105.19 | 67.62 | 155.16 | 87.37 | 82.59 | 122.77 | 62.86 |
| Low turbidity | 9.95 | 18.88 | 8.97 | 67.63 | 39.69 | 99.52 | 20.62 | 33.19 | 10.66 | 49.63 |
| High turbidity | 62.52 | 43.68 | 45.65 | 63.30 | 56.07 | 97.01 | 65.10 | 55.70 | 80.85 | 105.61 |
| **Flask 3** | | | | | | | | | | |
| Total | 78.48 | 48.47 | 39.67 | 292.76 | 231.02 | 301.75 | 243.04 | 199.47 | 218.62 | 87.62 |
| Low turbidity | 45.18 | 44.65 | 46.08 | 49.11 | 50.18 | 210.66 | 59.66 | 55.86 | 49.51 | 74.06 |
| High turbidity | 28.22 | 14.41 | 22.50 | 4.57 | 12.68 | 47.16 | 18.48 | 9.45 | 16.49 | 93.04 |

However, turbidity within the flasks increased with cell density, thus it is likely that the cultures became light-limited. Therefore, light availability within the flasks could have been a factor contributing to *P. parvum* pigment shifts, but due to pigments complex response further research is needed.

The coefficient of variation indicated inconsistent and sometimes high variation for the pigment ratios for *P. parvum*. Therefore, *P. parvum* pigment ratio variations could lead to variations in biomass determination provided by CHEMTAX. Schlüter et al. (2000) indicated similar shifts in pigment ratios of prymnesiophytes containing 19'hex-fucoxanthin (Table 4.3) when exposed to different light concentrations. However, Schlüter et al. (2000) also showed that the general trends of the phytoplankton community did not change when the varying pigment ratio files were loaded into CHEMTAX. Therefore, I concluded that CHEMTAX can be a reliable method to identify major phytoplankton groups in the presence of *P. parvum*. However, further research is needed to determine if CHEMTAX can precisely account for *P. parvum* within the prymnesiophyte category.

*4.2. Pigment identification*

Several studies have indicated that *P. parvum* contains the pigments Chl $c_3$, Chl $c_1$ and $c_2$, fucoxanthin, diadinoxathin, Chl a, and β-carotene (Wilhelm and Manns, 1991; Fawley, 1989; Zapata et al., 2004). Recently, an evaluation of the European (CCMP-708) and New Zealand (CS-345) strains of *P. parvum* pigments identified Chl $c_3$-MgDVP and Chl $c_2$-MgDG as new pigments. In addition, they identified similar pigment/Chl a ratios (fucoxanthin and Chl $c_3$) as reported in the current experiment (see

Table 4.3. Coefficient of variation for two prymnesiophytes with 19'hexFuco. Pigment/Chl *a* ratios obtained from Schlüter (2000, Table 2). COV was calculated from the averages of low light, medium light, and high light conditions. ND = pigments not determined. Variability is similar to differences found for *P. parvum*.

| | Pigments | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Prymnesiophytes | Chl $c_3$ | Chl c | 19'-butFuco | Fuco | 19'HFuco | Diad | Diat | β-Car |
| *Emiliania huxleyi* | ND | 15.11 | ND | 71.55 | 30.57 | 27.22 | 69.41 | 13.92 |
| *Phaeocystis* sp. | 4.37 | 2.90 | 62.62 | 23.97 | 50.43 | 7.41 | 40.97 | 13.27 |

appendix C). However, two new pigments, violaxanthin and zeaxanthin, were identified within the culture.

It is difficult to keep culture of *P. parvum* without the presences of bacteria, due to its mixtrophic ability. Therefore, these cultures did contain small amounts of bacteria. Prior to the experiment, an aliquot of the culture was counted using an epifluorescence microscope. Through this technique it was determined that the bacteria did not contain Chl *a*. However, due to the presence of this containment, we can not rule out the possibility that it maybe present within the pigment diagnostic. Therefore, further research is needed to examine an azenic culture of *P. parvum* for pigment analysis.

# CHAPTER V

# CONCLUSION

## 1. Summary

In this research, I suggest that *P. parvum* populations in Lake Possum Kingdom would not likely gain a selective advantage over other species when nutrients were not limiting (i.e., nutrient replete conditions). *P. parvum* did, however, gain an advantage during N- and P-limitation as indicated by toxicity, cell concentrations and bulk community shifts. *P. parvum* blooms in Lake Possum Kingdom would likely not be inhibited by barley straw extract application. Initial population densities affected the final population density, but only when initial populations were small. *P. parvum* pigments in the Texas strain were not conservative throughout the growth cycle; therefore, the application of CHEMTAX for estimating the relative abundance of *P. parvum* will have to relay on unique pigment ratio values depending on the growth stage of *P. parvum*.

## 2. Management recommendations

This research suggested that Lake Possum Kingdom becomes nitrogen limited during the winter. These seasons do not represent the optimal growth temperatures for *P. parvum* (Grover, *in prep*) though they do represent the optimal conditions of nutrient limitation for this species to produce toxins. Therefore, localized nutrient additions may be used as a mitigation tool during the fall, winter, and spring in order to deter *P. parvum* competition and minimize potential toxic effects. The application of nutrients in

enclosed areas, such as coves in lake systems where *P. parvum* blooms often initiate might prevent full bloom formation. A management strategy of localized nutrient additions, however, needs to be closely monitored so other phytoplankton species, such as chlorophytes or other non-desirable species overwhelm the current phytoplankton community.

**3. Future research needs**

Future research on *P. parvum* is needed in a number of areas, from physiochemical conditions of bloom areas to optimizing CHEMTAX for estimates of *P. parvum* relative abundance.

The presence of golden algae within the plankton community does not guarantee bloom formation. For example, four freshwater lakes in Texas that contain golden algae do not experience blooms or fish kills related to toxic events (R. Kiesling and D. Roelke, pers. comm.). Why then does *P. parvum* occur in some freshwater reservoirs and not cause detrimental effects, while in other lakes blooms can be devastating? Though it is known that *P. parvum* needs ions for the toxin to finishing formation (Joan Glass, pers. comm.), food web interactions, which in turn are affected by community structure, are important factors for bloom initiation. Phytoplankton community composition is different in brackish and saltwater lakes compared to freshwater lakes (Wetzel, 2001); this concept may provide an understanding of *P. parvum* bloom formation. I propose that blooms formations are affected by the physiochemical conditions and the structure of the phytoplankton community and these conditions warrant further study.

This research suggests that blooms may subside when nutrients are added into the system. However, this should be occurring in the winter and spring when Texas experiences its rainy season. But these seasons are historically the height of *P. parvum* blooms. Therefore, a central question is how are the nutrients getting into the system? If they are, are they available for biological use? A hypothesis to explain this maybe linked to theory of why *P. parvum* blooms occur in the brackish waters areas of Texas. The ions present in salt and brackish water have the ability to bind particles, therefore, causing them to sink to the floor of reservoirs. Thus, I suggest that the nutrients are entering into these systems, but they are not available for use, especially in deep reservoirs, such as Lake Possum Kingdom where the sediments are not re-suspended. Based on this theory studies are needed to look at the availability of nutrients (through direct loading as well as benthic storage) in the reservoirs and rivers.

Bloom termination is an important area of research and maybe the most important to determine mitigation strategies. Again, historically, blooms occur during the winter and early spring months, a time where most zooplankton species are senescent within the water column. Therefore, there maybe a link between the emergence of a predator (zooplankton), perhaps when *P. parvum* is non-toxic, that is able to terminate a bloom. Roelke et al. (*in prep*) suggests that competition could occur with bacteria for available resources when a liable carbon source, such as barley straw extract, is added. Additionally, termination may occur due to an introduction of a virus in bloom areas.

Besides *P. parvum* bloom dynamics, more information is needed on the basic physiological requirements of the species. *P. parvum* also demonstrates mixtrophic

capabilities (Nygaard and Tobiesen, 1993; Skovhaara and Hansen, 2003; and Martin-Cereceda et al., 2003). The life strategy of both producing energy through photosynthesis and consuming organisms for a similar purpose is not fully understood. It is possible that shift pigments occur during times of light stress and mixtrophy becomes an alternative at this point. Thus, the cellular concentrations of pigments are decreasing because they are no longer used for energy acquisition. If this is occurring, we could possibly determine light limitation of *P. parvum* based on the amount of mixtrophy occurring. If we can understand the mechanisms behind the apparent dynamics in pigments, we could possible optimize CHEMTAX based on these limitations.

Finally, based on this study new pigments have been identified in Texas's strain of *P. parvum.* Violaxanthin and zeaxanthin pigments are part of the xanthophylls cycle (Sapozhnikov et al., 1957 and Schubert et al., 1994) and have been shown to protect algae and plants against photodamage (Krinsky, 1971 and Schubert et al., 1994). Based on the proximity of Texas to tropical areas, where light is more predominate, is possible that speciation is occurring to account for the increase in light. DNA records need to be examined to determine if this is occurring, in addition to analysis of *P. parvum* cultures without the presences of bacteria.

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# APPENDIX A

FIGURE Page

FIGURE Page

FIGURE Page

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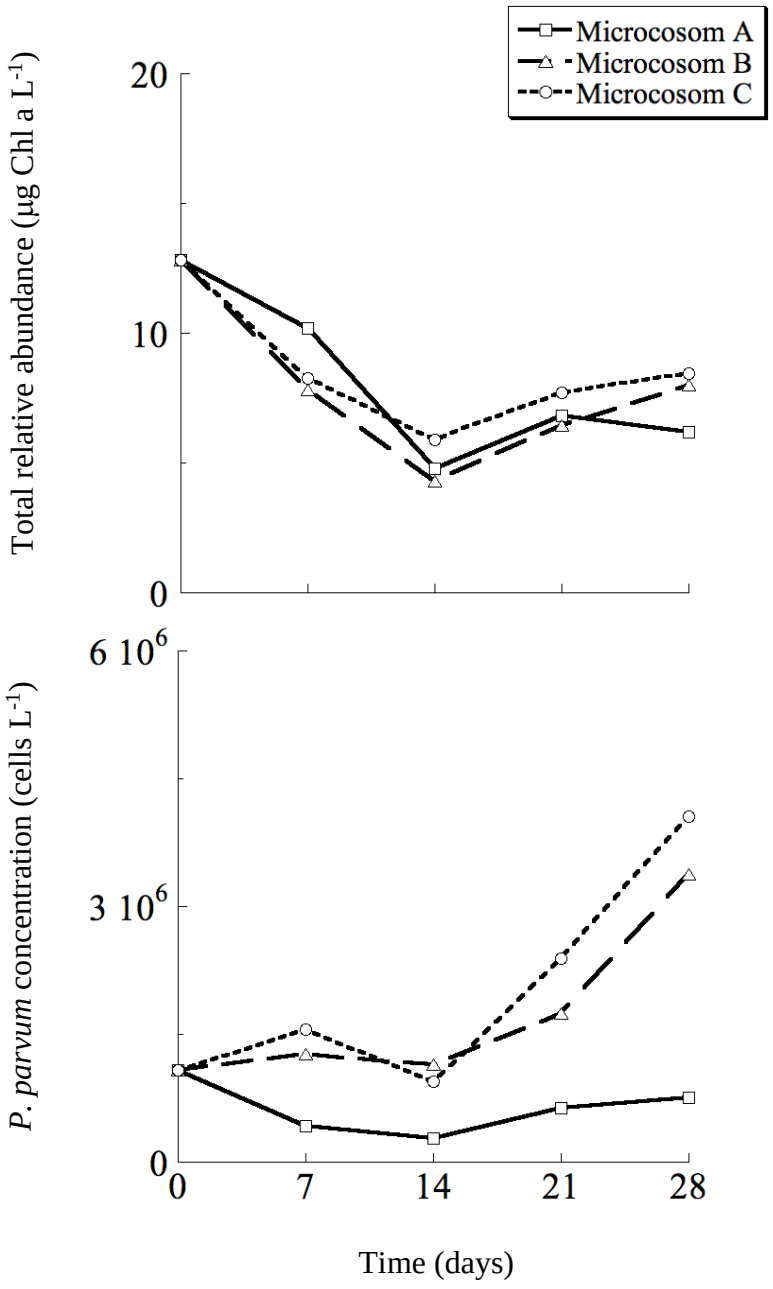
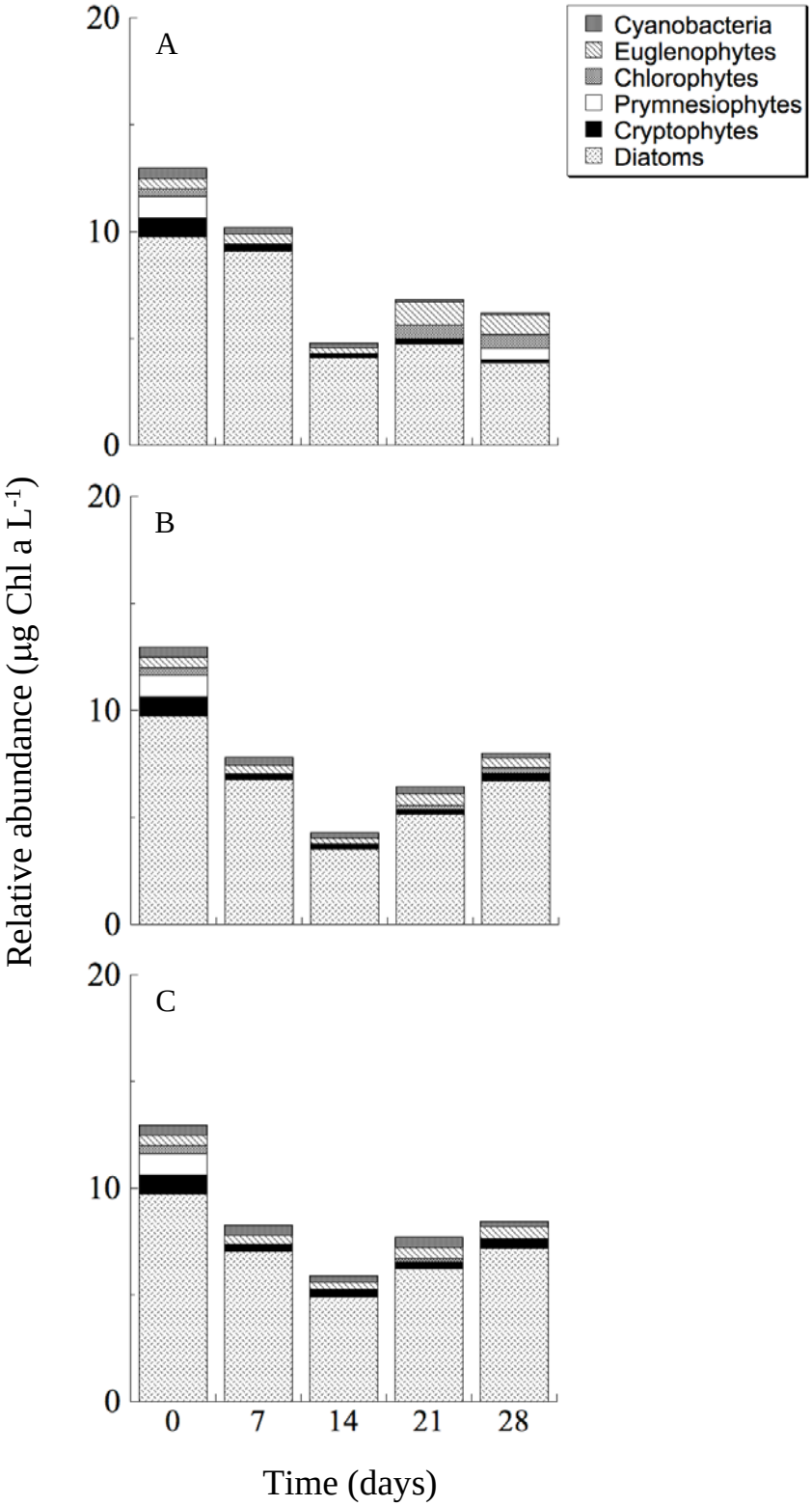
FIGURE Page

FIGURE Page

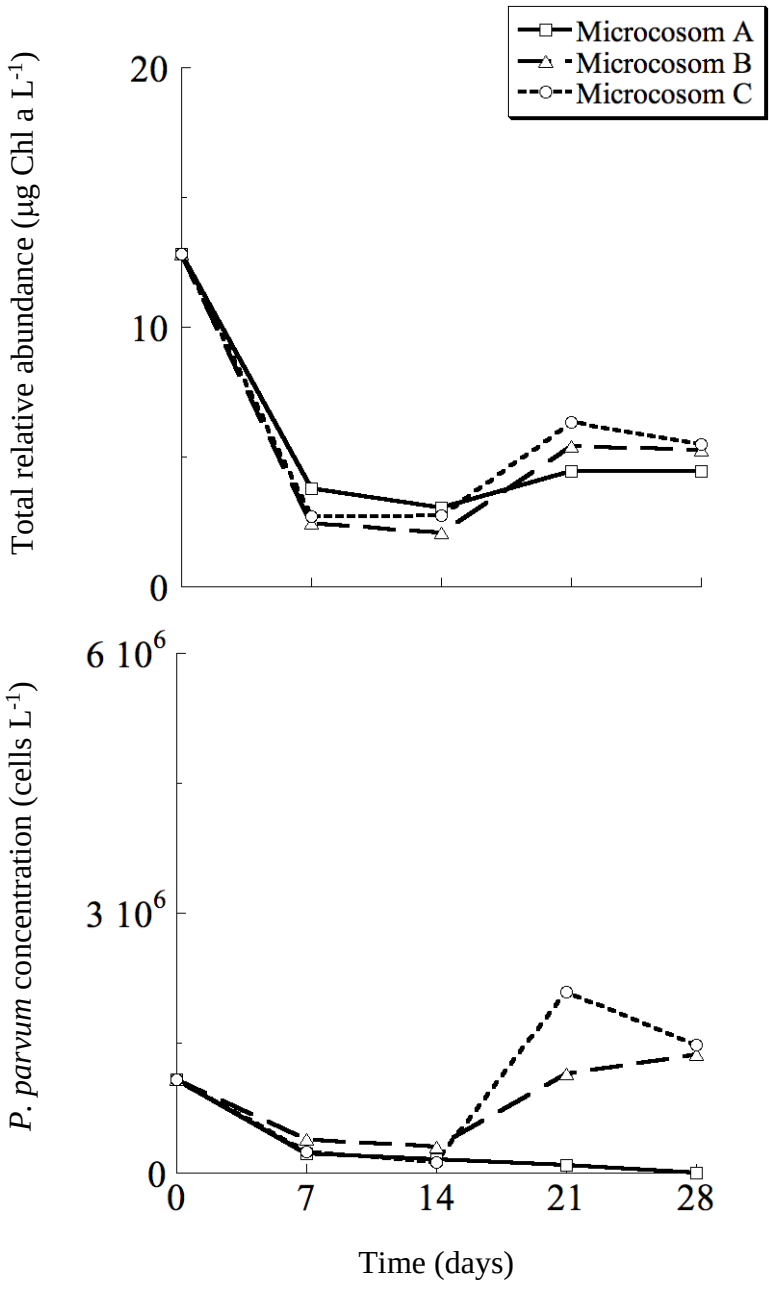
A-1
Fall 2004
Natural assemblage



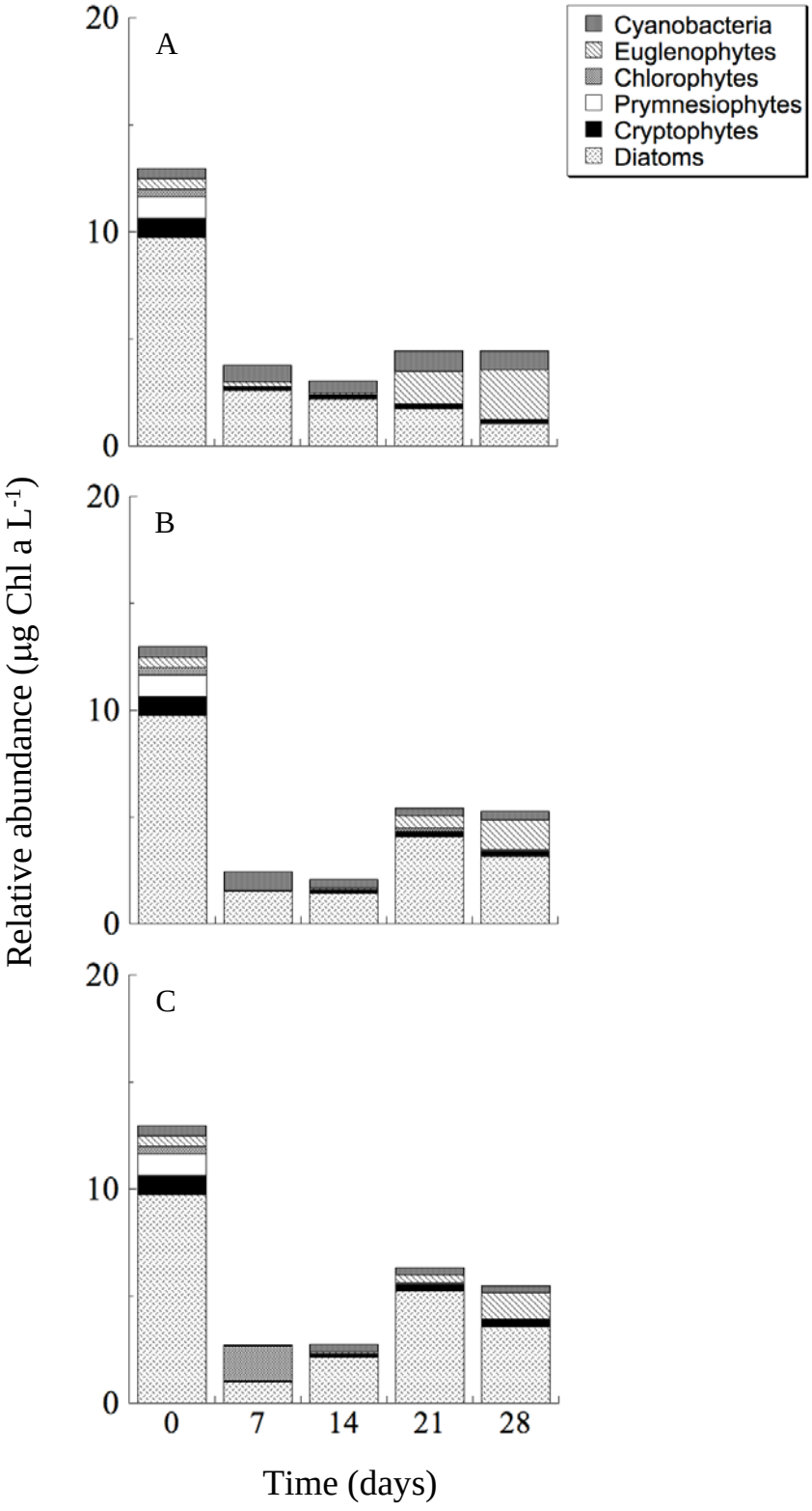
A-2
Fall 2004
Natural assemblage



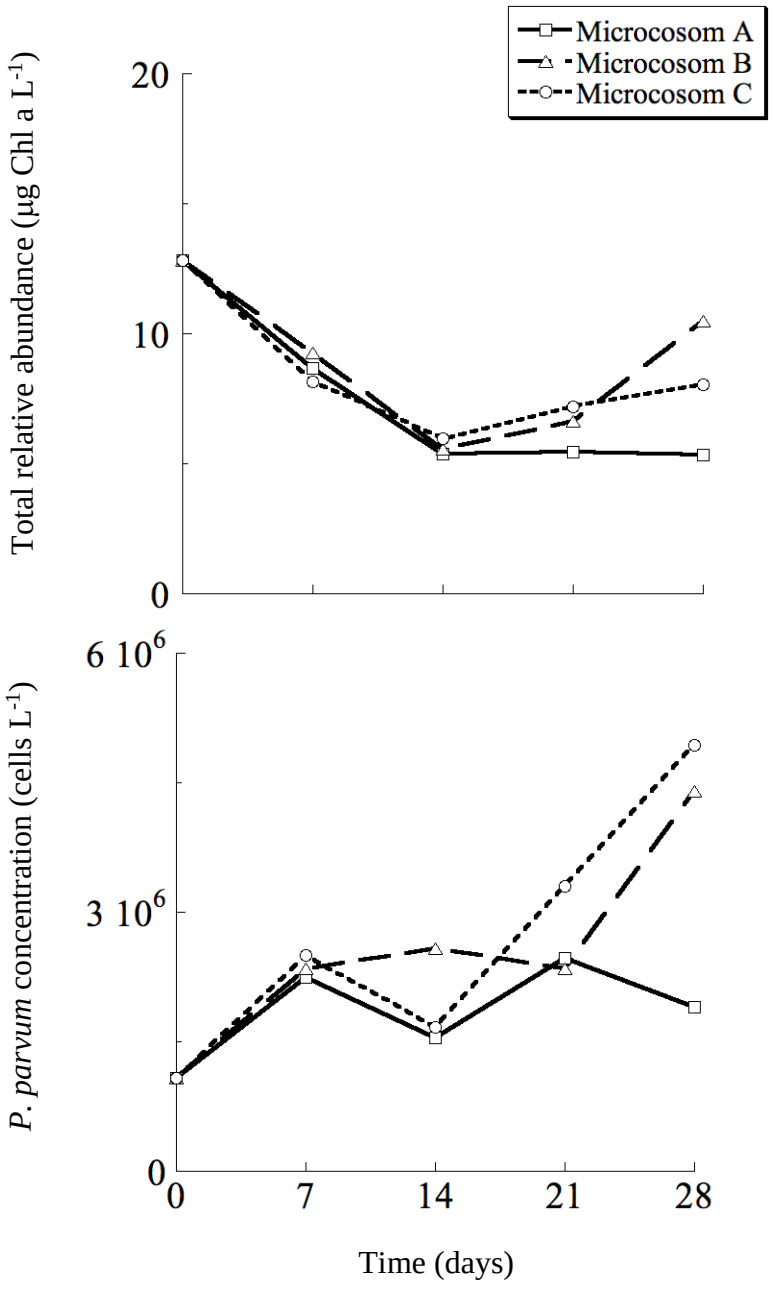
A-3
Fall 2004
Natural assemblage and BSE



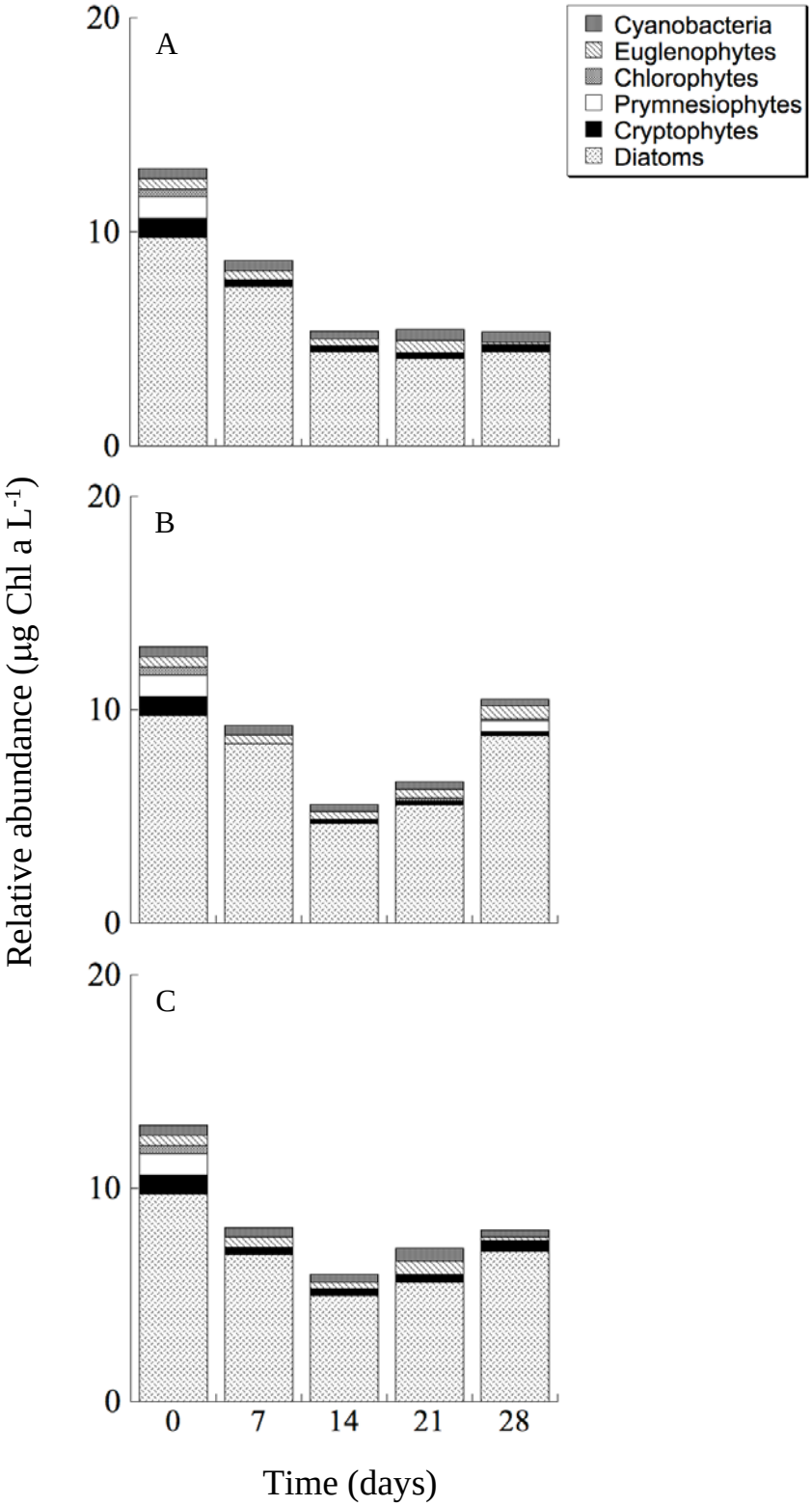
A-4
Fall 2004
Natural assemblage and BSE



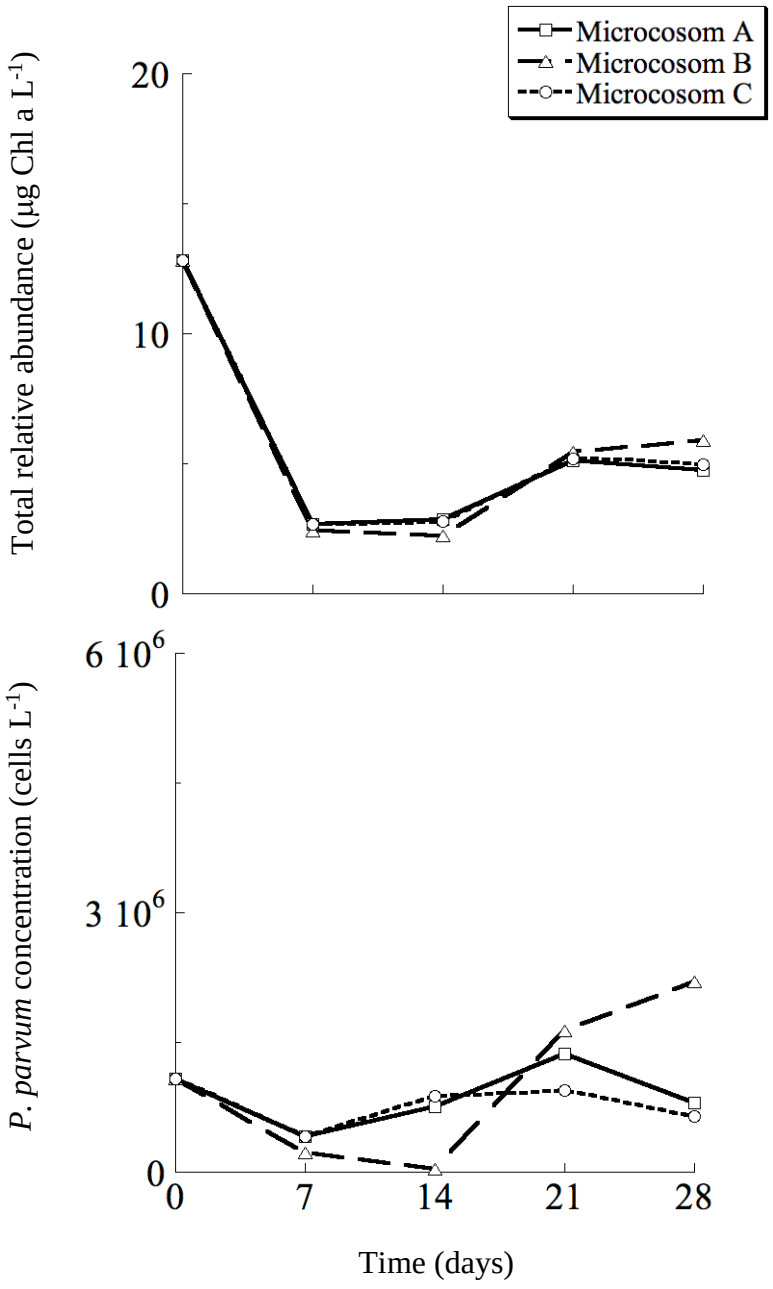
A-5
Fall 2004
Natural assemblage and *P. parvum*



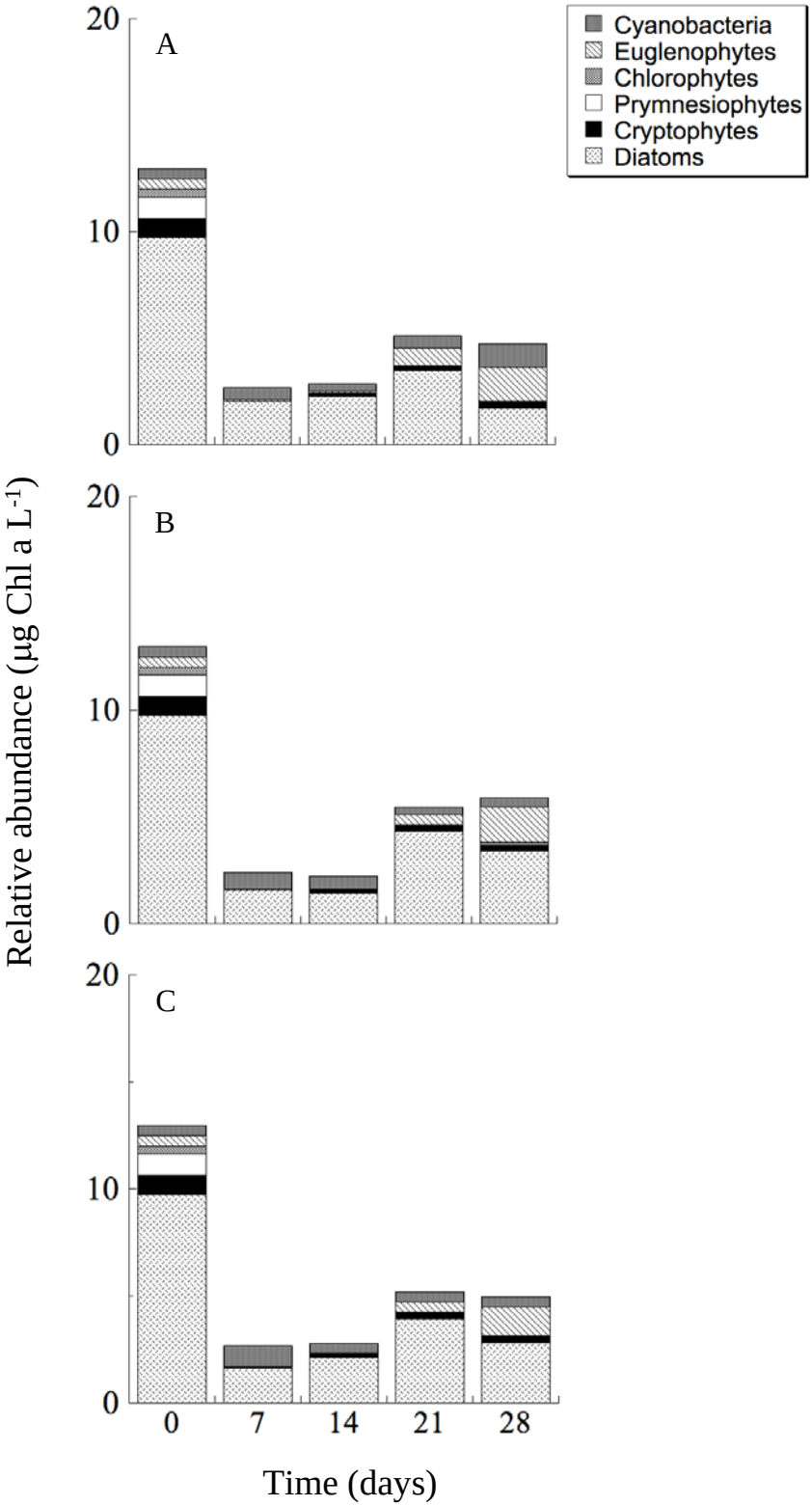
A-6
Fall 2004
Natural assemblage and *P. parvum*



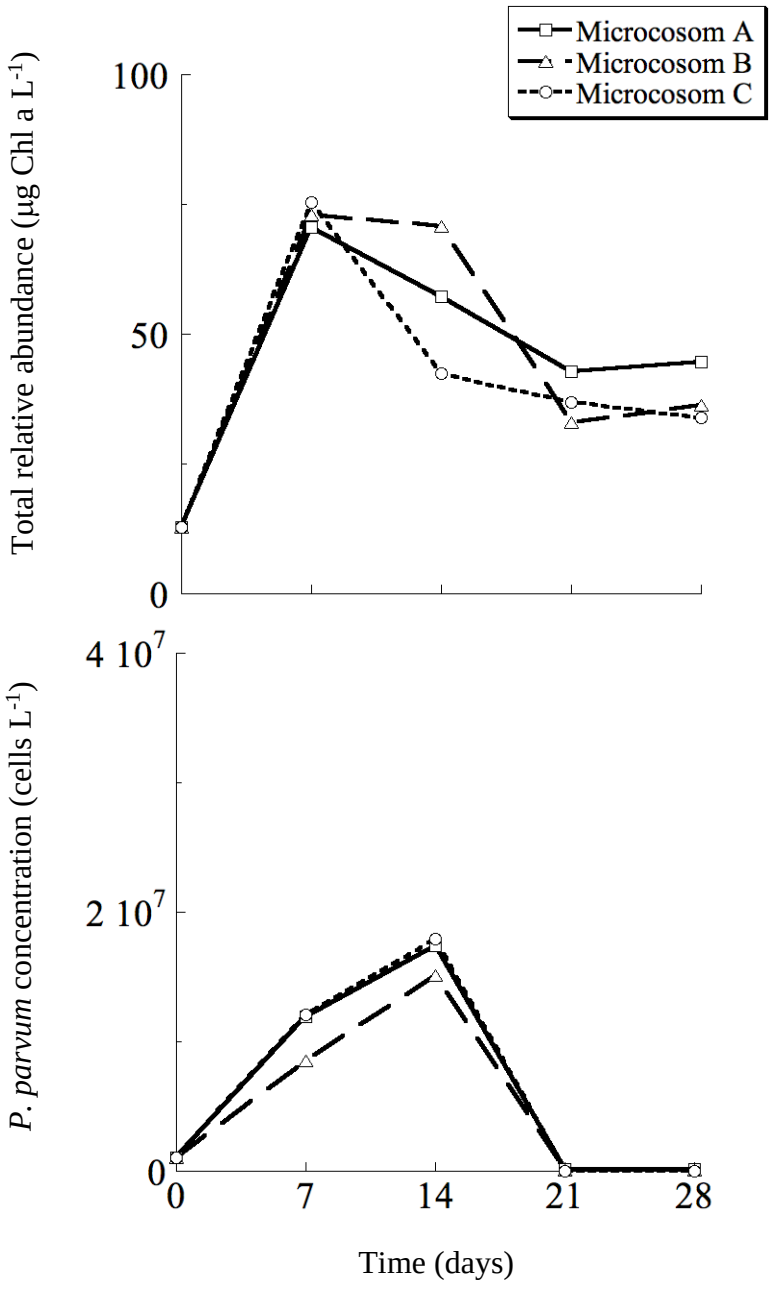
A-7
Fall 2004
Natural assemblage, BSE, and *P. parvum*



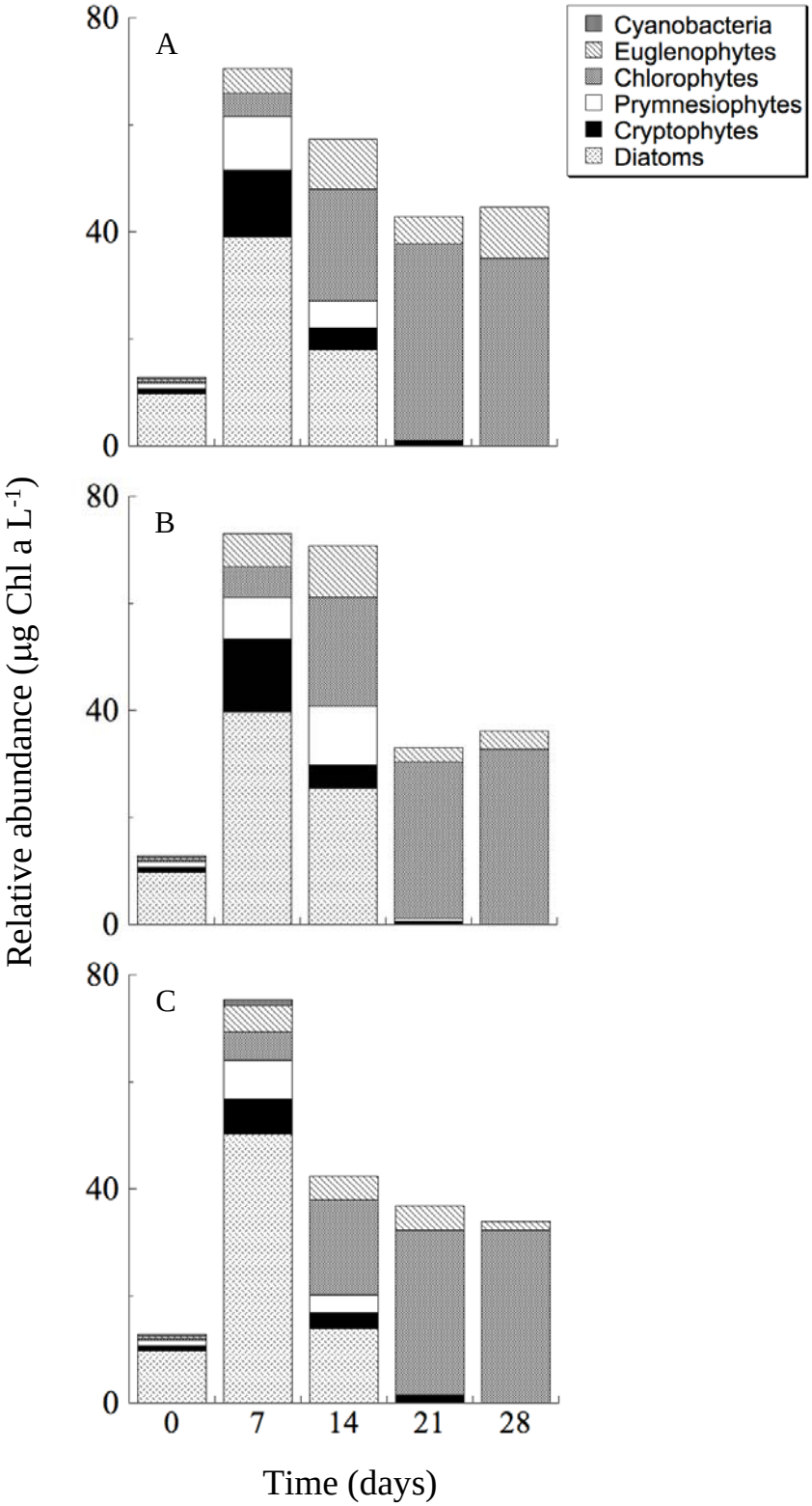
A-8
Fall 2004
Natural assemblage, BSE, and *P. parvum*



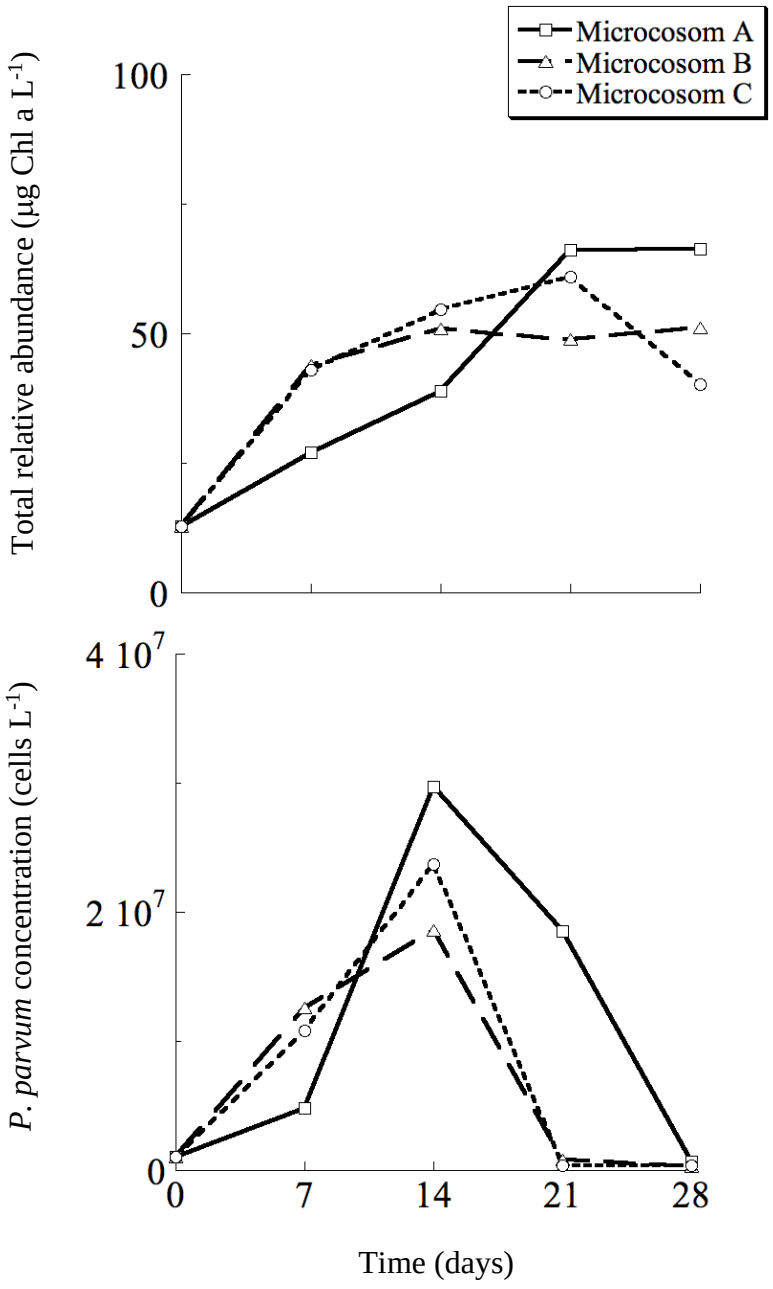
A-9
Fall 2004
Natural assemblage and f/2 nutrients



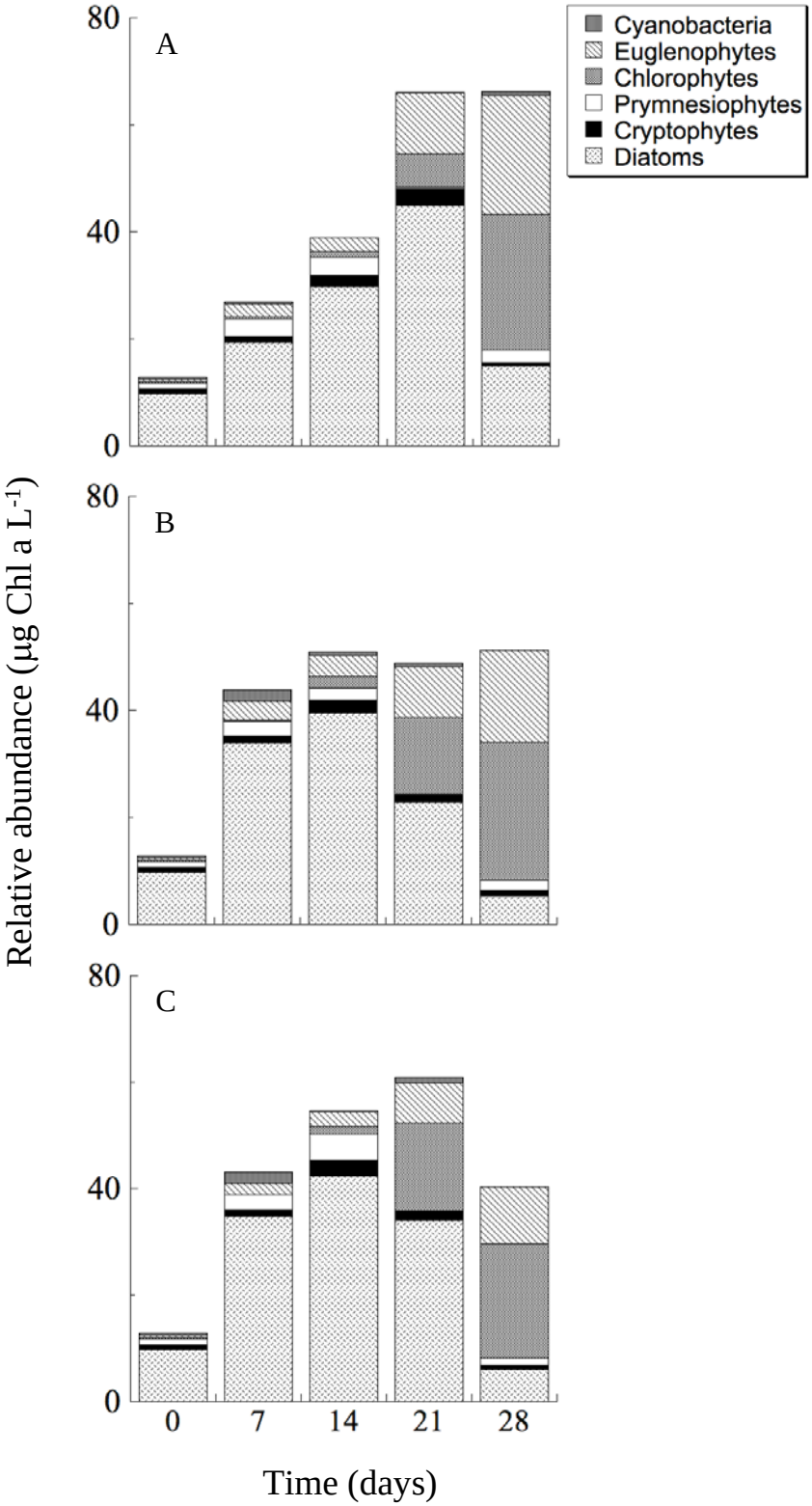
A-10
Fall 2004
Natural assemblage and f/2 nutrients



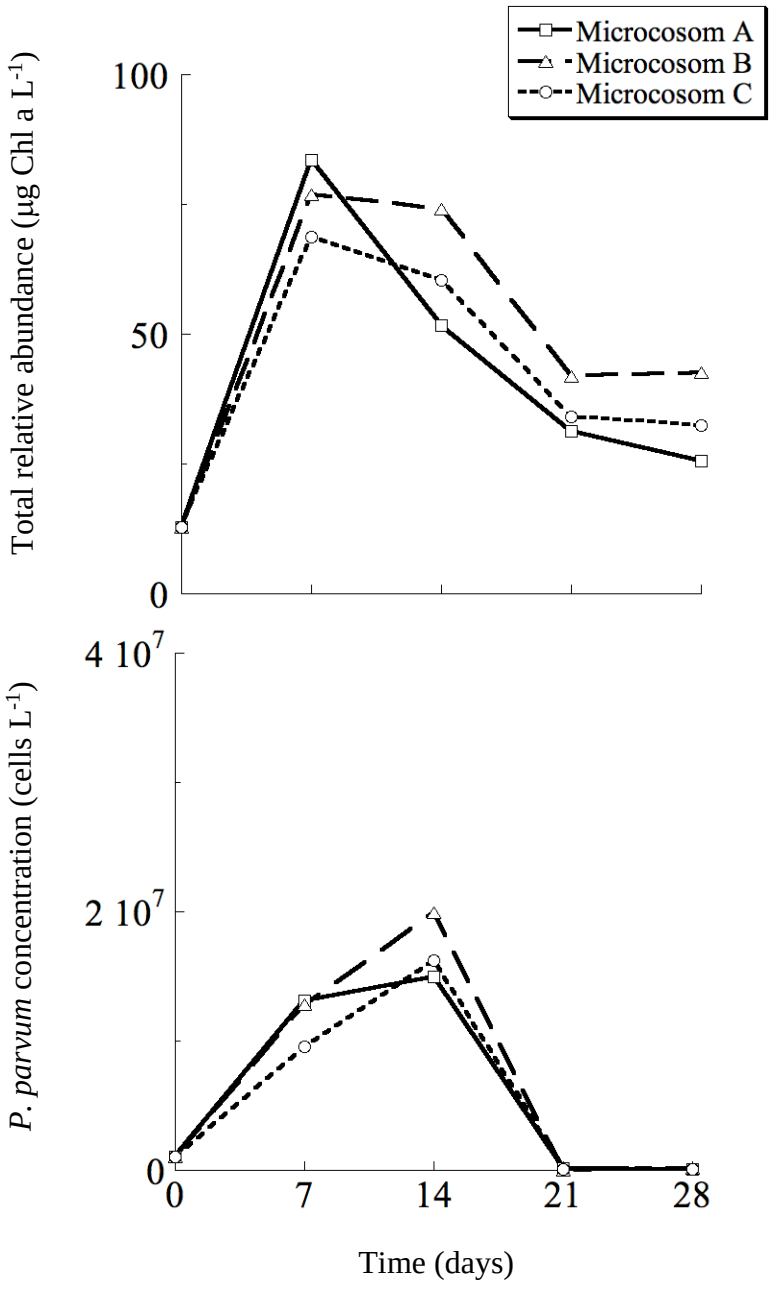
A-11
Fall 2004
Natural assemblage, f/2 nutrients, and BSE



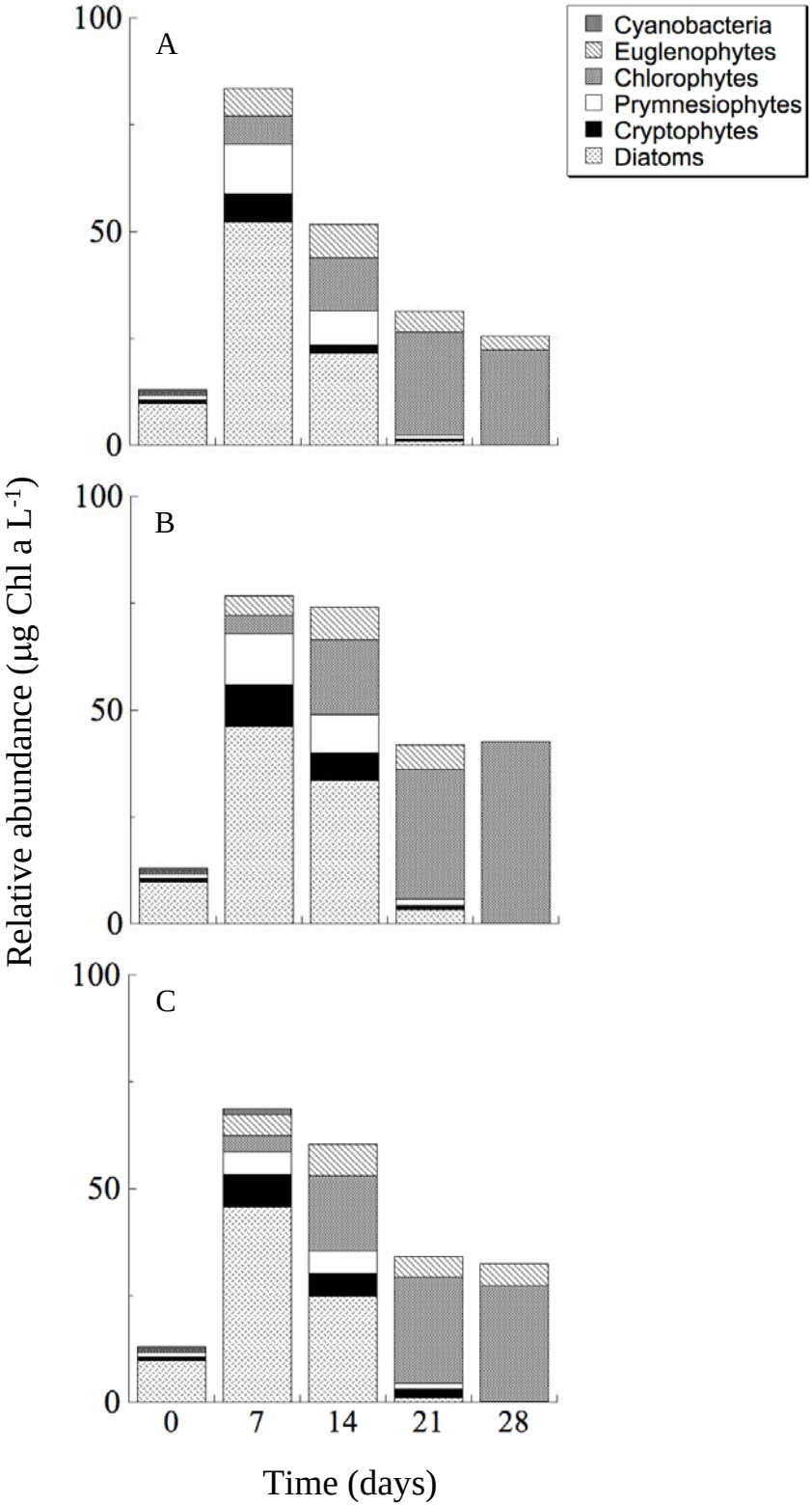
A-12
Fall 2004
Natural assemblage, f/2 nutrients, and BSE



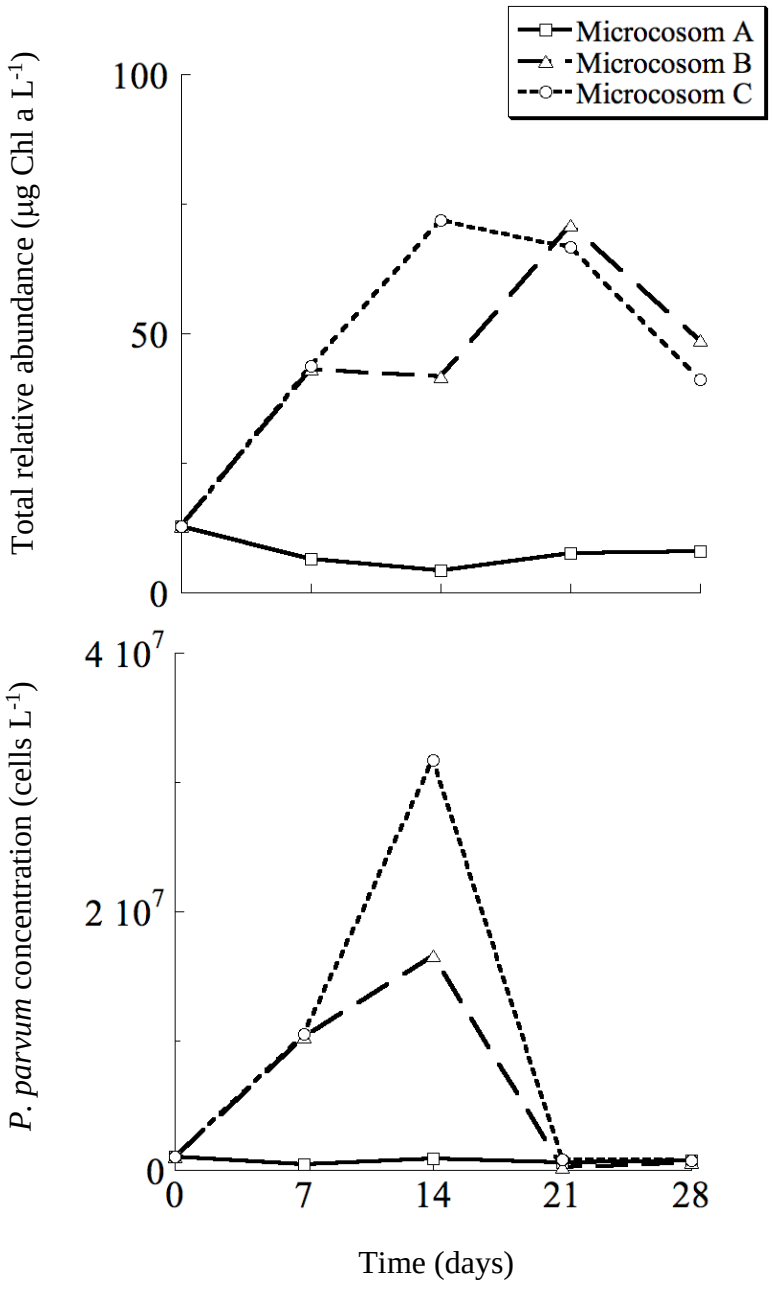
A-13
Fall 2004
Natural assemblage, f/2 nutrients, and *P. parvum*



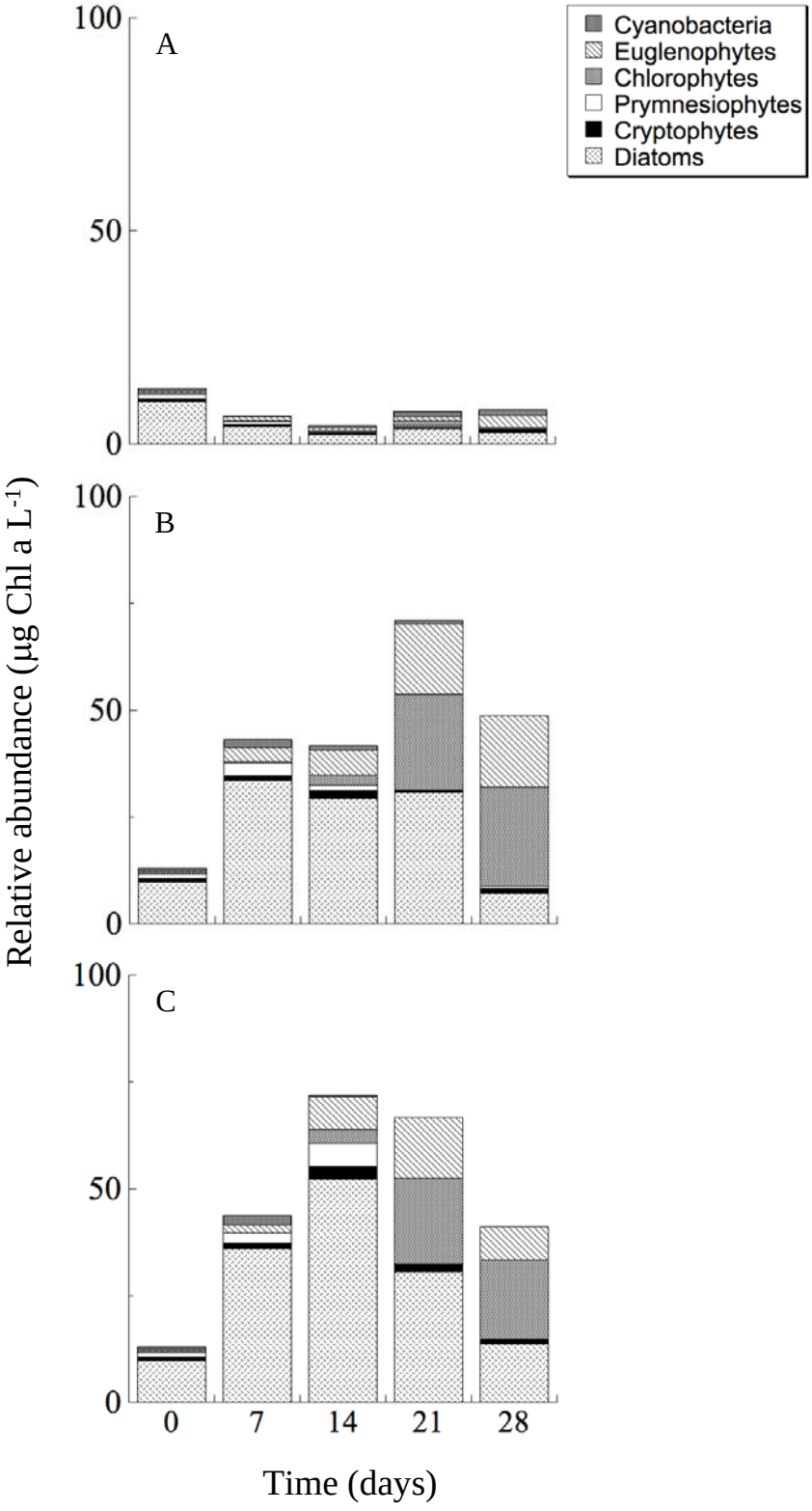
A-14
Fall 2004
Natural assemblage, f/2 nutrients, and *P. parvum*



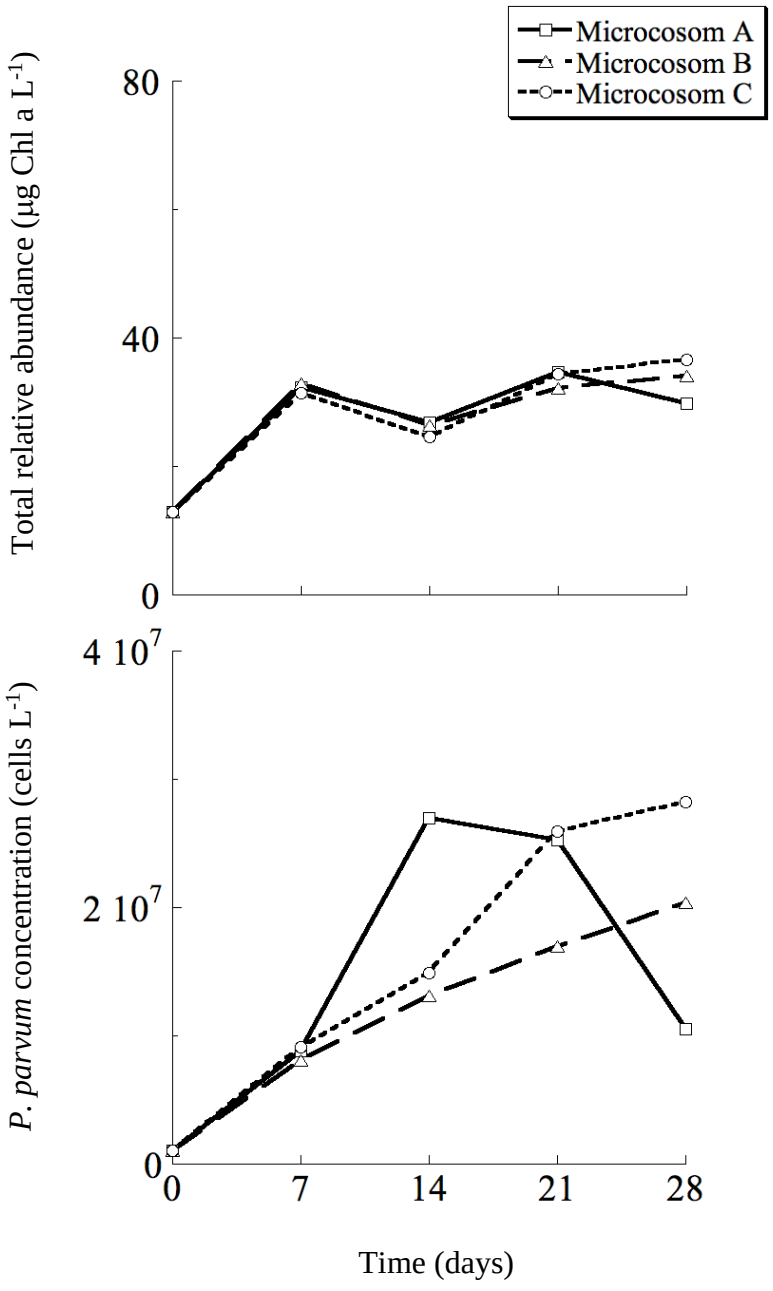
A-15
Fall 2004
Natural assemblage, f/2 nutrients, BSE, and *P. parvum*



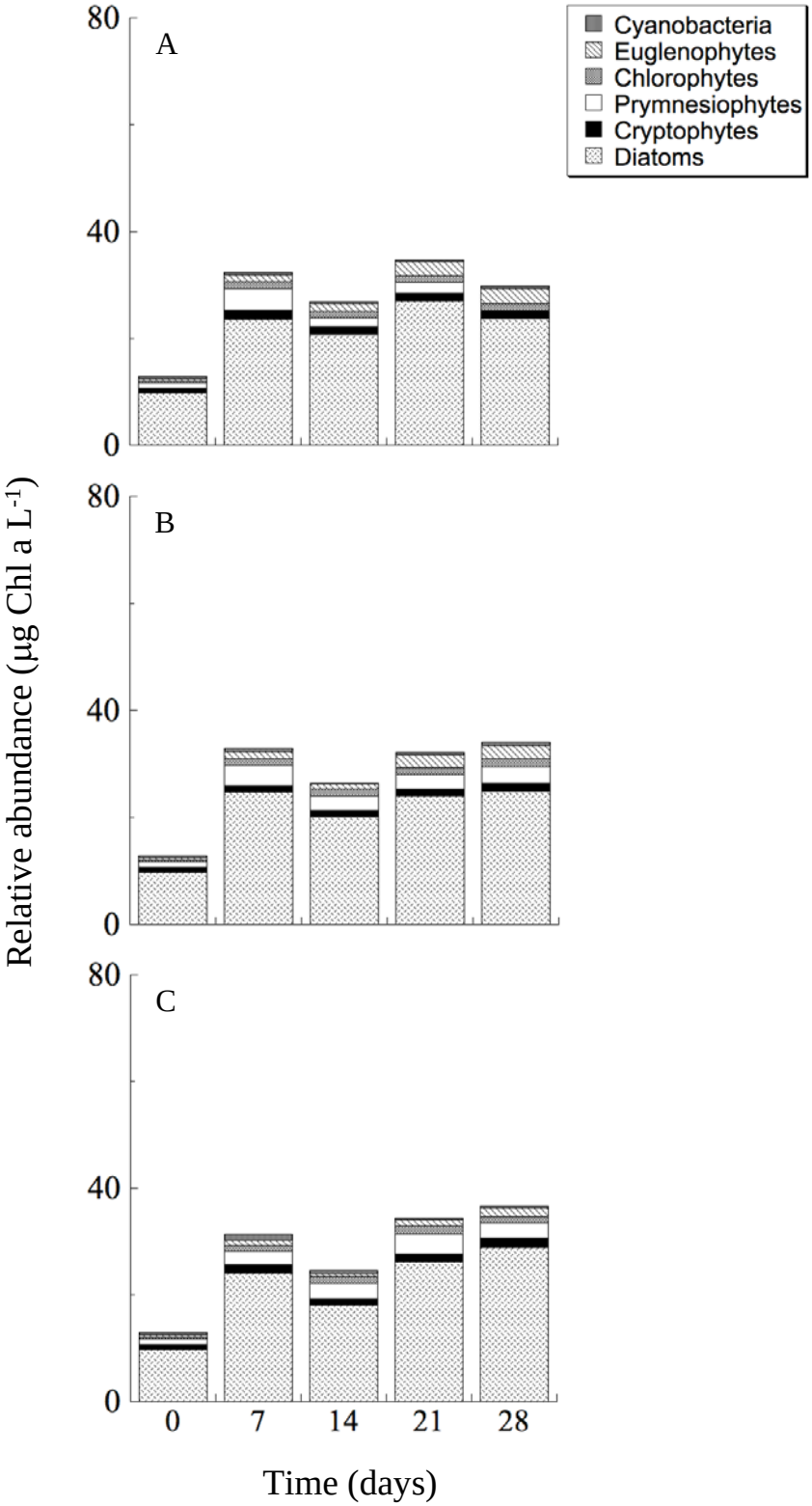
A-16
Fall 2004
Natural assemblage, f/2 nutrients, BSE, and *P. parvum*



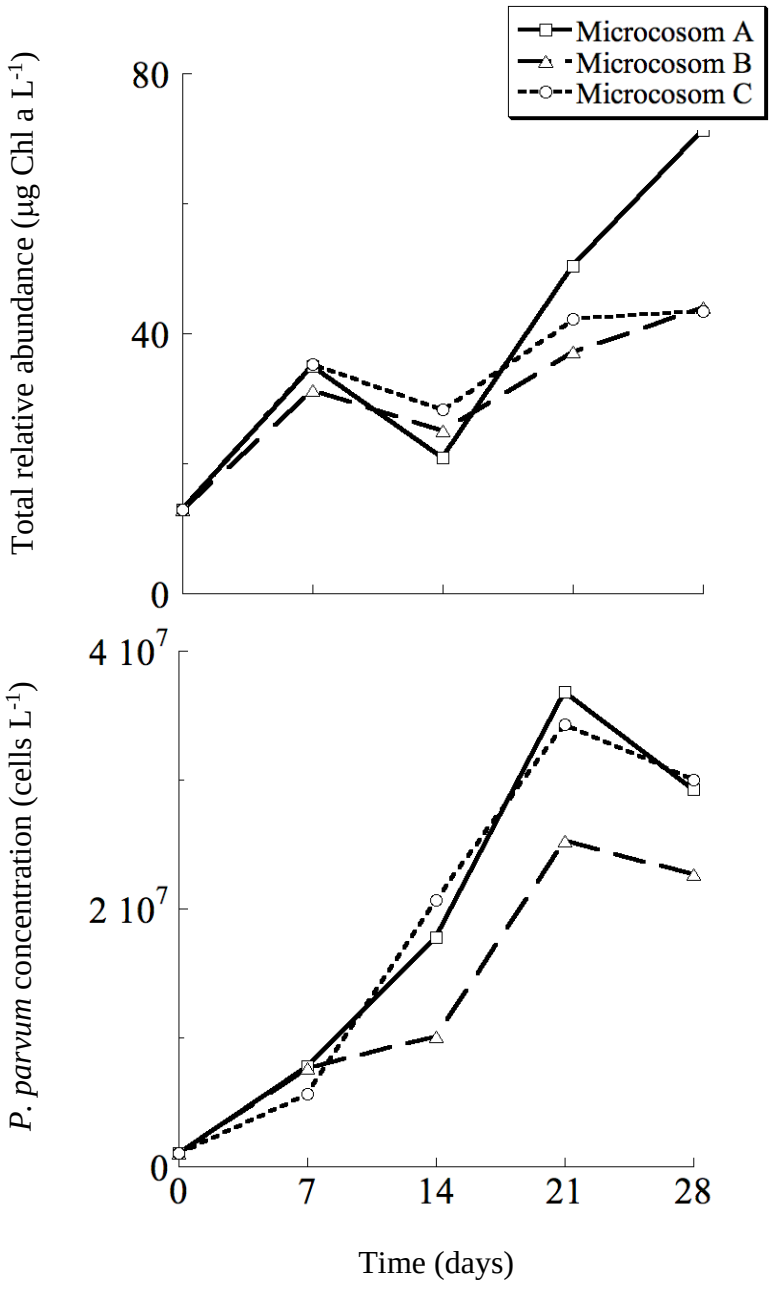
A-17
Fall 2004
Natural assemblage and nitrogen deplete f/2 nutrients



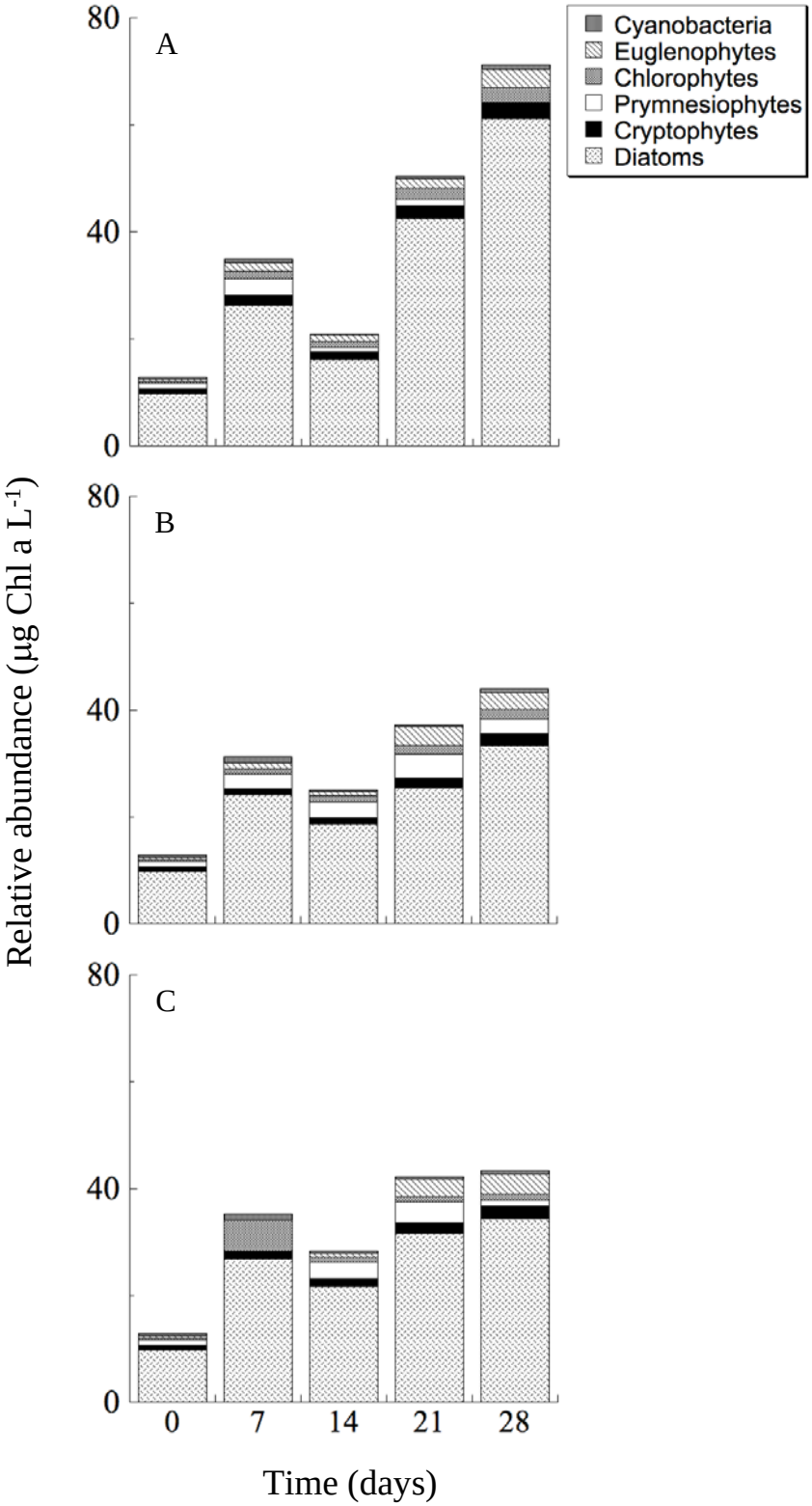
A-18
Fall 2004
Natural assemblage and nitrogen deplete f/2 nutrients



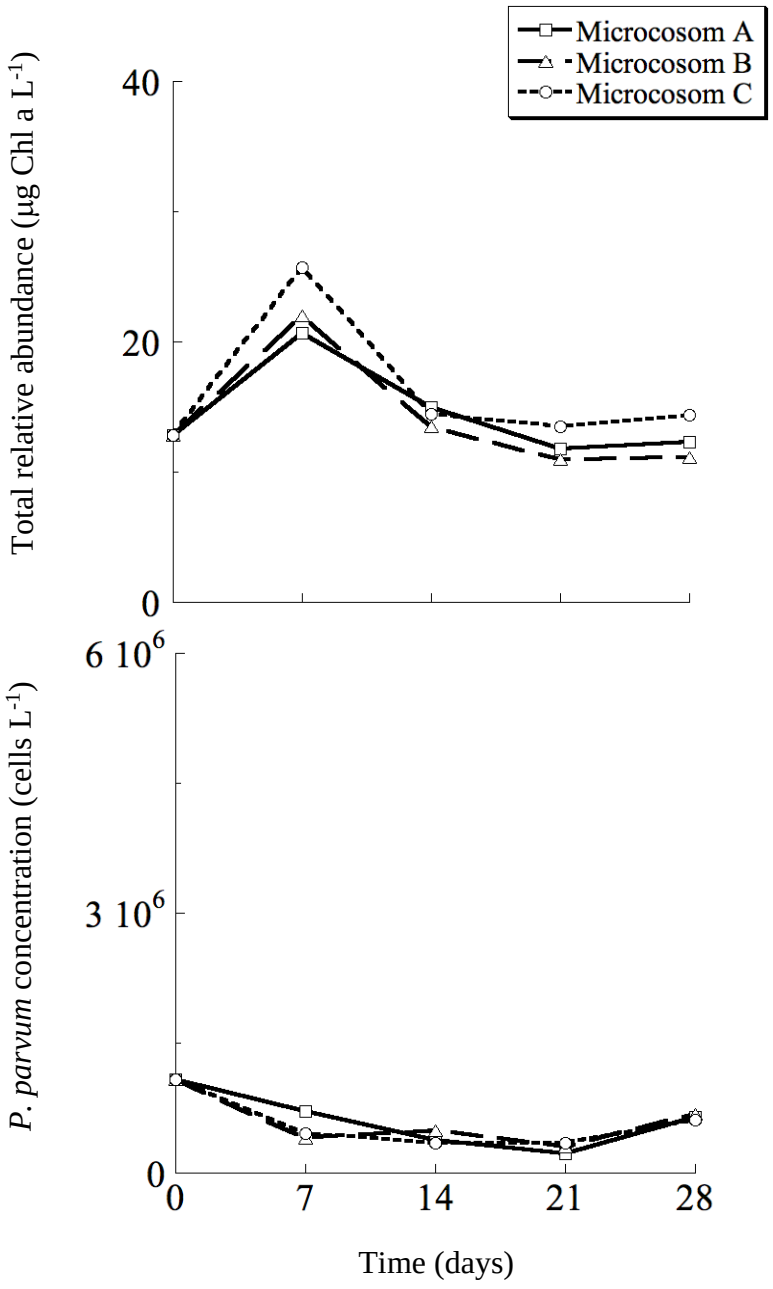
A-19
Fall 2004
Natural assemblage, nitrogen deplete f/2 nutrients and *P. parvum*



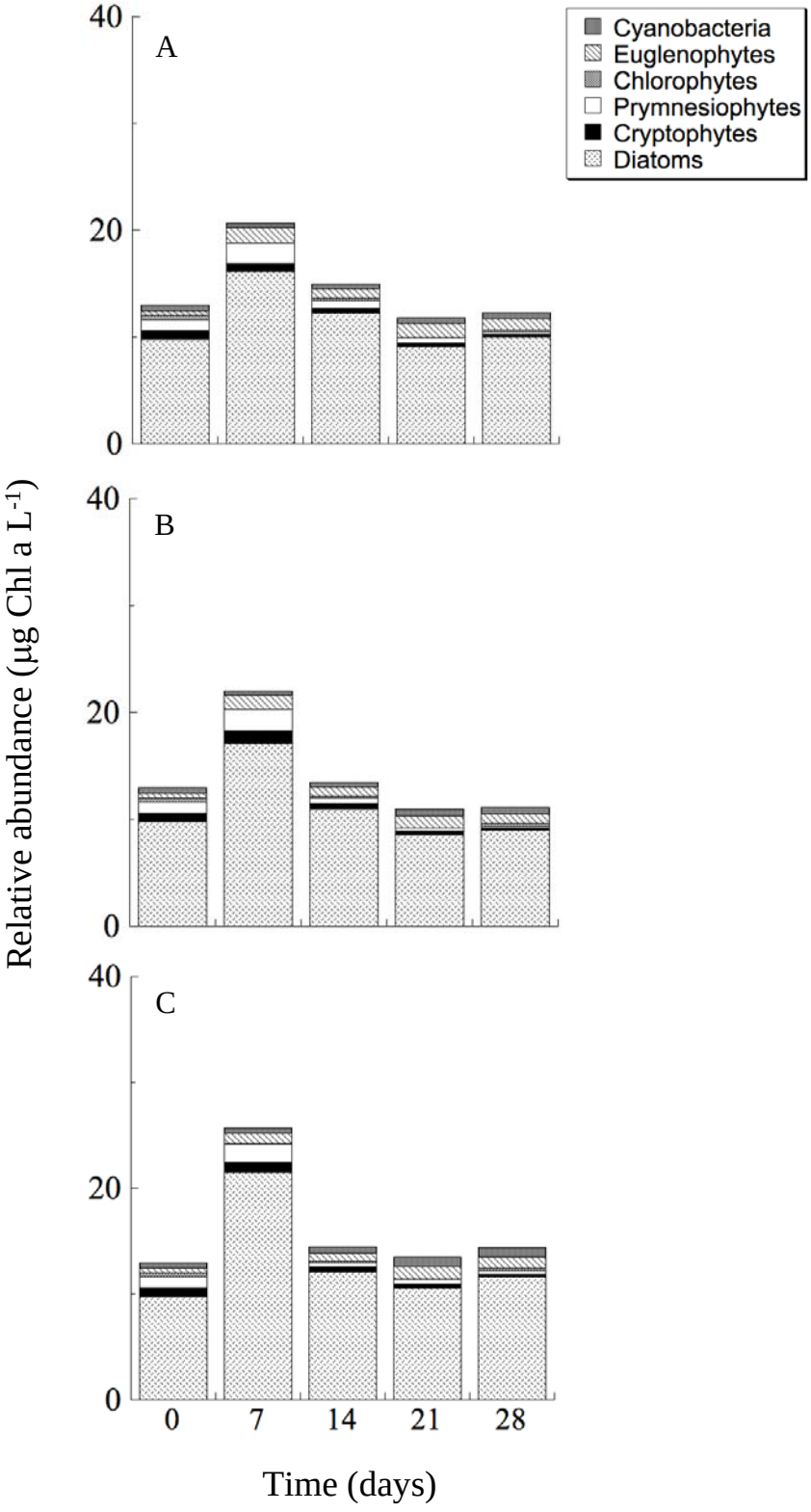
A-20
Fall 2004
Natural assemblage, nitrogen deplete f/2 nutrients, and *P. parvum*



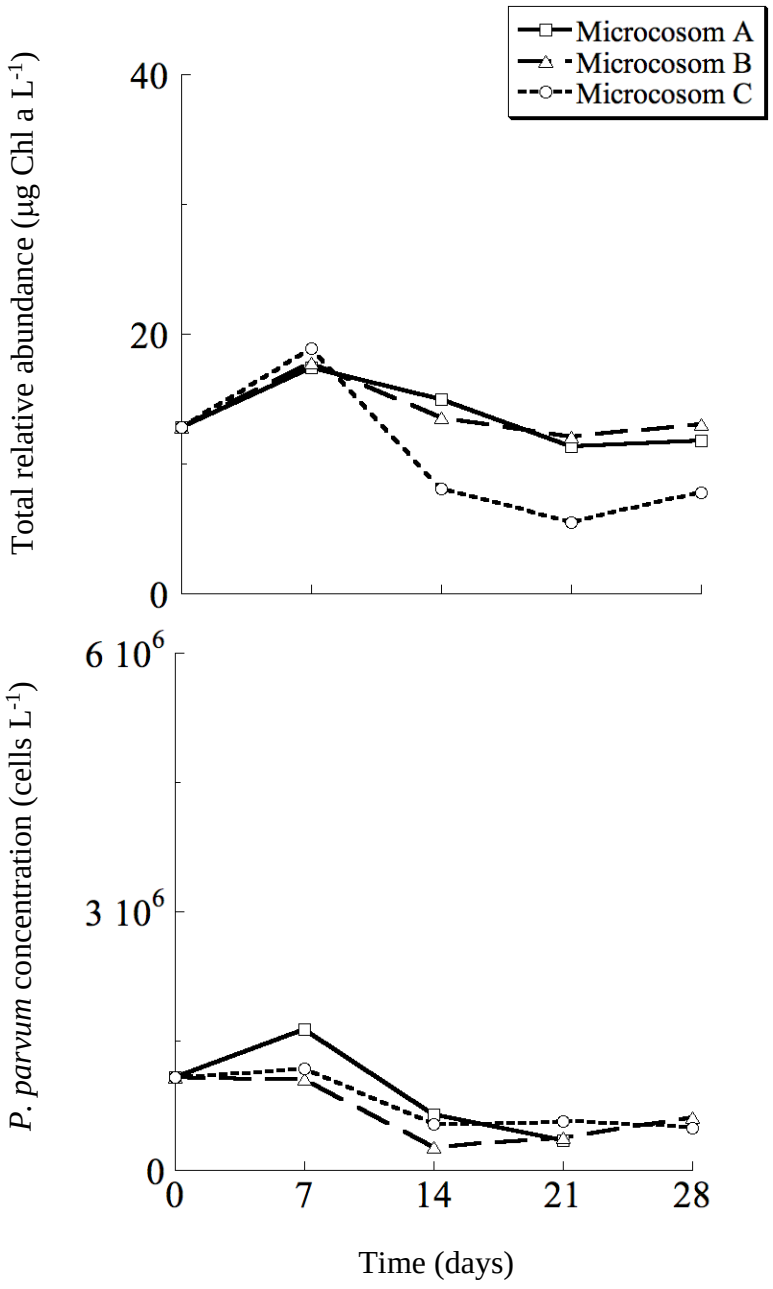
A-21
Fall 2004
Natural assemblage and phosphorus deplete f/2 nutrients



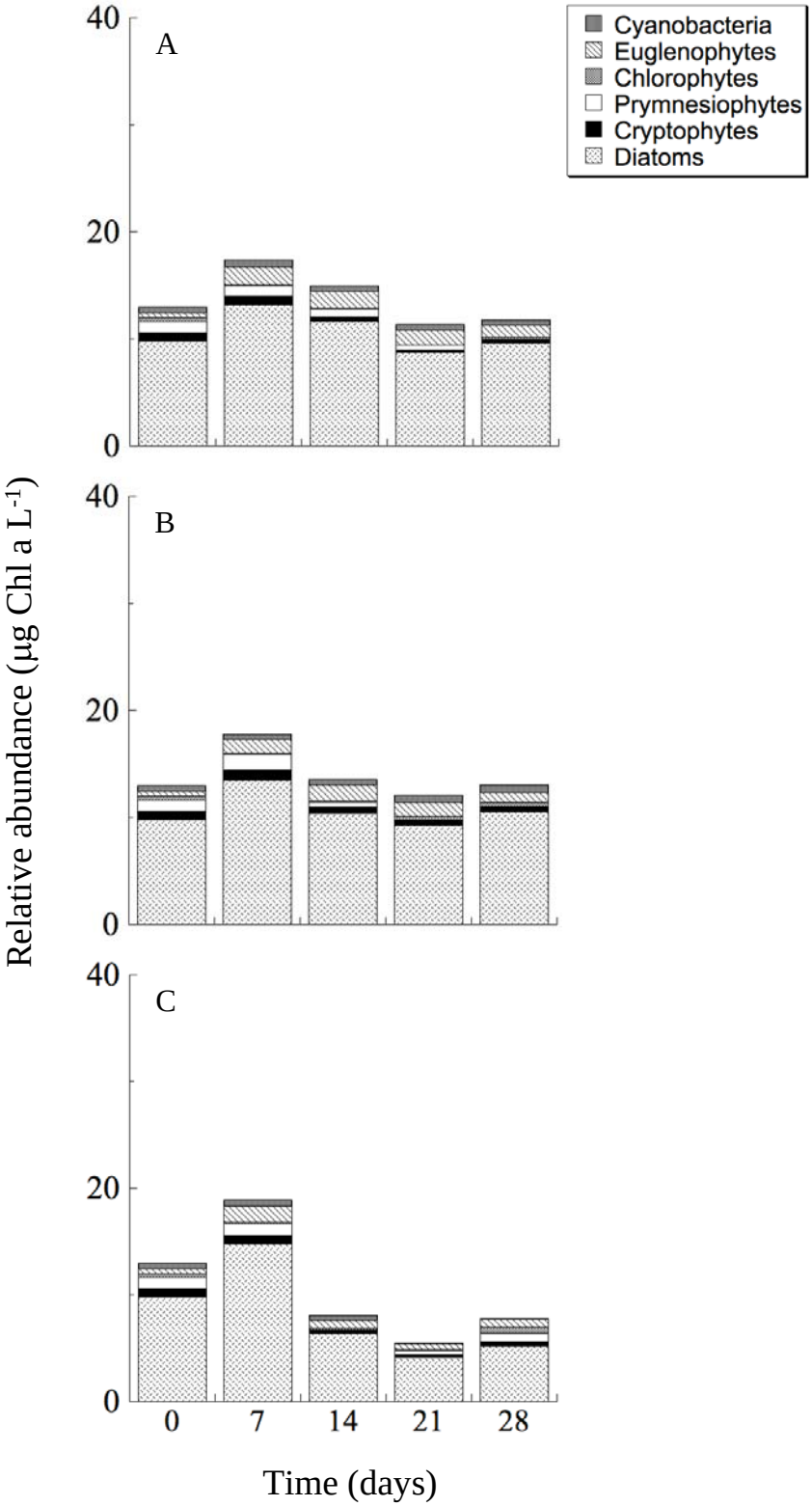
A-22
Fall 2004
Natural assemblage and phosphorus deplete f/2 nutrients



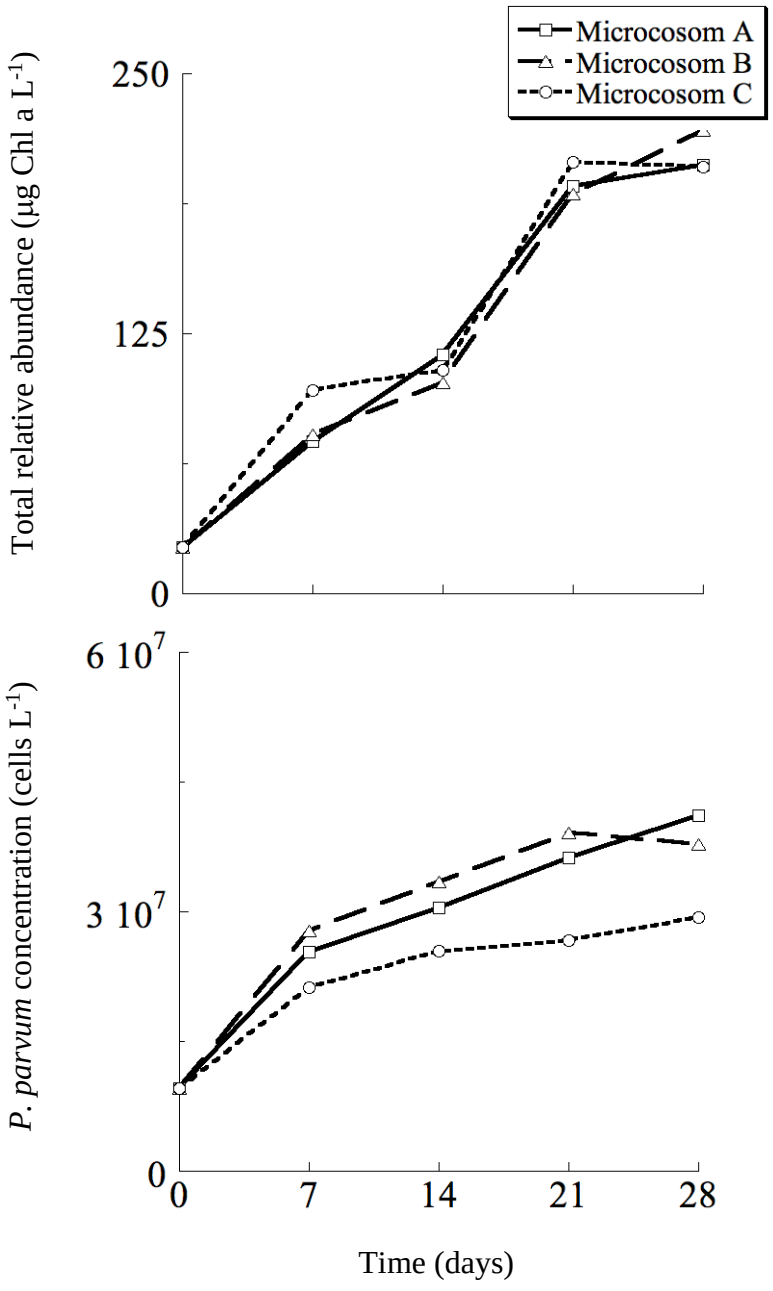
A-23
Fall 2004
Natural assemblage, phosphorus deplete f/2 nutrients, and *P. parvum*



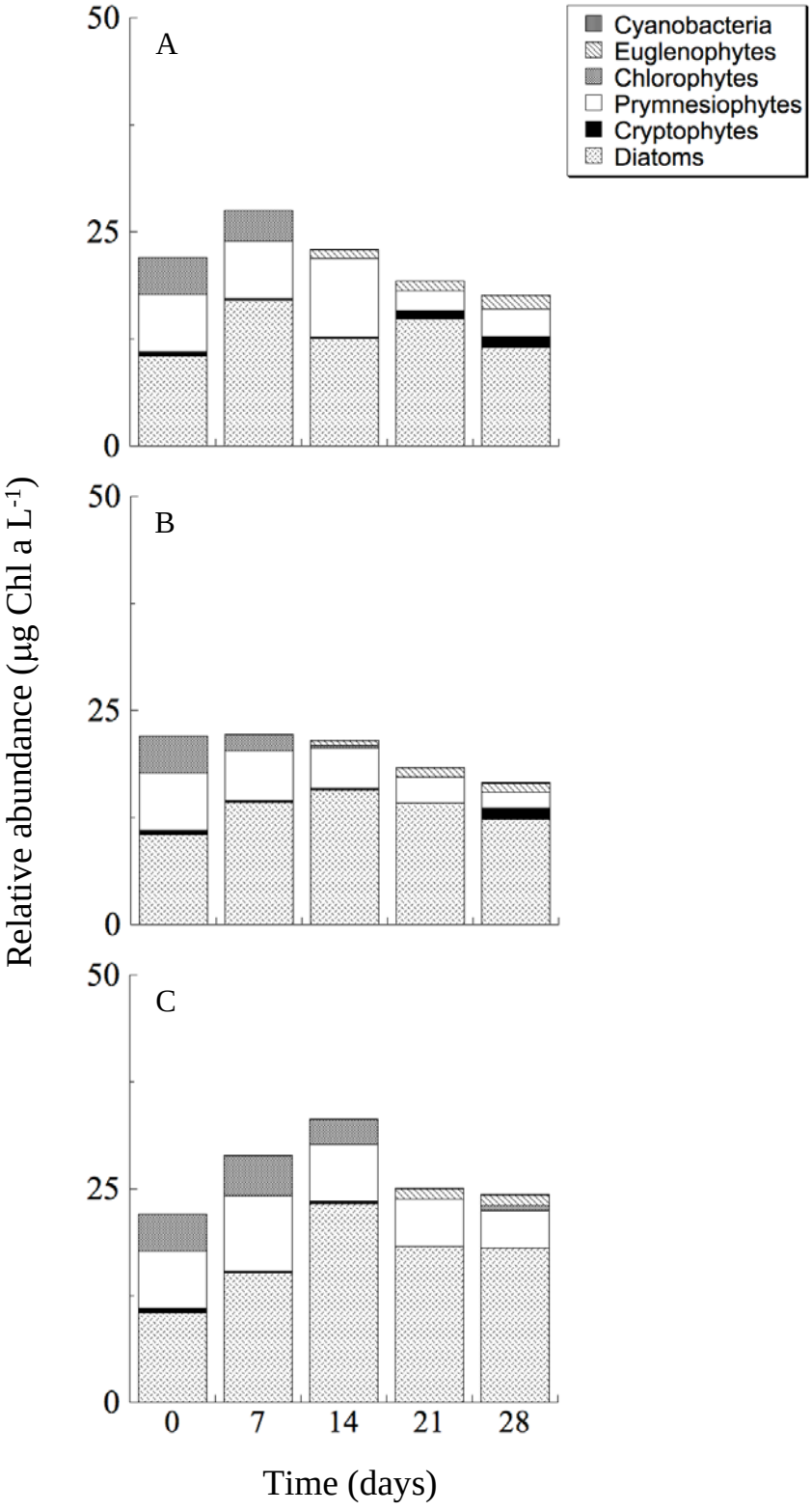
A-24
Fall 2004
Natural assemblage, phosphorus deplete f/2 nutrients, and *P. parvum*



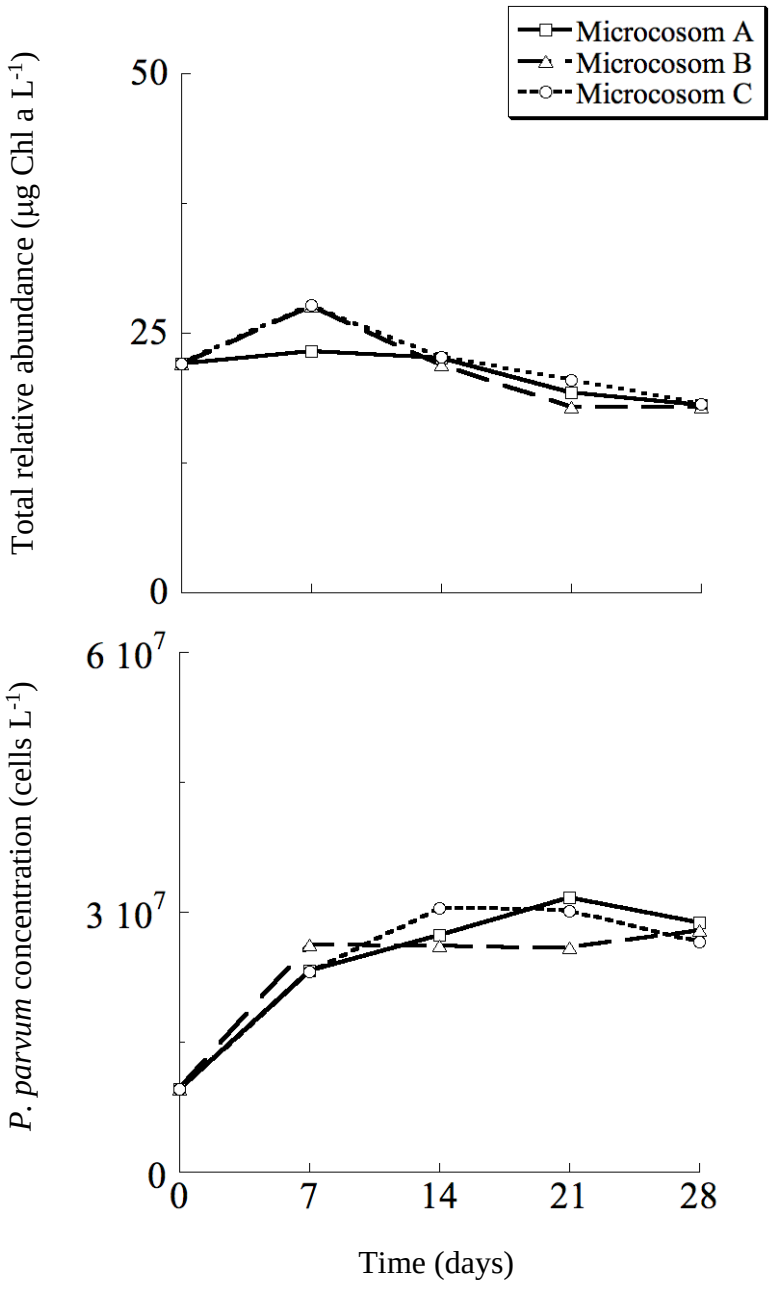
A-25
Winter 2005
Natural assemblage



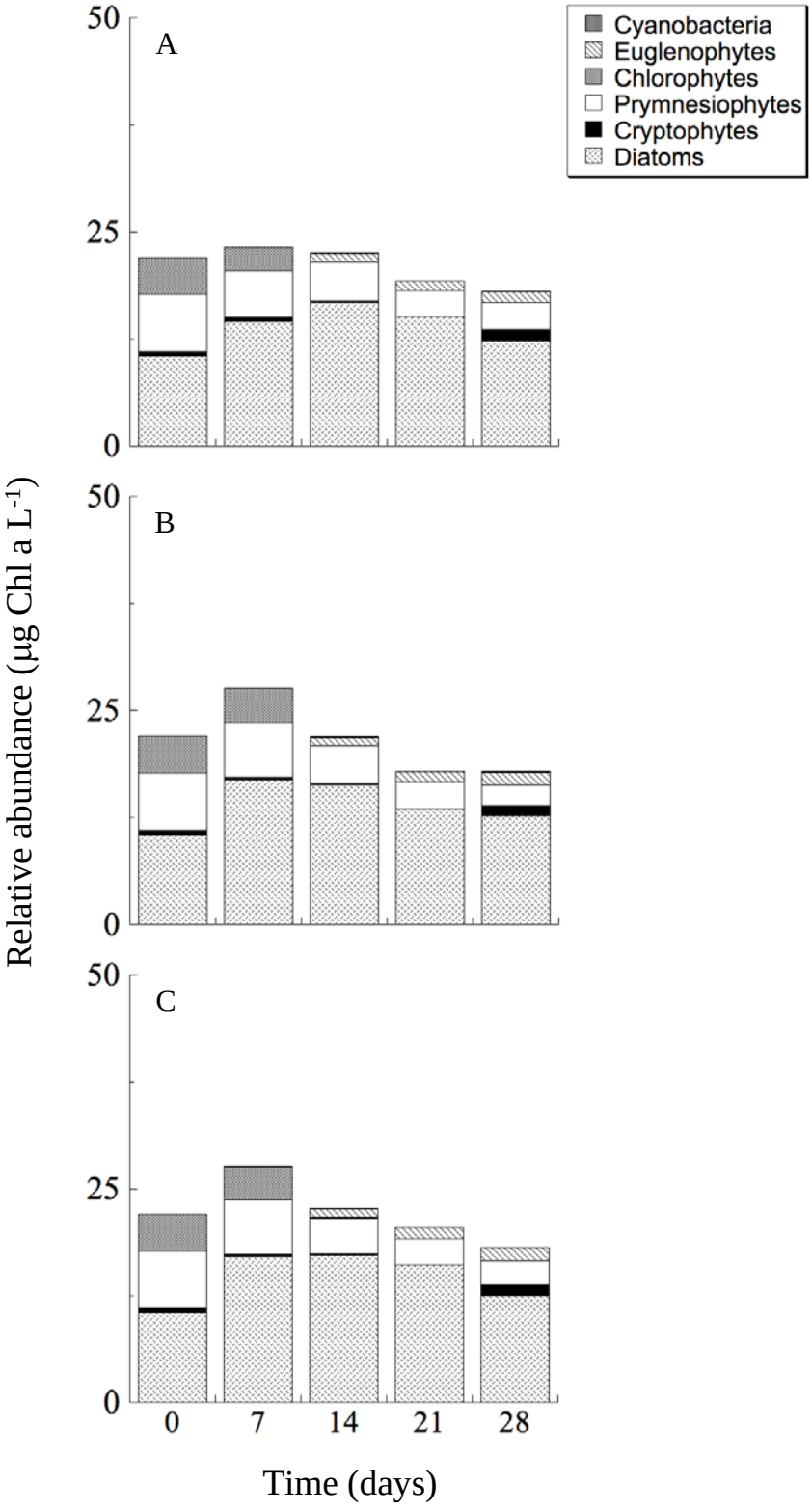
A-26
Winter 2005
Natural assemblage



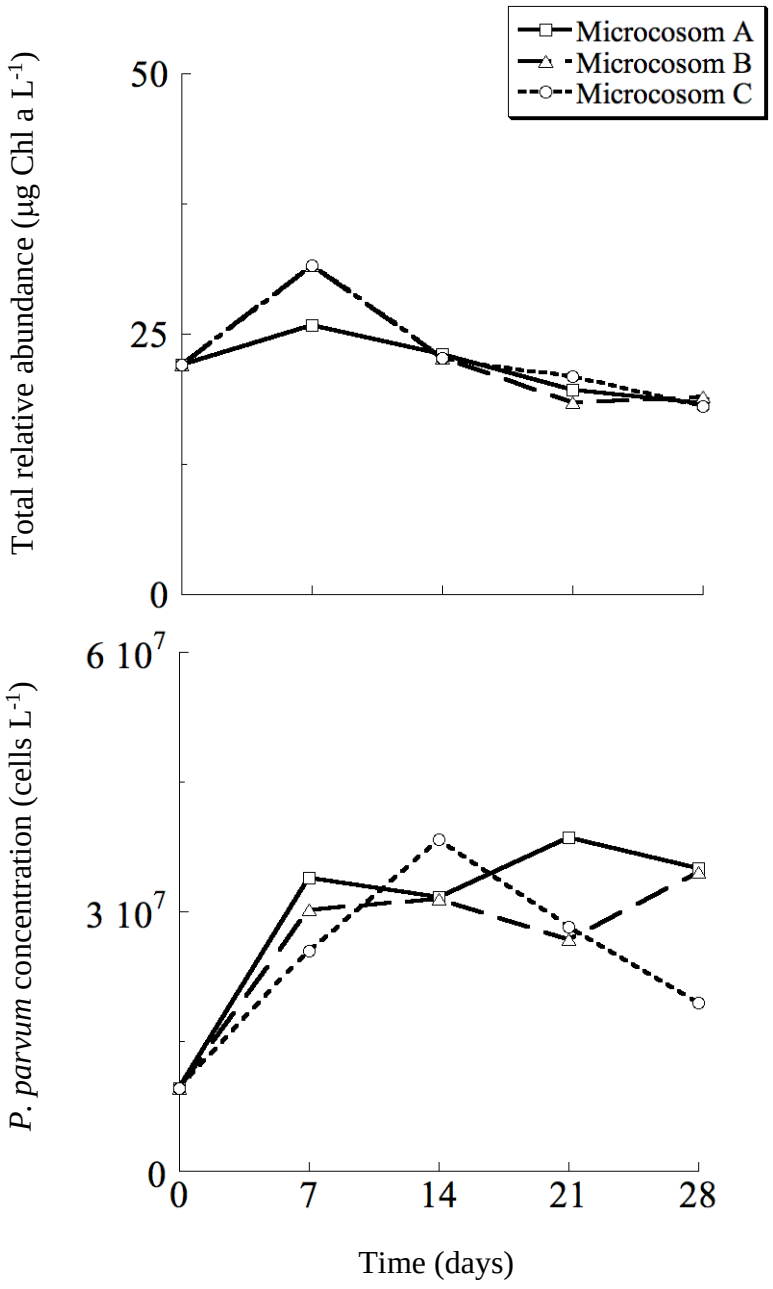
A-27
Winter 2005
Natural assemblage and BSE



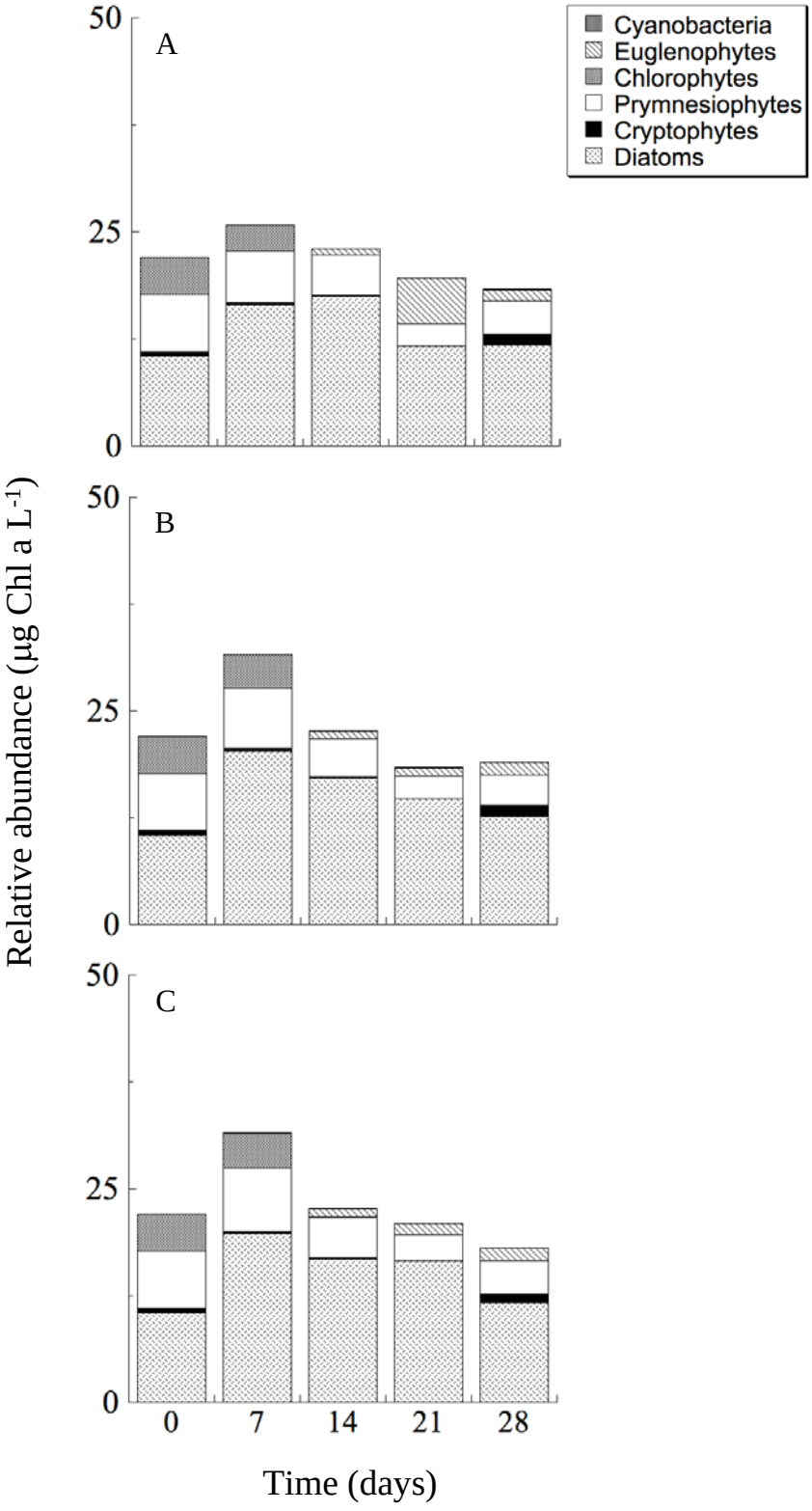
A-28
Winter 2005
Natural assemblage and BSE



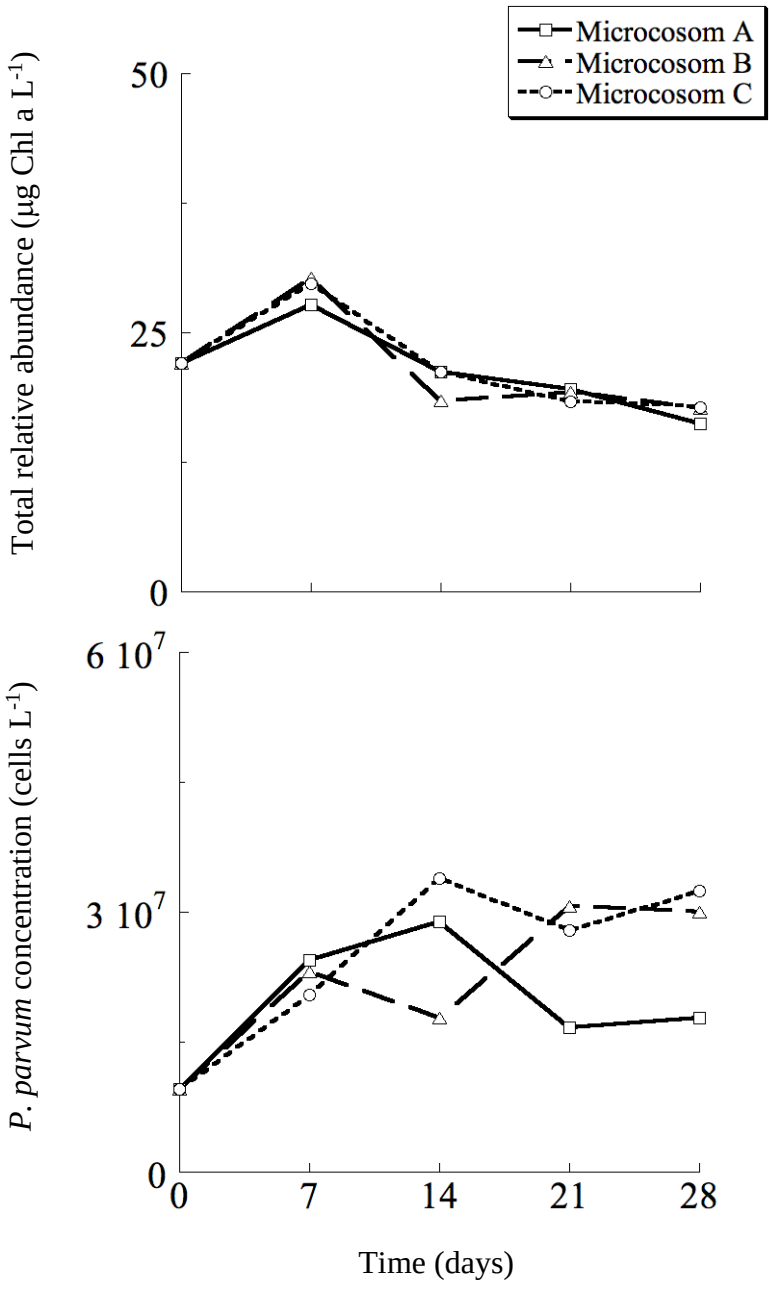
A-29
Winter 2005
Natural assemblage and *P. parvum*



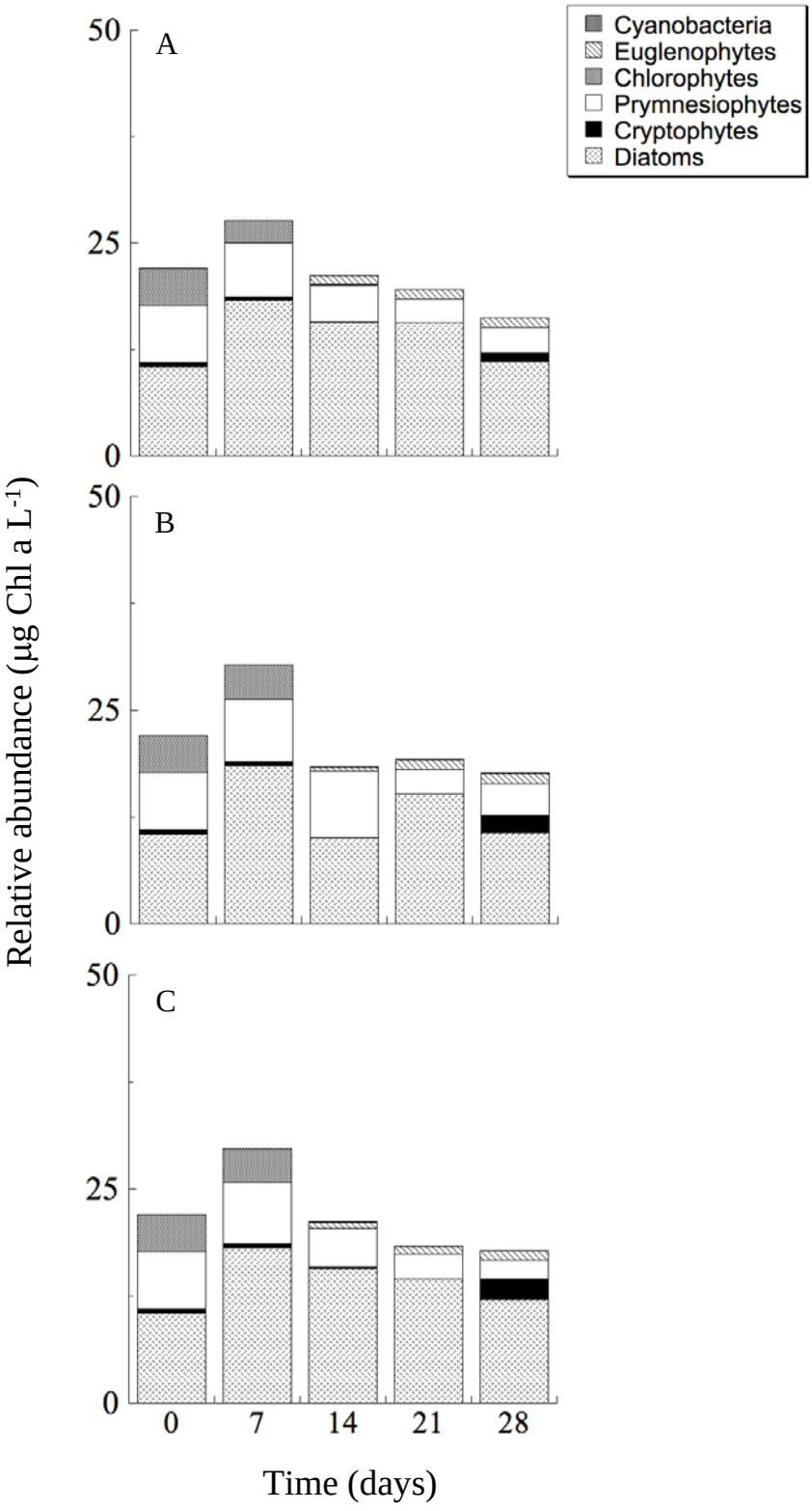
A-30
Winter 2005
Natural assemblage and *P. parvum*



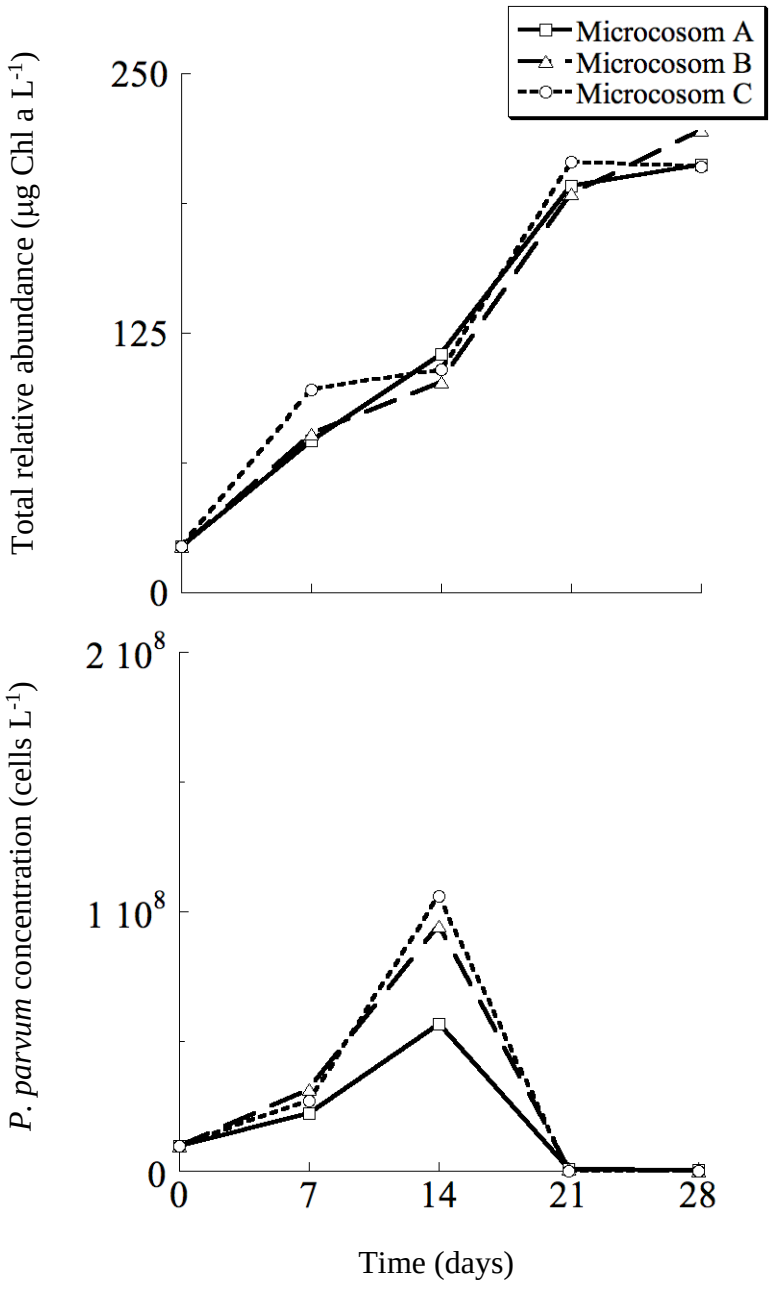
A-31
Winter 2005
Natural assemblage, BSE, and *P. parvum*



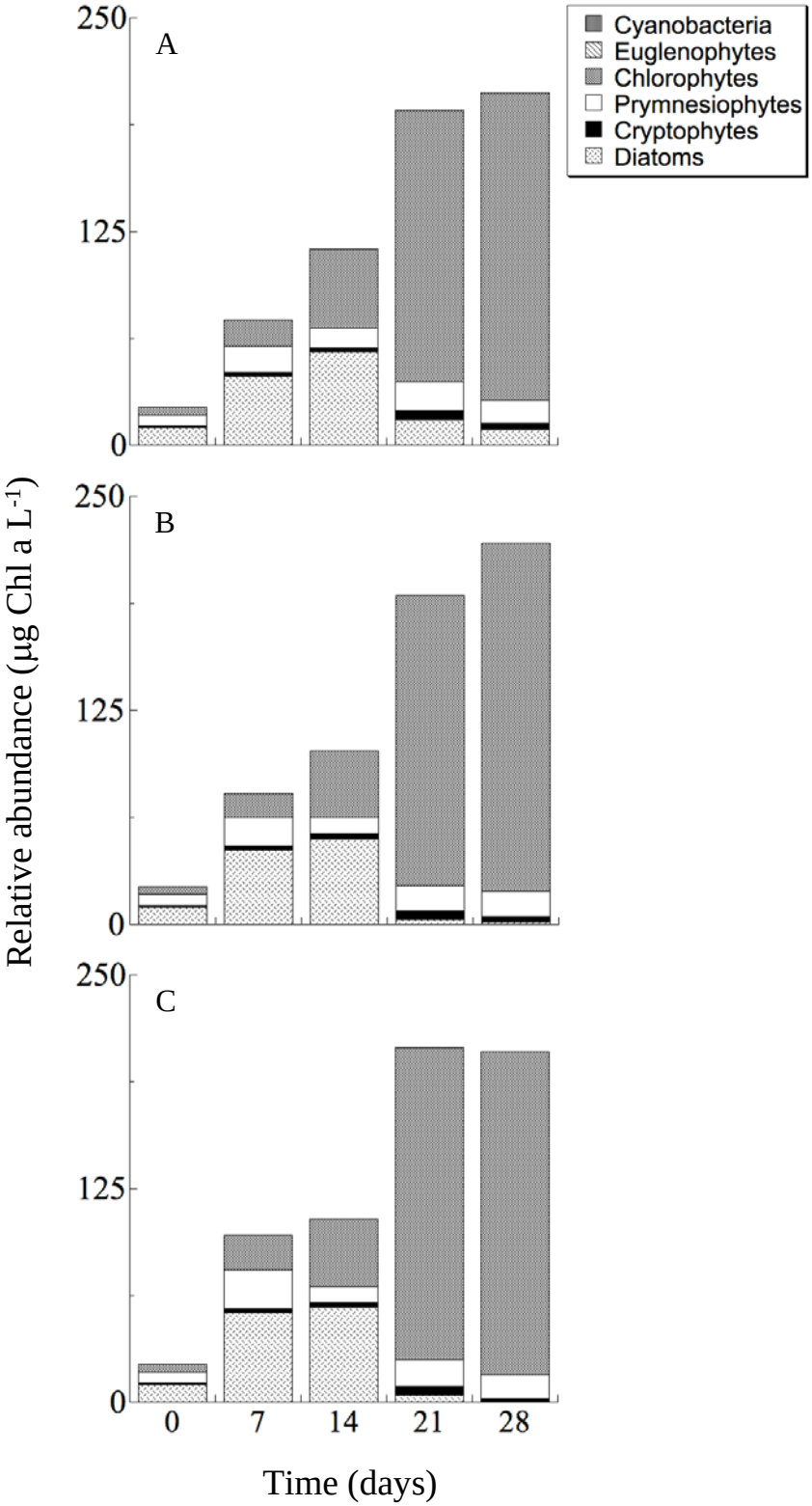
A-32
Winter 2005
Natural assemblage, BSE, and *P. parvum*



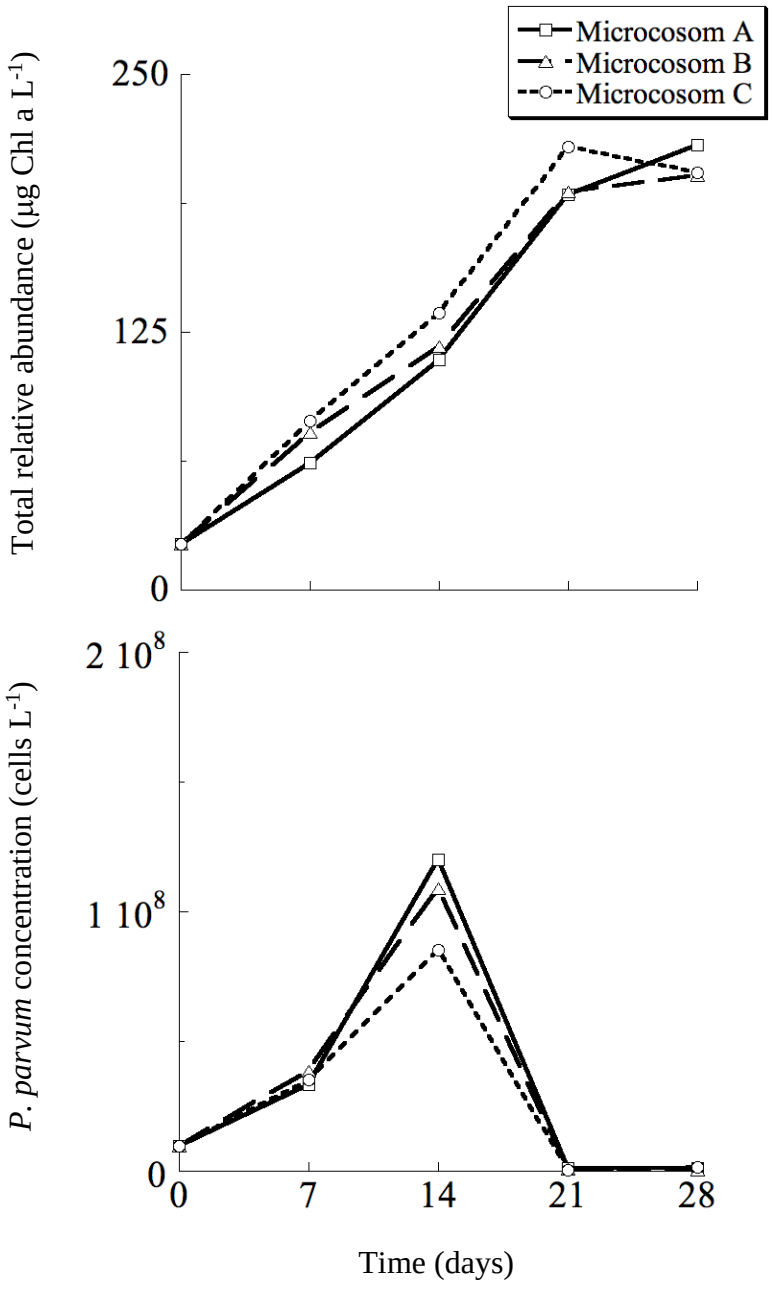
A-33
Winter 2005
Natural assemblage and f/2 nutrients



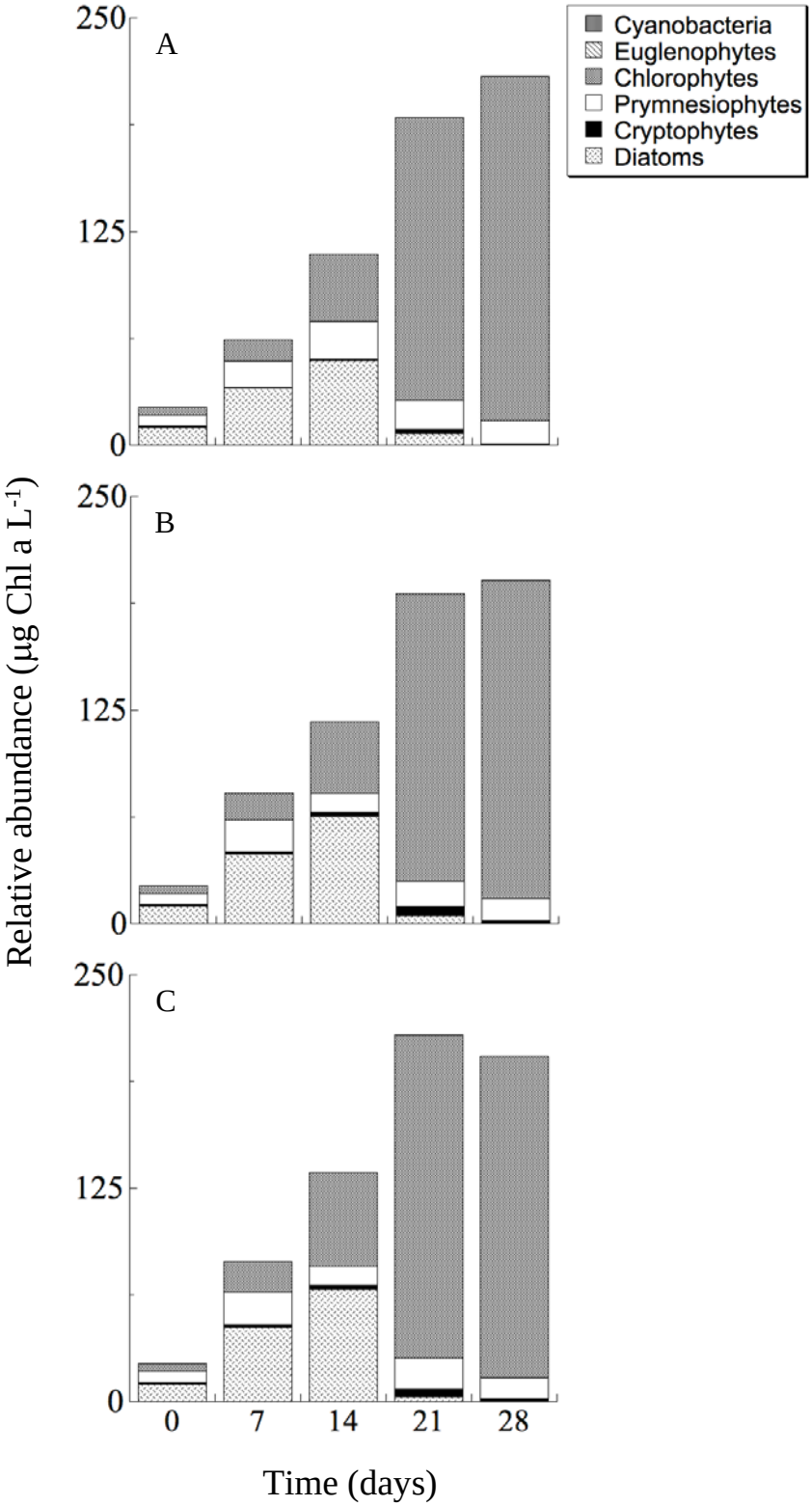
A-34
Winter 2005
Natural assemblage and f/2 nutrients



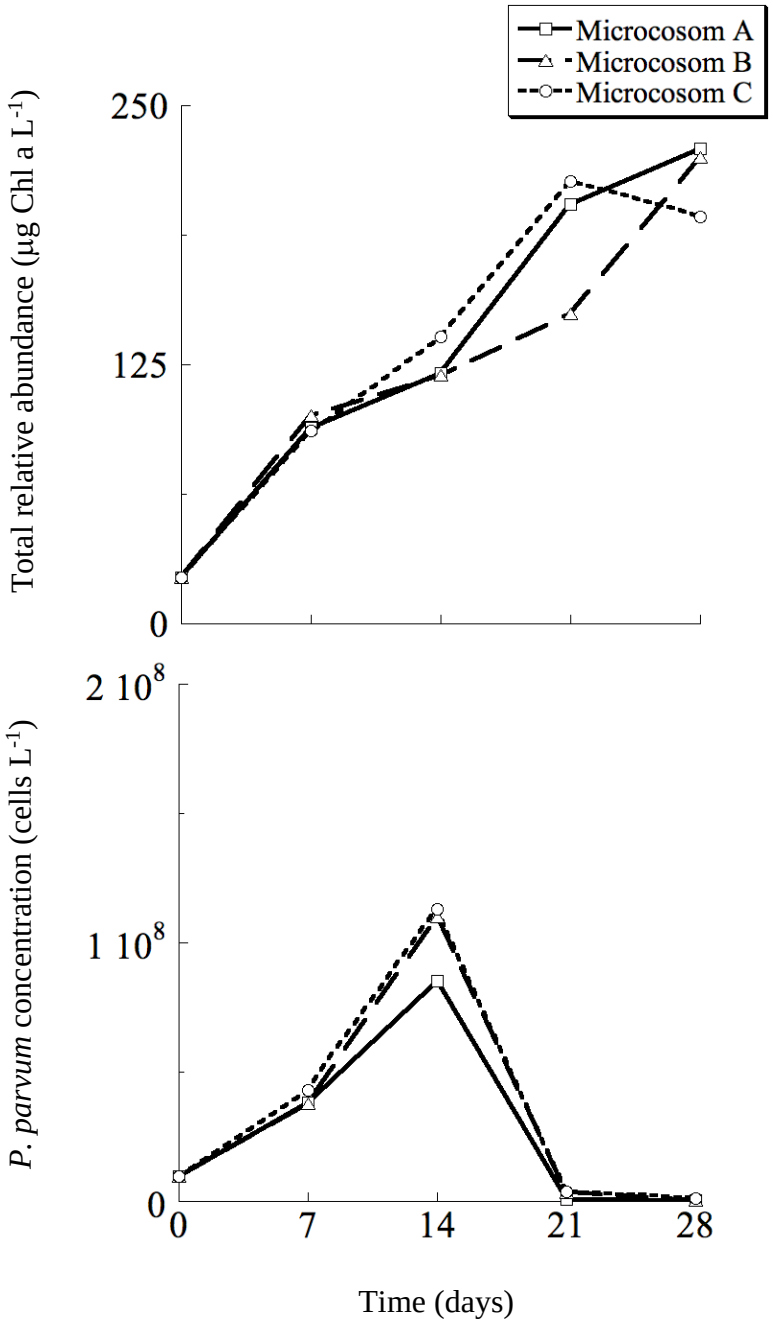
A-35
Winter 2005
Natural assemblage, f/2 nutrients, and BSE



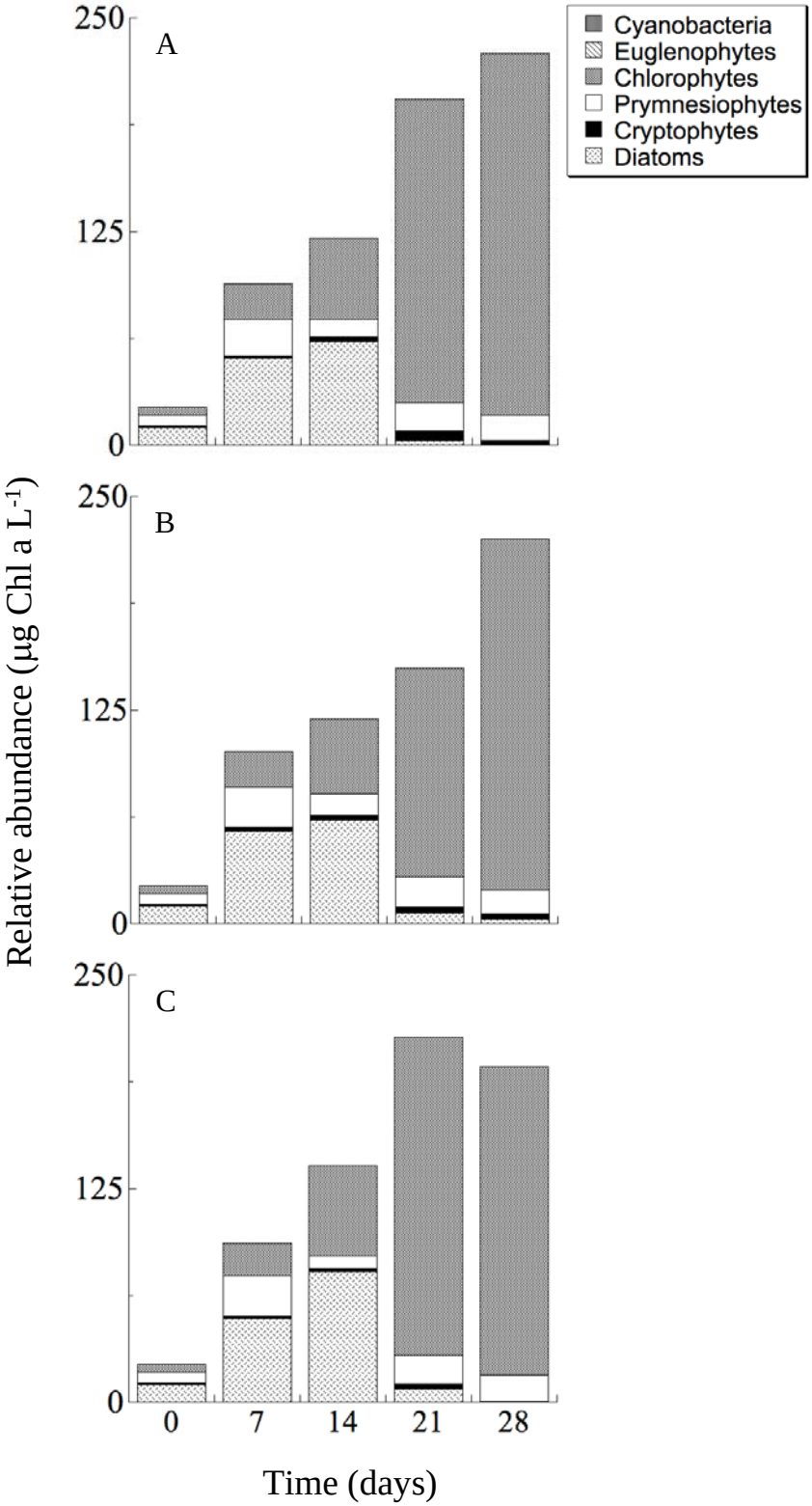
A-36
Winter 2005
Natural assemblage, f/2 nutrients, and BSE



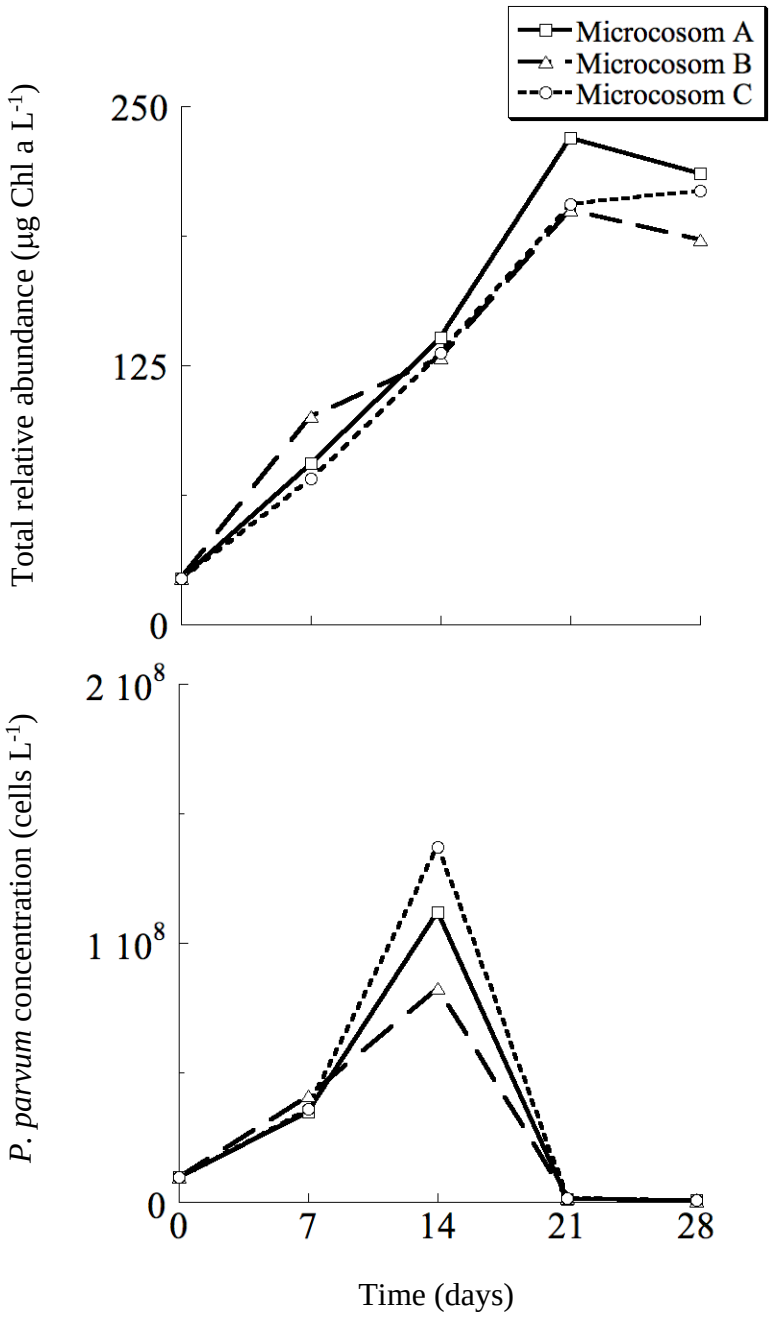
A-37
Winter 2005
Natural assemblage, f/2 nutrients, and *P. parvum*



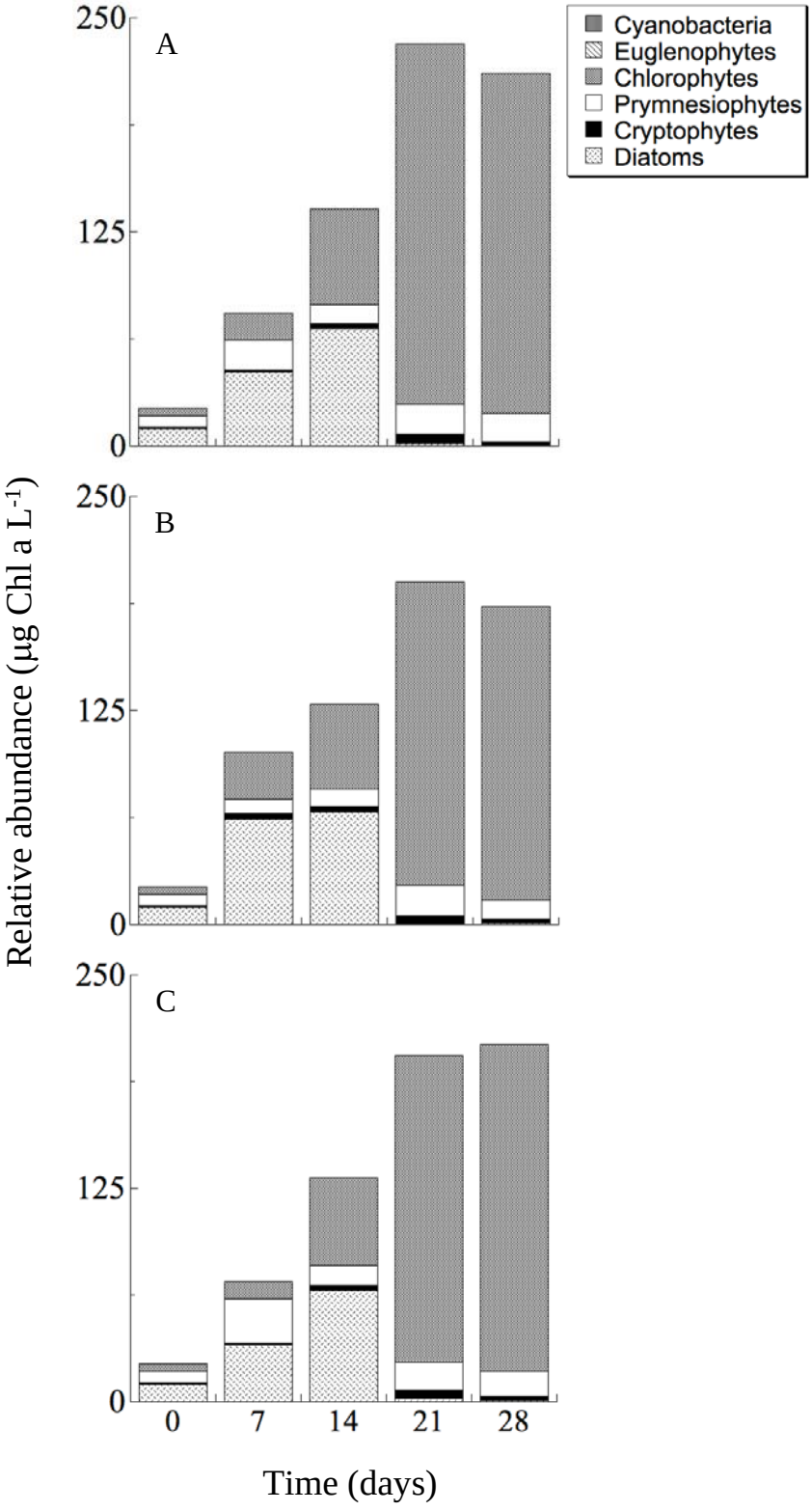
A-38
Winter 2005
Natural assemblage, f/2 nutrients, and *P. parvum*



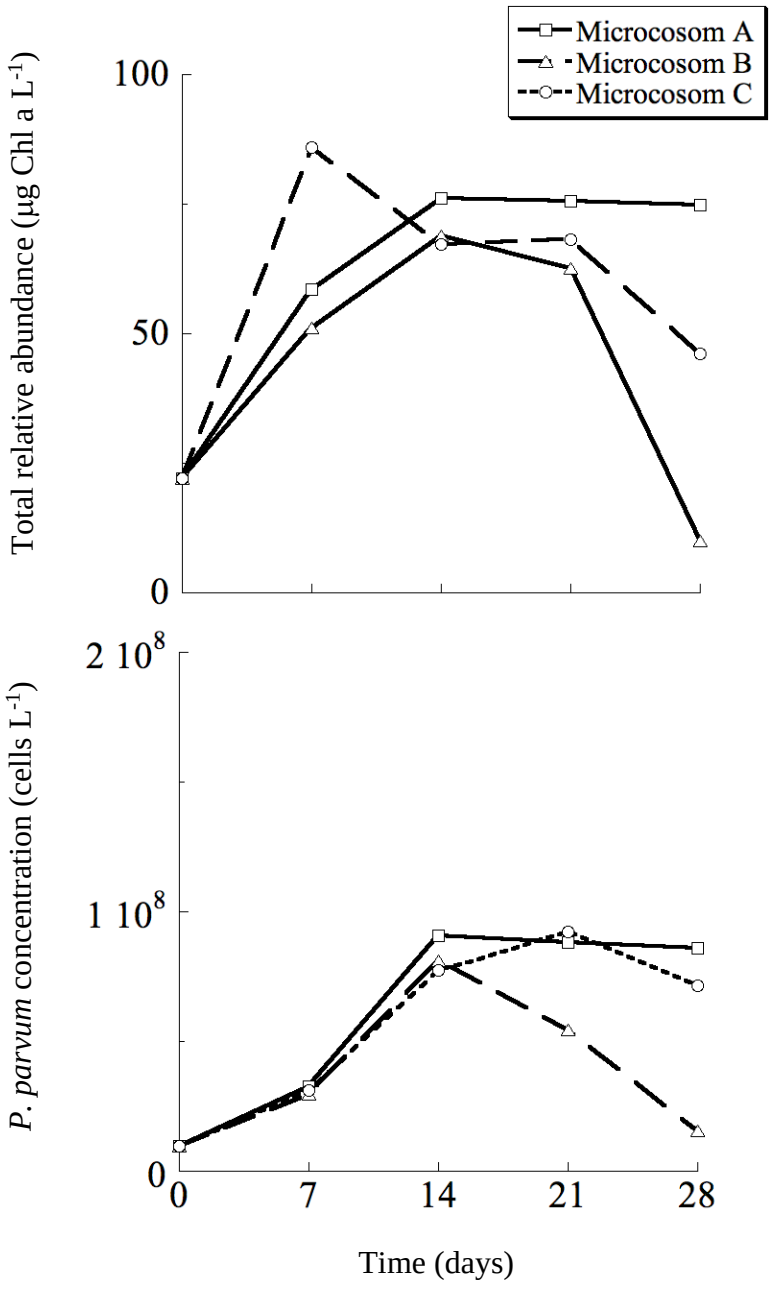
A-39
Winter 2005
Natural assemblage, f/2 nutrients, BSE, and *P. parvum*



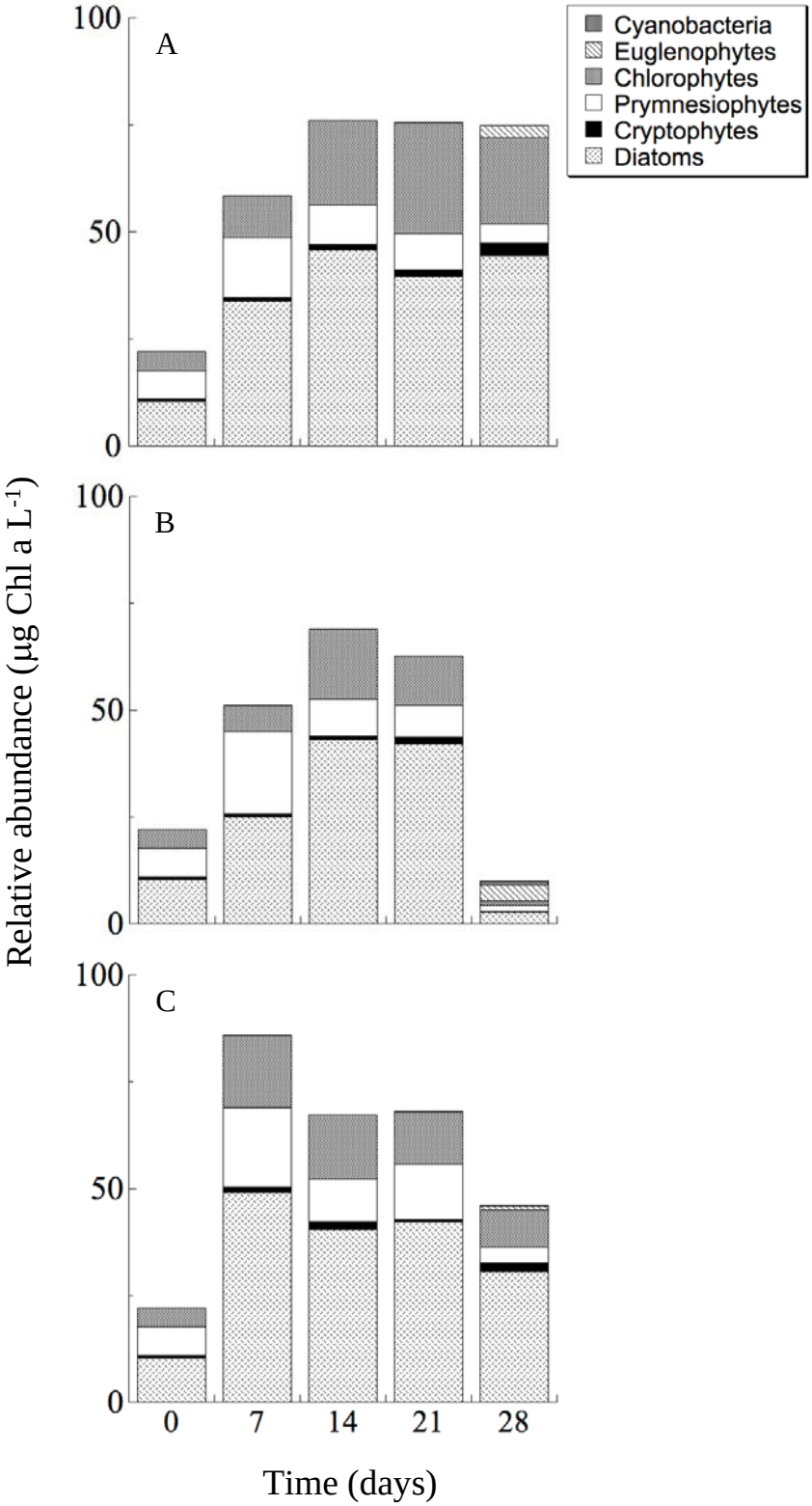
A-40
Winter 2005
Natural assemblage, f/2 nutrients, BSE, and *P. parvum*



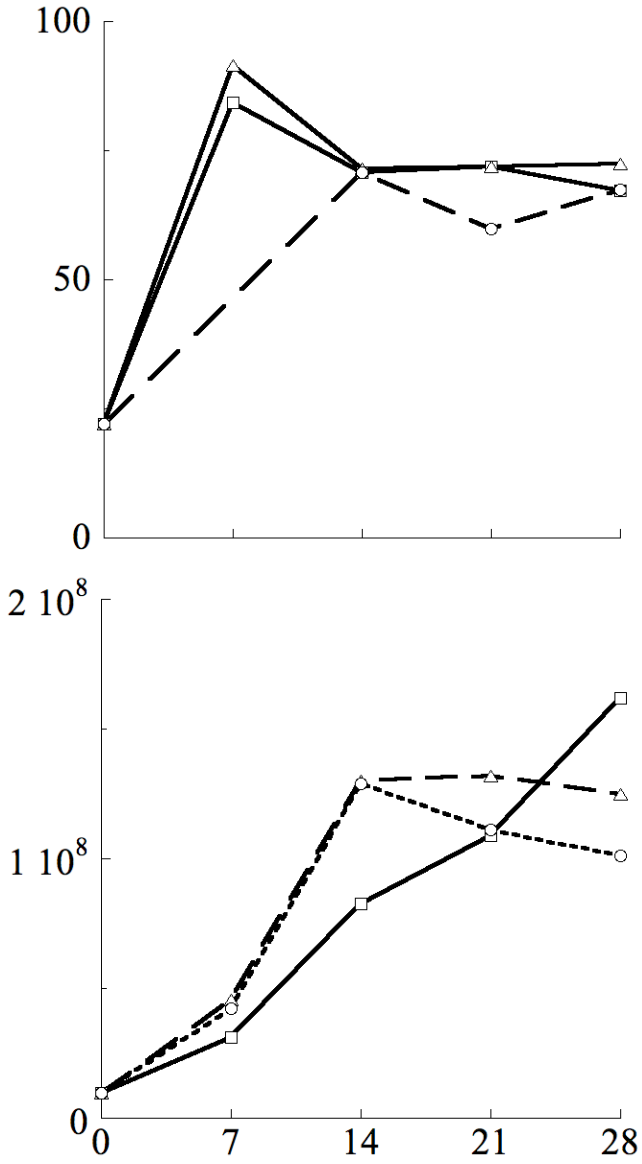
A-41
Winter 2005
Natural assemblage and nitrogen deplete f/2 nutrients



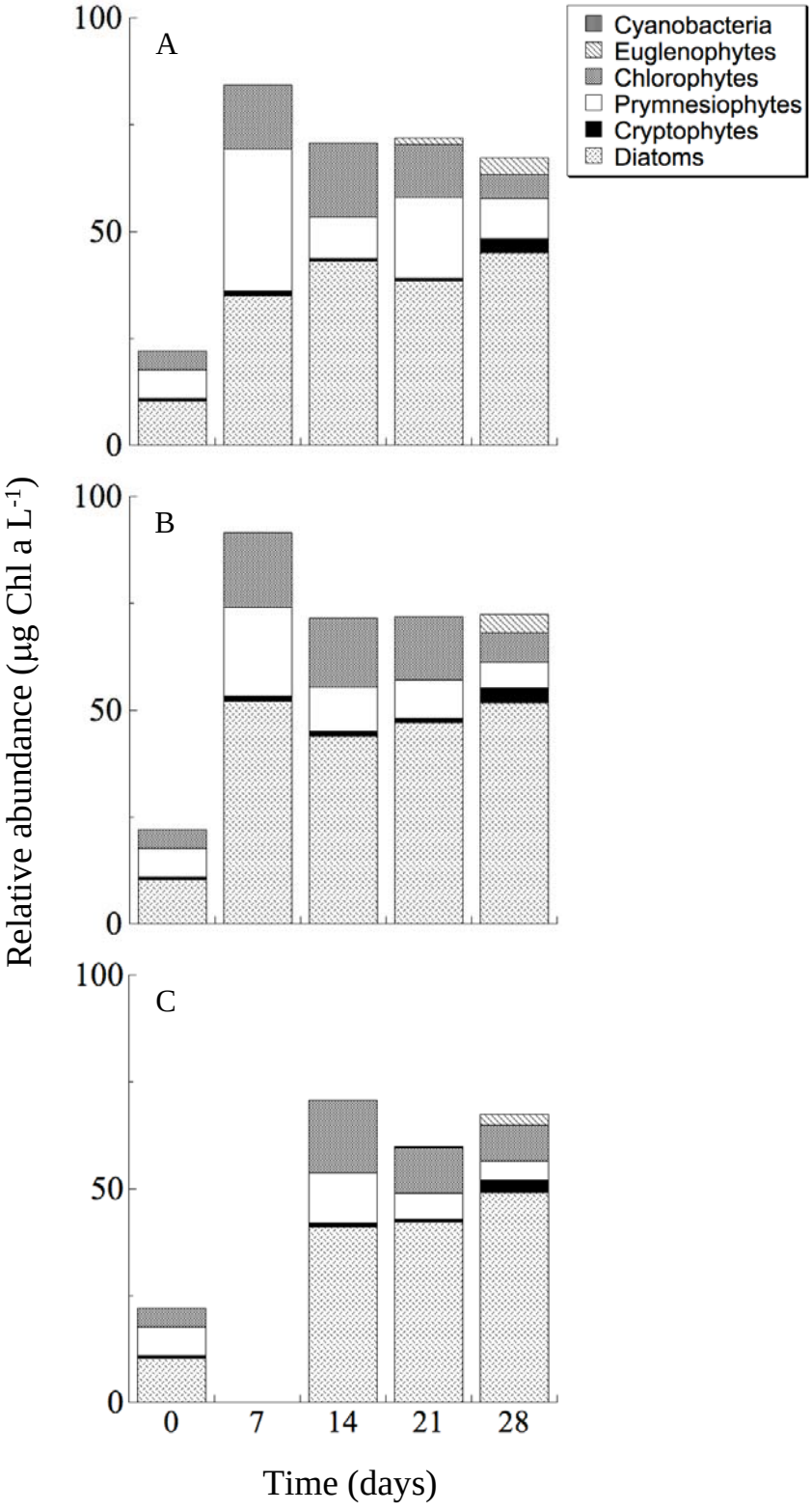
A-42
Winter 2005
Natural assemblage and nitrogen deplete f/2 nutrients



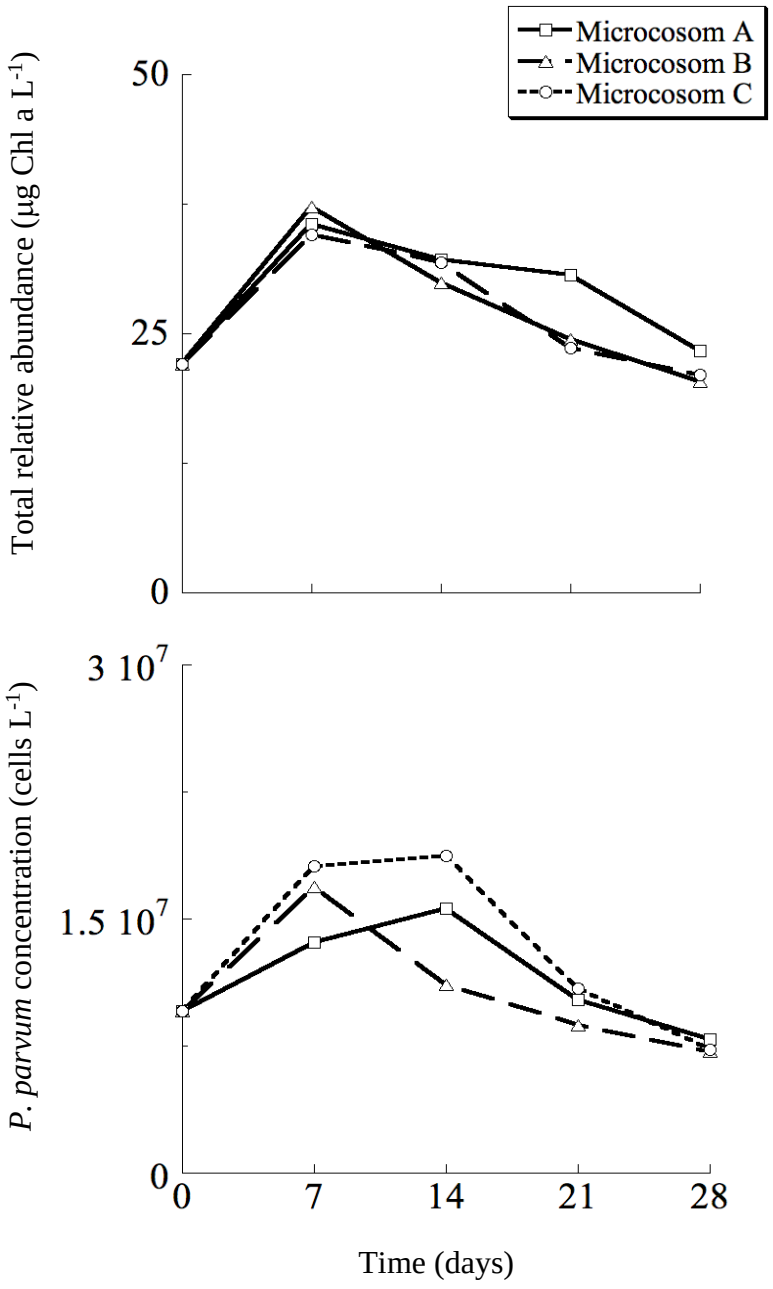
A-43
Winter 2005
Natural assemblage, nitrogen deplete f/2 nutrients, and *P. parvum*



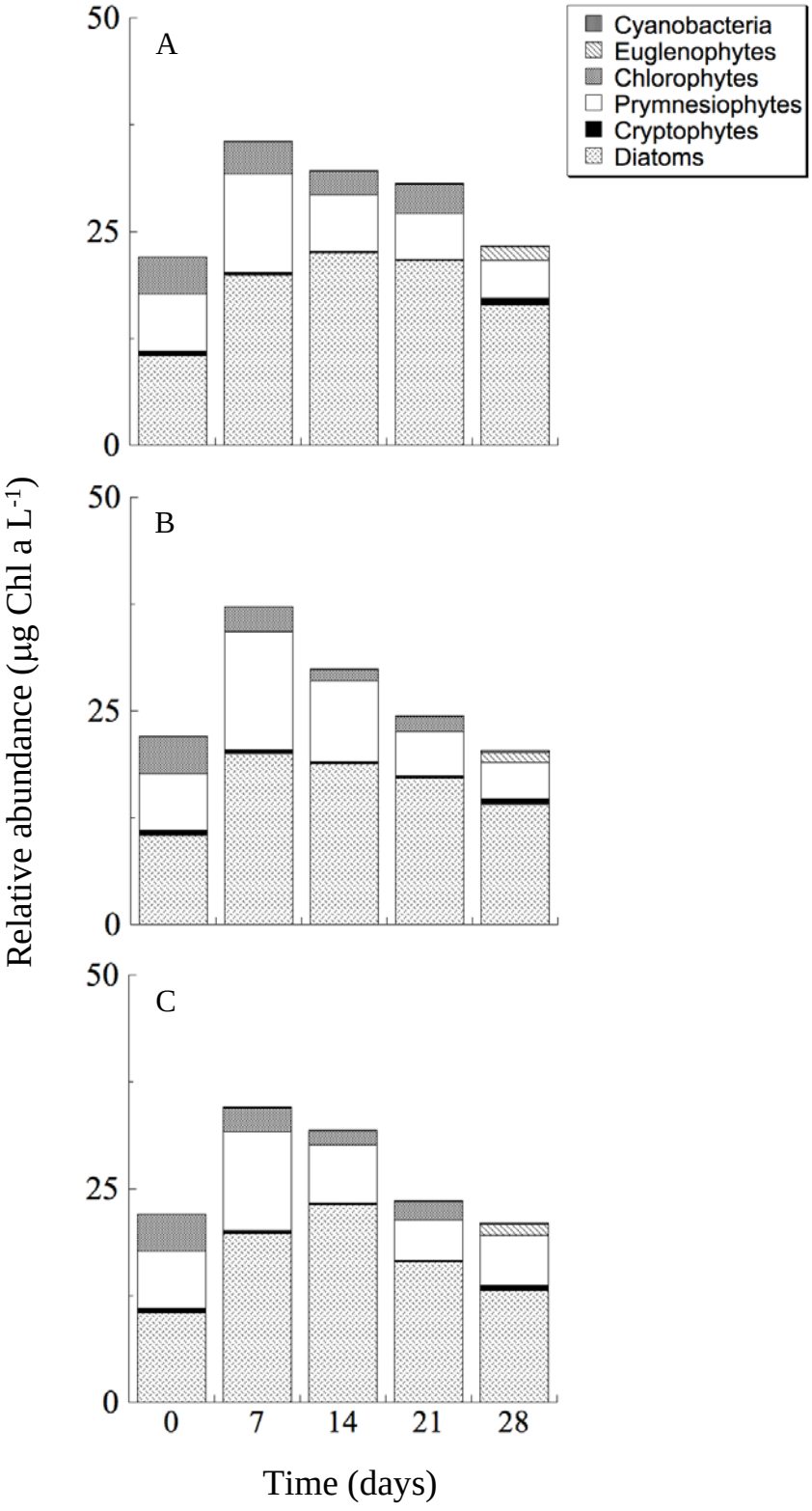
A-44
Winter 2005
Natural assemblage, nitrogen deplete f/2 nutrients, and *P. parvum*



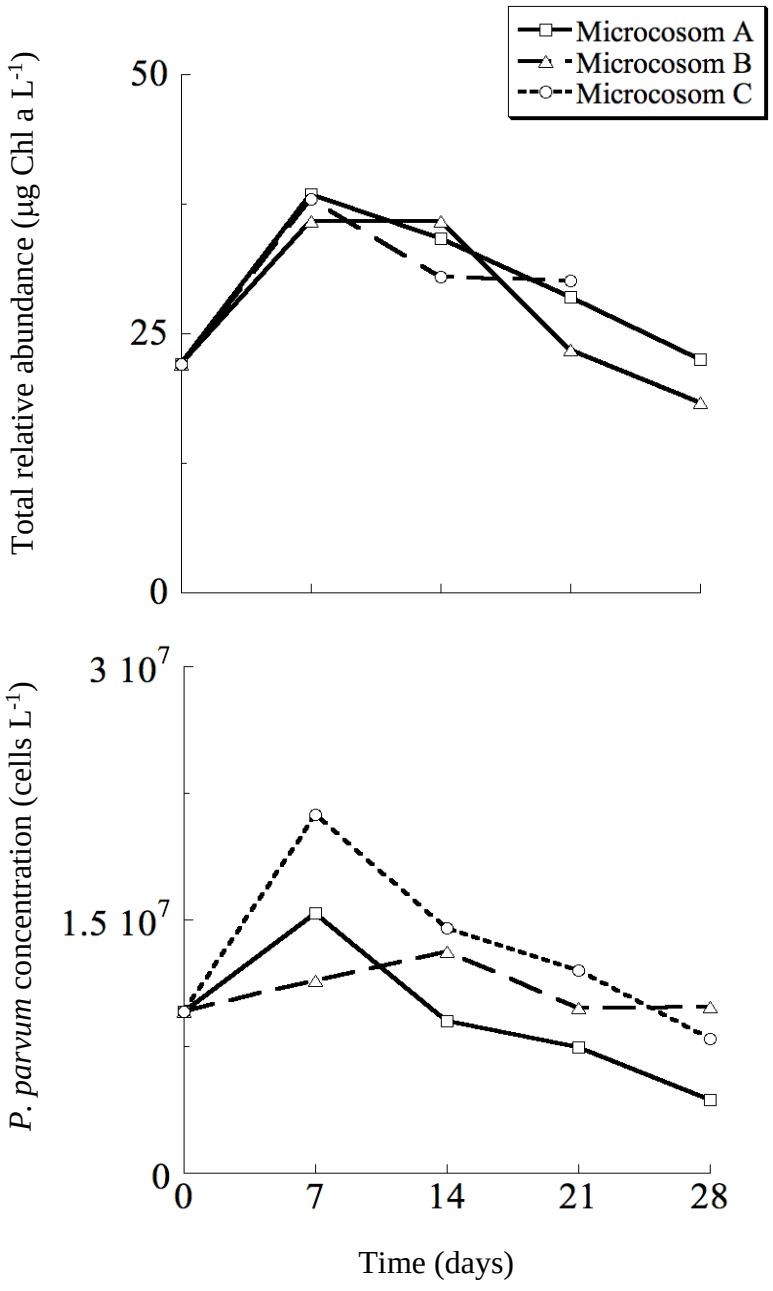
A-45
Winter 2005
Natural assemblage and phosphorus deplete f/2 nutrients



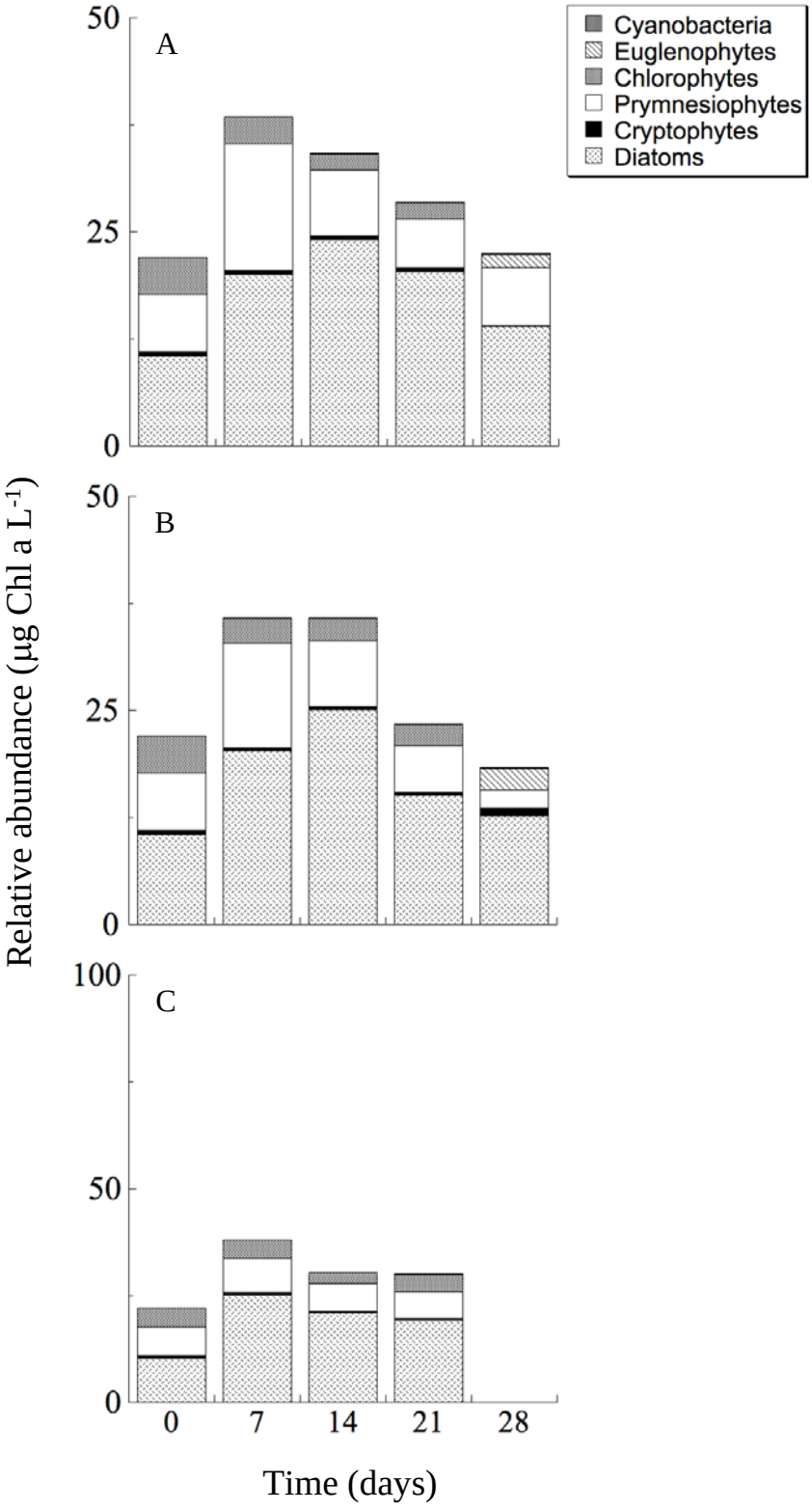
A-46
Winter 2005
Natural assemblage and phosphorus deplete f/2 nutrients



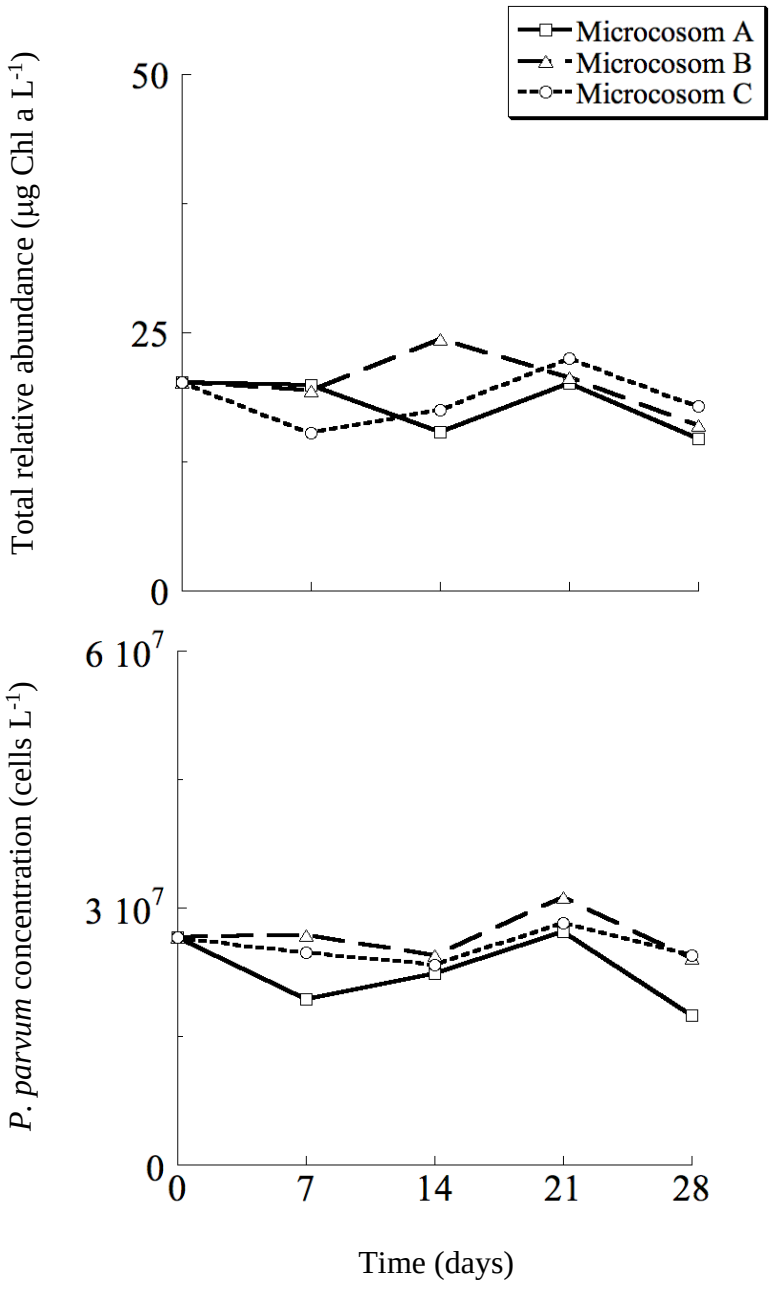
A-47
Winter 2005
Natural assemblage, *P. parvum*, and phosphorus deplete f/2 nutrients



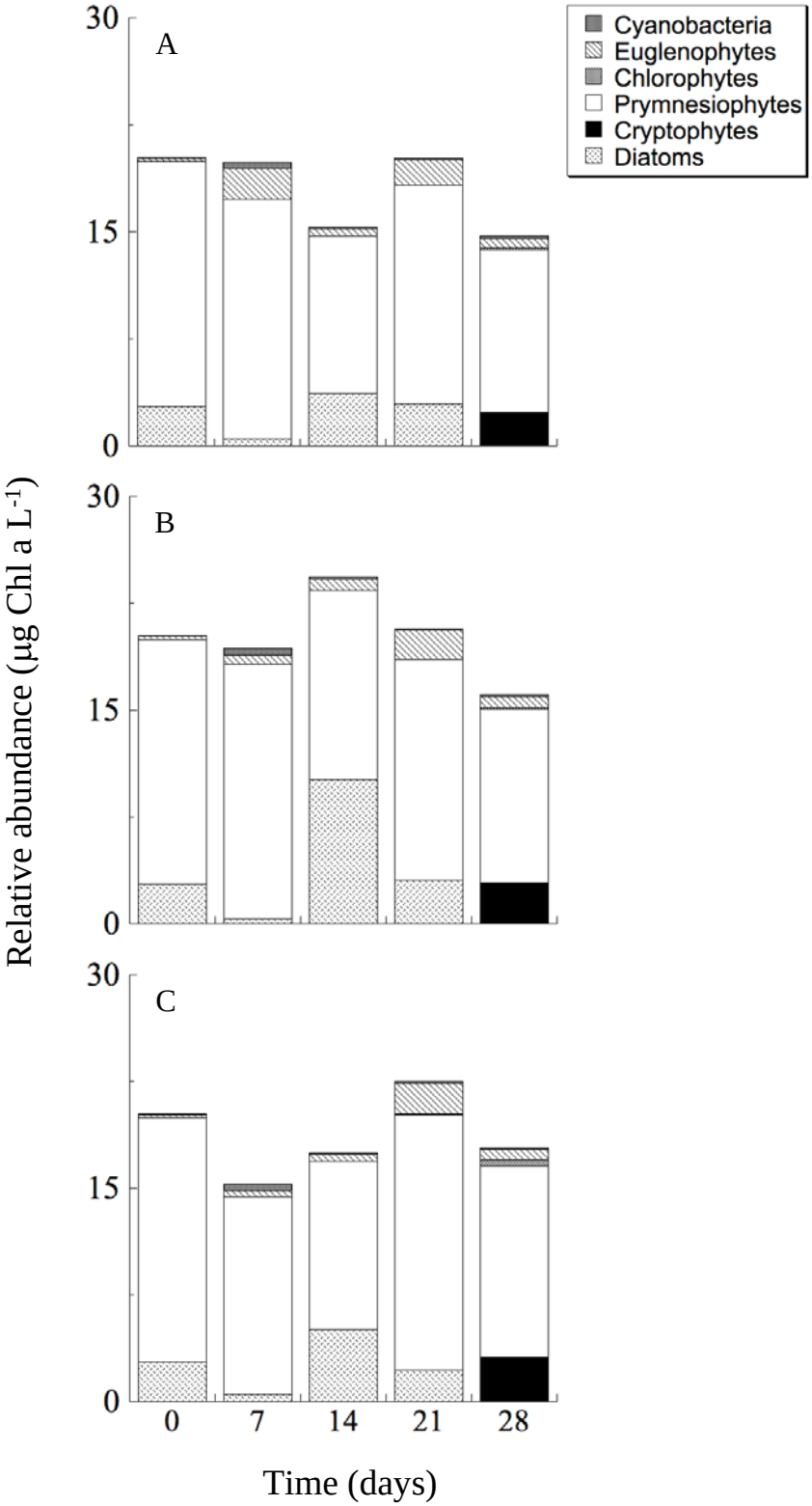
A-48
Winter 2005
Natural assemblage, phosphorus deplete f/2 nutrients, and *P. parvum*



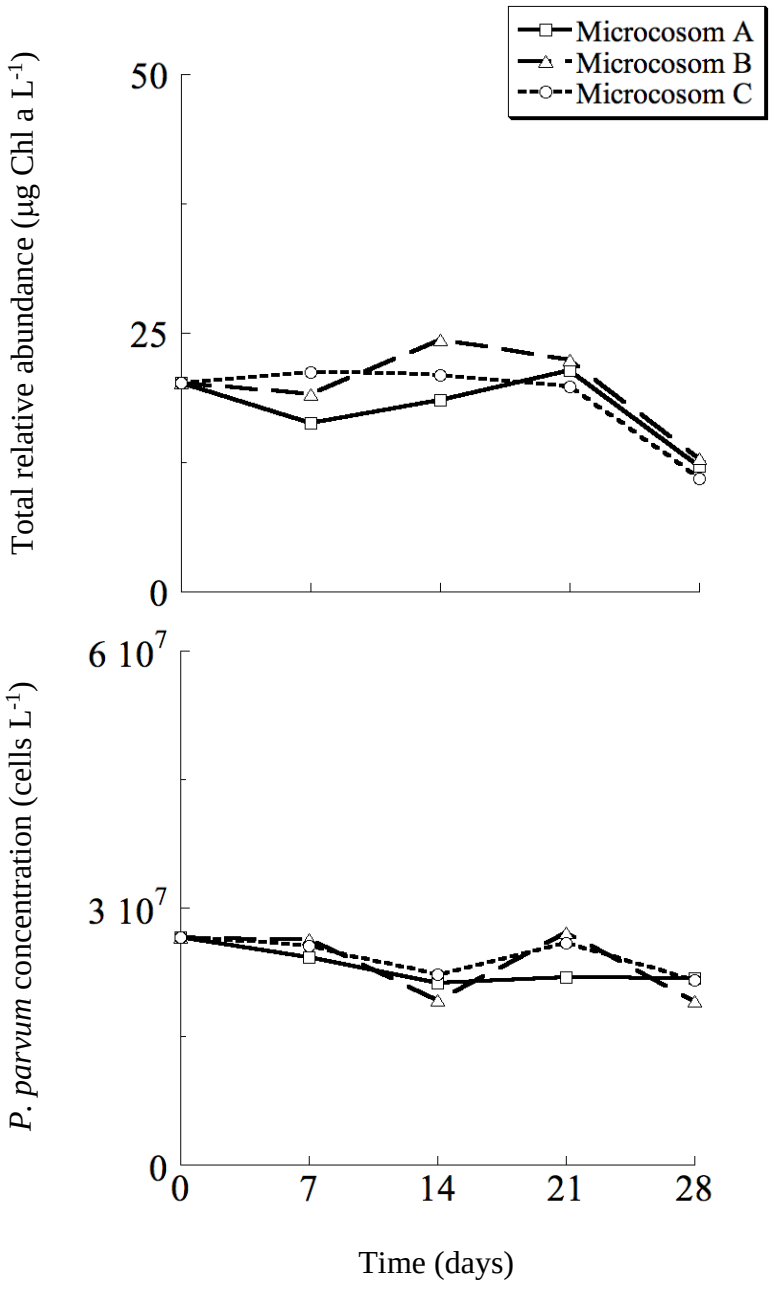
A-49
Spring 2005
Natural assemblage



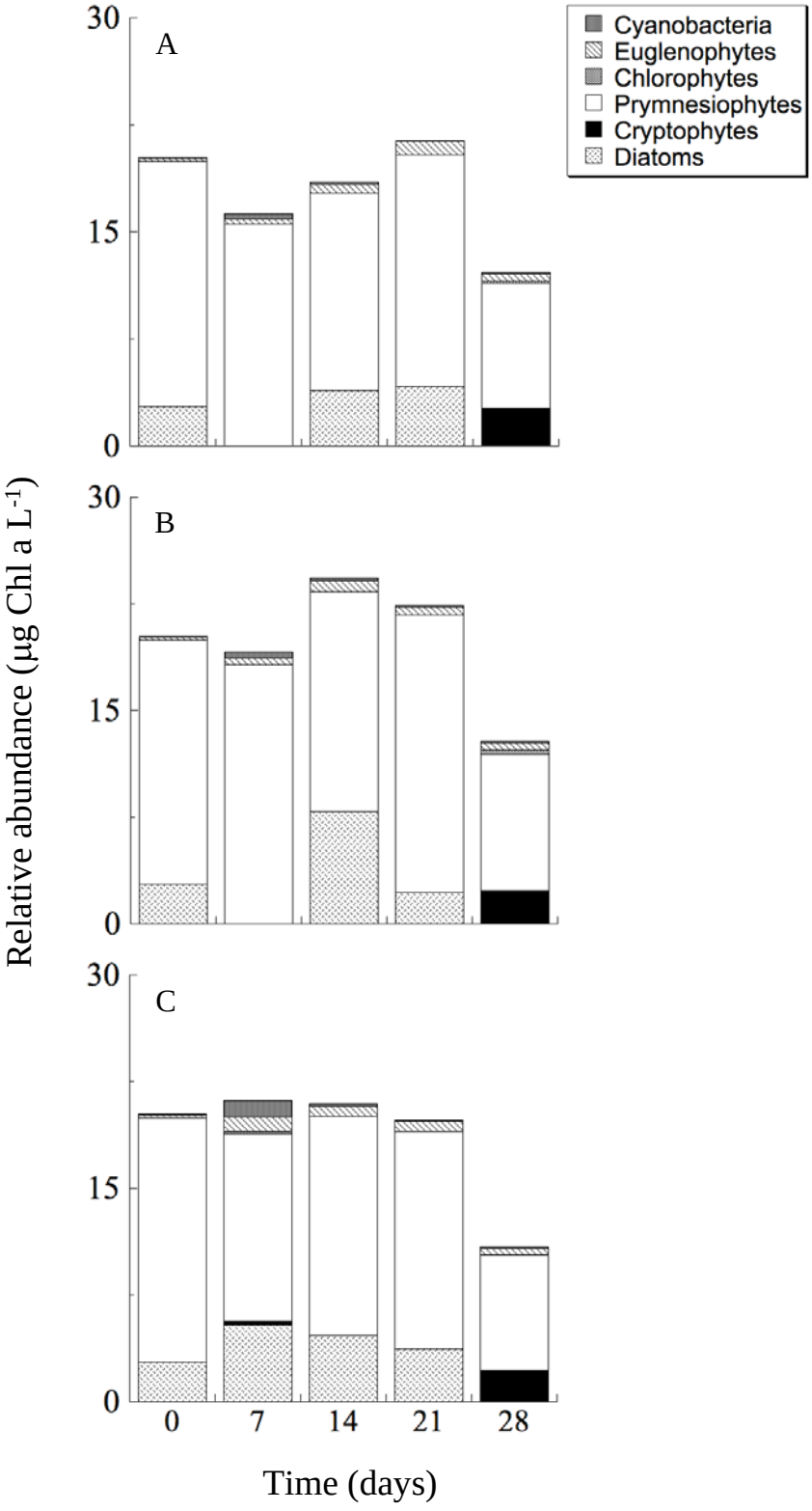
A-50
Spring 2005
Natural assemblage



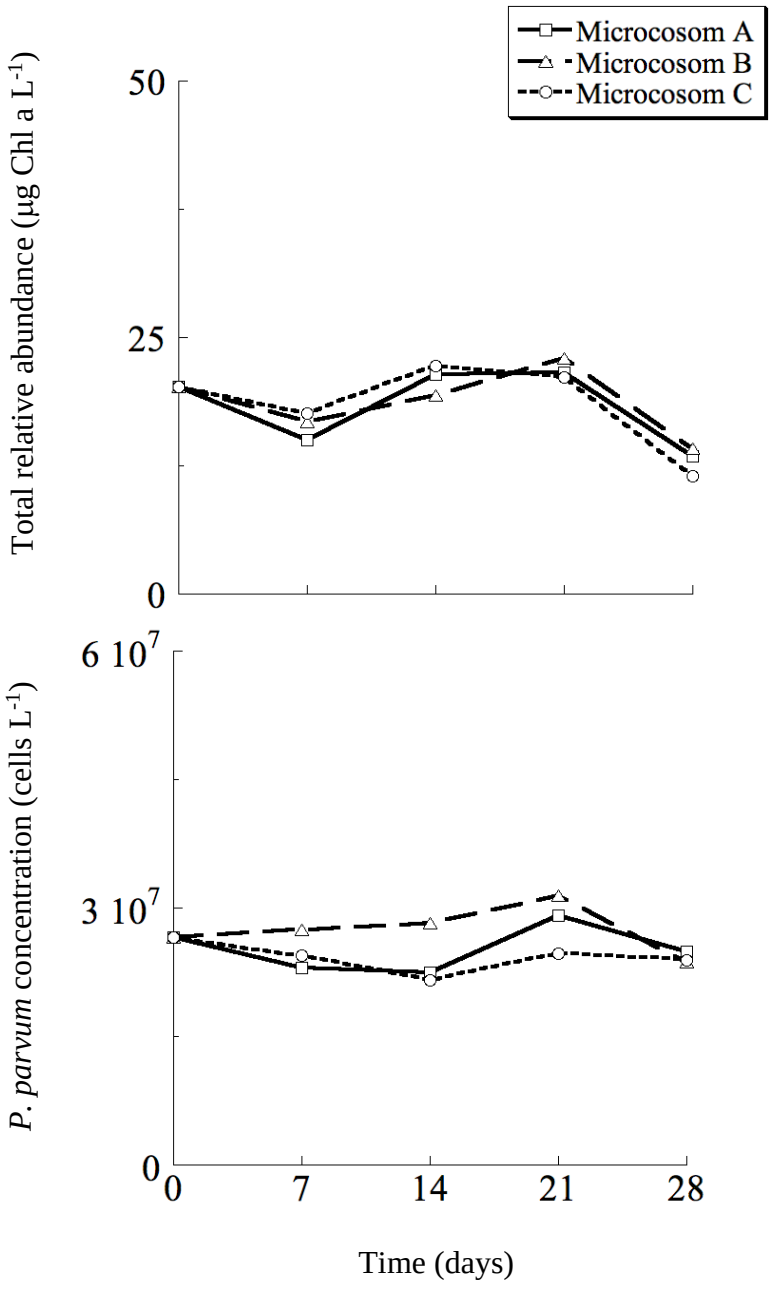
A-51
Spring 2005
Natural assemblage and BSE



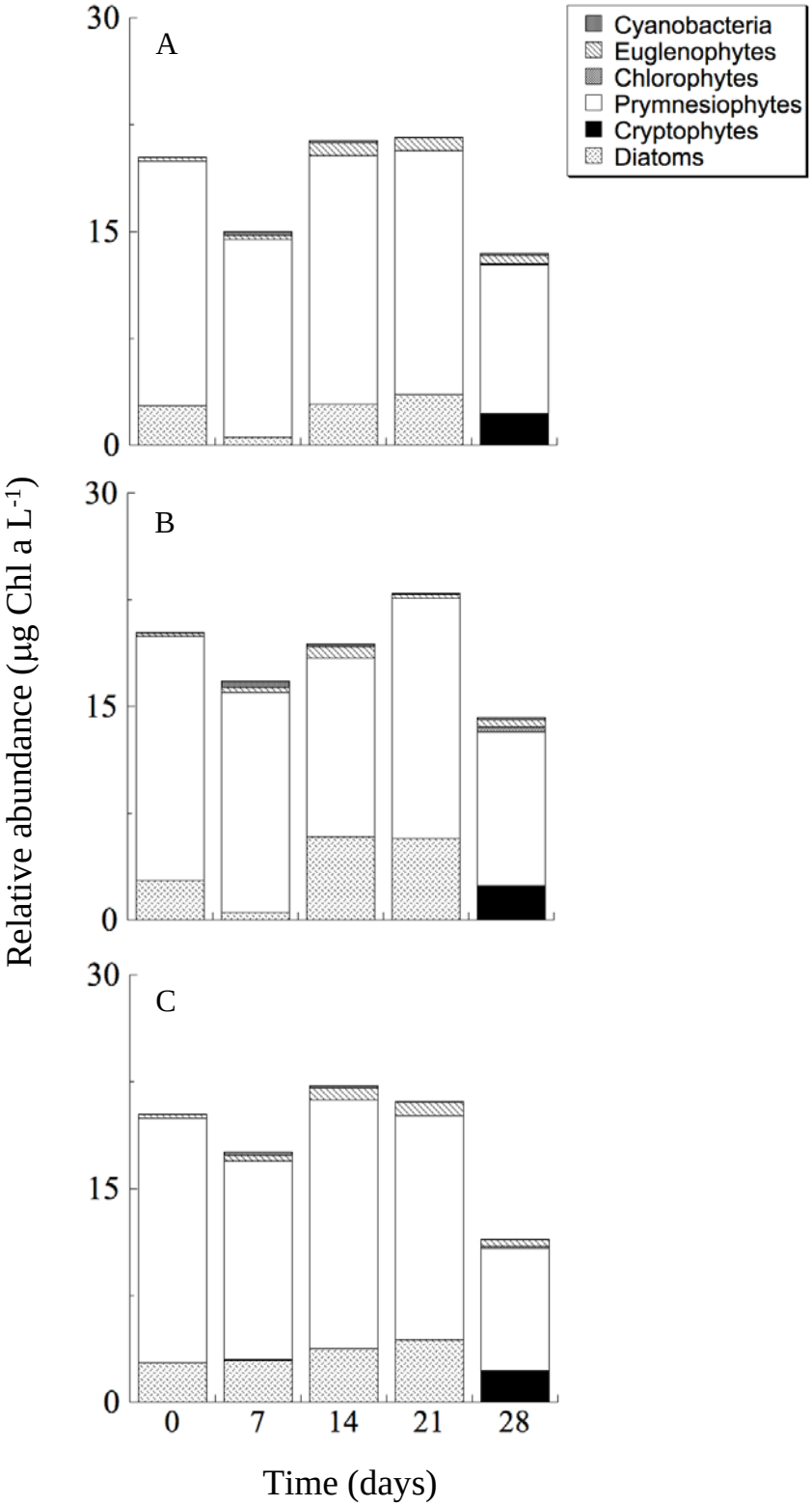
A-52
Spring 2005
Natural assemblage and BSE



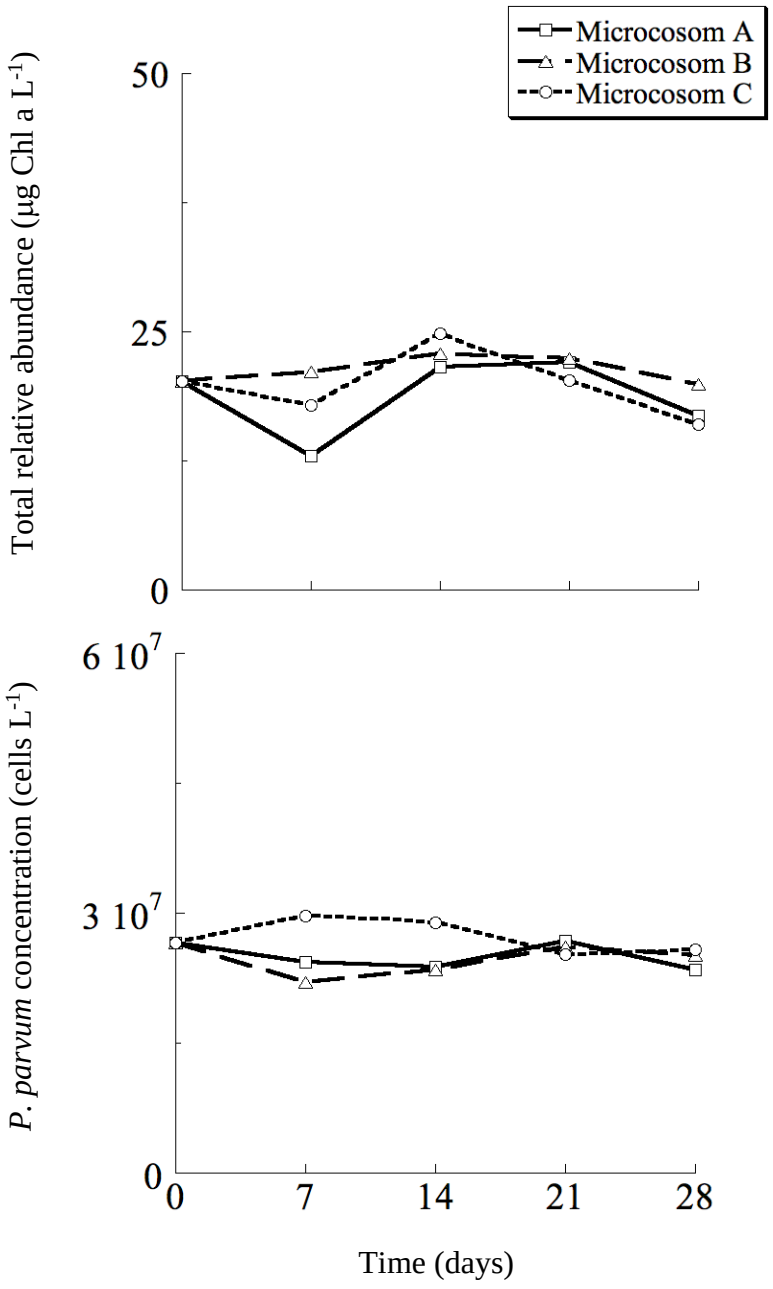
A-53
Spring 2005
Natural assemblage and *P. parvum*



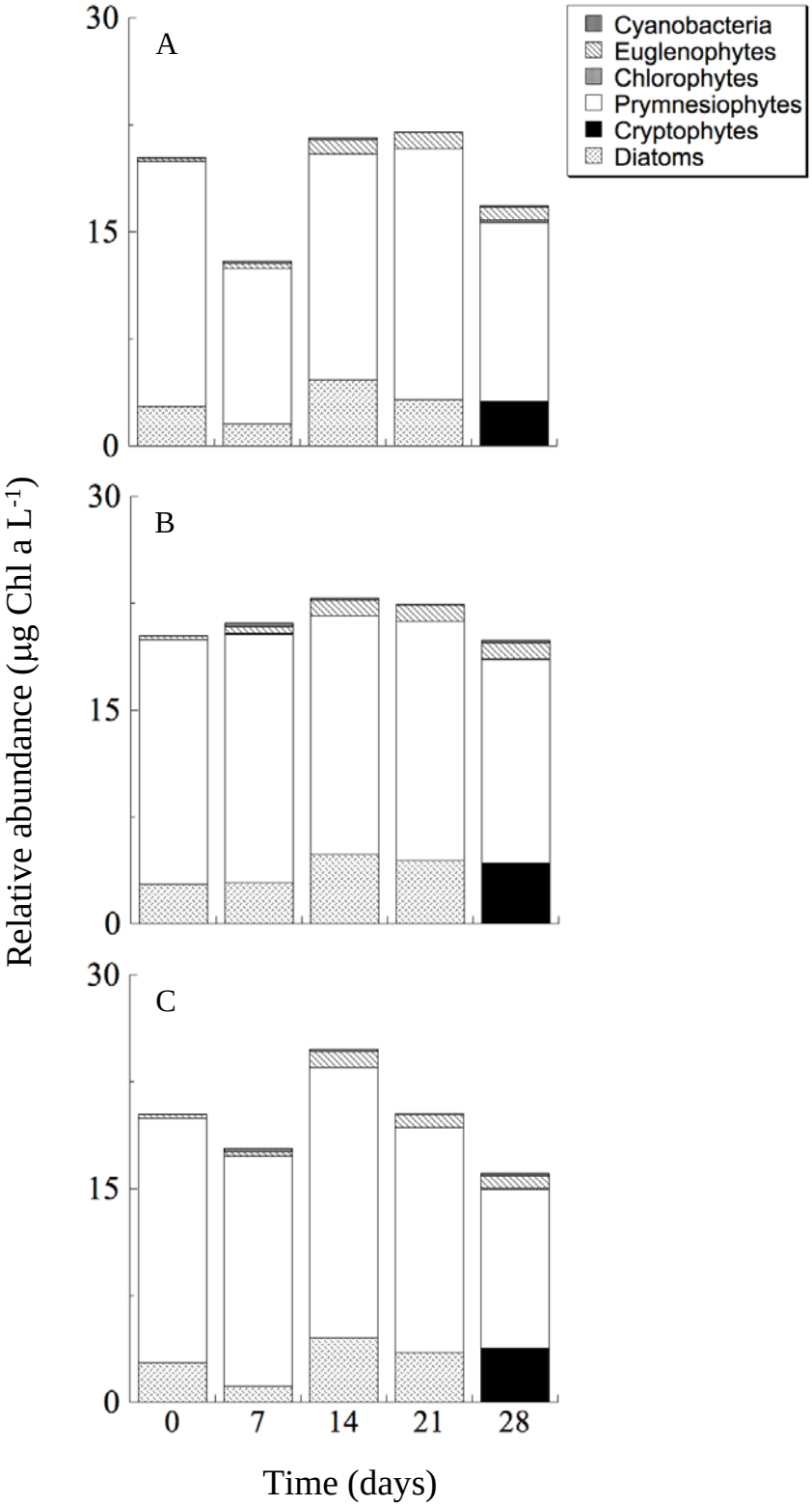
A-54
Spring 2005
Natural assemblage and *P. parvum*



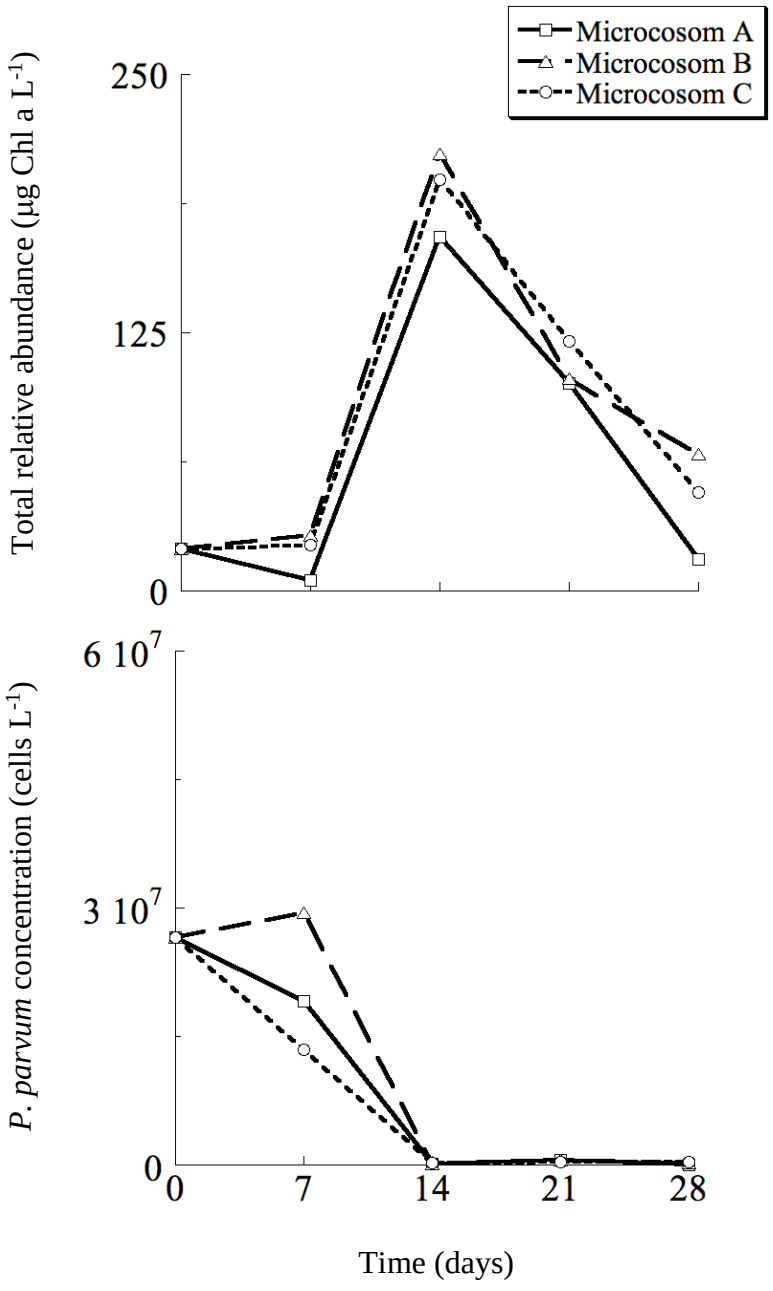
A-55
Spring 2005
Natural assemblage, BSE, and *P. parvum*



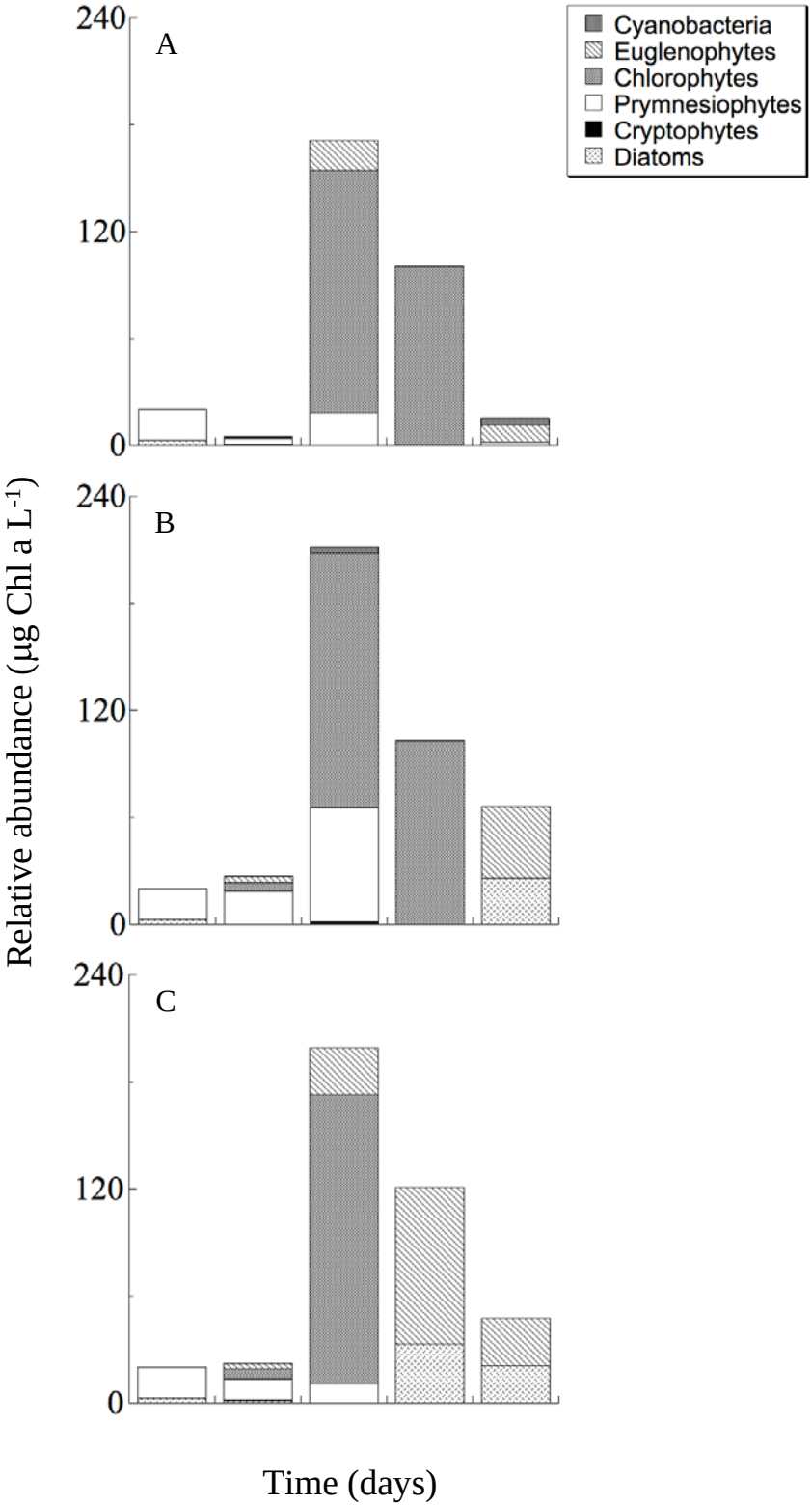
A-56
Spring 2005
Natural assemblage, BSE, and *P. parvum*



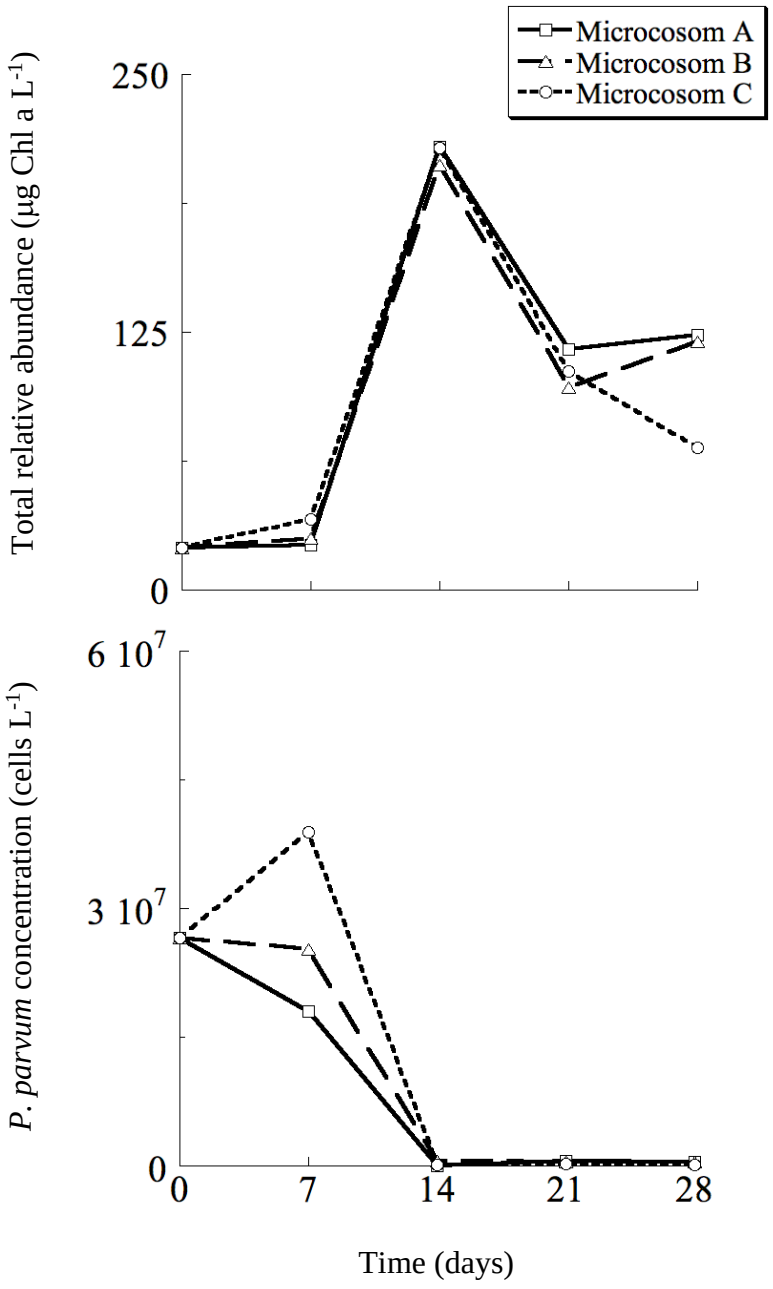
A-57
Spring 2005
Natural assemblage and f/2 nutrients



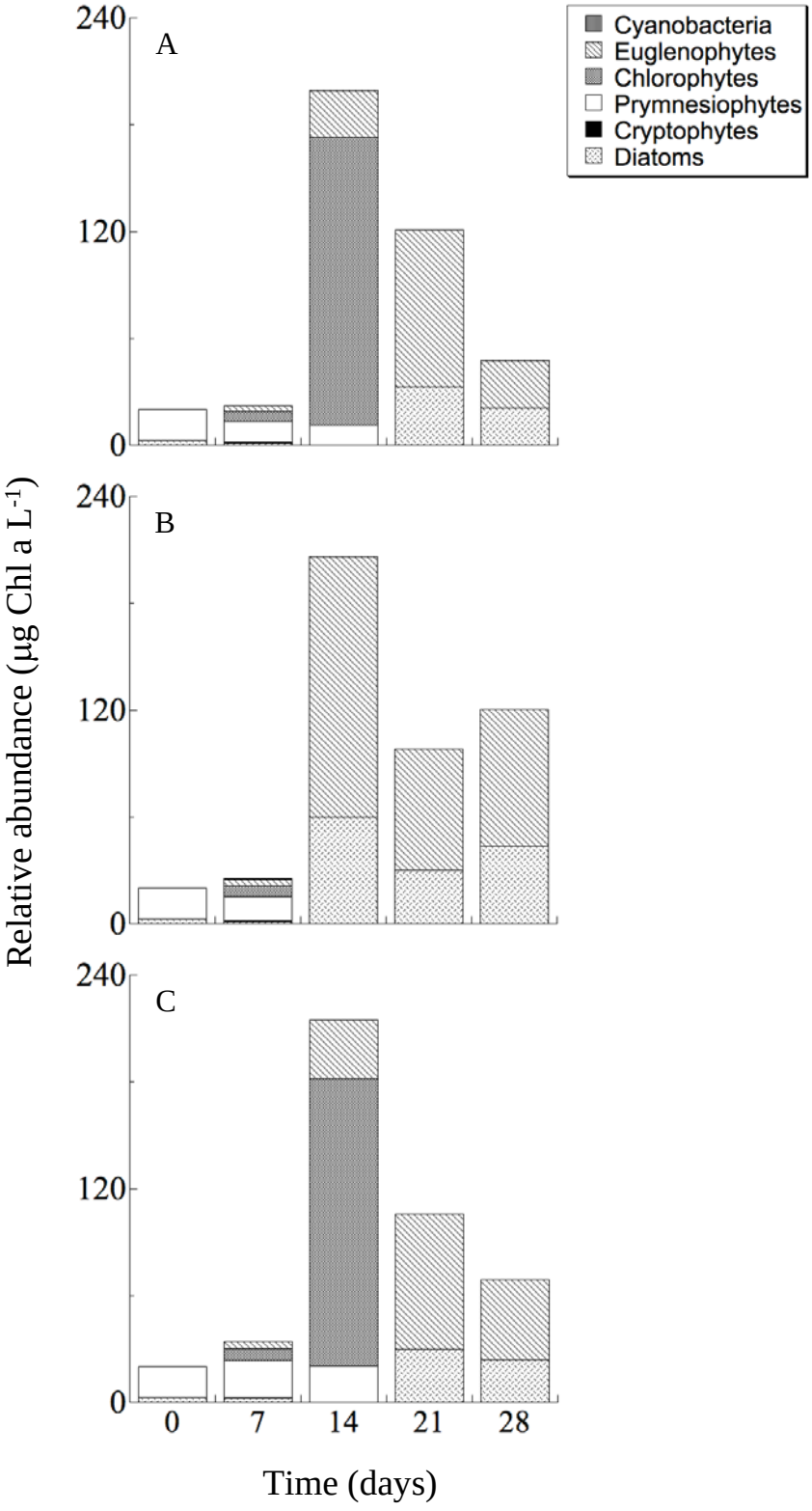
A-58
Spring 2005
Natural assemblage and f/2 nutrients



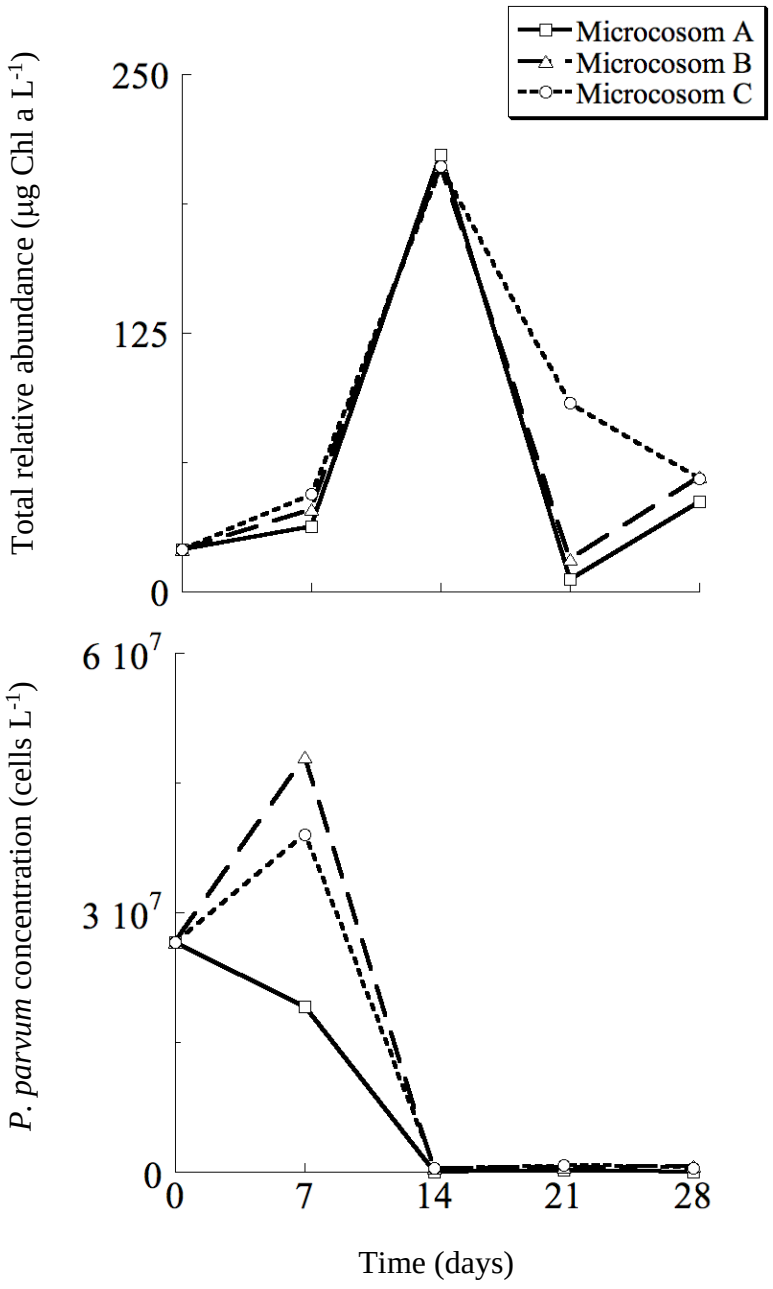
A-59
Spring 2005
Natural assemblage, f/2 nutrients, and BSE



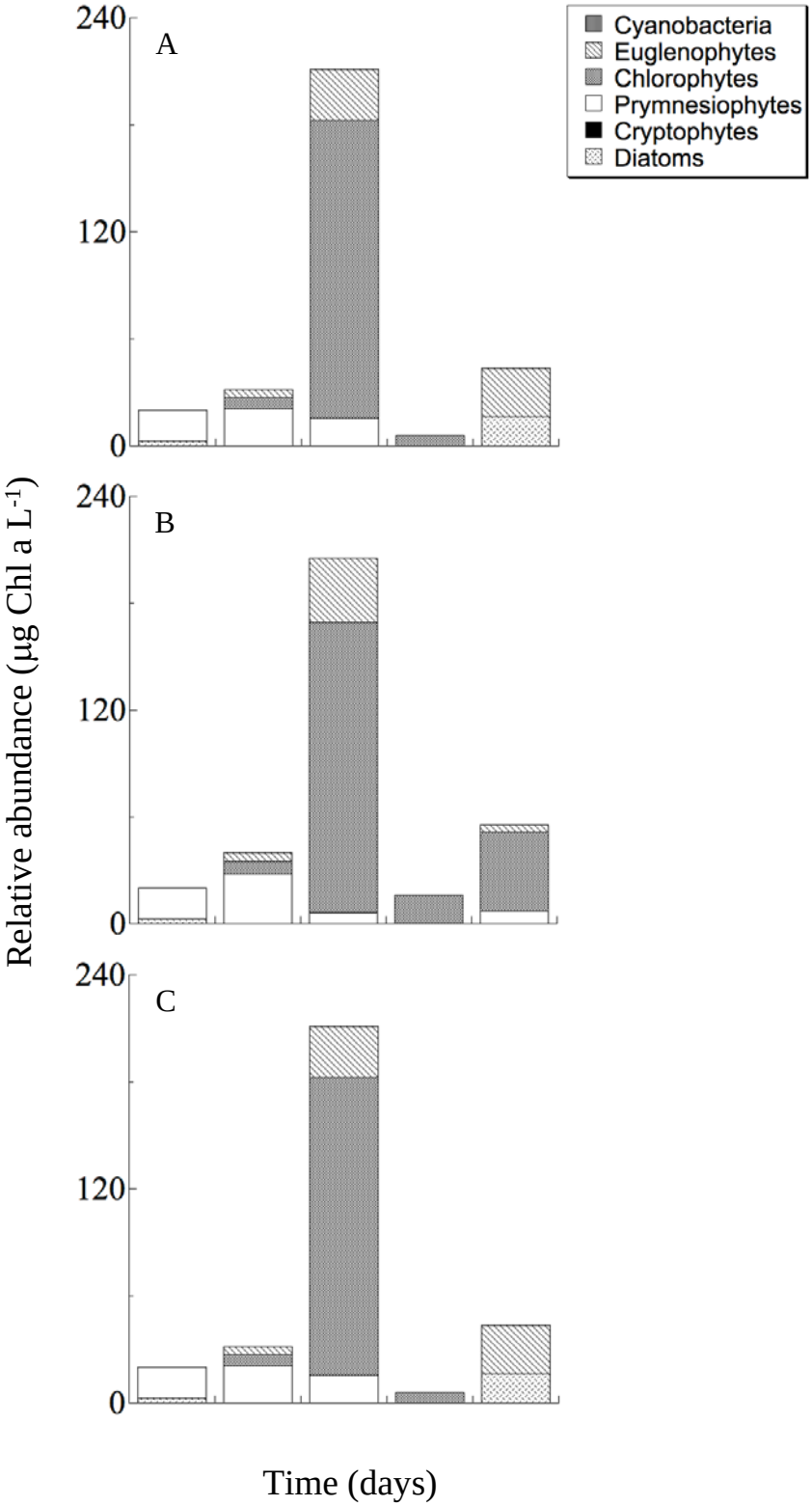
A-60
Spring 2005
Natural assemblage, f/2 nutrients, and BSE



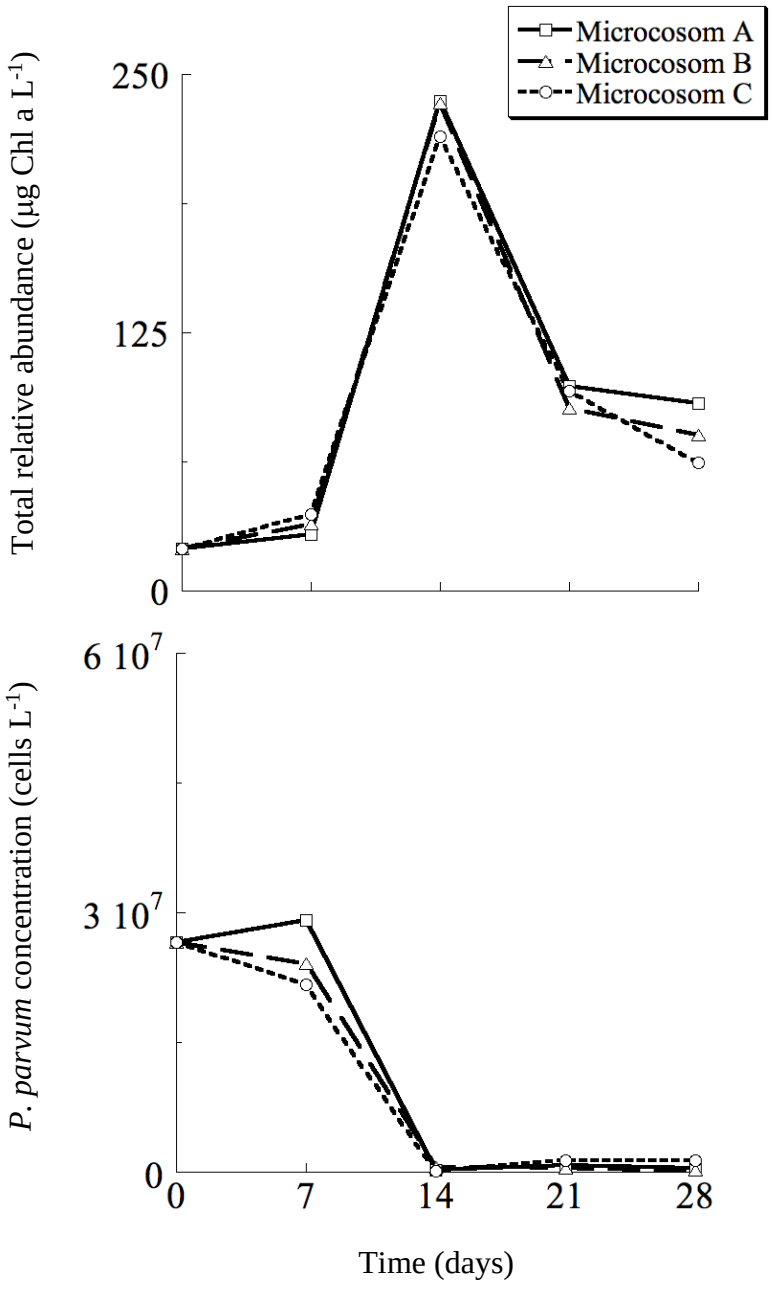
A-61
Spring 2005
Natural assemblage, f/2 nutrients, and *P. parvum*



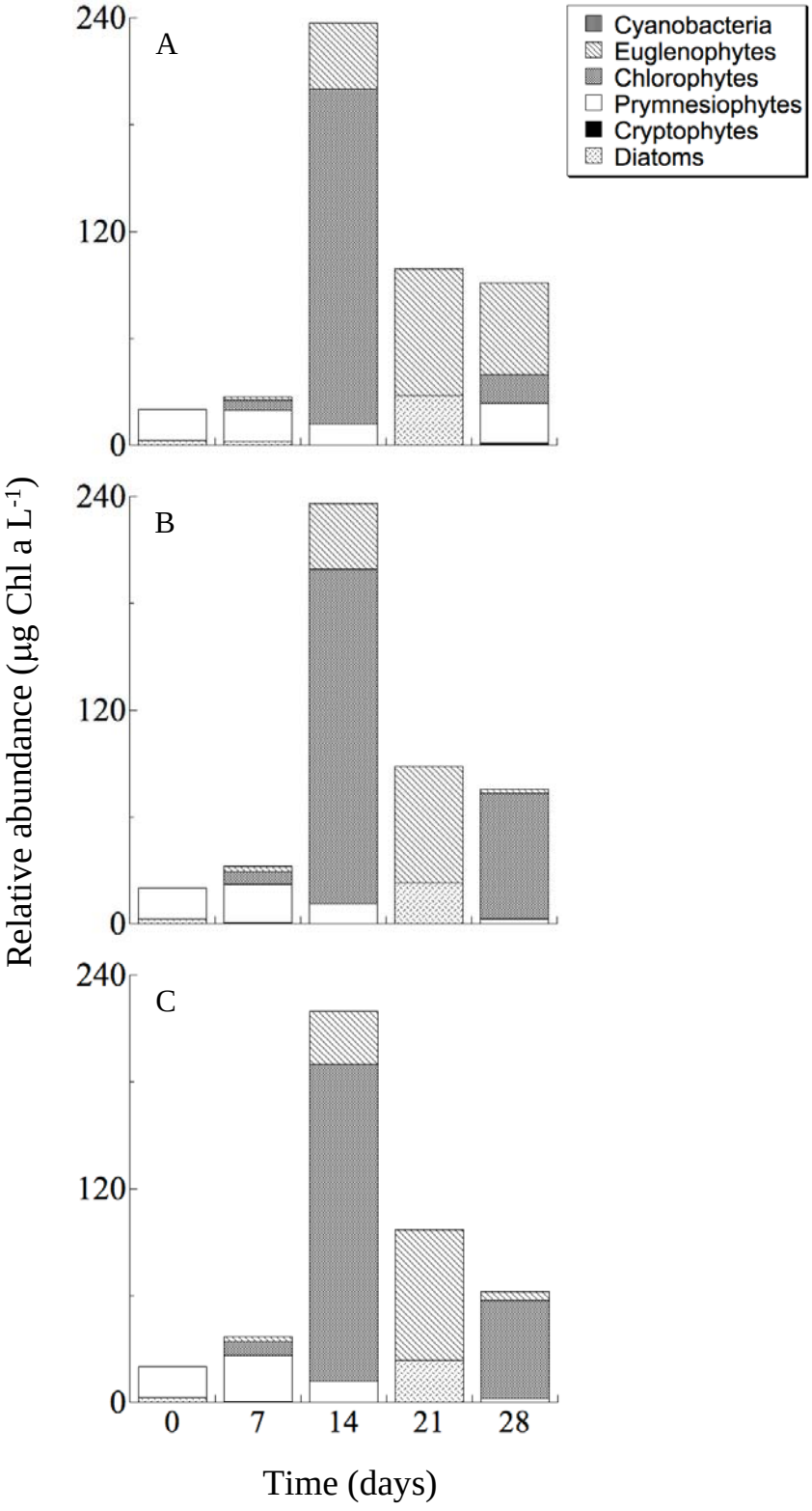
A-62
Spring 2005
Natural assemblage, f/2 nutrients, and *P. parvum*



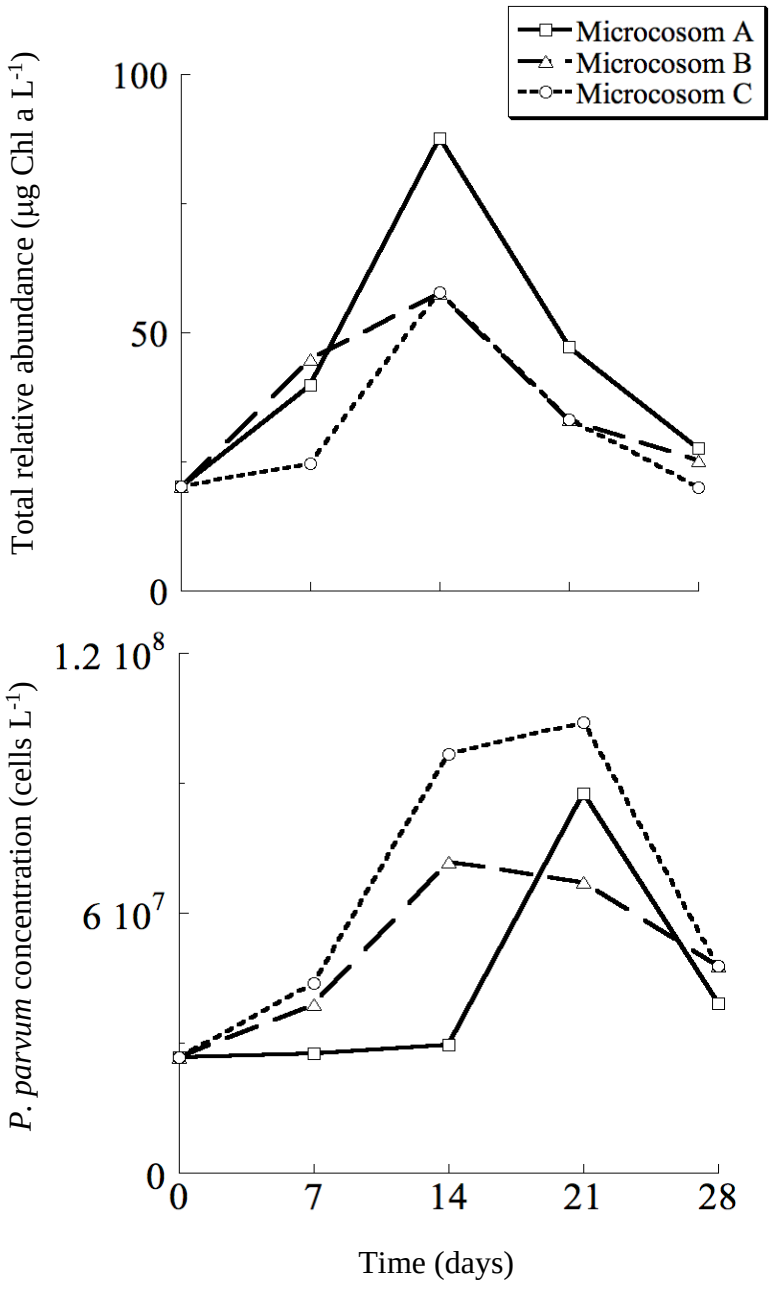
A-63
Spring 2005
Natural assemblage, f/2 nutrients, BSE, and *P. parvum*



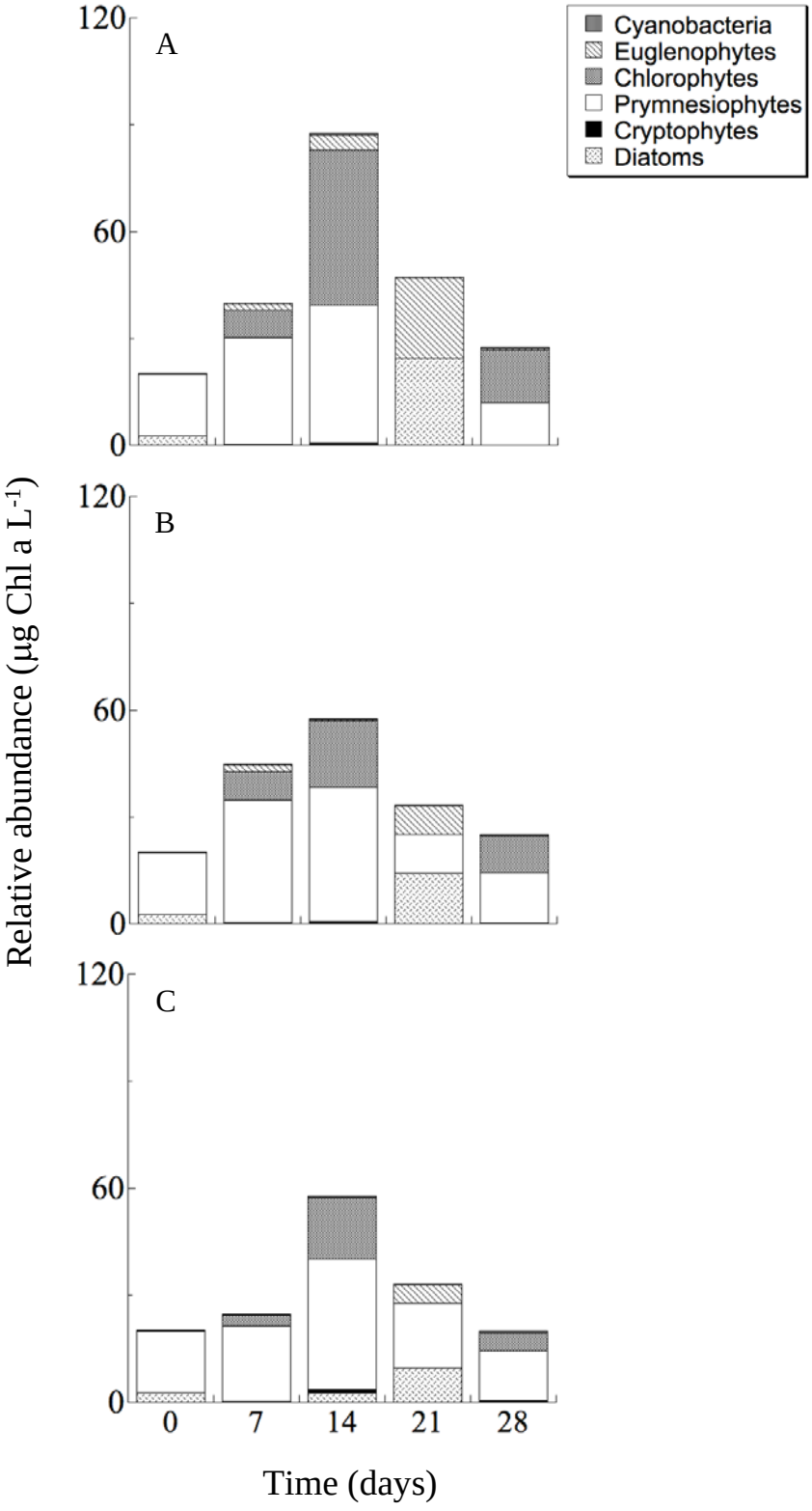
A-64
Spring 2005
Natural assemblage, f/2 nutrients, BSE, and *P. parvum*



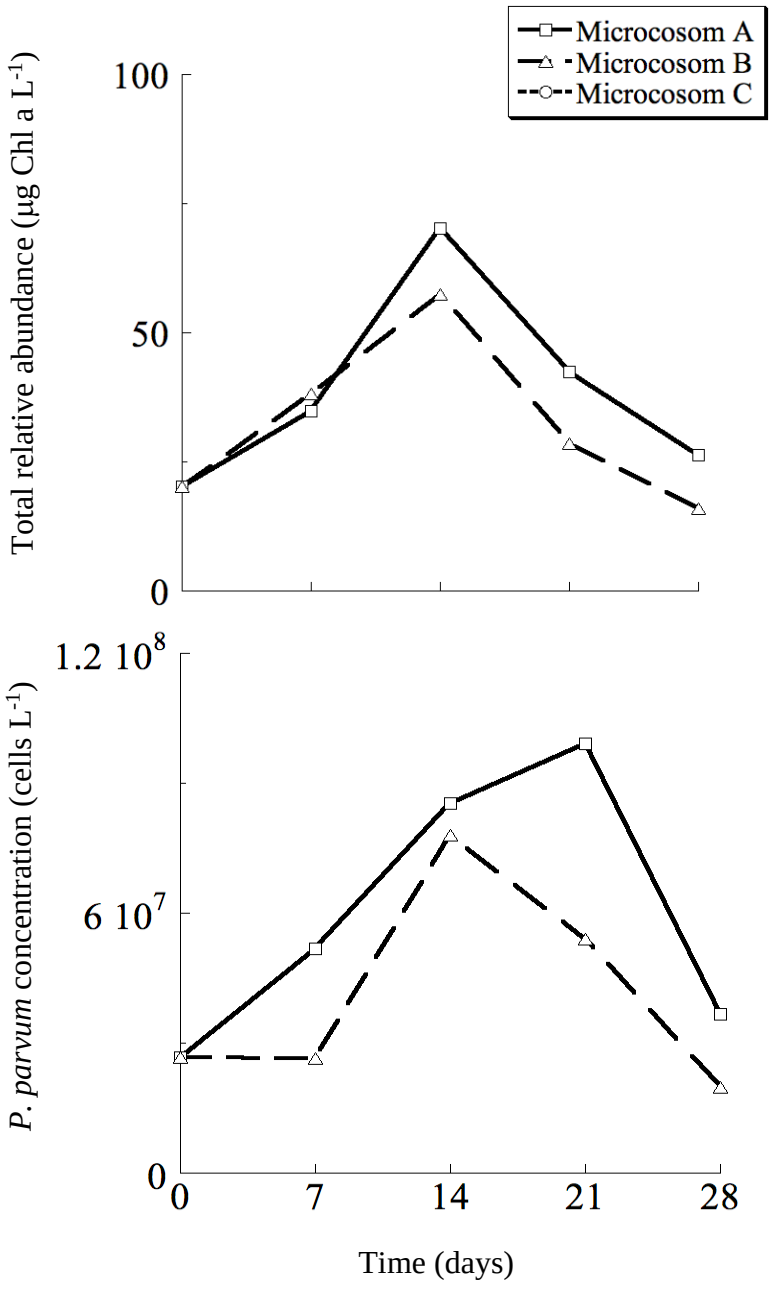
A-65
Spring 2005
Natural assemblage and nitrogen deplete f/2 nutrients



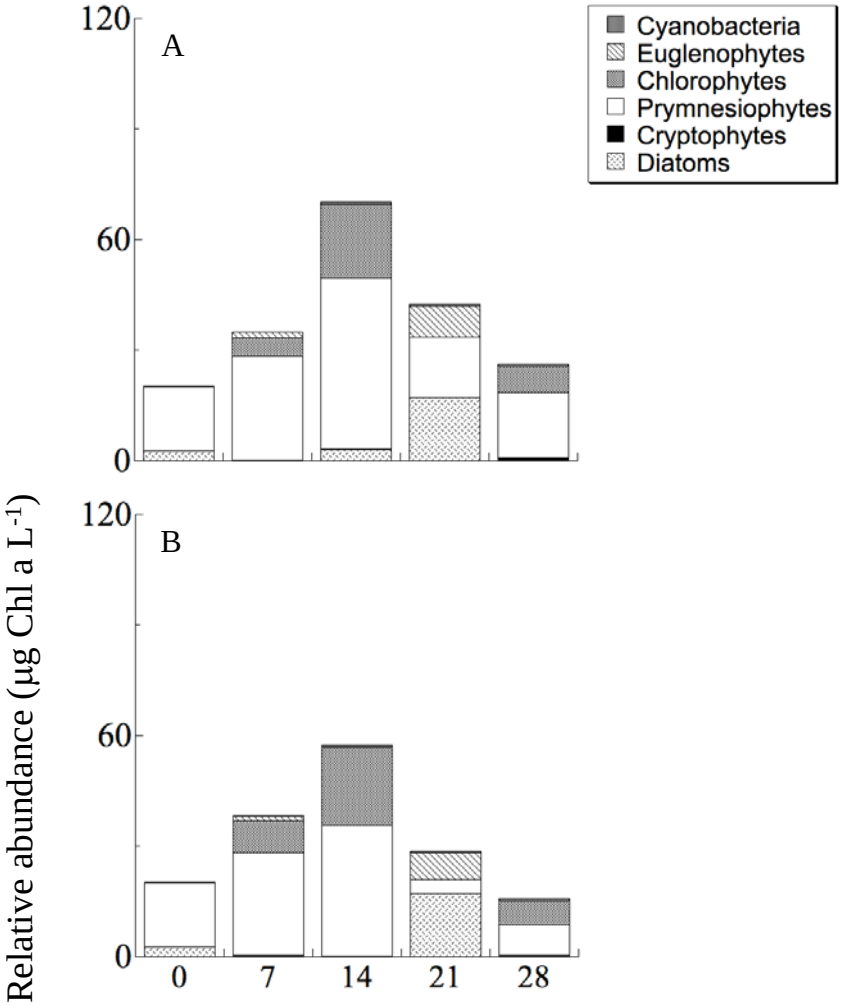
A-66
Spring 2005
Natural assemblage and nitrogen deplete f/2 nutrients



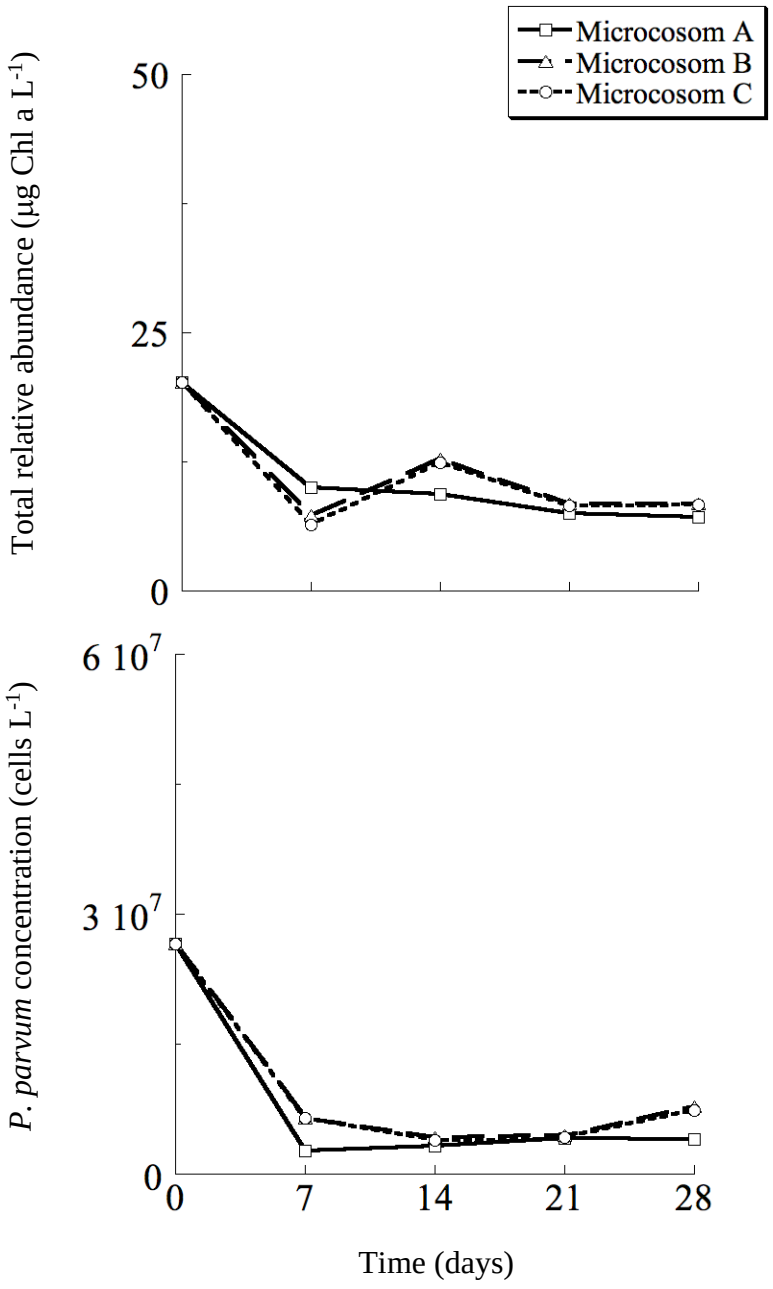
A-67
Spring 2005
Natural assemblage, nitrogen deplete f/2 nutrients, and *P. parvum*



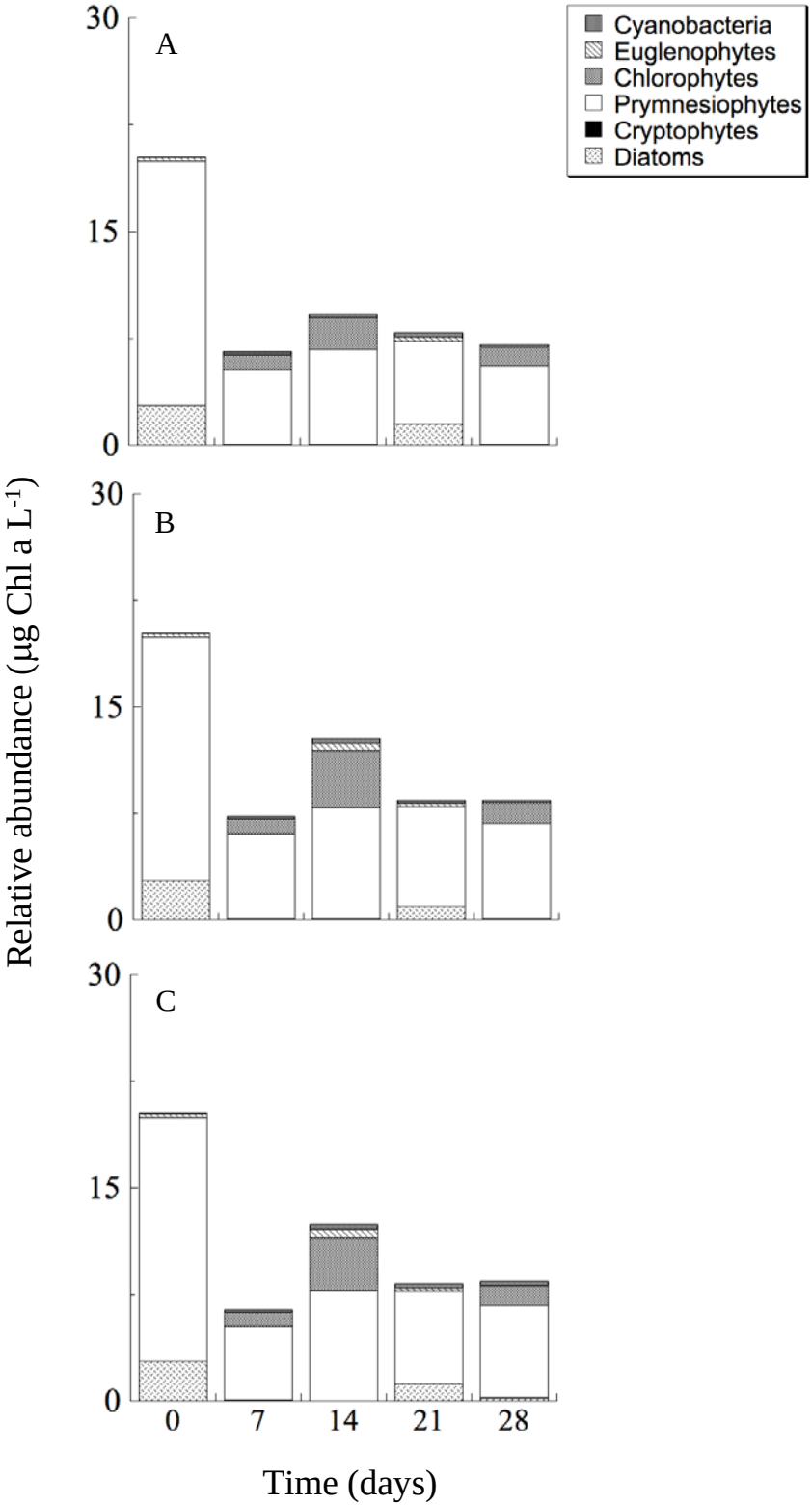
A-68
Spring 2005
Natural assemblage, nitrogen deplete f/2 nutrients, and *P. parvum*



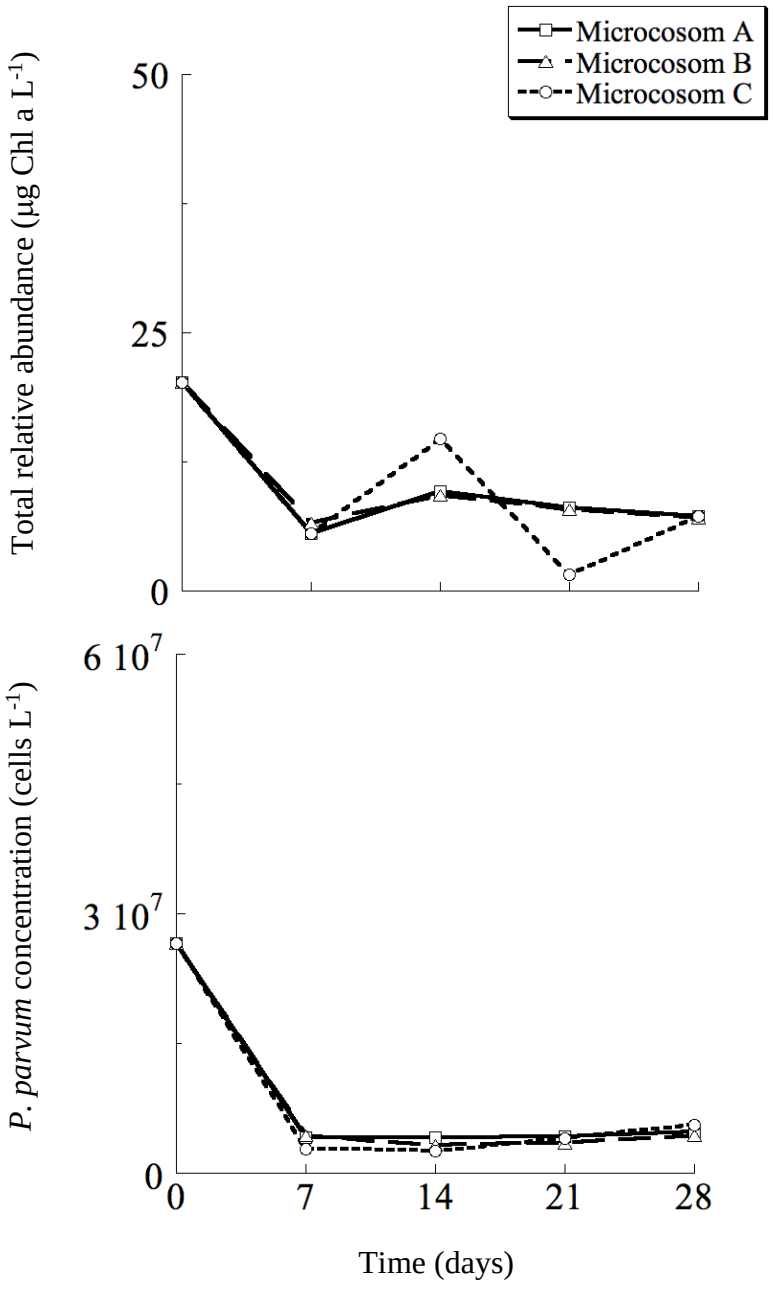
A-69
Spring 2005
Natural assemblage and phosphorus deplete f/2 nutrients



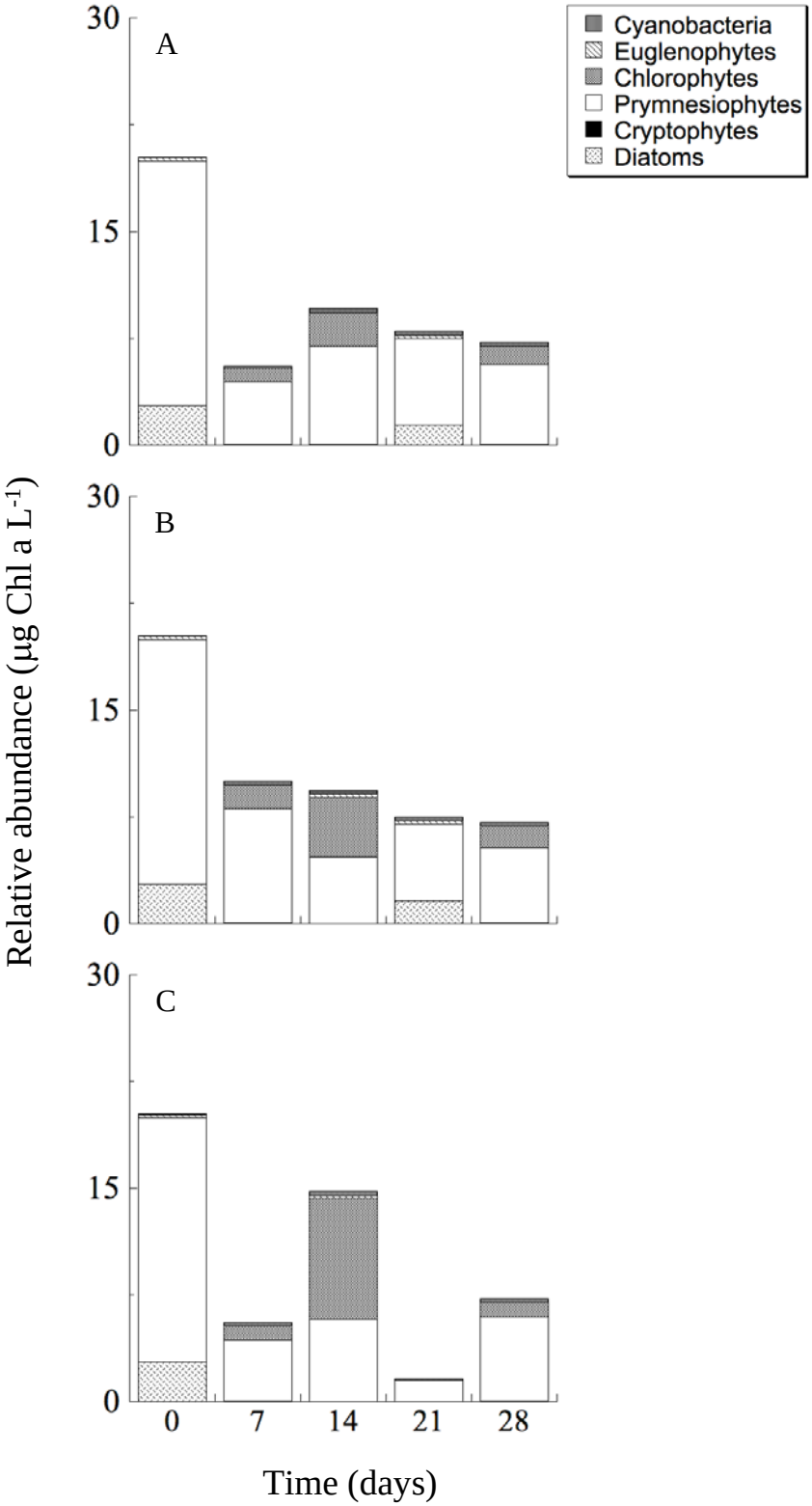
A-70
Spring 2005
Natural assemblage and phosphorus deplete f/2 nutrients



A-71
Spring 2005
Natural assemblage, phosphorus deplete f/2 nutrients, and *P. parvum*

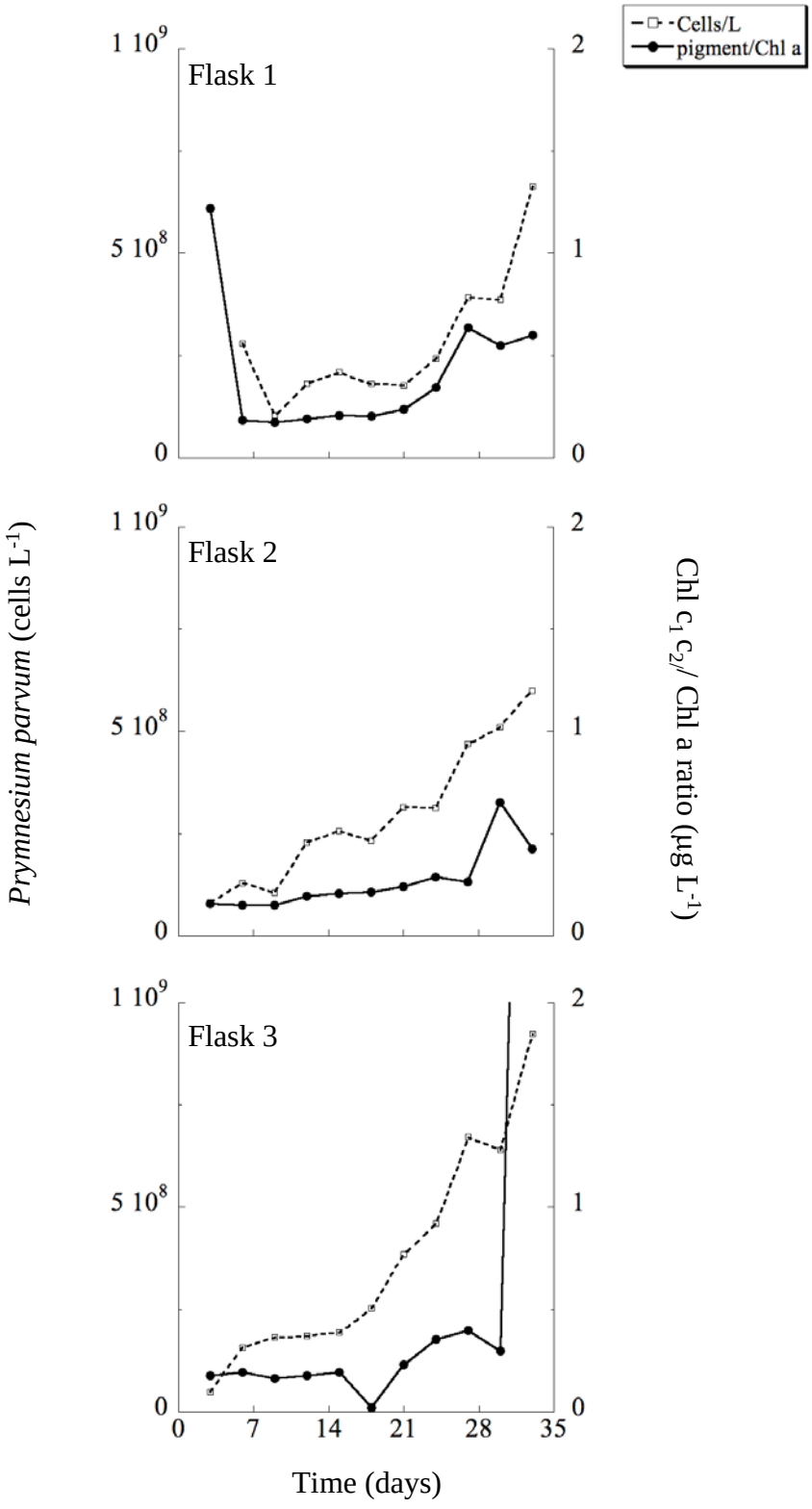


A-72
Spring 2005
Natural assemblage, phosphorus deplete f/2 nutrient, *P. parvum*

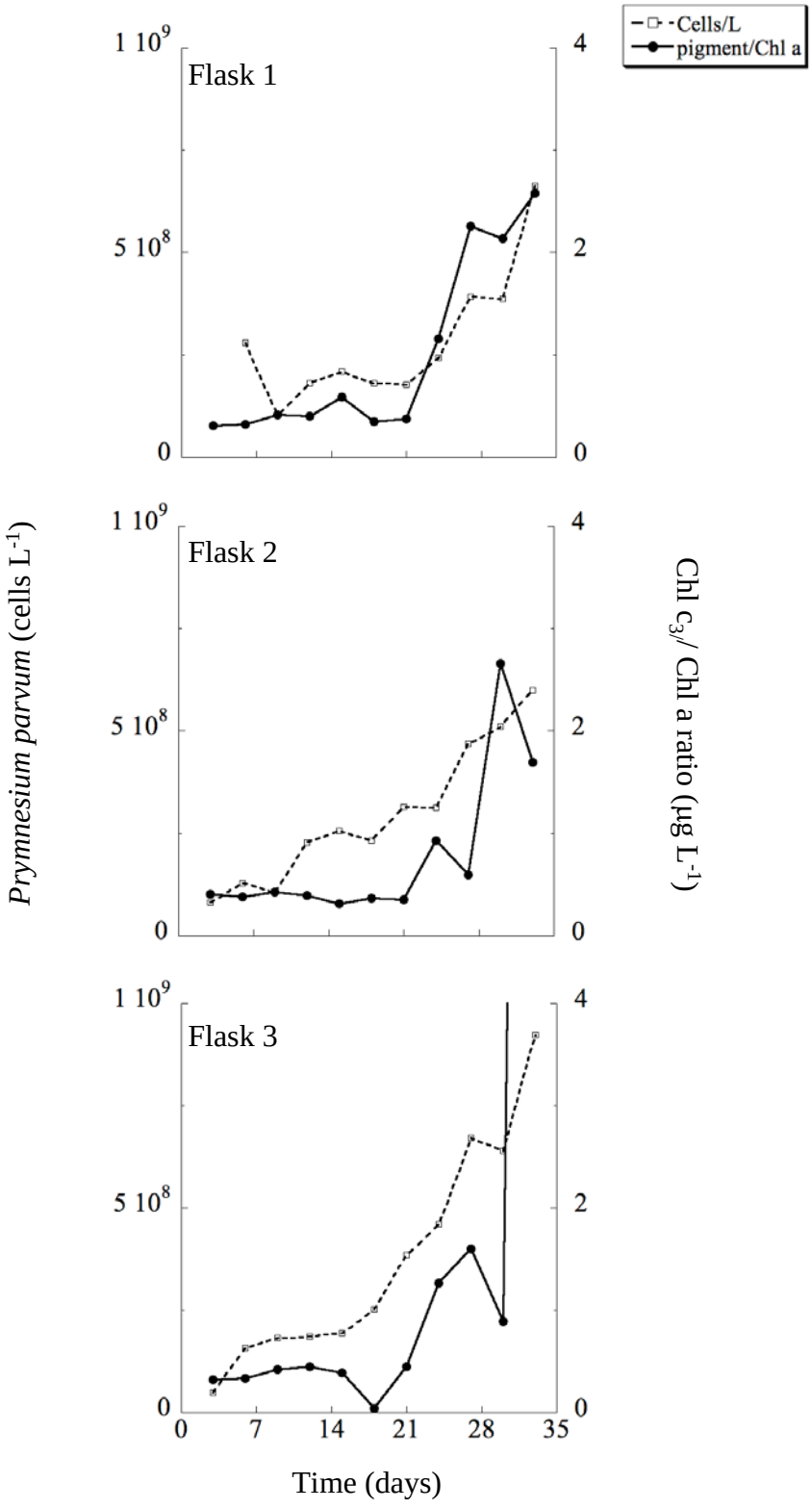


# APPENDIX B

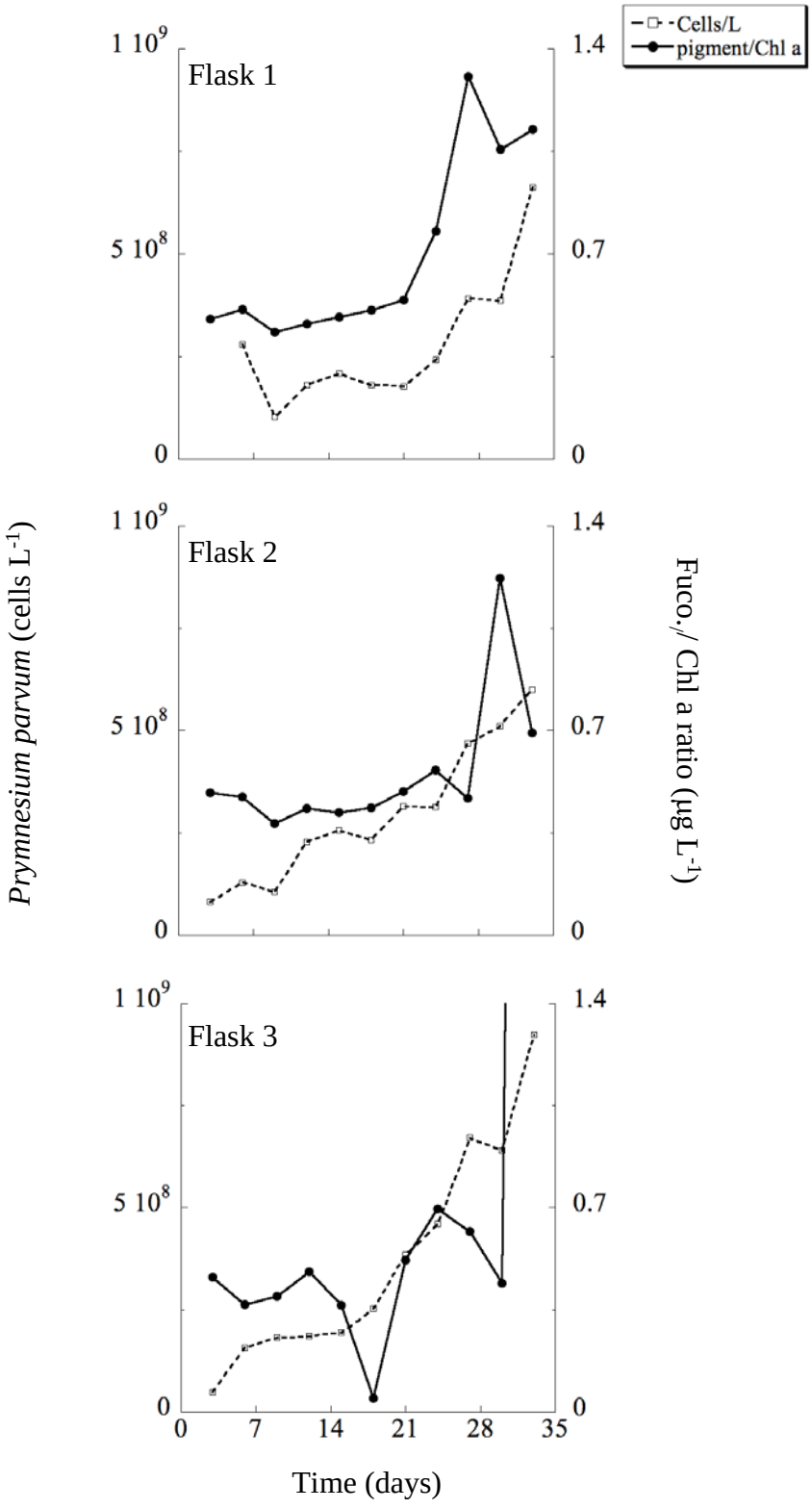
B-1
Pigment/Chlorophyll *a* ratios and *P. parvum* cell concentrations
Chlorophyll $c_1$ $c_2$



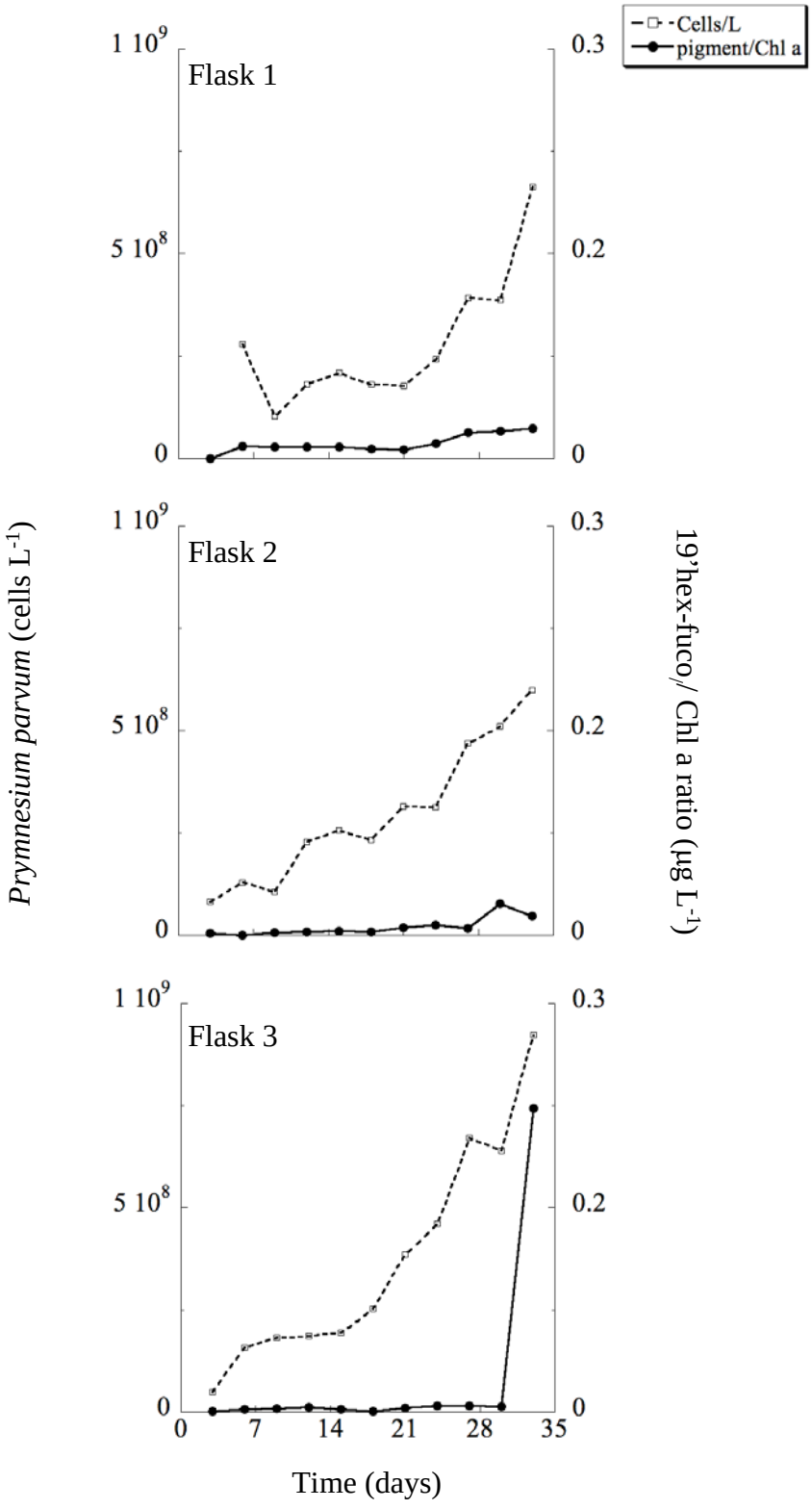
B-2
Pigment/Chlorophyll *a* ratios and *P. parvum* cell concentrations
Chlorophyll $c_3$



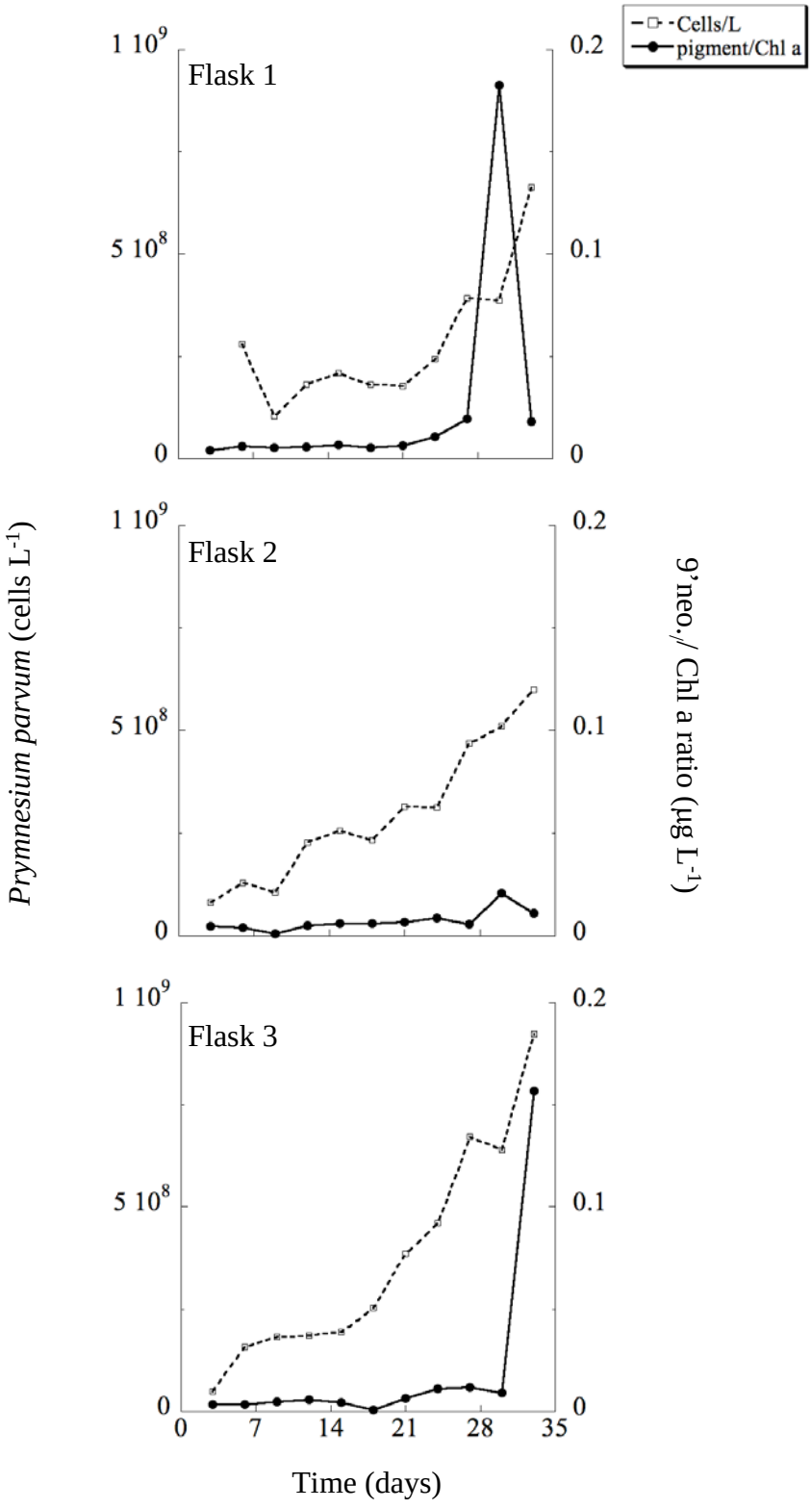
B-3
Pigment/Chlorophyll *a* ratios and *P. parvum* cell concentrations
Fucoxanthin



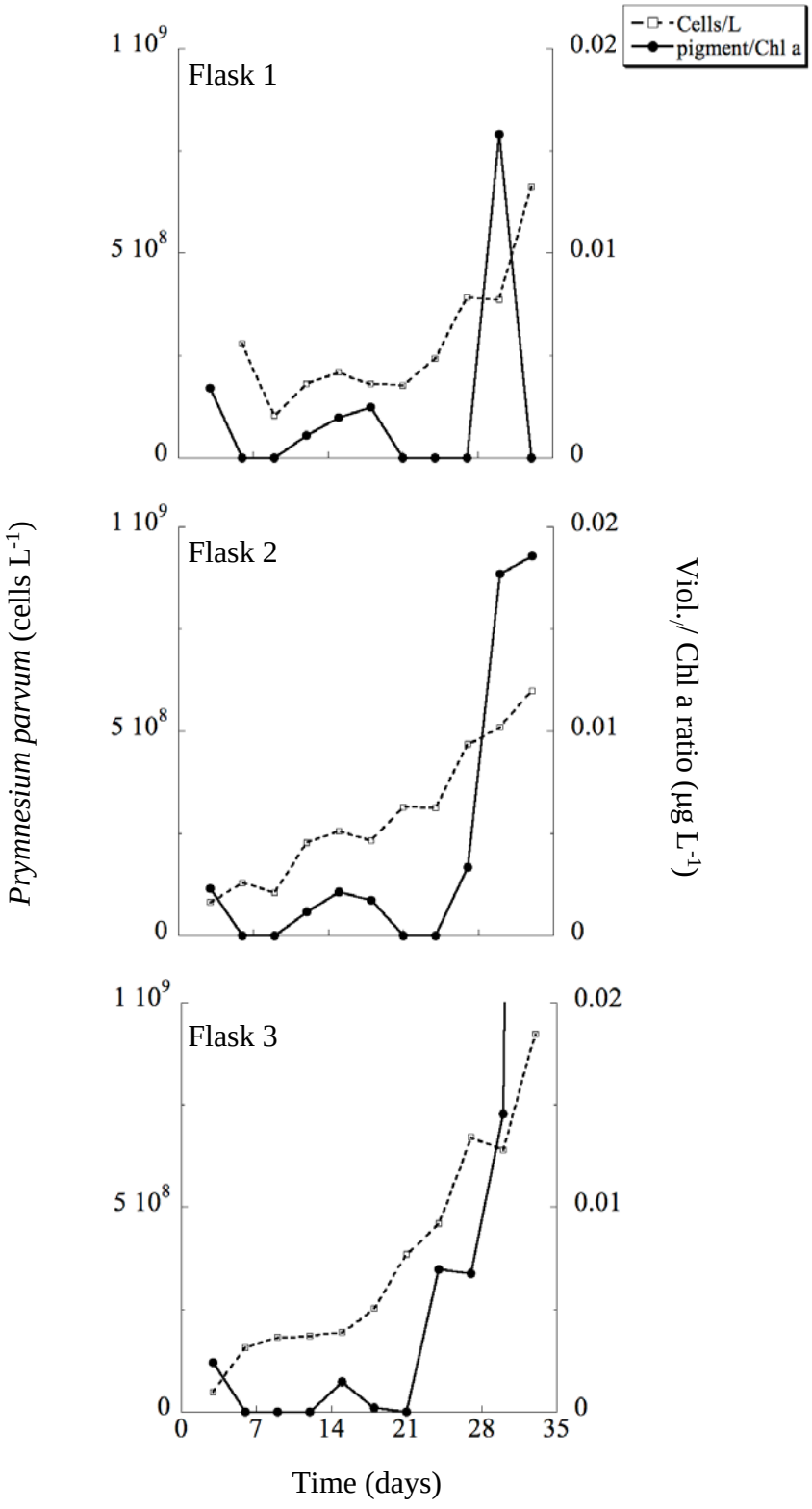
B-4
Pigment/Chlorophyll *a* ratios and *P. parvum* cell concentrations
19'-Hexanoyloxyfucoxanthin



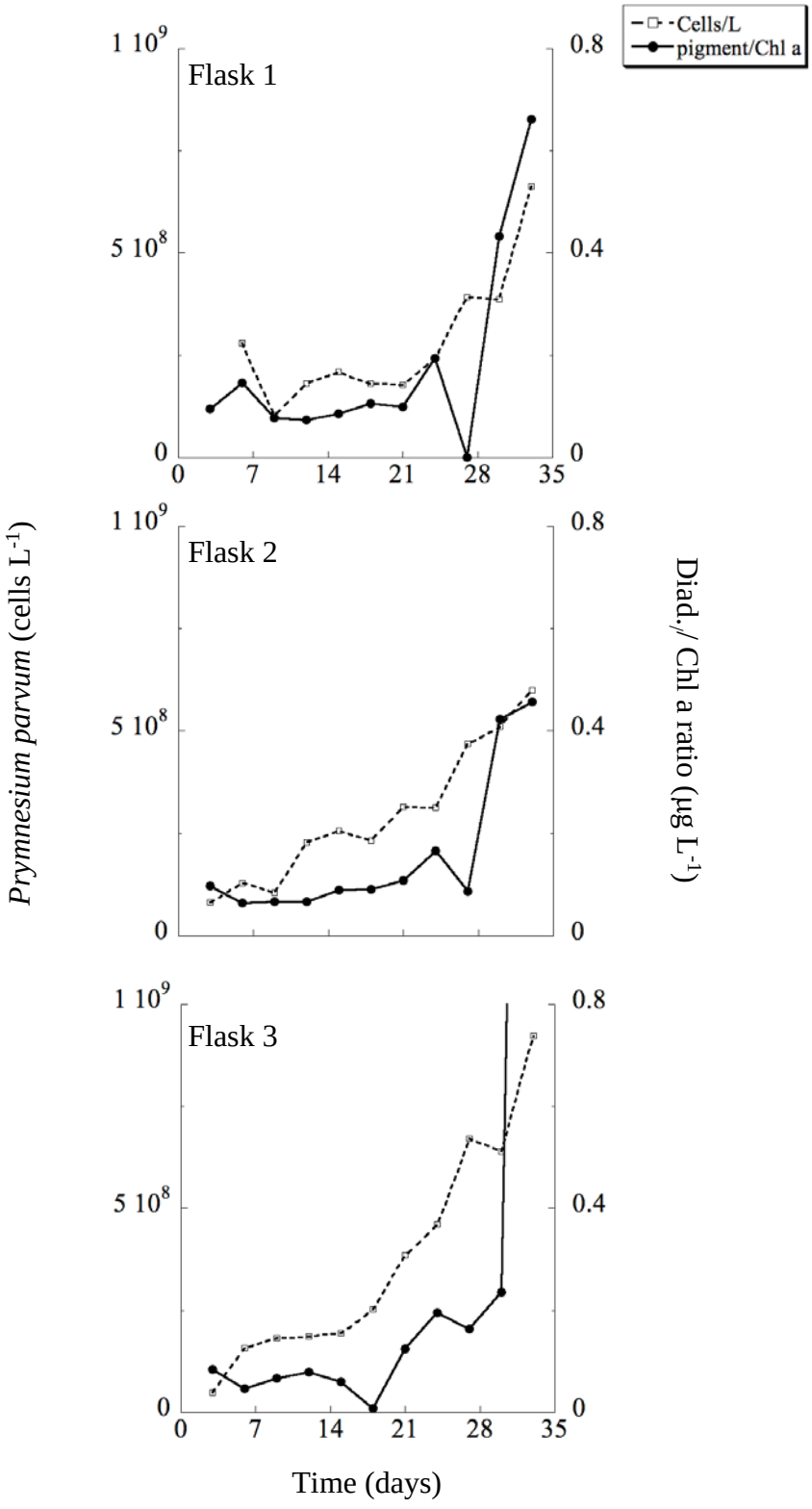
B-5
Pigment/Chlorophyll *a* ratios and *P. parvum* cell concentrations
9'-Neoxanthin



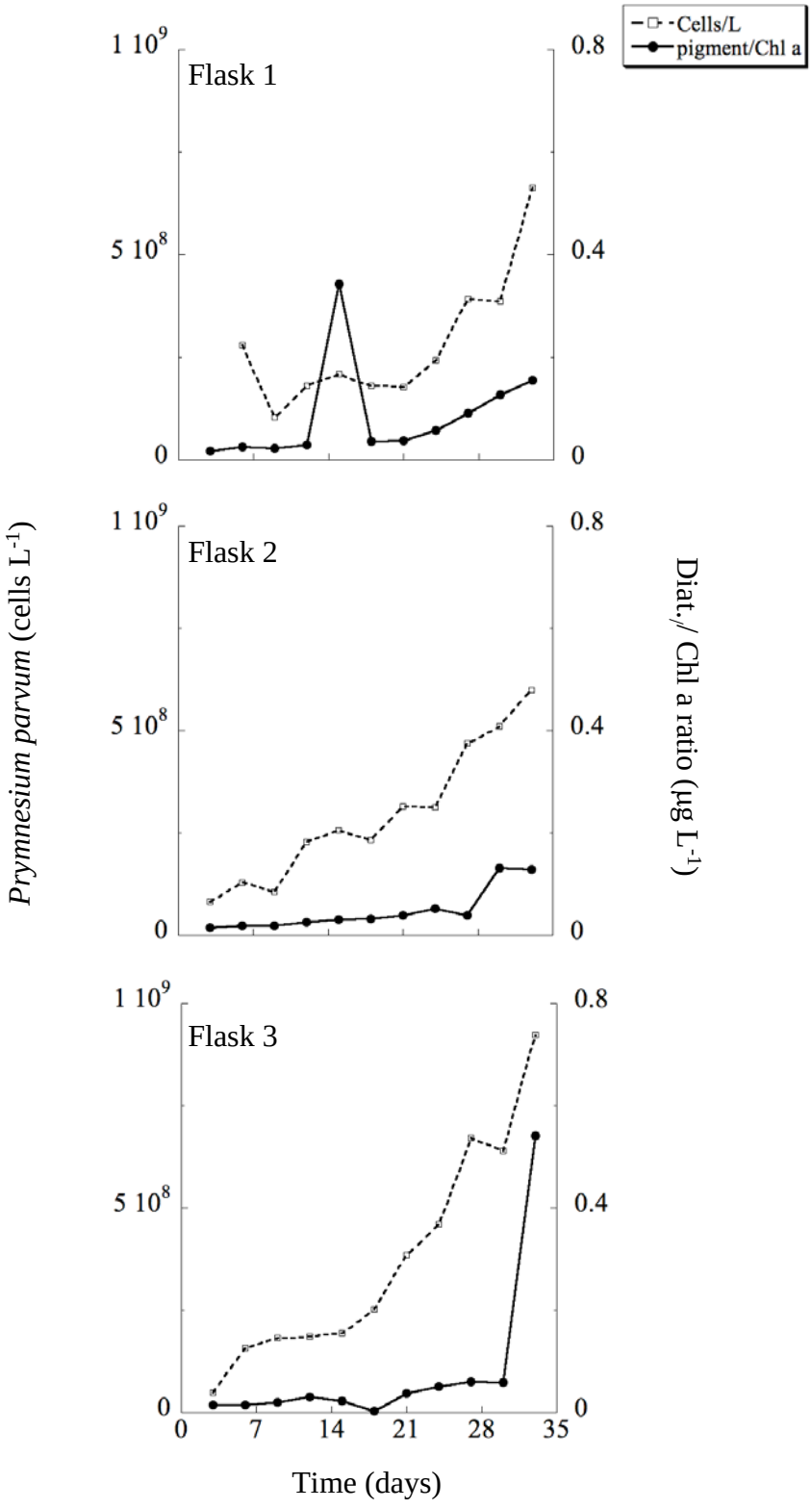
B-6
Pigment/Chlorophyll *a* ratios and *P. parvum* cell concentrations
Violaxanthin



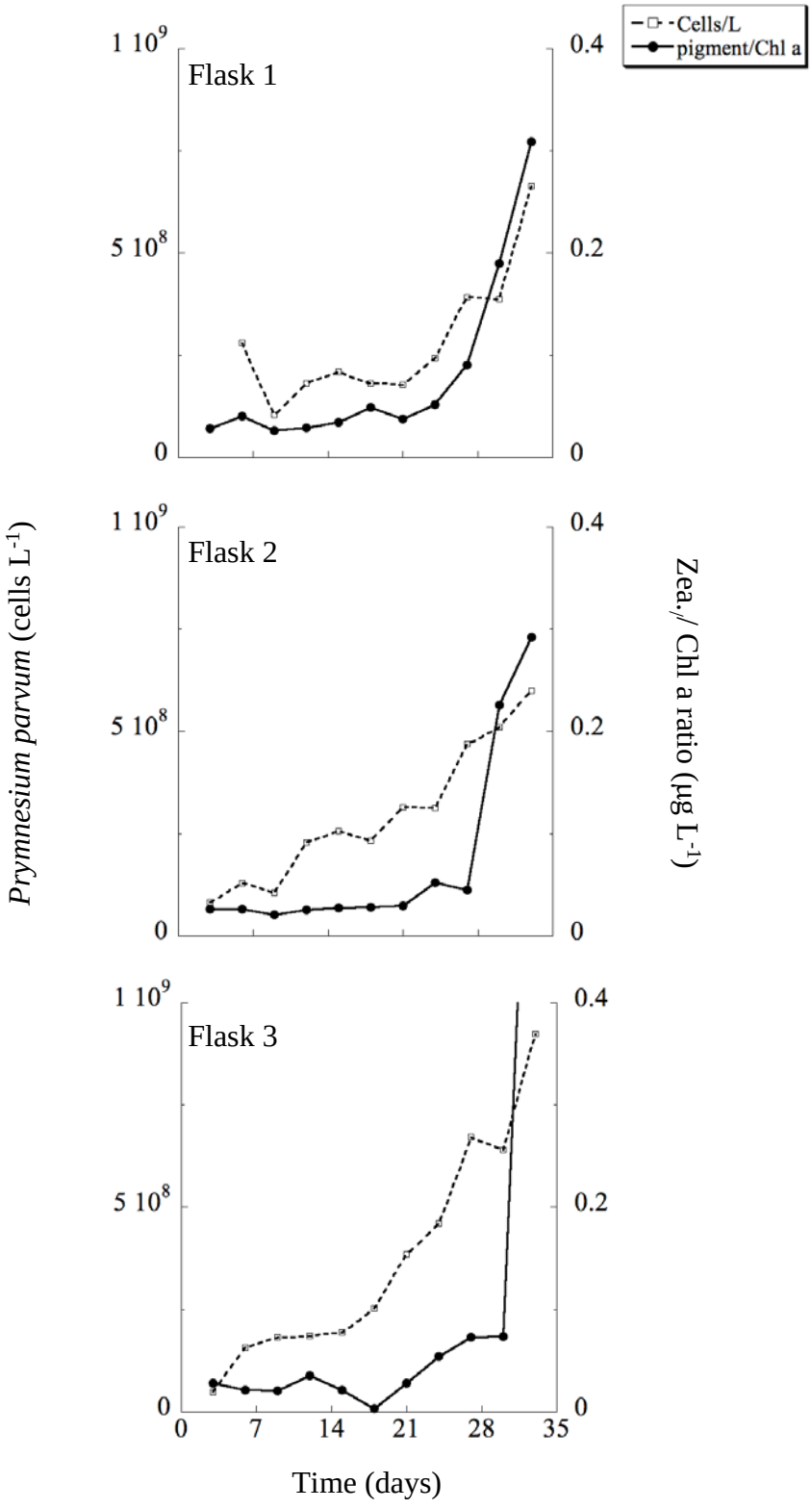
B-7
Pigment/Chlorophyll *a* ratios and *P. parvum* cell concentrations
Diadinoxanthin



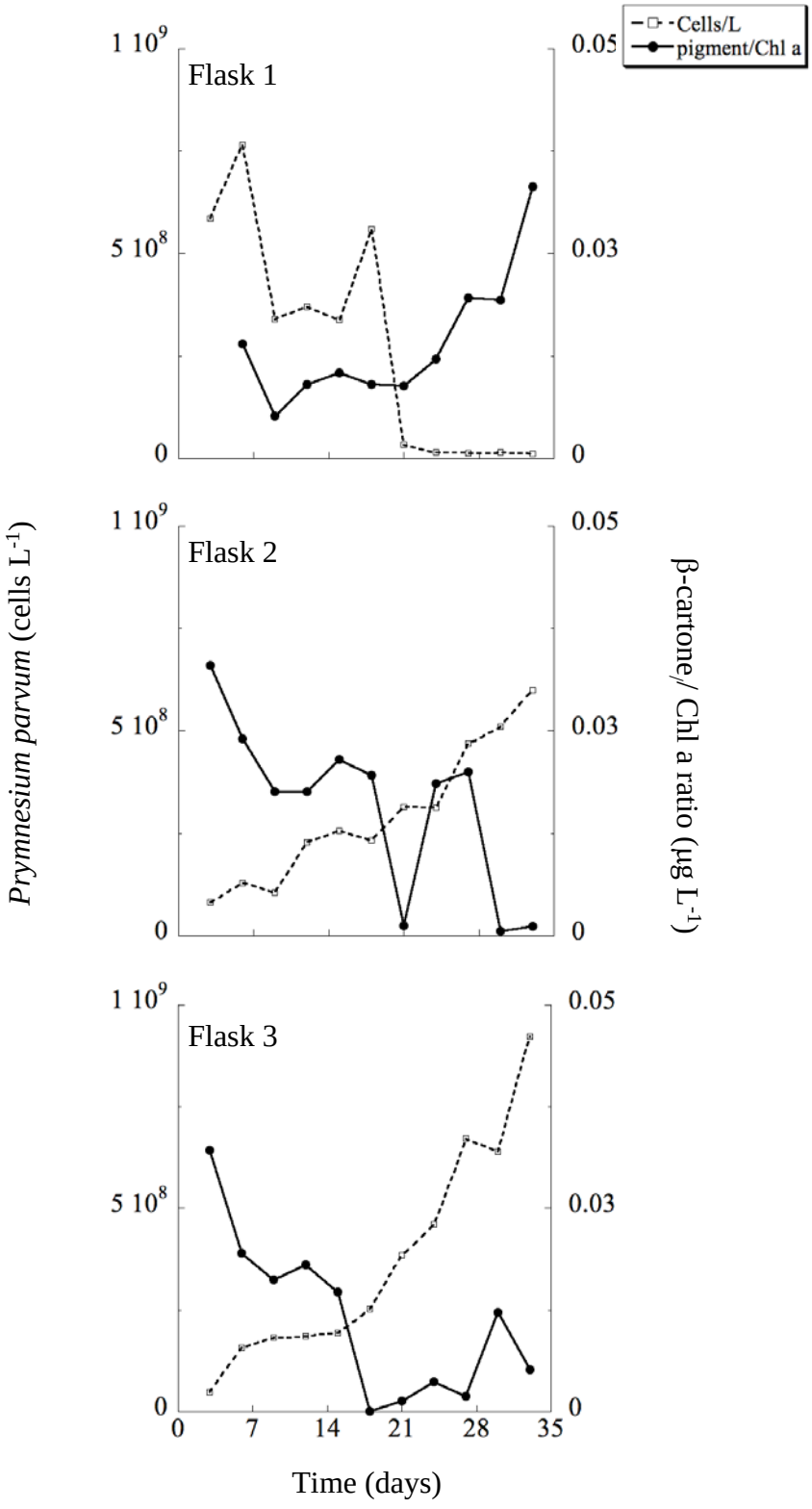
B-8
Pigment/Chlorophyll *a* ratios and *P. parvum* cell concentrations
Diatoxanthin



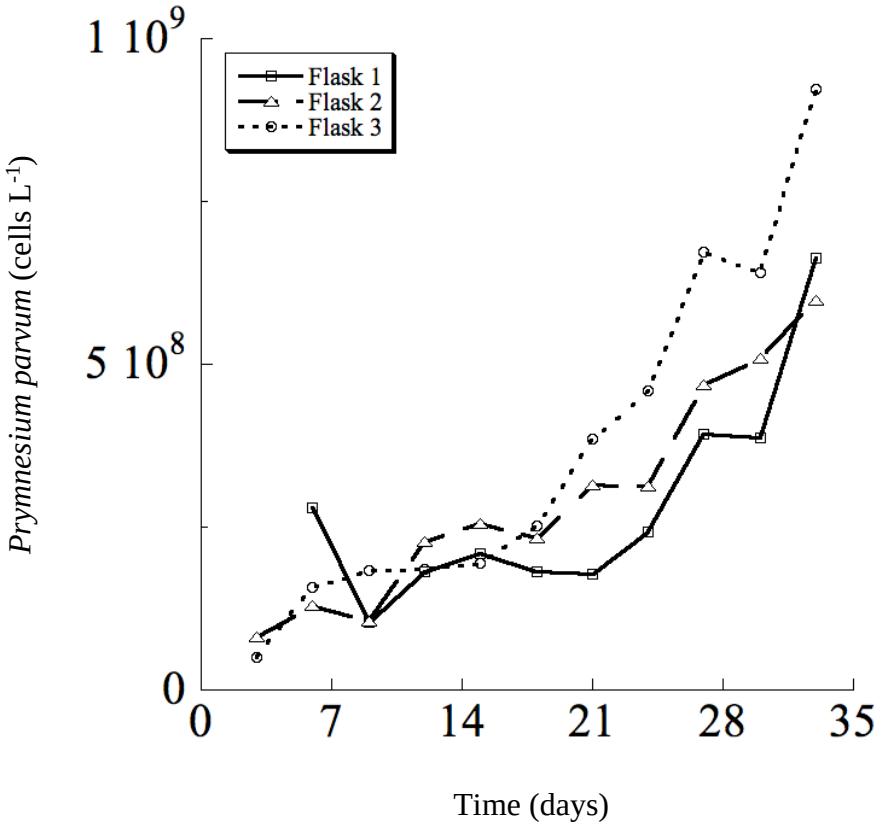
B-9
Pigment/Chlorophyll *a* ratios and *P. parvum* cell concentrations
Zeaxanthin



B-10
Pigment/Chlorophyll *a* ratios and *P. parvum* cell concentrations
β-cartone



B-11
*P. parvum* flask concentration



# APPENDIX C

TABLE Page

C-1

Fall experiment GLM repeated measures ANOVA with mean square

**A.**

| Total Chl *a* | 0-7 | | 0-14 | | 0-21 | | 0-28 | |
|---|---|---|---|---|---|---|---|---|
| | Mean sq. | F (*p*) | Mean sq. | F (*p*) | Mean sq. | F (*p*) | Mean sq. | F (*p*) |
| Nut. | 7165.810 | 189.297 (< 0.001) | 18629.970 | 127.391 (< 0.001) | 27574.600 | 106.342 (< 0.001) | 34019.500 | 107.974 (< 0.001) |
| *P. parvum* | 2.830 | 0.075 (< 0.001) | 5.900 | 0.040 (0.843) | 36.100 | 0.139 (0.714) | 144.230 | 0.458 (0.508) |
| BSE | 1594.830 | 42.130 (< 0.001) | 2079.160 | 14.217 (0.002) | 899.950 | 3.471 (0.081) | 603.730 | 1.916 (0.185) |
| Nut. x BSE | 879.110 | 23.223 (< 0.001) | 1115.570 | 7.628 (0.014) | 314.980 | 1.215 (0.287) | 115.560 | 0.367 (0.553) |
| Nut. x *P. parvum* | 1.660 | 0.044 (0.837) | 6.570 | 0.045 (0.835) | 32.280 | 0.124 (0.729) | 144.230 | 0.458 (0.508) |
| BSE x*P. parvum* | 21.070 | 0.557 (< 0.001) | 80.460 | 0.550 (0.469) | 104.100 | 0.401 (0.535) | 178.120 | 0.565 (0.463) |
| Nut. x BSE x*P. parvum* | 18.430 | 0.487 (0.495) | 68.540 | 0.469 (0.503) | 96.980 | 0.374 (0.549) | 165.770 | 0.526 (0.479) |

**B.**

| | 0-7 | | 0-14 | | 0-21 | | 0-28 | |
|---|---|---|---|---|---|---|---|---|
| Cells $L^{-1}$ | Mean sq. | F (*p*) | Mean sq. | F (*p*) | Mean sq. | F (*p*) | Mean sq. | F (*p*) |
| Nut. | 2.310E+14 | 63.718 (< 0.001) | 1.390E+15 | 73.528 (< 0.001) | 1.060E+15 | 57.672 (< 0.001) | 7.360E+14 | 48.303 (< 0.001) |
| BSE | 9.010E+07 | 4.181 (0.058) | 4.280E+12 | 0.127 (0.726) | 1.040E+13 | 0.002 (0.963) | 6.070E+12 | 0.035 (0.854) |
| *P. parvum* | 1.510E+13 | 0.000 (0.996) | 2.420E+12 | 0.225 (0.642) | 4.180E+10 | 0.567 (0.462) | 5.330E+11 | 0.399 (0.537) |
| Nut. x BSE | 2.130E+12 | 0.588 (0.454) | 3.330E+12 | 0.175 (0.681) | 1.830E+13 | 0.996 (0.333) | 2.780E+13 | 1.827 (0.195) |
| Nut. x *P. parvum* | 1.330E+12 | 0.368 (0.552) | 1.670E+13 | 0.877 (0.363) | 3.350E+13 | 1.820 (0.196) | 2.970E+13 | 1.948 (0.182) |
| BSE x*P. parvum* | 3.840E+12 | 1.063 (0.318) | 2.160E+13 | 1.136 (0.302) | 3.790E+13 | 2.059 (0.171) | 3.190E+13 | 2.094 (0.167) |
| Nut. x BSE x*P. parvum* | 8.290E+11 | 0.299 (0.639) | 1.050E+13 | 0.552 (0.468) | 1.920E+13 | 1.042 (0.323) | 1.330E+13 | 0.875 (0.364) |

C-2

Winter experiment GLM repeated measures ANOVA with mean square

| **A.** | 0-7 | | 0-14 | | 0-21 | | 0-28 | |
|---|---|---|---|---|---|---|---|---|
| LN Total Chl *a* | Mean sq. | F (*p*) | Mean sq. | F (*p*) | Mean sq. | F (*p*) | Mean sq. | F (*p*) |
| Nut. | 3.600 | 455.87 (< 0.001) | 15.407 | 1442.194 (< 0.001) | 38.868 | 2523.870 (< 0.001) | 68.067 | 3936.069 (< 0.001) |
| BSE | 0.015 | 1.905 (0.186) | 0.016 | 1.516 (0.236) | 0.010 | 0.665 (0.427) | 0.023 | 1.305 (0.27) |
| *P. parvum* | 0.050 | 6.36 (0.023) | 0.039 | 3.644 (0.074) | 0.023 | 1.496 (0.239) | 0.013 | 0.767 (0.394) |
| Nut. x BSE | 0.013 | 1.667 (0.215) | 0.003 | 0.248 (0.625) | 0.000 | 0.920 (0.352) | 0.015 | 0.855 (0.369) |
| Nut. x *P. parvum* | 0.000 | 0.035 (0.854) | 0.031 | 2.864 (0.11) | 0.029 | 1.860 (0.192) | 0.032 | 1.835 (0.194) |
| BSE x *P. parvum* | 0.000 | 0.059 (0.811) | 0.001 | 0.132 (0.721) | 0.000 | 0.023 (0.882) | < 0.000 | 0.002 (0.966) |
| Nut. x BSE x *P. parvum* | 0.000 | 0.018 (0.894) | 0.001 | 0.108 (0.747) | < 0.000 | 0.002 (0.962) | 0.000 | 0.013 (0.911) |

| **B.** | 0-7 | | 0-14 | | 0-21 | | 0-28 | |
|---|---|---|---|---|---|---|---|---|
| Cells $L^{-1}$ | Mean sq. | F (*p*) | Mean sq. | F (*p*) | Mean sq. | F (*p*) | Mean sq. | F (*p*) |
| Nut. | 2.712E+14 | 51.845 (< 0.001) | 1.308E16 | 142.960 (< 0.001) | 4.094E+15 | 53.597 (< 0.001) | 6.236E+14 | 8.651 (0.01) |
| BSE | 2.852E+11 | 0.055 (0.818) | 3.514E+13 | 0.384 (0.544) | 2.300E+12 | 0.030 (0.864) | 2.212E+12 | 0.031 (0.863) |
| *P. parvum* | 5.963E+13 | 11.398 (0.004) | 2.358E+14 | 2.578 (0.128) | 1.431E+14 | 1.873 (0.19) | 7.520E+13 | 1.043 (0.322) |
| Nut. x BSE | 3.906E+13 | 7.466 (0.015) | 3.121E+14 | 3.412 (0.083) | 3.363E+14 | 4.403 (0.052) | 3.885E+14 | 5.389 (0.034) |
| Nut. x *P. parvum* | 2.147E+13 | 4.103 (0.06) | 1.163E+14 | 1.271 (0.276) | 1.500E+14 | 1.964 (0.18) | 1.690E+14 | 2.344 (0.145) |
| BSE x *P. parvum* | 6.008E+13 | 11.484 (0.004) | 1.471E+14 | 1.608 (0.223) | 1.296E+14 | 1.697 (0.211) | 7.901E+13 | 1.096 (0.311) |
| Nut. x BSE x *P. parvum* | 4.025E+12 | 0.769 (0.393) | 1.467E+13 | 0.160 (0.694) | 1.145E+13 | 0.150 (0.704) | 2.182E+13 | 0.303 (0.59) |

C-3

Spring experiment GLM repeated measures ANOVA with mean square

| **A.** | 0-7 | | 0-14 | | 0-21 | | 0-28 | |
|---|---|---|---|---|---|---|---|---|
| LN Total Chl *a* | Mean sq. | F (*p*) | Mean sq. | F (*p*) | Mean sq. | F (*p*) | Mean sq. | F (*p*) |
| Nut. | 0.506 | 7.722 (0.013) | 11.029 | 167.118 (< 0.001) | 18.683 | 145.215 (< 0.001) | 34.133 | 219.639 (< 0.001) |
| BSE | 0.040 | 0.614 (0.445) | 0.065 | 0.982 (0.336) | 0.483 | 3.755 (0.071) | 1.129 | 7.265 (0.016) |
| *P. parvum* | 0.176 | 2.690 (0.120) | 0.003 | 0.053 (0.821) | 0.942 | 7.323 (0.016) | 0.755 | 4.860 (0.042) |
| Nut. x BSE | 0.020 | 0.311 (0.585 | 0.003 | 0.041 (0.843) | 0.276 | 2.147 (0.162) | 0.837 | 5.384 (0.034) |
| Nut. x *P. parvum* | 0.345 | 5.263 (0.036) | 0.004 | 0.061 (0.808) | 1.019 | 7.919 (0.012) | 0.983 | 6.324 (0.023) |
| BSE x *P. parvum* | 0.123 | 1.876 (0.190) | 0.122 | 1.844 (0.193) | 0.054 | 0.417 (0.527) | 0.374 | 2.407 (0.140) |
| Nut. x BSE x *P. parvum* | 0.119 | 1.815 (0.197) | 0.106 | 1.603 (0.224) | 0.040 | 0.314 (0.583) | 0.833 | 5.362 (0.034) |

| **B.** | 0-7 | | 0-14 | | 0-21 | | 0-28 | |
|---|---|---|---|---|---|---|---|---|
| Cells $L^{-1}$ | Mean sq. | F (*p*) | Mean sq. | F (*p*) | Mean sq. | F (*p*) | Mean sq. | F (*p*) |
| Nut. | 1.564E+13 | 0.558 (0.466) | 8.725E+14 | 39.844 (< 0.001) | 3.369E+15 | 181.873 (< 0.001) | 5.872E+15 | 354.086 (< 0.001) |
| BSE | 4.800E+11 | 0.017 (0.898) | 9.010E+11 | 0.041 (0.842) | 7.112E+12 | 0.384 (0.544) | 5.850E+12 | 0.353 (0.561) |
| *P. parvum* | 3.434E+13 | 1.225 (0.285) | 4.506E+13 | 2.058 (0.171) | 3.788E+13 | 2.045 (0.172) | 5.352E+13 | 3.227 (0.091) |
| Nut. x BSE | 6.453E+12 | 0.230 (0.638) | 2.589E+12 | 0.118 (0.735) | 3.839E+11 | 0.021 (0.887) | 6.910E+11 | 0.042 (0.841) |
| Nut. x *P. parvum* | 2.269E+13 | 0.809 (0.382) | 4.579E+12 | 0.209 (0.654) | 3.264E+12 | 0.176 (0.680) | 7.360E+09 | 0.000 (0.983) |
| BSE x *P. parvum* | 6.348E+13 | 2.265 (0.152) | 2.690E+13 | 1.228 (0.284) | 1.526E+13 | 0.824 (0.378) | 8.893E+12 | 0.536 (0.475) |
| Nut. x BSE x *P. parvum* | 4.641E+13 | 1.656 (0.216) | 4.803E+13 | 2.193 (0.158) | 4.013E+13 | 2.166 (0.160) | 3.748E+13 | 2.260 (0.152) |

C-4

Fall experiment GLM repeated measures ANOVA with mean square

| A. | 0-7 | | 0-14 | | 0-21 | | 0-28 | |
|---|---|---|---|---|---|---|---|---|
| Total Chl *a* | Mean sq. | F (*p*) | Mean sq. | F (*p*) | Mean sq. | F (*p*) | Mean sq. | F (*p*) |
| Nutrient class | 2412.463 | 235.278 (< 0.001) | 5346.688 | 203.815 (< 0.001) | 6132.085 | 113.828 (< 0.001) | 7216.298 | 67.527 (< 0.001) |
| *P. parvum* | 0.369 | 0.036 (0.852) | 1.132 | 0.043 (0.838) | 2.005 | 0.037 (0.85) | 2.396 | 0.022 (0.883) |
| Nut _ class x *P. parvum* | 11.023 | 1.075 (0.389) | 30.548 | 1.164 (0.356) | 59.423 | 1.092 (0.383) | 167.327 | 1.566 (0.239) |
| | Nuts N None P | | Nuts N None P | | Nuts N None P | | Nuts N None P | |

| B. | 0-7 | | 0-14 | | 0-21 | | 0-28 | |
|---|---|---|---|---|---|---|---|---|
| Cells $L^{-1}$ | Mean sq. | F (*p*) | Mean sq. | F (*p*) | Mean sq. | F (*p*) | Mean sq. | F (*p*) |
| Nutrient class | 2.246E+14 | 120.000 (< 0.001) | 4.033E+14 | 88.754 (< 0.001) | 8.329E+14 | 97.240 (< 0.001) | 1.330E+15 | 147.473 (< 0.001) |
| *P. parvum* | 3.583E+11 | 0.574 (0.462) | 6.154E+10 | 0.014 (0.909) | 1.210E+13 | 1.413 (0.252) | 3.247E+13 | 3.599 (0.076) |
| Nut _ class x *P. parvum* | 4.121E+12 | 2.202 (0.128) | 3.726E+12 | 0.820 (0.502) | 1.770E+12 | 0.207 (0.89) | 9.242E+12 | 1.024 (0.408) |
| | Nuts N None P | | Nuts N None P | | N Nuts None P | | N Nuts None P | |

C-5

Winter experiment GLM repeated measures ANOVA with mean square

| A. | 0-7 | | 0-14 | | 0-21 | | 0-28 | |
|---|---|---|---|---|---|---|---|---|
| Total Chl a | Mean sq. | F (*p*) | Mean sq. | F (*p*) | Mean sq. | F (*p*) | Mean sq. | F (*p*) |
| Nutrient class | 2608.167 | 68.386 (< 0.001) | 30457.369 | 350.191 (< 0.001) | 33590.037 | 401.144 (< 0.001) | 67277.733 | 613.423 (< 0.001) |
| *P. parvum* | 298.517 | 7.827 (0.014) | 408.067 | 14.076 (0.002) | 238.857 | 2.853 (0.112) | 442.598 | 4.036 (0.064) |
| Nut _ class x *P. parvum* | 62.785 | 1.646 (0.221) | 333.164 | 3.831 (0.032) | 58.802 | 0.702 (0.565) | 158.499 | 1.445 (0.272) |
| | Nuts N P None | | | | Nuts N P None | | Nuts N P None | |

| B. | 0-7 | | 0-14 | | 0-21 | | 0-28 | |
|---|---|---|---|---|---|---|---|---|
| Cells $L^{-1}$ | Mean sq. | F (*p*) | Mean sq. | F (*p*) | Mean sq. | F (*p*) | Mean sq. | F (*p*) |
| Nutrient class | 2.238E+14 | 23.923 (< 0.001) | 5.258E+15 | 49.475 (< 0.001) | 9.880E+15 | 77.876 (< 0.001) | 1.687E+16 | 104.608 (< 0.001) |
| *P. parvum* | 1.290E+14 | 13.793 (0.002) | 7.033E+14 | 6.618 (0.02) | 1.203E+15 | 9.479 (0.007) | 2.396E+15 | 14.858 (0.001) |
| Nut _ class x *P. parvum* | 2.259E+13 | 2.415 (0.104) | 1.856E+14 | 1.747 (0.198) | 5.017E+14 | 3.954 (0.028) | 1.558E+15 | 9.661 (0.001) |
| | N Nuts None P | | Nuts N None P | | | | | |

C-6

Spring experiment GLM repeated measures ANOVA with mean square

| A. | 0-7 | | 0-14 | | 0-21 | | 0-28 | |
|---|---|---|---|---|---|---|---|---|
| Total Chl a | Mean sq. | F (*p*) | Mean sq. | F (*p*) | Mean sq. | F (*p*) | Mean sq. | F (*p*) |
| Nutrient class | 465.578 | 21.951 (< 0.001) | 18235.626 | 250.021 (< 0.001) | 22931.316 | 133.100 (< 0.001) | 23806.556 | 105.718 (< 0.001) |
| *P. parvum* | 55.547 | 2.619 (0.126) | 98.793 | 1.355 (0.263) | 179.697 | 1.043 (0.323) | 137.842 | 0.612 (0.446) |
| Nut _ class x *P. parvum* | 95.304 | 4.493 (0.019) | 165.583 | 2.270 (0.122) | 100.862 | 0.585 (0.634) | 38.180 | 0.170 (0.915) |
| | | | Nuts N None P | | Nuts N None P | | Nuts N None P | |

| B. | 0-7 | | 0-14 | | 0-21 | | 0-28 | |
|---|---|---|---|---|---|---|---|---|
| Cells $L^{-1}$ | Mean sq. | F (*p*) | Mean sq. | F (*p*) | Mean sq. | F (*p*) | Mean sq. | F (*p*) |
| Nutrient class | 5.485E+14 | 15.204 (< 0.001) | 3.468E+15 | 32.370 (< 0.001) | 8.583E+15 | 56.783 (< 0.001) | 8.091E+15 | 12.102 (< 0.001) |
| *P. parvum* | 4.975E+13 | 1.379 (0.259) | 1.298E+14 | 1.212 (0.288) | 4.792E+13 | 0.317 (0.582) | 5.384E+14 | 0.805 (0.384) |
| Nut _ class x *P. parvum* | 3.829E+13 | 1.062 (0.395) | 4.209E+13 | 0.393 (0.76) | 1.965E+13 | 0.130 (0.941) | 4.131E+14 | 0.618 (0.614) |
| | N Nuts None P | | N None Nuts P | | N None Nuts P | | N None P Nuts | |

C-7

*P. parvum* photopigments

| | Time (days) | *P. parvum* (cells L $^{-1}$) | Pigment (µg L$^{-1}$) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Chl $C_3$ | Chl $c_1c_2$ | Fuco | 19'HFuco | 9'cis-Neo | Viola | Diad | Diat | Zeax | Total Chl *a* | B-Car |
| | 3 | NA | 22.94 | 91.17 | 35.69 | 0.00 | 0.29 | 0.25 | 7.11 | 1.31 | 2.11 | 74.72 | 2.19 |
| | 6 | 2.80E+08 | 27.13 | 15.40 | 42.98 | 0.77 | 0.52 | 0.00 | 12.26 | 2.10 | 3.41 | 84.29 | 3.22 |
| | 9 | 1.04E+08 | 26.24 | 11.05 | 27.57 | 0.56 | 0.33 | 0.00 | 4.91 | 1.42 | 1.64 | 63.56 | 1.08 |
| | 12 | 1.81E+08 | 30.25 | 14.42 | 35.02 | 0.65 | 0.42 | 0.08 | 5.58 | 2.24 | 2.17 | 75.77 | 1.40 |
| | 15 | 2.09E+08 | 49.66 | 17.64 | 41.15 | 0.74 | 0.57 | 0.17 | 7.24 | 28.98 | 2.87 | 84.61 | 1.43 |
| Flask 1 | 18 | 1.81E+08 | 29.05 | 17.13 | 42.53 | 0.60 | 0.46 | 0.21 | 8.83 | 2.98 | 4.10 | 83.69 | 2.34 |
| | 21 | 1.78E+08 | 31.40 | 19.71 | 45.17 | 0.55 | 0.52 | 0.00 | 8.20 | 3.15 | 3.10 | 82.91 | 0.14 |
| | 24 | 2.43E+08 | 75.33 | 22.40 | 50.77 | 0.72 | 0.69 | 0.00 | 12.71 | 3.76 | 3.38 | 65.22 | 0.05 |
| | 27 | 3.92E+08 | 78.80 | 22.20 | 45.55 | 0.67 | 0.68 | 0.00 | 0.00 | 3.17 | 3.15 | 34.86 | 0.02 |
| | 30 | 3.87E+08 | 114.59 | 29.45 | 56.67 | 1.07 | 9.79 | 0.85 | 23.24 | 6.85 | 10.18 | 53.66 | 0.04 |
| | 33 | 6.63E+08 | 155.24 | 36.17 | 67.76 | 1.32 | 1.08 | 0.00 | 39.84 | 9.37 | 18.61 | 60.27 | 0.03 |
| | 3 | 8.25E+07 | 36.47 | 14.10 | 43.16 | 0.15 | 0.41 | 0.20 | 8.65 | 1.35 | 2.32 | 88.70 | 2.92 |
| | 6 | 1.29E+08 | 27.35 | 10.78 | 33.67 | 0.00 | 0.29 | 0.00 | 4.58 | 1.35 | 1.86 | 71.06 | 1.71 |
| | 9 | 1.06E+08 | 32.06 | 11.29 | 28.79 | 0.15 | 0.08 | 0.00 | 5.08 | 1.39 | 1.58 | 75.39 | 1.32 |
| | 12 | 2.28E+08 | 38.82 | 19.11 | 42.39 | 0.26 | 0.50 | 0.11 | 6.58 | 2.49 | 2.51 | 97.99 | 1.72 |
| | 15 | 2.56E+08 | 40.11 | 26.46 | 53.81 | 0.36 | 0.77 | 0.28 | 11.58 | 3.89 | 3.56 | 128.00 | 2.76 |
| Flask 2 | 18 | 2.33E+08 | 43.94 | 25.77 | 51.95 | 0.33 | 0.73 | 0.21 | 10.83 | 3.87 | 3.38 | 119.30 | 2.34 |
| | 21 | 3.15E+08 | 39.86 | 27.07 | 55.34 | 0.63 | 0.75 | 0.00 | 12.15 | 4.31 | 3.34 | 112.45 | 0.14 |
| | 24 | 3.13E+08 | 99.58 | 30.79 | 60.51 | 0.82 | 0.94 | 0.00 | 17.83 | 5.59 | 5.63 | 107.35 | 2.00 |
| | 27 | 4.69E+08 | 75.12 | 33.50 | 59.24 | 0.67 | 0.74 | 0.42 | 10.98 | 4.97 | 5.69 | 126.63 | 2.53 |
| | 30 | 5.10E+08 | 128.16 | 31.39 | 58.82 | 1.10 | 1.00 | 0.85 | 20.39 | 6.34 | 10.86 | 48.17 | 0.03 |
| | 33 | 5.99E+08 | 175.80 | 44.17 | 71.72 | 1.45 | 1.13 | 1.93 | 47.28 | 13.25 | 30.24 | 103.64 | 0.12 |
| | 3 | 4.94E+07 | 29.01 | 16.14 | 41.67 | 0.05 | 0.30 | 0.22 | 7.65 | 1.31 | 2.53 | 90.44 | 2.91 |
| | 6 | 1.58E+08 | 23.05 | 13.31 | 25.20 | 0.13 | 0.22 | 0.00 | 3.25 | 1.00 | 1.49 | 68.51 | 1.33 |
| | 9 | 1.83E+08 | 33.61 | 13.12 | 31.41 | 0.19 | 0.36 | 0.00 | 5.33 | 1.58 | 1.66 | 79.33 | 1.29 |
| | 12 | 1.85E+08 | 43.31 | 17.25 | 46.31 | 0.33 | 0.57 | 0.00 | 7.63 | 2.96 | 3.45 | 96.56 | 1.74 |
| | 15 | 1.94E+08 | 33.25 | 16.65 | 31.27 | 0.16 | 0.37 | 0.13 | 5.19 | 1.92 | 1.85 | 85.30 | 1.26 |
| Flask 3 | 18 | 2.53E+08 | 32.59 | 17.77 | 38.79 | 0.40 | 0.46 | 0.17 | 6.31 | 2.58 | 2.55 | 833.53 | 0.09 |
| | 21 | 3.85E+08 | 54.00 | 27.65 | 62.53 | 0.38 | 0.77 | 0.00 | 14.97 | 4.41 | 3.34 | 119.98 | 0.16 |
| | 24 | 4.60E+08 | 124.41 | 35.03 | 68.48 | 0.42 | 1.09 | 0.68 | 19.20 | 4.99 | 5.33 | 98.42 | 0.36 |
| | 27 | 6.72E+08 | 152.81 | 38.11 | 59.03 | 0.43 | 1.11 | 0.65 | 15.56 | 5.76 | 6.98 | 95.50 | 0.18 |
| | 30 | 6.40E+08 | 177.69 | 59.32 | 87.39 | 0.82 | 1.81 | 2.90 | 46.93 | 11.87 | 14.62 | 198.88 | 2.43 |
| | 33 | 9.23E+08 | 160.80 | 35.20 | 81.82 | 1.29 | 0.91 | 2.08 | 19.05 | 3.13 | 4.58 | 5.79 | 0.03 |

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*Education*

| | |
|---|---|
| May 2006 | **Texas A&M University**, Master of Science, Wildlife and Fisheries Sciences |
| May 2002 | **Trinity University**, Bachelor of Science, Biology and History |

*Professional Experience*

| | |
|---|---|
| Aug 2003 - Present | Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX, *Research Assistant* |
| Jan 2003 - Aug | HBC/Terracon, Inc., Dallas, TX, *Environmental Professional* |
| May 2002 - Sept | Texas Commission of Environmental Quality, San Antonio, TX, *Mickey Leland Intern, Department of Defense Specialist* |
| Jan 2002- May | Nature Conservancy, San Antonio, TX**,** *Government Relations Intern* |

*Grantsmanships and Honors*

| | |
|---|---|
| 2005/06 | Texas Water Resource Institute, Mills Scholarship |
| 2005/06 | Ethel Ashworth-Tsutsui Memorial Research Award for Research |
| 2004/05 | Outstanding Master's Student, Wildlife and Fisheries Sciences |

*Scholastic Memberships*

| | |
|---|---|
| 2005-P | Phi Kappa Phi, National Honor Society |
| 2005-P | Gamma Sigma Delta, Honor Society of Agriculture |

*Memberships*

| | |
|---|---|
| 2005-P | Texas River and Reservoir Management Society |
| 2005-P | Graduate Student Council Representative |
| 2004-P | American Society of Limnology and Oceanography |
| 2004/05 | Ecological Integration Symposium Committee |
| 2003-P | Association of Graduate Students of Wildlife and Fisheries Sciences |