

DEEP WATER MIXING PREVENTS HARMFUL ALGAL BLOOM FORMATION:
IMPLICATIONS FOR MANAGED FISHERIES REFUGIA

A Thesis

by

NATANYA JEANNE HAYDEN

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

August 2011

Major Subject: Wildlife and Fisheries Sciences

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Managed Fisheries Refugia

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Approved by:

Chair of Committee,	Daniel Roelke
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ABSTRACT

Deep Water Mixing Prevents Harmful Algal Bloom Formation: Implications for
Managed Fisheries Refugia. (August 2011)

Natanya Jeanne Hayden, B.S., Lake Erie College

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Inflows affect water quality, food web dynamics, and even the incidence of harmful algal blooms. It may be that inflows can be manipulated to create refuge habitat for biota trying to escape poor environmental conditions, such as fish populations in lakes during times of toxic *Prymnesium parvum* blooms. Water availability sometimes can be an issue, especially in arid climates, which limits this approach to management. Utilizing source water from deeper depths to displace surface waters, however, might effectively mimic inflow events. I test this notion by conducting in-lake mesocosm experiments with natural plankton communities where I manipulate hydraulic flushing. Results show that *P. parvum* cell density is reduced by 69%, and ambient toxicity completely ameliorates during pre-bloom conditions in the lake. During conditions of bloom development, population density is reduced by 53%, toxicity by 57%, and bloom proportions are never reached. There is minimal effect of these inflows on total phytoplankton and zooplankton biomass, and little effect on water quality. Shifts toward more rapidly growing phytoplankton taxa are observed, as are enhanced copepod nauplii. In other words, while inflows using deep waters suppress *P. parvum* bloom

initiation and development, they are benign to other aspects of the lower food web and environment. The results from using deep lake water to suppress harmful algal blooms indicate this may be a promising management approach and further studies are recommended to test whether this mitigating effect can translate to a large-scale in-lake treatment.

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NOMENCLATURE

Chl- <i>a</i>	Chlorophyll <i>a</i>
DIN	Dissolved Inorganic Nitrogen
HAB	Harmful Algal Bloom
N	Nitrogen
P	Phosphorus
PO ₄	Phosphate

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CHAPTER I
INTRODUCTION: THE IMPORTANCE OF FRESHWATER INFLOWS ON
SYSTEM DYNAMICS

Freshwater inflows have many resounding effects that can shape the productivity, diversity, and food web structure of an aquatic system. The magnitude and intensity of inflow events have a direct effect on abiotic water quality parameters, such as salinity, turbidity, and thermal stratification (Mitrovic et al. 2011; Roelke and Pierce 2011). In addition, dissolved inorganic nutrients (i.e., nitrogen and phosphate) are susceptible to shifts induced by inflow loads and flushing rates (Sommer 1984; Barbiero et al. 1999; Buyukates and Roelke 2005; Roelke et al. 2007). Altered nutrient load in a lake can be linked to successional shifts in phytoplankton and zooplankton community composition, as well as primary productivity rates (Havens 1991a; Havens et al. 2003; Roelke et al. 2004; Miller et al. 2008).

Inorganic nutrient enrichment and timing of hydraulic flushing have various effects on lower food web dynamics, such as influencing growth rates and resource competition (Floder and Sommer 1999; Chicharo et al. 2006; Roelke et al. 2004, 2007). For example, systems with a constant inflow may select for fast-reproducing, generalist species, which can recover from disturbances quickly (Miller et al. 2008), and pulsed inflows may increase diversity by allowing both specialist (slow-growing) and generalist

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species to co-dominate (Tilman et al. 1982; Grover 1997; Miller et al. 2008).

Competitive selection, coupled with physical disturbance, and flushing cell losses associated with hydraulic inflow events can aid in promoting species diversity and offer a potential management technique for circumventing harmful algal blooms (HABs) (Havens 1991a; Lawrence et al. 2004; Miller et al. 2008), such as *Prymnesium parvum*.

P. parvum has been present in estuarine and brackish water worldwide (Johnsen et al. 2010); however, within recent decades it has become an invasive species in freshwater systems as well (Roelke et al. 2007; Errera et al. 2008). Resulting in the formation of toxic blooms outside of optimal growth conditions (Baker et al. 2007, 2009), this has been observed since 1985 in Texas inland lakes during the wintertime (James and De La Cruz 1989; Roelke et al. 2007; Sager et al. 2008). Baker et al. (2007) characterized optimal growth performance at 27°C and salinity levels near 22 psu. However, winter conditions in Texas inland lakes are drastically different, where temperature typically ranges between 7-15°C. Furthermore, salinity levels range between 2-4 psu and blooms have been associated with low precipitation years (Roelke et al. 2010). The maximum growth rate of *P. parvum*, a slow-growing species, is 0.94 d⁻¹ under optimal conditions (Baker et al. 2007); therefore, when this growth rate is reduced, such as suboptimal temperature and salinity levels, *P. parvum* becomes dependant on toxin production to gain dominance over faster-growing phytoplankton taxa, such as chlorophytes and diatoms (Tilman et al. 1982; Grover 1989; Grover et al. 1999). Toxin compounds function in suppressing grazing zooplankton, competing phytoplankton, and have a lethal effect on fish (Granéli and Johansson 2003a, 2003b).

There are several biotic and abiotic factors that have been linked to *P. parvum* growth rate and toxin compound production, such as light, temperature, and inorganic nutrients (Granéli and Johansson 2003a; Granéli and Salomon 2010). All of these environmental variables play a role in bloom formation and many previous studies have focused on determining what influences suboptimal conditions and subsequent increased toxicity. Despite many years of research and a strong understanding of system dynamics leading to bloom formation and toxicity, some of the previous findings have been contradictory. This highlights the need for further research regarding management techniques within natural systems, such as freshwater lakes. A review of previous studies that identify bloom-influencing factors follows, particularly light, temperature, salinity, and inorganic nutrient availability.

Hagström and Granéli (2005) have observed increased toxin production correlated with reduced photo availability. Alternatively, Baker et al. (2007) found no significant difference in toxin levels due to light availability with regard to acute ambient toxicity to fish. Water temperature is directly related to the reproductive growth rate of *P. parvum*, where growth rate is drastically reduced at 5°C compared to 25°C and results in a unimodal curve (Baker et al. 2007). Conditions characterized by low water temperature produce lower cell densities (Grover et al. 2007) and interestingly some studies have concluded an increase in toxicity (Baker et al. 2007). Additionally, high toxicity of *P. parvum* has been associated with salinity extremes (i.e., low salinity ≤ 7.5 psu or high salinity ≥ 35 psu; Hagström and Granéli 2005; Baker et al. 2007). Brackish conditions, similar to the systems in Europe and the Middle East, where *P. parvum*

originated from, have not been correlated with increased toxicity or allelopathic suppression. Instead, toxic blooms in these systems have been mainly due to eutrophication (van Rijn and Shilo 1989; Amsinck et al. 2005).

The impact that nutrient availability has on *P. parvum* bloom proliferation and toxin production is extremely complex. Many previous studies have concluded that in the absence of N and P availability, *P. parvum* experiences “stressful” conditions and toxin compound production is stimulated (Johansson and Granéli 1999; Barkoh et al. 2003; Granéli and Johansson 2003a; Grover et al. 2007; Kurten et al. 2007). Furthermore, unbalanced N:P ratios within a system compared to Redfield ratio (N:P=16:1), even when inorganic nutrients are initially available, ultimately lead to either N or P deficiency and increase *P. parvum* toxin production (Granéli and Salomon 2010). Unbalanced nutrient ratios are characteristic of eutrophic systems (Granéli and Salomon 2010), and may in part explain toxic blooms both witnessed in Texas and Europe. An effective management technique for toxicity mitigation could be preventing nutrient extremes, both depleted conditions with low nutrient availability and unnatural nutrient ratios that are observed with eutrophication. In addition, N and P availability is particularly important for the survivorship of competing phytoplankton, thus limiting the dominance of *P. parvum*.

Nutrient addition into systems are often associated with hydraulic inflow events (Turner and Rabalais 1994; Eldridge and Roelke 2010) and can have strong implications regarding HAB prevalence. Some marine systems receiving nitrate rich inflows have suffered from toxic diatom blooms (*Pseudo-nitzschia* spp.; MacIntyre et al. 2011). While on the other hand, some freshwater systems experiencing eutrophication and reduced inflow rates have suffered from increased cyanobacteria blooms (*Anabaena* spp.; Hudnell et al. 2010). Additionally, inflow events have been linked to bloom mitigation and termination. For example, mixing induced by hydraulic inflows may lead to a reduction in stratification, which has subsequently increased turbidity in some systems and alleviated cyanobacteria blooms (*Anabaena circinalis*; Mitrovic et al. 2011). Similarly, freshwater inflow events have coincided with bloom termination and cessation of toxin production during *P. parvum* blooms (Roelke et al. 2007; Schwierzke-Wade et al. 2011). Dilution of existing *P. parvum* cells and toxin compounds, as well as increased nutrient availability and subsequent lowered toxin production were acting mechanisms in bloom termination (Roelke et al. 2007; Schwierzke-Wade et al. 2011).

All of these factors influence the flow of energy to zooplankton, secondary productivity, and overall system sustainability. Pulsed flow conditions are positively correlated with increased nutrient availability, due to the system being replenished with nitrogen and phosphate (Sommer 1989; Roelke 2000; Miller et al. 2008). This enables phytoplankton to take up more nutrients than are immediately necessary to maintain maximum growth rates (Roelke 2000). Which result in higher nutrient levels being ingested by grazers and subsequently elevate secondary productivity levels (Buyukates

and Roelke 2005), despite the temporary flushing effect that large inflows may have on slow-growing grazers (i.e., copepods; Reynolds 1984; Sommer et al. 1986; Havens 1991a, 1991b; Miller et al. 2008).

The minimum magnitude and frequency of inflows required to maintain the health of inland lakes is far from understood and very difficult to discern. Previous studies document evidence that lack of freshwater inflow events lead to reduced diversity of phytoplankton (Tilman 1982; Grover 1997; Miller et al. 2008), increased prevalence and toxicity of HABs (Mitrovic et al. 2011; Roelke et al. 2007, 2011; Schwierzke-Wade et al. 2011), and lowered secondary productivity (Reynolds 1984; Sommer et al. 1986; Havens 1991a, 1991b; Roelke 2000; Miller et al. 2008). This relationship between inflows and ecosystem health illustrates the importance of increasing our knowledge regarding the necessity of inflow magnitudes and timing, particularly when considering global climate change (Cai and McCarl 2009; Roelke et al. 2011), and increased sequestration of water with a rising human population.

OVERARCHING GOAL AND SPECIFIC OBJECTIVES

The primary goal of this research was to construct and facilitate two mesocosm experiments to determine the effect of hydraulic flushing on *P. parvum* pre-bloom and bloom development factors. This is important because a more developed understanding regarding pulsed inflows using in-lake water has the potential to lead to management strategies for *P. parvum* bloom mitigation and prevention, improved system health and water quality, as well as provide refuge habitat for valuable fish populations. This will be achieved by accomplishing the following specific objectives:

- 1) Determine what level of pulsed flushing magnitude that was tested (if any) was capable of circumventing, mitigating, or terminating these lethal blooms.
- 2) Analyze multiple response variables, including abiotic parameters, inorganic nutrient levels, and phytoplankton and subsequent zooplankton composition to determine if flushing treatments had any negative effect on water quality or food web structure.
- 3) Identify the acting mechanisms responsible for bloom mitigation under pre-bloom and bloom development conditions.

CHAPTER II

DEEP WATER MIXING PREVENTS HARMFUL ALGAL BLOOM FORMATION: IMPLICATIONS FOR MANAGED FISHERIES

INTRODUCTION

Disturbances can threaten ecosystem form and function, such as toxic harmful algal blooms that cause high mortality rates among aquatic species. To offset such deleterious disturbances, management techniques can be employed to create refuge areas for populations negatively impacted. Refuge habitat can buffer the effect of deleterious disturbances, increase species resilience, and lower population losses (Sedell et al. 1990; Schlosser and Angermeier 1995; Magoulick and Kobza 2003). The importance of refuge habitat has been emphasized in conservation studies of many endangered animals (Barton and Roth 2007; Chacon-Chaverri and Eckert 2007; Gannon et al. 2007), and is believed essential for maintaining some populations of natural living resources, such as commercial fish (Tuck and Possingham 2000; Baird and Flaherty 2005; Relini et al. 2008).

A variety of refugia have been implemented in marine systems. Practical management techniques include marine protected areas (Quinn et al. 1993; Roberts 1997; Allison et al. 1998; Tuck and Possingham 2000), preserved nursery habitats (Roberts 1997), and “man-made” refugia (Relini et al. 2008). For example, mangrove conservation efforts protected nursery habitat and resulted in improved juvenile fish survivorship, increasing population sustainability (Manson et al. 2005). In other parts of

the world, such as Laos along the Mekong River, an effort has been made to identify vital areas to support fish population health and implement “fish conservation zones” or no take areas, these areas are critical to local economies that are heavily dependent on small scale fisheries (Baird and Flaherty 2005). Created habitat, such as offshore artificial reefs, provide valuable nursery areas for commercial species and have led to successful increases in overall fish yield and diversity (Relini et al. 2008).

However, similar management strategies for inland water bodies are less developed (Hedges et al. 2010). Advancing our knowledge is paramount when considering lake systems, as they are particularly vulnerable to deleterious disturbances (Freeman and Freeman 1985; Magoulick and Kobza 2003). The presence of refuge habitat may be a requirement for resilience of some populations that otherwise might become extirpated (Sedell et al. 1990; Magoulick and Kobza 2003).

There is an increasing need for active fisheries management and creation of refugia in recent decades. Global climate change and increasing water sequestration due to human population growth may have a resounding impact on water availability, inflow events, and nutrient loading (Roelke et al. 2011). Climate change impacts are projected to make aquatic species more vulnerable to extirpation (Heino et al. 2009), with some regions expected to experience up to 60% reduction in precipitation events (i.e., Texas, USA; Cai and McCarl 2009). Inflows have a direct impact on water quality parameters, such as salinity, turbidity, pH (in response to production: respiration dynamics), and stratification (Mitrovic et al. 2011; Roelke and Pierce 2011; Valenti et al. *In press*), dissolved inorganic nutrients (Sommer et al. 1984; Barbiero et al. 1999; Buyukates and

Roelke 2005; Roelke et al. 2007), and successional changes in lower food web dynamics, such as altered phytoplankton and zooplankton composition (Havens 1991; Havens et al. 2003; Roelke et al. 2004; Miller et al. 2008).

As inflow frequency and magnitude decrease, deterioration of habitat quality with accompanying fish-killing harmful algal blooms (HABs) will likely follow, specifically in water bodies susceptible to *Prymnesium parvum* blooms. This mixotrophic, haptophyte has caused numerous harmful blooms worldwide and in recent years has expanded to southern regions of the USA and as far north as Pennsylvania and West Virginia (Sager et al. 2008; Roelke et al. 2011; Brooks et al. *In press*). *P. parvum* produces toxins that are lethal to fish, causing a change in the selective permeability of gill epithelial cells, thus inhibiting respiration. These devastating blooms have killed over 34 million fish in Texas alone, resulting in a conservative estimate of \$13-million value (Southard et al. 2010). In addition, competing phytoplankton are suppressed through allelopathy by these chemicals, and zooplankton grazing rates are reduced due to lethal and sublethal effects across many taxonomic groups (Granéli and Johansson 2003b; Barreiro et al. 2005; Brooks et al. 2010).

Increased nutrient availability tends to be associated with inflow events (Sommer 1989; Roelke 2000; Miller et al. 2008). Particularly in developed landscapes, inflows carry an increased level of nutrients due to agriculture and urbanization (Schindler 2006). In addition, human water demands have led to longer residence time in most lake systems (Rabalais et al. 2010), which sometimes allow undesirable environmental conditions to arise, such as HABs.

Nutrients can become depleted when hydraulic residence times are long as well. In the absence of inflow events, vertical mixing can enable phytoplankton to utilize nutrients already present in a system that were below the photic zone and previously inaccessible (Jager et al. 2010). This could offer a potential nutrient management technique in arid climates, which are vulnerable to drought conditions.

Under high nutrient availability conditions, phytoplankton are commonly able to take up more nutrients than are immediately necessary for growth, resulting in higher nutritional value of phytoplankton to grazers, subsequently elevating secondary productivity (Roelke 2000, Buyukates and Roelke 2005). However, when inflows are stifled, nutrients can become depleted and the nutritional value of phytoplankton diminished. Under suboptimal conditions such as these, *P. parvum* increases its rate of toxin production enabling bloom formation and persistence. Conversely, bloom formation and onset of toxicity may be interrupted by nutrient additions (Barkoh et al. 2003; Grover et al. 2007; Kurten et al. 2007; Roelke et al. 2007, 2010; Errera et al. 2008; Baker et al. 2009).

Inflow events can also lead to the displacement of cells through hydraulic flushing. *P. parvum* has a low maximum specific growth rate during the time of year when it forms blooms, considerably lower than competing phytoplankton species, such as chlorophytes and diatoms (Tilman et al. 1982; Grover 1989; Grover et al. 1999; Baker et al. 2009). Consequently, it is vulnerable to flushing losses. And without the competitive edge gained through production of allelopathic and grazer-inhibiting chemicals because of nutrient loading, other phytoplankton groups are able to

outcompete *P. parvum* (Roelke et al. 2007, Errera et al. 2008). Indeed, bloom-terminating inflow events have been observed in multiple lake systems (Roelke et al. 2010, 2011; Schwierzke-Wade et al. 2011) and even in streams with low water dams (Brooks et al. *In press*).

Managing flows may offer an ecologically benign approach for mitigation of *P. parvum* blooms, particularly in cove areas of lakes where the system is more confined and controllable. The shallow waters of coves are known habitat for juvenile and small fish and are important refuge areas because they offer an escape from large piscivores (Schlosser 1987; Ryer et al. 2010). The deeper areas of coves might also provide an environment where larger fish could escape from blooms occurring in open areas of the lake. If fish populations can be protected in coves during bloom periods by preventing the bloom from occurring in the coves, then lake-wide recovery of populations would be accelerated after blooms have subsided.

In this research, we tested the hypothesis that blooms can be prevented through managed hydrology. To achieve this objective, we performed in-lake mesocosm experiments on natural plankton assemblages during a pre-bloom period, and a period when a bloom was developing. Mesocosms served as an important intermediate research experiment, where small portions of the cove were isolated. This enabled us to test the effect of flushing in an experimental area and substantiate whether toxicity and cell proliferation could be mitigated. Experimental results furthered our understanding of how managed flushing could potentially reduce the harmful effect of blooms. We used deep water from the lake, free of *P. parvum* cells, to displace surface waters,

thereby simulating inflow events. This approach to bloom management is particularly appealing in watersheds of arid landscapes where access to source water outside the lake is limited.

METHODS

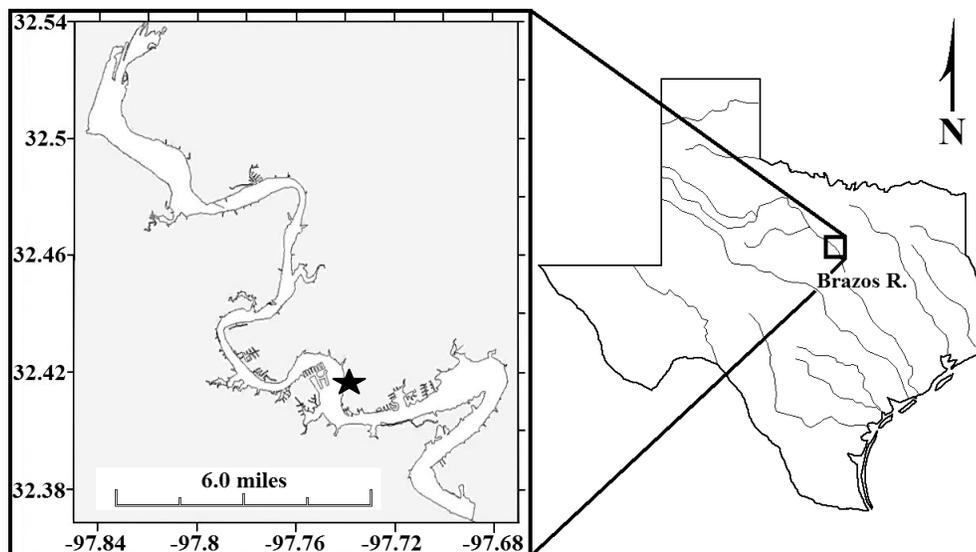
Lake Granbury, a subtropical lake along the Brazos River in Texas (USA), served as the study site for this experiment (Figure 1). Built in 1969, this narrow lake is sinuous, following the historical meandering river channel; averaging 0.6-km wide, ~5-m deep, and ~45-km long. The total surface area is 34-km² and storage capacity is 188×10^6 m³.

Two in-lake experiments were performed in mesocosms placed in a cove area with cove waters (natural assemblages) being used to initiate the experiments. The first experiment was timed to coincide with pre-bloom conditions during the winter (initiated 24 February 2010) and the second experiment was timed to coincide with bloom development (initiated 31 March 2010). Each experiment ran for three weeks with sampling conducted every seven days. Experiments were performed in 18 cylindrical mesocosms made from impermeable, translucent material, with a closed bottom (Aquatic Research Instruments©). The dimensions were 1-m diameter, 2-m depth, with a volume of 1.57 m³. Each mesocosm was filled with surface lake water from the cove and suspended from floating frames that prevented waves from breaking into the experimental enclosures while remaining uncovered to allow for natural solar irradiation and gas exchange.

The treatments were conducted in triplicate and included three levels of pulsed flushing with magnitudes of 5, 10, and 30% water displacement every seventh day. For a control, nine mesocosms were used that received no water displacement. For the pulsed flushing treatments, water used for the source was obtained from a depth of seven meters from a location in the open area of the lake adjacent to the cove. Since Lake Granbury is an unstratified system during this time of year, there was no large temperature difference between cove conditions and deeper waters. Compared to mesocosm water, deeper source water had very low *P. parvum* density (most samples during the pre-bloom experiment resulted in zero cells L⁻¹ observed), no ambient toxicity and similar dissolved inorganic nutrient concentrations compared to surface waters.

The experimental units were sampled every 7th day along with source and cove waters. Initial samples were taken after the first water exchange; representing the beginning of the experiment (i.e., T0). For each of the following weeks (T7, T14 and T21) samplings were collected prior to water exchange.

Figure 1: Study site—Lake Granbury, Texas (U.S.A.). A riverine, subtropical reservoir formed within the Brazos River system. A black star indicates the test cove.



Cell density of *P. parvum* was enumerated for each mesocosm from a 100-mL water sample collected at 0.5-m depth (glutaraldehyde was used to preserve cells; 5% v/v). The sample was well mixed and a 1-mL subsample was extracted with a pipette and settled for 24-h. Settling procedure followed Utermöhl (1958). *Prymnesium parvum* were counted in ~20 randomly selected fields of view at 400x using an inverted, phase-contrast light microscope (Leica Microsystem), and this technique yielded approximately 200 cells per sample.

Phytoplankton biomass was assessed through chlorophyll-*a* (chl-*a*) samples. Triplicate 50-mL samples were collected from 0.5-m lake depth and filtered through Whatman 25-mm GF/F filters. Fluorometer methodology was used for analysis (Strickland and Parsons 1968). Taxonomic groups of phytoplankton were determined through phytopigment concentration measurements as described by Pinckney et al.

(1998). Phytopigments were filtered through Whatman GF/F filters (25-mm) and sonicated in 3-mL of 100% acetone for 30-s, then extracted in the dark for ~24-h at -20°C. Extracts were filtered through Nucleopore 0.2- μ m and injected with 300- μ l into an HPLC system equipped with reverse-phase C₁₈ columns in series (Rainin Microsorb-MV, 0.46- 10-cm, 3-mm, Vydac 201 TP, 0.46- 25-cm, 5-mm). A nonlinear binary gradient, adapted from Van Heukelem et al. (1994), was followed for pigment separation procedures. Solvent A was composed of 80% methanol and 20% ammonium acetate (0.5-M, adjusted to pH 7.2). Solvent B consisted of 80% methanol and 20% acetone. Measured phytopigment concentrations were employed to estimate biomasses of higher taxonomic phytoplankton groups through CHEMTAX, a matrix factorization program (Mackey et al. 1996; Wright et al. 1996). Aggregated assemblage composition accounted for included: cyanobacteria, euglenophytes, chlorophytes, haptophytes, cryptophytes, diatoms, and dinoflagellates, which were selected due to their historical prevalence in Lake Granbury during the winter and spring seasons.

Zooplankton samples were collected using a 12-L Schindler trap, which was concentrated to 50 mL. Samples were preserved in 2% buffered formalin. Subsamples of ~9–12-mL aliquots were extracted and settled for 24-h. Slides were counted at 40x and 200x to account for the size difference between taxonomic groups. Enumerations were performed using an inverted, phase-contrast microscope (Leica Microsystem). This technique resulted in approximately 200 individuals per slide being counted and measured (length and width). Biovolume was calculated through determining the corresponding geometric shape of each zooplankton genera and measuring dimensions

of individuals as described in Wetzel and Likens (1991). Density and biovolume were calculated for each of the following aggregated genera categories: cladocerans, adult copepods, copepod nauplii, rotifers, and protozoa.

Abiotic water quality parameters were collected, such as temperature, salinity, Secchi depth, and pH. A Hydrolab Quanta multiprobe was used to record temperature, salinity and pH at 0.5 and 7-m depths. Light transparency was determined by estimating Secchi depth.

Chemical variables including inorganic nutrients and total phosphorus were collected and filtered through Whatman GF/F filters (pore size $\sim 0.7\text{-}\mu\text{m}$). Samples were immediately frozen, transported to the laboratory, then analyzed using an autoanalyzer (OI Analytical, Flow Solutions IV) method described by Armstrong and Sterns (1967) and Harwood and Kuhn (1970). Results included levels of nitrate and nitrite (NO_x), ammonia (NH_4), and orthophosphates (PO_4). For some of our analysis, we refer to dissolved inorganic nitrogen (DIN), which is the sum of NO_x and NH_4 .

Ambient water toxicity was determined by estimating acute toxicity to juvenile fathead minnows (*Pimephales promelas*). Standardized 24-h static toxicity assays were performed according to the United States Environmental Protection Agency recommendations for determining ambient toxicity of surface waters (US EPA 2002). Samples were collected from each experiment unit ($\sim 1200\text{-mL}$), as well as a lake sample from the cove, and transported to the laboratory where tests were initiated within 24-h. Samples were diluted following a 0.5 dilution series with reconstituted hard water (RHW) (APHA 1998); RHW functioned as the control treatment water in all toxicity

assays. Alkalinity (mg l^{-1} as CaCO_3) and hardness (mg l^{-1} as CaCO_3) of RHW were measured potentiometrically and by colorimetric titration, respectively, prior to initiation of acute studies (APHA et al. 1998). Dissolved oxygen (mg l^{-1}), conductance ($\mu\text{S cm}^{-1}$), and pH of RHW were assessed prior to initiation of toxicity tests with common, calibrated meters and probes. *Pimephales promelas* cultures and all subsequent studies were performed within climate controlled chambers ($25 \pm 1^\circ\text{C}$), with a 16:8-h light:dark cycle. Larvae (<48-h old) were used and fed newly hatched *Artemia* nauplii 2-h prior to the initiation of toxicity assays (US EPA 2002). LC_{50} values for fathead minnow toxicity tests were estimated at percentage of ambient sample using Probit (Finney 1971) or Trimmed Spearman Karber (Hamilton et al. 1977) methodology, depending on which technique was appropriate for corresponding data.

Statistical Analysis

Repeated-measures general linear models (post-hoc Tukey HSD tests) were used to test for differences in response variables among treatment magnitudes and the control (GLM©, SPSS©). A one-way ANOVA (non-repeated measures) was used to analyze acute lethal toxicity to fish in the first experiment (pre-bloom) because samples only had detectable toxic activity on one occasion (T21). A repeated measure ANOVA was used to analyze acute fish toxicity in the second experiment (bloom development), since toxic activity was observed throughout the experiment. Results from 9 control replicates were used in statistical analysis of response variable, with the exception of acute fish toxicity, which had 3 control replicates. Data was additionally analyzed using concentration

change calculations and statistical significance was evaluated using repeated-measures ANOVA (see Appendix A). Results were considered significant if $P \leq 0.05$.

Exploration of multivariate relationships among response variables was achieved through nonmetric multidimensional scaling (NMS; PC-ORD Version 5.1; McCune and Mefford 1999). NMS is an ordination method well suited for non-normally distributed data sets and does not assume linear relationships among variables, making it an ideal statistical technique for the diverse responses measured in this study. A final solution was achieved based on the lowest stress obtained using a Monte-Carlo test after 100 runs in a cascade procedure using a stability criterion of 0.0001 (McCune and Grace 2002).

RESULTS

Cove Conditions

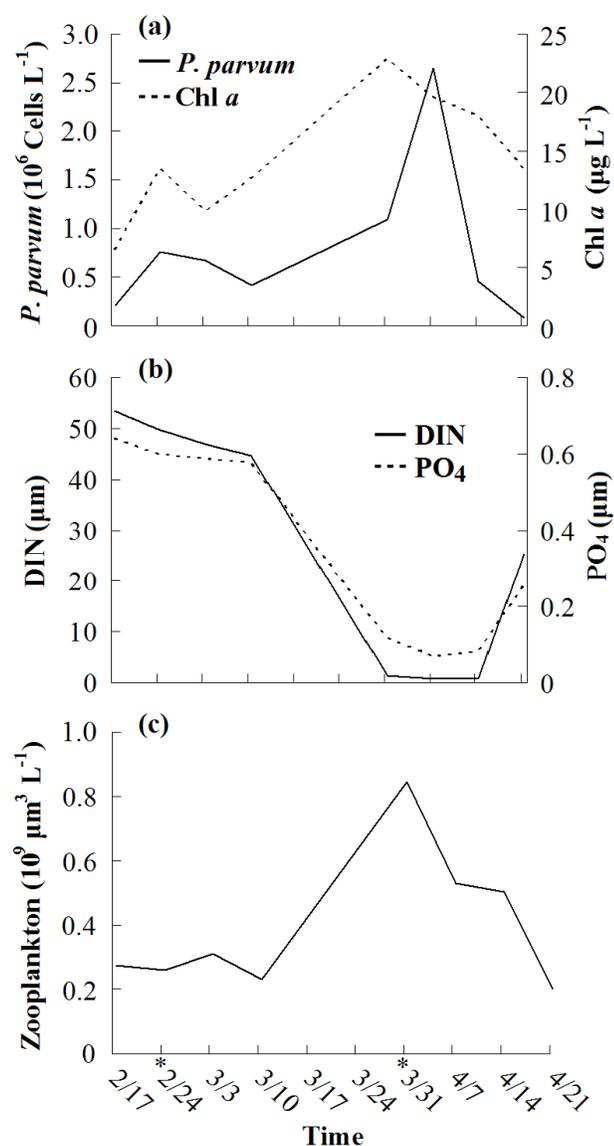
In our selected cove, a week before the initiation of our first experiment (17 February 2010), *P. parvum* cell density was 0.21×10^6 cells L^{-1} (Figure 2a) and acute lethal toxicity to fish was not detected (data not shown). The following week, when the first experiment was initiated, *P. parvum* had tripled to 0.76×10^6 cells L^{-1} and waters were still not detectably toxic to fish. Approximately one month later, *P. parvum* population reached 2.65×10^6 cells L^{-1} and waters were acutely toxic to fish (LC_{50} was 77% dilution of ambient waters). From these observations, it was apparent that a *P. parvum* bloom was imminent at the start of our first experiment. So, we refer to our first experiment as “pre-bloom” conditions. It is also apparent from these observations that

our second experiment was initiated just prior to the observed *P. parvum* population maxima and onset of acute toxicity to fish. So, we now refer to our second experiment as “bloom development” conditions.

Increased *P. parvum* density coincided with haptophyte dominance as estimated from phytopigment analysis, which is expected because *P. parvum* is the only representative of the haptophyte phylum in this region. Before the pre-bloom experiment, haptophytes dominated the phytoplankton, comprising 3.83 of the total 6.53 $\mu\text{g chl-}a\text{ L}^{-1}$. Haptophyte dominance continued through 7 April 2010, when it reached its maxima of 15.77 of the total 22.90 $\mu\text{g chl-}a\text{ L}^{-1}$. After this, haptophytes decreased abruptly, falling to 4.51 of the total 10.03 $\mu\text{g chl-}a\text{ L}^{-1}$.

Dissolved inorganic nitrogen (DIN) and PO_4 levels were highest before and during the initiation of the pre-bloom experiment, where DIN was 53.6 μM and PO_4 was 0.64 μM (Figure 2b). As the *P. parvum* population developed in the cove, nutrients were depleted. At the start of the bloom development experiment, they were 1.3 μM for DIN and 0.12 μM for PO_4 . The dissolved N:P ratio changed from 84:1 to 11:1 between the initiation of the pre-bloom and bloom development experiments.

Figure 2: Cove conditions throughout 17 February 2010 to 21 April 2010. This represents well-mixed lake conditions leading to initiation of isolated mesocosm experiments under “pre-bloom” and “bloom development” phases. *Prymnesium parvum* density and chl-*a* levels (a); dissolved inorganic nitrogen (DIN) and phosphate levels (b); and total zooplankton biovolume (c) in Lake Granbury, Texas. The asterisks on the x-axis denote the beginning (T0) of pre-bloom and bloom development experiments.



Zooplankton total biovolume increased along with *P. parvum* population density and total phytoplankton biomass, reaching a peak of $0.85 \times 10^9 \mu\text{m}^3 \text{L}^{-1}$ on 31 March, but then decreased to $0.53 \times 10^9 \mu\text{m}^3 \text{L}^{-1}$ as ambient waters became toxic. Zooplankton did not recover during our period of study, decreasing further to $0.20 \times 10^9 \mu\text{m}^3 \text{L}^{-1}$ by the conclusion of the bloom development experiment (Figure 2c). Rotifers, copepod adults, and copepod nauplii dominated the cove zooplankton assemblage.

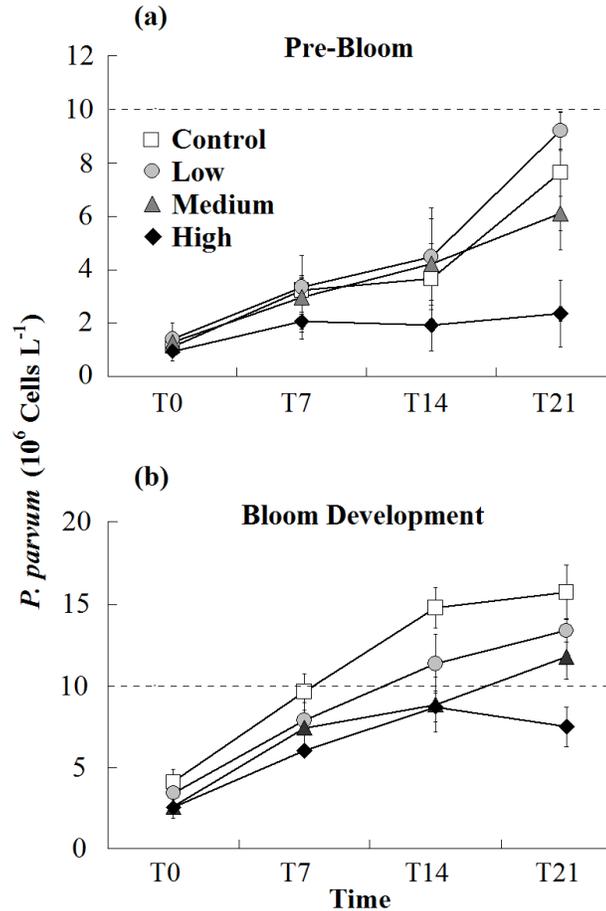
Some abiotic parameters showed large variation during our period of study, while others changed little. For example, temperature followed a predictable seasonal trend, warming from 7.7°C to 18.9°C throughout February and April (data not shown). Light penetration generally inversely varied with phytoplankton biomass, where Secchi depth ranged from 41-53 cm between 17 February and 31 March, then increased to 103-115 cm between 7 April and 21 April (data not shown). Little variation was observed in pH, as it remained between 7.8 and 8.1 (data not shown). Similarly, salinity was low during this period, ranging between 0.3 and 0.7 PSS.

Experimental Results

Generally, flushing slowed the accumulation of *P. parvum* cells, with the magnitude of flushing related to the degree of population suppression. During the pre-bloom experiment, high magnitude flushing resulted in significantly lower *P. parvum* density (P -value <0.001), with 69% fewer cells by experiment completion (Figure 3a). The low and intermediate levels of flushing were not statistically significant from the control. During the bloom development experiment, all flushing levels produced *P.*

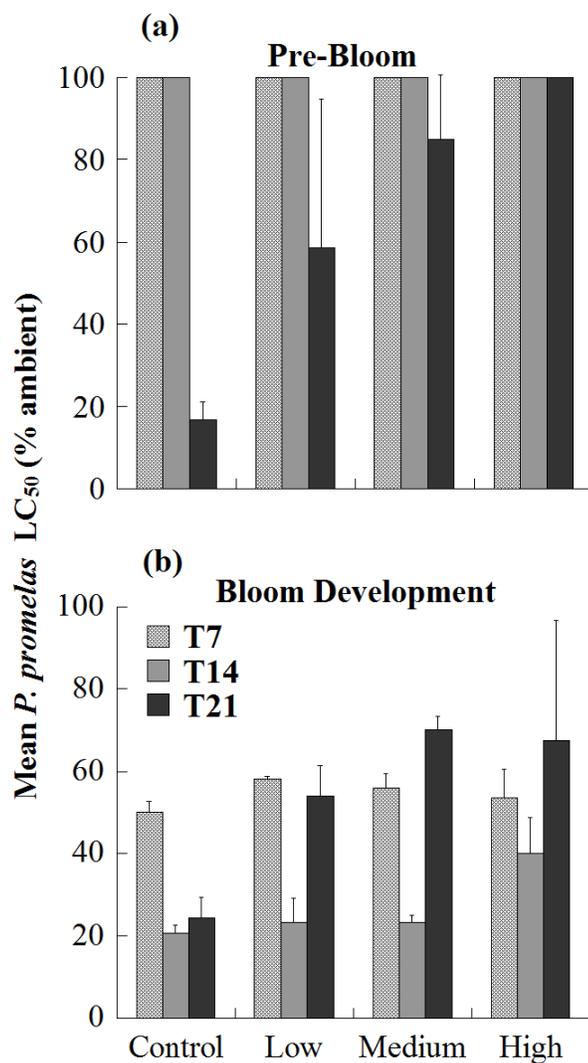
parvum densities significantly lower than the control (P -value ≤ 0.003). Furthermore, the high-level flushing prevented population densities from reaching bloom proportions, with 53% fewer cells by experiment termination (Figure 3b).

Figure 3: *Prymnesium parvum* density during pre-bloom (a) and bloom development (b) at Lake Granbury, Texas during 2010. The treatment levels (low, medium, and high) refer to pulsed flushing magnitudes: low=5%, medium=10%, and high=30% water exchange in test mesocosms. High flushing significantly reduced *P. parvum* density in both experiments (P -value < 0.001) and prevented bloom proportions from being reached during bloom development. Bloom proportions are represented by the dotted line.



Ambient acute toxicity to fish was greatly reduced by medium and high flushing in both experiments. During the pre-bloom experiment, no samples from any treatment were detectably toxic to fish from initiation through T14; however, by the end of the experiment (T21), samples from the control mesocosms were extremely toxic to fish (Figure 4a). Results from our bioassays of acute mortality showed that medium and high flushing treatments were significantly less toxic than controls at T21, with the high flushing treatment not detectably toxic to fish (medium flushing P -value=0.024, high flushing P -value=0.008). During the bloom development experiment, all mesocosms started out with moderate acute toxicity to fish through T7 and within the successive week (T14), control, low flushing, and medium flushing treatments increased in toxicity to fish at a similar rate. However, the high flushing treatment diverged from this trend and exhibited a minimal increase in the magnitude of toxicity to fish from T7 to T14 (Figure 4b). At experiment completion (T21), both the medium and high treatments were significantly less toxic than the control (medium flushing P -value=0.012, high flushing P -value=0.004).

Figure 4: Ambient acute toxicity to *Pimephales promelas* measured as LC₅₀ during pre-bloom (a) and bloom development (b) experiments. Acute toxicity was significantly reduced at T21 by medium and high flushing during both experiments, and toxic conditions were never reached in high flushing mesocosms during the pre-bloom experiment. 100% LC₅₀ bars signify non-toxic conditions.



Inorganic nutrients decreased during the pre-bloom experiment (Figures 5a, 6a).

Nitrogen levels started out ranging 32.6-46.8 μM , then decreased by T14 to 1.2-1.7 μM and remained depleted. All flushing treatments maintained significantly higher levels of

DIN (P -value <0.001), due to replenishment of nutrients by source water. The greatest difference in concentration between the control and treatment mesocosms was observed at T7, when low, medium, and high flushing maintained 27, 32, and 43% higher DIN levels (Figure 5a). PO_4 levels were not significantly affected by any treatment (Figure 6a), and probably represent the limiting nutrient during pre-bloom conditions, where dissolved N:P was 83:1 at experiment initiation. However, by the conclusion of the experiment N:P ratios decreased to a range of 11:1 to 16:1. Declining nitrogen levels coincided with phytoplankton biomass increases, which reached maximal levels at T7 ($28\text{-}32\ \mu\text{g L}^{-1}$), then decreased to $19\text{-}22\ \mu\text{g L}^{-1}$ at T21 following nutrient depletion (Figure 7a). There was no significant effect of treatments on chl-*a* levels throughout the pre-bloom experiment.

A different trend for inorganic nutrients was observed for the bloom development experiment. DIN and PO_4 were both low at experiment initiation (Figure 5b, 6b). These nutrient levels remained low and relatively consistent throughout the experiment.

Figure 5: Dissolved inorganic nitrogen (DIN) concentration (comprised of NO_2 , NO_3 , and NH_4) during pre-bloom (a) and bloom development (b) experiments. All flushing levels increased DIN compared to the control during the pre-bloom experiment. Overall levels decrease from T0-T14 of the pre-bloom experiment and remained low to T21 of bloom development.

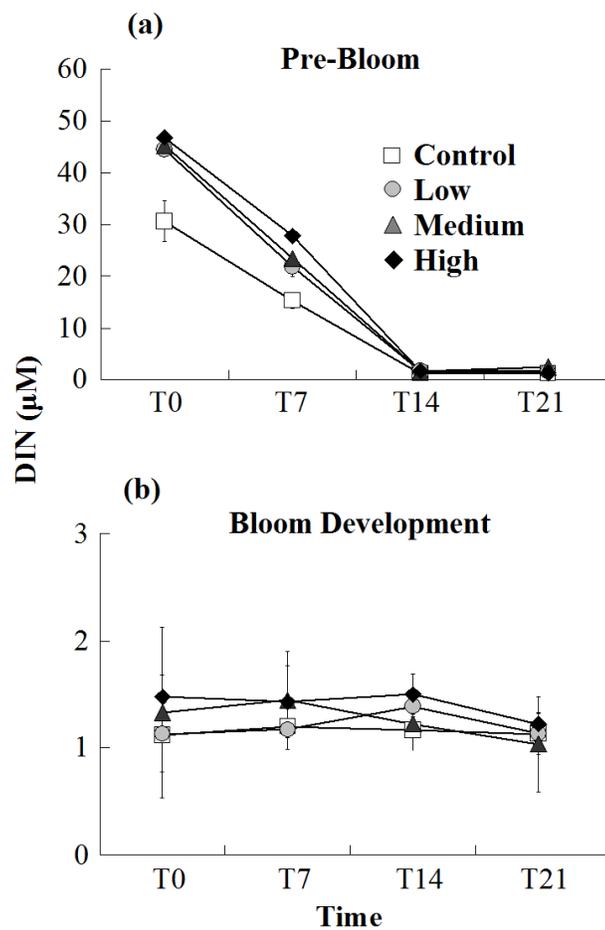
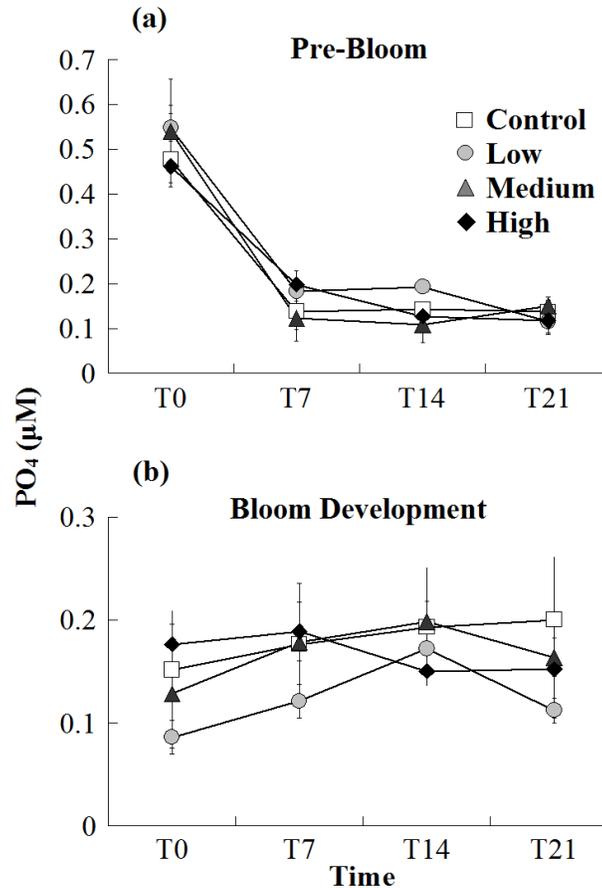


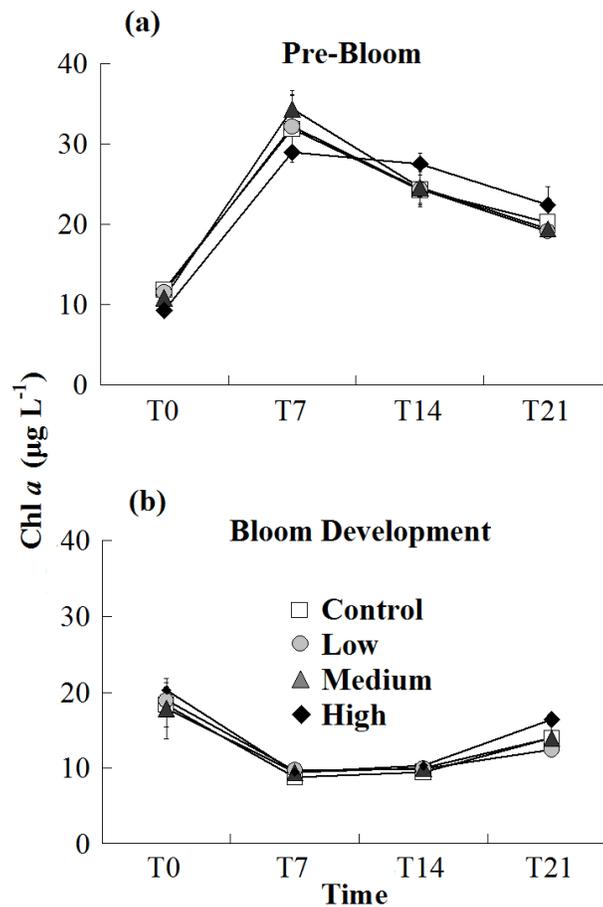
Figure 6: Soluble reactive phosphorus (SRP) concentration (as PO_4) for pre-bloom (a) and bloom development (b) experiments. SRP seemed to have a minimal effect from flushing treatments.



Total phytoplankton biomass (as chl-*a*) decreased as ambient water toxicity increased during the bloom development experiment (Figure 7b). Levels were highest at the beginning of the experiment (moderate toxicity), and then decreased to T14 (increased toxicity). A modest recovery was observed by T21 as toxicity lessened, particularly in high flushing mesocosm, which had significantly higher chl-*a* levels (*P*-

value=0.035). There was no significant effect of low or medium flushing treatments on chl-*a* levels throughout the bloom development experiment.

Figure 7: Total phytoplankton biomass estimated with chlorophyll *a* (chl-*a*) during pre-bloom (a) and bloom development (b) experiments. Flushing treatments had a benign effect on chl-*a* levels, which were inversely proportional to ambient toxicity.

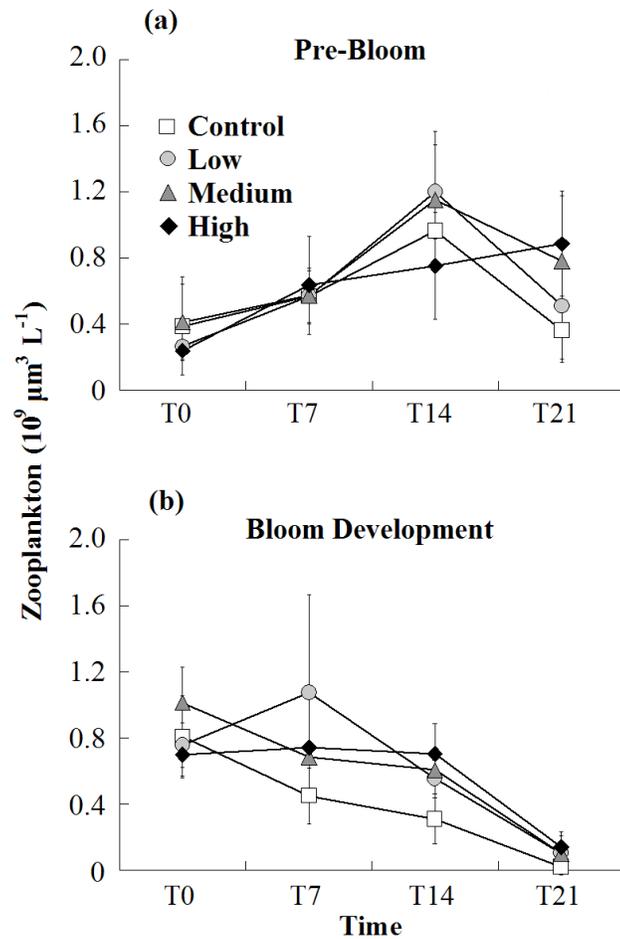


In both experiments, there were flushing effects on phytoplankton assemblage composition. In the pre-bloom experiment, significant effects were observed in high and medium flushing treatments, which included higher levels of chlorophytes (P –

value<0.001) and prasinophytes (P -value=0.010), and lower levels of cyanobacteria (P -value=0.005), dinoflagellates (P -value=0.037), and diatoms (P -value=0.007; see Appendix B). Medium flushing only produced a significant increase in chlorophytes (P -value=0.001) and low flushing treatments produced no detectable change from controls. During the bloom development experiment, significantly higher chlorophytes (P -value=0.001), prasinophytes (P -value=0.038), cryptophytes (P -value=0.003), and diatoms (P -value=0.010) were observed with the high flushing level (see Appendix B). Dinoflagellates were significantly lower at all levels of flushing (high P -value=0.001; medium P -value=0.013; low P -value=0.016). There was no flushing effect on cyanobacteria during the bloom development experiment.

Zooplankton biovolume was initially low for the pre-bloom experiment and peaked in most treatments by T14 following an increase in phytoplankton biomass. Zooplankton then decreased along with nutrients and phytoplankton by T21 (Figure 8a). The exception was the high flushing treatments. In those, zooplankton progressively increased for the duration of the experiment. By the experiment's conclusion, the high flushing treatments had the highest densities of zooplankton (which were also the least acutely toxic to fish) and the controls had the lowest zooplankton densities (which were the most acutely toxic to fish). In our repeated measures analysis, however, this trend was not statistically significant. Zooplankton community composition was comprised mostly of rotifers, copepod adults, and nauplii. Community shifts in zooplankton caused by flushing were not apparent, as statistically significant differences for copepod adults and nauplii, cladocerans, rotifers and protozoa were not observed between treatments.

Figure 8: Total zooplankton biovolume during pre-bloom (a) and bloom development (b) experiments. Flushing treatments did not alter zooplankton levels.



Zooplankton biovolume decreased throughout the bloom development experiment (Figure 8b). Flushing treatments had a slower rate of decline during times of reduced toxicity to fish (T14 and T21), but this observation was not statistically significant. Again, zooplankton community composition was comprised mostly of rotifers, copepod adults, and nauplii. A community shift in zooplankton caused by

flushing was apparent, as copepod nauplii were significantly increased by medium and high flushing treatments (P -value <0.001 ; see Appendix B).

Analysis of abiotic water quality parameters showed little effect of flushing. The exceptions were that during the pre-bloom experiment, salinity was significantly lower in medium and high flushing versus the control, with values of 0.39 and 0.35 PSS for medium and high flushing compared to 0.43 PSS by experiment conclusion (medium flushing P -value=0.035; high flushing P -value <0.001 ; see Appendix B). Additionally, light penetration was significantly lower resulting from high flushing with a Secchi depth of 60.3 compared to 68.7 cm (P -value <0.001 ; see Appendix B). Temperature and pH showed no deviation from the control. There were no statistically significant differences observed in abiotic water quality parameters throughout the bloom development experiment.

Our NMS analysis generally supported these observations, where the high-flushing treatment plotted opposite *P. parvum* and toxicity for the pre-bloom experiment (Figure 9a), and to a lesser degree during the bloom development experiment (Figure 9b). Cyanobacteria and dinoflagellates were separated in ordination space from our high flushing observations (Figure 9a, b). Additionally, rotifer density was strongly correlated to flushing treatments, and negatively correlated to *P. parvum* density and toxicity in both experiments (Figure 9a, b).

Figure 9: Nonmetric multidimensional scaling (NMS) results for flushing treatments compared to the control in pre-bloom (a) and bloom development (b); along with sampling time during pre-bloom (c) and bloom development (d) experiments.

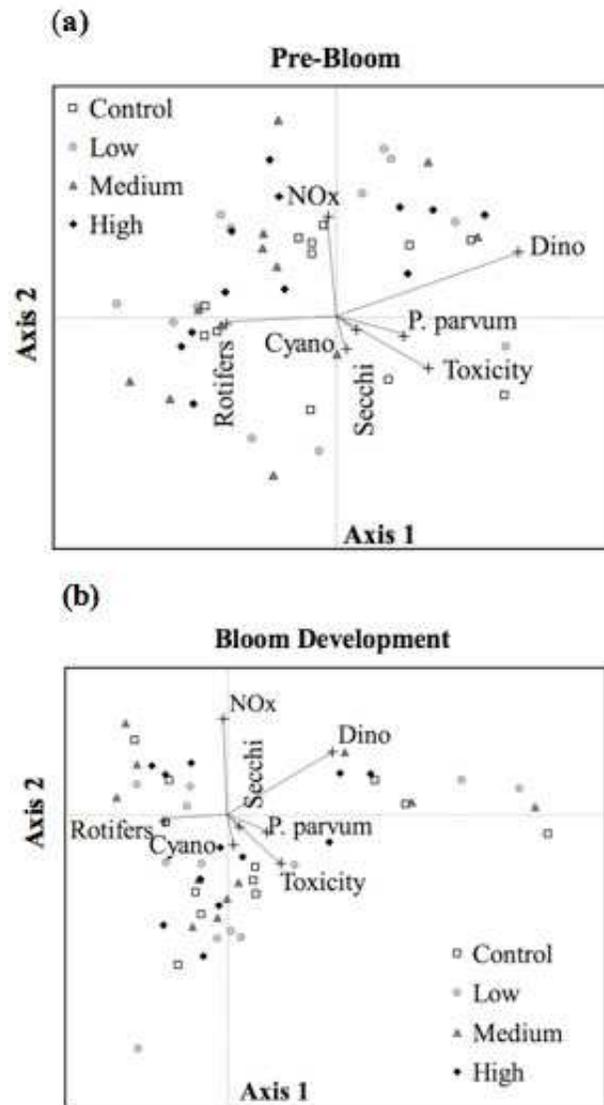
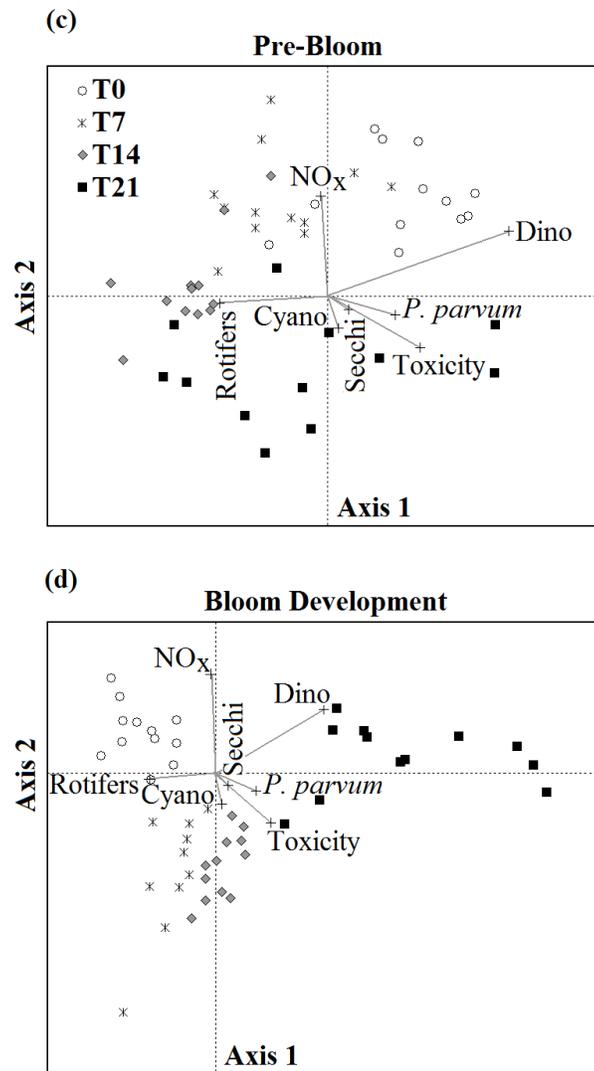


Figure 9 continued.



NMS analysis between sampling times illustrated a strong temporal separation of response variables. During pre-bloom conditions, T0 and T7 were most closely associated with NO_x and negatively correlated with *P. parvum* and toxicity, particularly flushing treatments (Figure 9 a, c). Rotifer biovolume was associated with T14 samples, and controls at T21 were correlated most strongly to Secchi depth, ambient toxicity and

P. parvum density (Figure 9a, c). During bloom development, T0 and T7 associated most closely with rotifer biovolume, T14 with toxicity, and T21 with *P. parvum* and dinoflagellate concentration (Figure 9d).

DISCUSSION

In this research, we set out to test the notion that in-lake source waters from deeper depths could be used to flush surface waters, thereby circumventing a *P. parvum* bloom, or reducing the impact of a developing bloom. Results from both our experiments support this idea, showing that reduction in *P. parvum* density and ambient toxicity to fish were strongly related to flushing magnitude. Furthermore, there was no significant effect of flushing on phytoplankton biomass, with the exception of reduced toxicity by high flushing during bloom development, no lasting change in biovolume or community composition of zooplankton, and little effect on water quality parameters. In other words, while flushing mitigated *P. parvum* bloom initiation and development, it was benign to other aspects of the lower food web and environment. This observation, combined with not having to rely on a source of water external to the system, makes flushing an appealing management approach. An explanation of the mechanisms likely underpinning our experimental observations follows.

In the pre-bloom experiment, DIN levels decreased with phytoplankton growth, but were significantly higher at all flushing levels. It is likely that nitrogen was replenished with the displacement of surface waters with deeper waters. It is also likely that phosphorus was replenished with the deep water flushing, but elevated

concentrations were not detectable, as phosphorus appeared to be the nutrient more limiting to growth. In other words, it was used as soon as it was added. This nutrient addition with deep water flushing would have led to less stressful conditions for *P. parvum*, an environmental condition that would reduce toxicity (Johansson and Granéli 1999; Baker et al. 2009), thereby diminishing its competitive advantage. *P. parvum* bloom initiation may be interrupted by alleviating nutrient limitation, as suggested previously (Barkoh et al. 2003; Grover et al. 2007; Kurten et al. 2007; Roelke et al. 2007, 2010).

This same mechanism likely influenced the flushing treatments during the bloom development experiment. In this experiment, initial nutrients were lower than the previous experiment, and appeared to be more limited by nitrogen (dissolved N:P = 11:1). It is likely that elevated nitrogen concentrations were not detectable with deep-water additions because they were quickly used by phytoplankton during the seven-day period between flushing treatments and sampling (refer to methods). Similar experiments have shown increased phytoplankton production in response to nutrient enrichment within one to five days and by the fifth day dissolved N and P concentrations were reduced (Järvinen and Salonen 1998). It is also likely that elevated phosphorus concentrations were not detectable with deep-water additions because they were quickly sequestered through the process of luxury consumption (Davies and Morales 1998; Schelske et al. 1999; Revilla and Weissing 2008).

Inflow events causing displacement of phytoplankton cells can change assemblage composition and subsequent succession (Roelke 2000, Roelke et al. 2010).

In addition, dilution of existing *P. parvum* cells and toxin compounds in a system has been linked with bloom termination, even in the absence of system outflow (Schwierzke-Wade et al. 2011). The pulsed flushing treatments in both experiments involved a percentage of water be removed from mesocosm every seven days. In other words, a percentage of *P. parvum* cells and existing toxin compounds were also removed during each treatment. The source water that refilled mesocosm had no toxin compounds and extremely low levels of *P. parvum* cells L⁻¹ (a photophilic and flagellated organism); therefore, providing a dilution effect on toxicity and reducing the existing number of cells capable of producing more toxins. These factors increased the survivorship of competing phytoplankton, and immigration rates of other phytoplankton taxa were higher in source water compared to *P. parvum* density. Furthermore, pulsed flushing created disturbances, which selected for taxonomic groups with higher reproductive growth rates because they can recover more quickly (Buyukates and Roelke 2005; Miller et al. 2008). *P. parvum* has a low reproductive growth rate during wintertime (Grover et al. 2007, Baker et al. 2007, 2009), while competing phytoplankton groups, such as chlorophytes, have a higher specific growth rate (Tilman et al. 1982; Grover 1989; Grover et al. 1999; Roelke et al. 2007; Baker et al. 2009). Therefore, biomass was expected to increase for competing phytoplankton groups while *P. parvum* declined. Additionally, in the absence of high toxic activity some phytoplankton groups (i.e., chlorophytes and euglenophytes) have been able to outperform *P. parvum*, with succession patterns leading to their dominance (Roelke et al. 2007).

Following the trend observed in these previous studies, results from this study showed significantly different phytoplankton assemblages emerged as a result of flushing. During the pre-bloom experiment at the high level of flushing, *P. parvum* was outcompeted by chlorophytes and prasinophytes, with cyanobacteria, dinoflagellates, and diatoms performing poorly. During the bloom development experiment at the medium and high levels of flushing, *P. parvum* was outcompeted by chlorophytes, prasinophytes, cryptophytes and diatoms, with dinoflagellates performing poorly.

Zooplankton grazing by copepod nauplii may have been enhanced because of flushing, at least for the bloom development experiment. While zooplankton populations were decreasing in all treatments during this experiment, in the flushing treatments they were decreasing to a lesser degree. This resulted either because of higher survivorship with reduced toxicity or perhaps elevated immigration with the addition of deep waters. Whereas toxins produced by *P. parvum* are inhibitory to many zooplankton taxa, some appear able to feed on *P. parvum* while it is producing toxins (Schwierzke-Wade et al. 2010). It may be that the copepod nauplii observed here were resistant to *P. parvum* toxins to some degree, and that their grazing aided in further reducing the *P. parvum* and water toxicity under pulsed flushing conditions.

Reduced inflows to aquatic systems with climate change and human population growth may prove crippling to environmental integrity, as suggested for the southcentral region of the USA (Roelke et al. 2011), and other areas predicted to become drier in the near future that are plagued by HABs (Webster et al. 1995; Bormans et al. 1997; Sherman et al. 1998; Liu and Buskey 2000; Oliver et al. 2000; Buskey et al. 2001;

Mitrovic et al. 2003, 2011). There is a need for management strategies to address this threat, and our findings suggest an approach. Manipulating inflows to aquatic systems may not always be practical because of the associated cost of infrastructure and stakeholders competing for water rights. Manipulating waters already present in a system, however, may be feasible. Displacement of surface waters with deeper water can be controlled, producing effects on plankton communities similar to a natural inflow event (Hudnell et al. 2010). The flushing magnitude most effective in reducing toxicity and *P. parvum* cell proliferation was a 30% exchange in mesocosm; therefore, proportions similar to 30% of surface water would be recommended for testing cove water displacement with deeper water. Regarding our study region, pulsed flushing of surface waters could be performed at times of the year when blooms are known to develop, i.e., during late winter/early spring (Roelke et al. 2011), and areas to be manipulated could target smaller confined water bodies, such as shallow coves, which were previously identified as sources for fish populations (Schlosser 1987; Ryer et al. 2010). The benefits of such an innovation would be the creation of refuge habitat for living natural resources, such as fisheries, which would accelerate their recovery after bloom events subside.

The results from this experiment offer a better understanding of potential fisheries management strategies in response to the increase of HABs (i.e., toxic *P. parvum* blooms) in lakes. Creating refugia would provide metapopulations with areas of source habitat, and have been suggested as a valuable fisheries management tool in freshwater (Quinn et al. 1993; Roberts 1997; Allison et al. 1998; Tuck and Possingham

2000) and marine environments (Sedell et al. 1990; Schlosser and Angermeier 1995; Magoulick and Kobza 2003), and is particularly important for sustaining harvested species (Tuck and Possingham 2000). Oftentimes, it is not possible to completely alleviate the consequences of deleterious disturbances, such as HABs. But management efforts practiced at a smaller-scale, such as discussed here, might enable better mitigation of these disturbances. This approach may be applicable to other systems as well, where the incidences of HABs are linked to inflow events.

CHAPTER III

CONCLUSION: SIGNIFICANCE OF RESEARCH

The prevalence of harmful algal blooms (HABs) has been increasing in recent decades throughout the world (Hallegraeff 1993; Sunda et al. 2006; Roelke et al. 2007; Baker et al. 2009). In many systems this has been attributed in part to reduced freshwater inflows (Mitrovic et al. 2011; Roelke et al. 2007, 2011; Schwierzke-Wade et al. 2011), posing increasing uncertainty for the recovery of these systems; and therefore, the implications of this experiment are extremely important. The methods outlined in this study, particularly utilizing water already present in a lake, offers a management technique without further complicating eutrophication and water utilization concerns. The benefits from these innovations could greatly impact the control of invasive and harmful *P. parvum* blooms, which are continuing to spread across North America (Sager et al. 2008; Roelke et al. 2011; Brooks et al. *In press*), and could possibly translate to treatments for other HABs worldwide. Additionally, if successful, these treatments could improve food web sustainability, through promoting biodiversity of phytoplankton (Tilman 1982; Grover 1997; Miller et al. 2008), increasing secondary productivity (Roelke 2000, Buyukates and Roelke 2005), and mitigating the detrimental loss of fish populations. Additionally, this could provide a realistic method to maintain water quality for recreation and other human benefits.

The results from this experiment offer a better understanding of the direct mechanisms involved in controlling pre-bloom and bloom development conditions;

building on previous studies to outline possible techniques for circumventing and terminating blooms within a natural system or selected cove areas. The high flushing treatment outlines a method for producing significant mitigation of blooms and perhaps a way to circumvent toxicity production altogether. The recommendation highlighted by this study involves testing a high level flushing treatment (30% exchange) within cove areas of an inland lake with HABs (i.e., *P. parvum* blooms). This would determine if these experimental results can be replicated in an in-lake demonstration and translate beyond a mesocosm experiment. The simulated pulsed flushing could be contained to times of the year when blooms have been historically present (i.e., during late winter/early spring; Roelke et al. 2011), and controlling conditions in a cove versus a lake-wide system would allow for evaluation of juvenile fish population impacts. These fish refuge zones would ensure overall population sources and continued generations of commercial fish communities, benefiting both local and statewide economies.

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APPENDIX A

CONCENTRATION CHANGE OF RESPONSE VARIABLES

Concentration change of response variables were calculated and compared against the control to account for flushing losses (i.e., induced by physical removal of mesocosm water and subsequent addition of source water). Change in concentration of *P. parvum* cells, inorganic nutrients, chl-*a* levels, phytoplankton composition and density, zooplankton biovolume and density (i.e., total and taxonomic composition), and abiotic water quality parameters were adjusted to account for water exchange experienced during each treatment. Treatments took place every 7 days and occurred at T0, T7, and T14 (see methods). The magnitude of dilution (5, 10, and 30%) and measurements of each of the above parameters collected from flushing source water were used to calculate: 1) reduced concentration due to water being removed 2) increased concentration due to addition of deeper waters 3) the overall change in concentration throughout each week of the experiment (a week response time following each treatment) to allow for time series data analysis, and 4) treatment change compared against the control. Sampling during T7, T14, and T21 (the weeks of observable change) occurred before the successive weeks flushing treatment; therefore, measured data represents the change in mesocosm concentration one week after treatments and not initial concentrations. However, initial concentrations were calculated for the beginning of each week using the previous weeks sampling data. For example, to calculate the

concentrations from the beginning of T14 (which was initiated by performing treatments directly after T7 sampling) for a 10% exchange, the measurements collected during T7 sampling represented 90% of the initial concentration and measurements collected from source water represented 10%. Once beginning values were established, change in concentration over time was determined (Example: T14 end concentration (measured) minus T14 beginning concentration (calculated), and then divided by T14 beginning concentration). This calculation was also applied to control (measured) values and yielded a technique to compare flushing concentration changes against the control throughout the experiment. In addition, once dilution and flushing losses were accounted for, then changes in concentration could be attributed to other possible factors, such as nutrient enrichment, toxin cessation effects, disturbance dynamics, and altered assemblage composition (i.e., phytoplankton groups with a higher growth rate or zooplankton grazing pressure).

Concentration change was used in statistical analysis for both treatment and control values. A repeated-measures general linear model was used to analyze significance and maintain consistency (GLM©, SPSS©). Results were significant if $P \leq 0.05$.

RESULTS AND DISCUSSION

P. parvum cell density was significantly reduced by high flushing during the bloom development experiment compared to the control (P -value=0.027). The greatest difference between concentration changes was observed between T14 and T21, where

control mesocosms experienced a 30% increase in *P. parvum* cells L⁻¹ compared to a 19% increase in high flushing mesocosms. The results observed with high flushing during bloom development are extremely important and indicate that there are other acting mechanisms responsible for reduced *P. parvum* cell density besides physical flushing loss of cells. For example, reduced toxin production due to nutrient availability and lower cells L⁻¹ present to produce more toxin compounds may have led to higher grazing rates by copepod nauplii. All other flushing treatments in the pre-bloom experiment, as well as low and medium flushing treatments in the bloom development experiment failed to produce a significantly different rate of increase in *P. parvum* cell density. Therefore, most of the difference in cell density within these mesocosms can be attributed to physical flushing loss of *P. parvum* cells during water exchange. These results indicate that one of the most important mechanisms in controlling cell proliferation is physical reduction in cell number (due to removal of *P. parvum* rich mesocosm water and replacement by source water that consistently had very low or no *P. parvum* cells present).

Ambient toxicity of source water was not tested; therefore, concentration change calculations could not be performed. DIN concentration change was strongly effected by flushing in the pre-bloom experiment after accounting for source water exchange. Even though all flushing treatments maintained overall higher levels of NO_x and NH₄ throughout the experiment, particularly from T0 to T14, a faster rate of decrease was documented in these nutrient levels compared to the control. All flushing levels led to a significantly faster rate of phytoplankton taking up NO_x throughout the pre-bloom

experiment (low P -value=0.017; medium P -value=0.016; high P -value=0.022), and measured samples (not accounting for water exchange) still maintained significantly higher NO_x levels throughout the experiment compared to control mesocosms (note: measured samples were collected following a seven day lag-time after flushing treatments took place; P -values reported in Chapter II results). This trend of faster nutrient uptake was the strongest from T14 to T21, when NO_x levels in the control decreased by 16% compared to a 91, 95, and 98% decrease due to nitrogen uptake in low, medium, and high flushing mesocosms. Results from both measured samples (not accounting for source water exchange), which indicated higher NO_x levels throughout our experiment and results from concentration change calculations (accounting for water exchange), which illustrated a faster rate of NO_x uptake, confirm that a higher level of NO_x was present in flushing mesocosms. This difference can be attributed to nitrogen replenishment by source water.

Similarly, high flushing treatments in the pre-bloom experiment also led to a significantly faster rate of NH_4 uptake (P -value<0.001) compared to the control. For example, between T14 and T21, control mesocosms experienced almost no change in NH_4 (<1%); however, NH_4 decreased due to nitrogen uptake by 67% in high flushing mesocosms.

During the pre-bloom experiment, all flushing treatments resulted in a significantly faster rate of PO_4 uptake by phytoplankton (P -value \leq 0.001). This trend was most pronounced from T7-T21. For example, between T7 and T14, the control mesocosms experienced a <1% uptake in PO_4 , whereas low, medium, and high flushing

resulted in a 10, 43, and 67% rate of phosphate uptake. Furthermore, from T14-T21 the control decreased by 11%, compared to a 48, 13, and 62% uptake rate documented in the low, medium, and high flushing mesocosms. No significant change in inorganic nutrients was observed during the bloom development experiment.

These findings further support nutrient addition from source water as a key mechanism in mitigating bloom conditions. Replenishment of both N and P seem to play a critical role in alleviating suboptimal conditions that stimulate toxin production, and subsequently keep *P. parvum* densities low (Barkoh et al. 2003; Grover et al. 2007; Kurten et al. 2007; Roelke et al. 2007, 2010). Furthermore, rapid decrease of nutrient concentrations observed after flushing treatments support the theory of luxury consumption (Davies and Morales 1998; Schelske et al. 1999; Revilla and Weissing 2008). The rapid uptake of nutrients was not detected by our measured concentrations, because sampling only took place a week after flushing treatments and elevated N and P levels were quickly sequestered, rendering our sampling technique insensitive to these changes. However, this method highlights the importance of increased nutrient levels when initial weekly concentrations were calculated (i.e., accounting for nutrient replenishment).

High flushing resulted in significantly higher chl-*a* levels compared to the control in both pre-bloom and bloom development experiments (pre-bloom P -value=0.004; bloom development P -value=0.021). This difference was apparent between T0 and T7 of the pre-bloom experiment, where chl-*a* increased by 180% in the control compared to a 213% increase under high flushing conditions. From T7-T14, the control decreased by

25%, while high flushing mesocosms continued to increase by 25%. This trend was observed in the bloom development experiment, albeit to a lesser extent. During bloom development, an increase in chl-*a* levels was documented only from T14 to T21, the control mesocosms experienced an increase of 34%, and high flushing conditions experienced an increase of 63%. This trend was not observed at lower flushing levels. These results indicate that after accounting for the addition of deep flushing water, which contained relatively no chl-*a*, high flushing treatments still maintained equal or higher chl-*a* levels within mesocosms compared to the control. This is likely due to more N and P availability compared to the control, which resulted in higher levels of photosynthesis and primary productivity.

Pigment samples representing taxonomic phytoplankton assemblage for flushing water were not analyzed in the pre-bloom experiment, although they were analyzed in the bloom development experiment allowing calculations accounting for water exchange and then statistical analysis to be performed. During the bloom development experiment and after accounting for addition of source water into mesocosms, only haptophyte concentrations were significantly affected by flushing treatments (low *P* – value=0.050; medium *P* –value=0.035; high *P* –value=0.001). It is important to note that *P. parvum* is the only representative species in Lake Granbury. The strongest difference between concentration changes was from T14-T21, where haptophytes increased by 74% in the control, compared to haptophytes increasing by only 6% in low flushing mesocosms, 26% in medium flushing, and 13% in high flushing mesocosms after accounting for dilution effects. Since this methodology accounts for the physical

removal of cells, results may be due to other acting mechanisms, such as toxin dilution and nutrient availability, which allow other phytoplankton groups to successfully compete against *P. parvum*.

There were no significant differences in concentration change observed for other phytoplankton groups (cyanobacteria, euglenophytes, chlorophytes, prasinophytes, dinoflagellates, cryptophytes, or diatoms) versus the control. Therefore, most of the differences in taxonomic assemblage can be accounted for by mesocosm water removal and deeper water addition. Similarly, there were no significant differences in zooplankton density or biovolume after accounting for dilution effects in flushing mesocosms compared to the control. This included total zooplankton, as well as community composition (cladocerans, protozoa, rotifers, copepod adults and nauplii). Additionally, no abiotic water quality parameters showed a significant change due to flushing treatments when compared to the control.

APPENDIX B

RESULTS FOR ADDITIONAL RESPONSE VARIABLES AND SUPPLEMENTARY
DATA

Additional figures to supplement the results portion in Chapter II are provided here. Including response variables, such as NO_x and NH₄ analysis, taxonomic phytoplankton assemblage, total zooplankton density and community composition (i.e., density and biovolume), and abiotic water quality parameters.

NO_x (NO₂ + NO₃) and NH₄, were analyzed together as DIN in Chapter II (Figure 5a, b). During the pre-bloom experiment, NO_x and NH₄ decreased from T0 to T14 and remained at low levels to experiment conclusion. Despite rapid decrease, NO_x and NH₄ maintained significantly higher levels in all flushing mesocosms compared to the control (*P*-value < 0.001; Figures 10a, 11a). During the bloom development experiment, inorganic nutrients remained fairly constant and all flushing treatments resulted in no observed change to DIN (Figure 5b). However, when NO_x and NH₄ were separated, NO_x was significantly increased under high flushing conditions (*P*-value = 0.005; Figure 10b). Medium and low flushing had no detectable effect on NO_x, and NH₄ was not significant in any treatment (Figures 10b, 11b).

Figure 10: NO_x ($\text{NO}_2 + \text{NO}_3$) levels during pre-bloom (a) and bloom development (b) experiments.

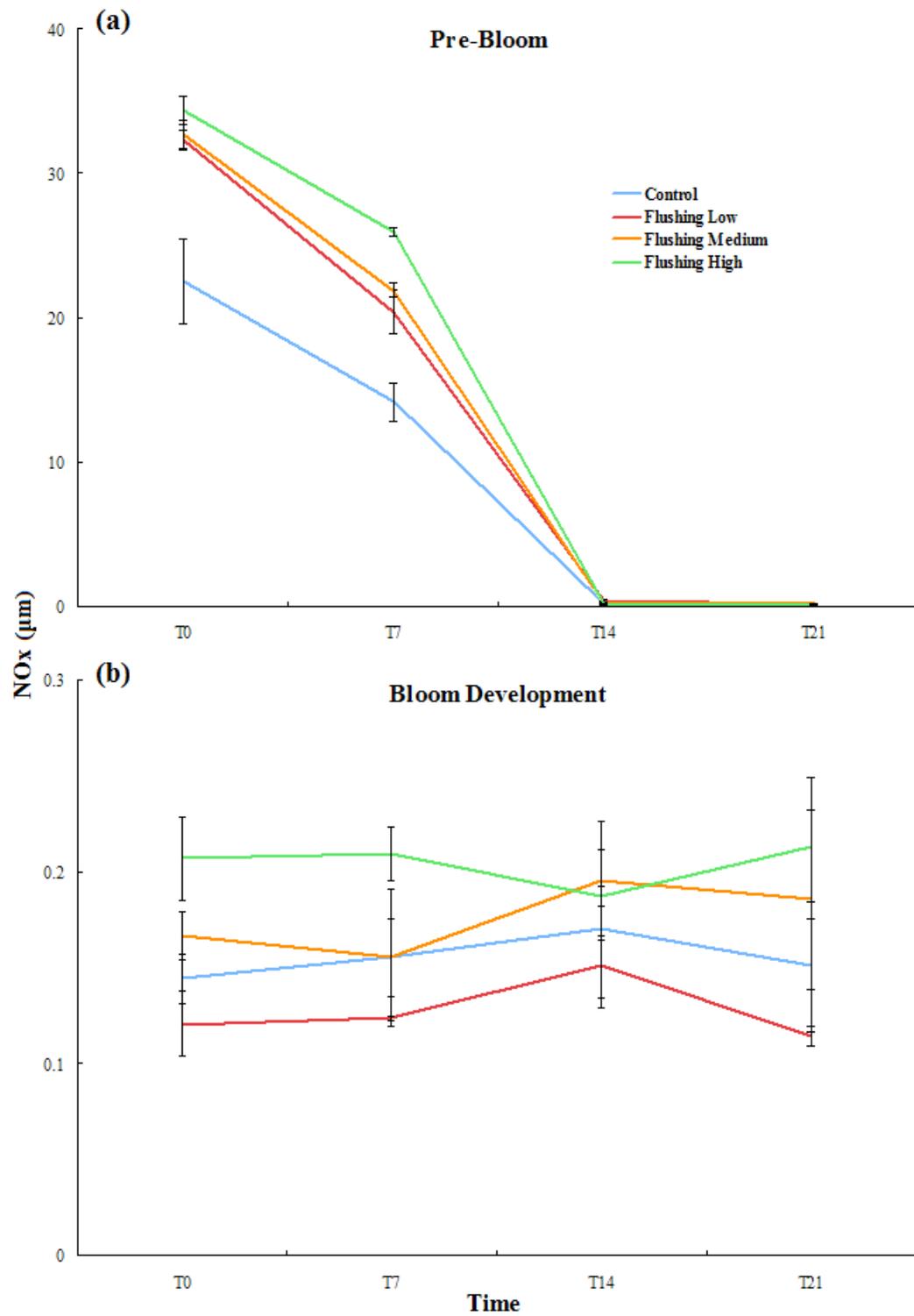
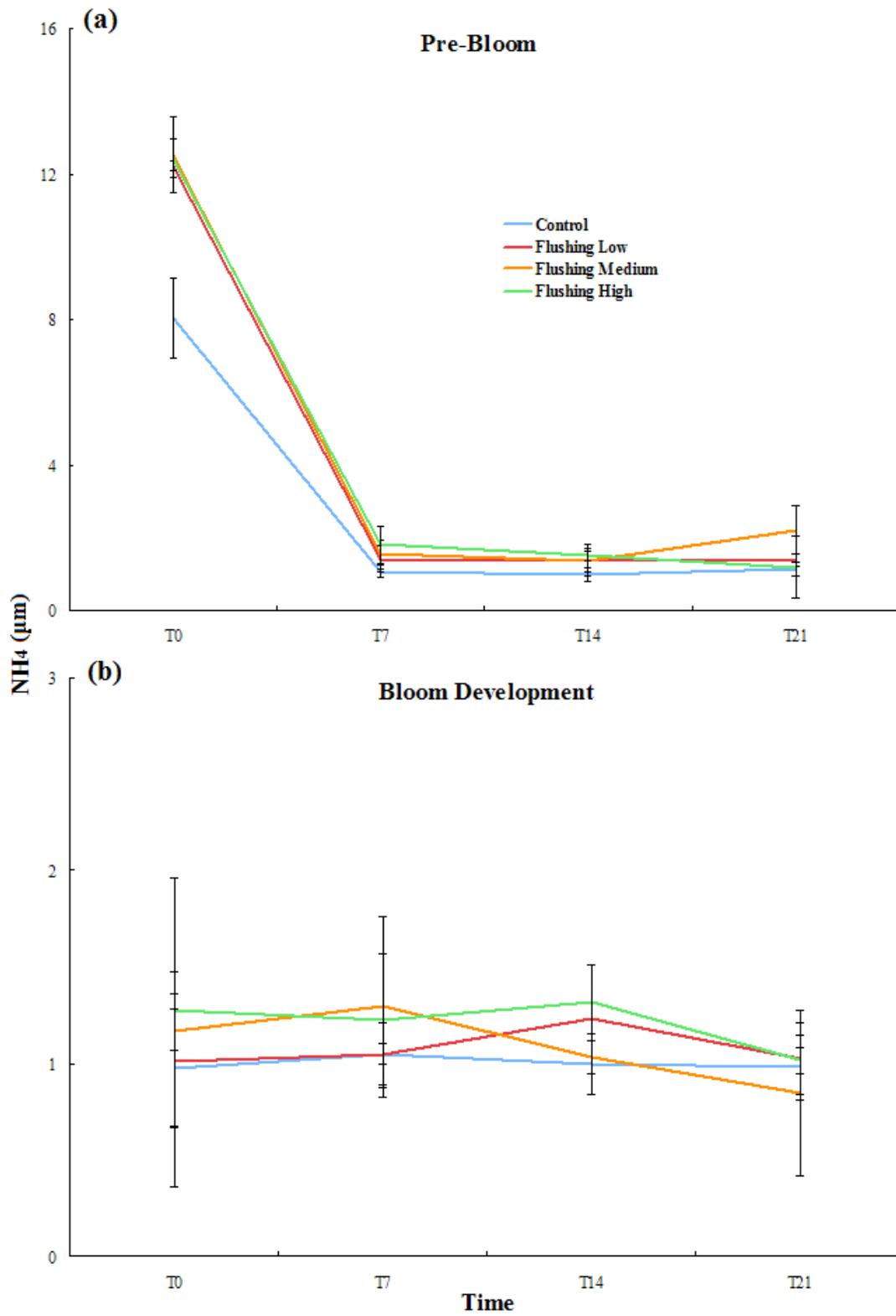


Figure 11: NH_4 levels during pre-bloom (a) and bloom development (b) experiments.

In both experiments, there was an effect on taxonomic group composition of phytoplankton biomass based on pigment analysis. In the pre-bloom experiment, the most significant effects were observed in high flushing treatments, including lower levels of cyanobacteria (P -value=0.005). Levels were initially $0.1 \mu\text{g chl-}a \text{ L}^{-1}$ and increased to $0.7 \mu\text{g chl-}a \text{ L}^{-1}$ in both control and flushing treatments by experiment conclusion, however high flushing cyanobacteria levels were observably lower during T7 and T14 (Figure 12a). Euglenophytes had no significant change during the pre-bloom experiment. Starting out at $2.0 \mu\text{g chl-}a \text{ L}^{-1}$ and decreasing to zero by T21. Levels were increased at T7 due to low flushing ($6.2 \mu\text{g chl-}a \text{ L}^{-1}$) compared to the control ($3.3 \mu\text{g chl-}a \text{ L}^{-1}$; Figure 13a). Chlorophytes were significantly increased by medium and high flushing compared to the control (medium P -value=0.001; high P -value<0.001). All levels started out around $0.4 \mu\text{g chl-}a \text{ L}^{-1}$, however by T21, control levels reached $1.1 \mu\text{g chl-}a \text{ L}^{-1}$, medium levels were $1.8 \mu\text{g chl-}a \text{ L}^{-1}$, and high levels were $2.8 \mu\text{g chl-}a \text{ L}^{-1}$ (Figure 14a). Similarly, high flushing significantly elevated prasinophyte levels (P -value=0.010). This trend was clear at T7, where high flushing levels increased from 0.4 to $2.6 \mu\text{g chl-}a \text{ L}^{-1}$ and the control increased from 0.3 to $1.1 \mu\text{g chl-}a \text{ L}^{-1}$, high flushing levels remained elevated compared to the control through T21 (Figure 15a). Dinoflagellates were significantly lower in high flushing treatments (P -value=0.037); however, all levels were extremely low. Maximal levels of $0.015 \mu\text{g chl-}a \text{ L}^{-1}$ were reached in the control at T7 compared to zero dinoflagellates detected in high flushing mesocosms, but by T14 and T21 all levels were zero (Figure 16a). Haptophytes had no detectable change throughout either experiment (Figure 17a, b); *P. parvum* is the

only representative species in Lake Granbury. However, it is important to note that CHEMTAX is not always sensitive to detecting changes in this group and an error is likely, based on microscope counts (see methods) and subsequent significant decreases observed in *P. parvum* cell density (refer to results, chapter II). There was no significant change to cryptophytes in any level of flushing (Figure 18a). Diatoms were significantly lower in high flushing (P -value= 0.007). All levels were around 0.8-1.0 $\mu\text{g chl-}a \text{ L}^{-1}$ at T0, then the control increased to 4.9 $\mu\text{g chl-}a \text{ L}^{-1}$ by T21 compared to the high flushing treatment, which only reached 3.5 $\mu\text{g chl-}a \text{ L}^{-1}$ by T21 (Figure 19a).

During the bloom development experiment there was no significant change in cyanobacteria, and levels remained between 0.7 and 1.1 $\mu\text{g chl-}a \text{ L}^{-1}$ (Figure 12b). Again, there was no significant change to euglenophytes as a result of flushing treatments. Euglenophyte levels started out higher in the control mesocosms (0.9 $\mu\text{g chl-}a \text{ L}^{-1}$) compared to the treatment mesocosms, which ranged from zero to 0.4 $\mu\text{g chl-}a \text{ L}^{-1}$, then peaked at T14 for all mesocosms and decreased to zero by experiment conclusion (Figure 13b). Similar to the pre-bloom experiment, Chlorophytes were significantly increased by high flushing (P -value= 0.001). All levels were around 0.7 $\mu\text{g chl-}a \text{ L}^{-1}$ at experiment initiation and then decreased to zero at T7, but by experiment conclusion the control mesocosms remained at zero, whereas high flushing levels had increased to 0.3 $\mu\text{g chl-}a \text{ L}^{-1}$ (Figure 14b). Prasinophytes were significantly higher in high flushing mesocosms compared to the control (P -value= 0.038), however this trend is likely due to higher initial levels. Control levels were 1.1 $\mu\text{g chl-}a \text{ L}^{-1}$ at T0, whereas high flushing started out at 1.7 $\mu\text{g chl-}a \text{ L}^{-1}$, then both mesocosms followed a similar

trend, that is decreasing to 0.4-0.5 $\mu\text{g chl-}a \text{ L}^{-1}$ at T7 and T14 and increasing to 1.4 $\mu\text{g chl-}a \text{ L}^{-1}$ at experiment end (Figure 15b). There was a significantly lower level of dinoflagellates in all treatment levels (high flushing P -value=0.001; medium flushing P -value=0.013; low flushing P -value=0.016). Although, all levels were low throughout the experiment and initiated at zero, control levels reached maxima at T21 (0.03 $\mu\text{g chl-}a \text{ L}^{-1}$) and treatment levels ranged from 0.007 to 0.017 $\mu\text{g chl-}a \text{ L}^{-1}$ (Figure 16b).

Chryptophytes were increased by high flushing (P -value= 0.003), however this was a modest increase, where control levels remained relatively constant from T0 to T21 (0.7 $\mu\text{g chl-}a \text{ L}^{-1}$) and high flushing remained around 0.8 $\mu\text{g chl-}a \text{ L}^{-1}$ (Figure 18b). Diatoms were higher due to high flushing (P -value= 0.010), where levels started out at 2.0 $\mu\text{g chl-}a \text{ L}^{-1}$ and reached a peak of 2.8 $\mu\text{g chl-}a \text{ L}^{-1}$ in high flushing, but only reached 2.2 $\mu\text{g chl-}a \text{ L}^{-1}$ in the control (Figure 19b).

Figure 12: Cyanobacteria pigment levels as represented by CHEMTAX analysis during pre-bloom (a) and bloom development (b) experiments.

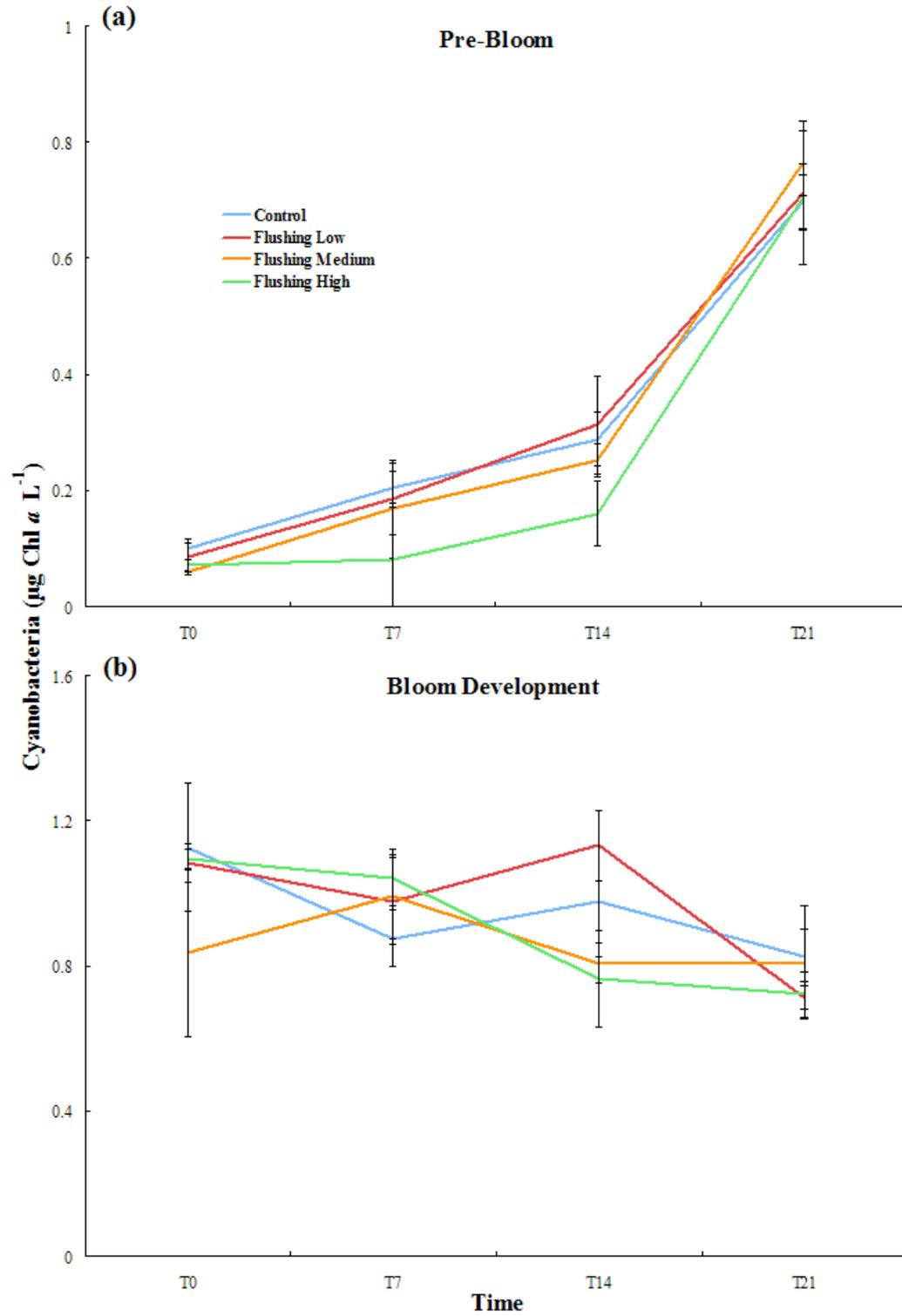


Figure 13: Euglenophyte pigment levels as represented by CHEMTAX analysis during pre-bloom (a) and bloom development (b) experiments.

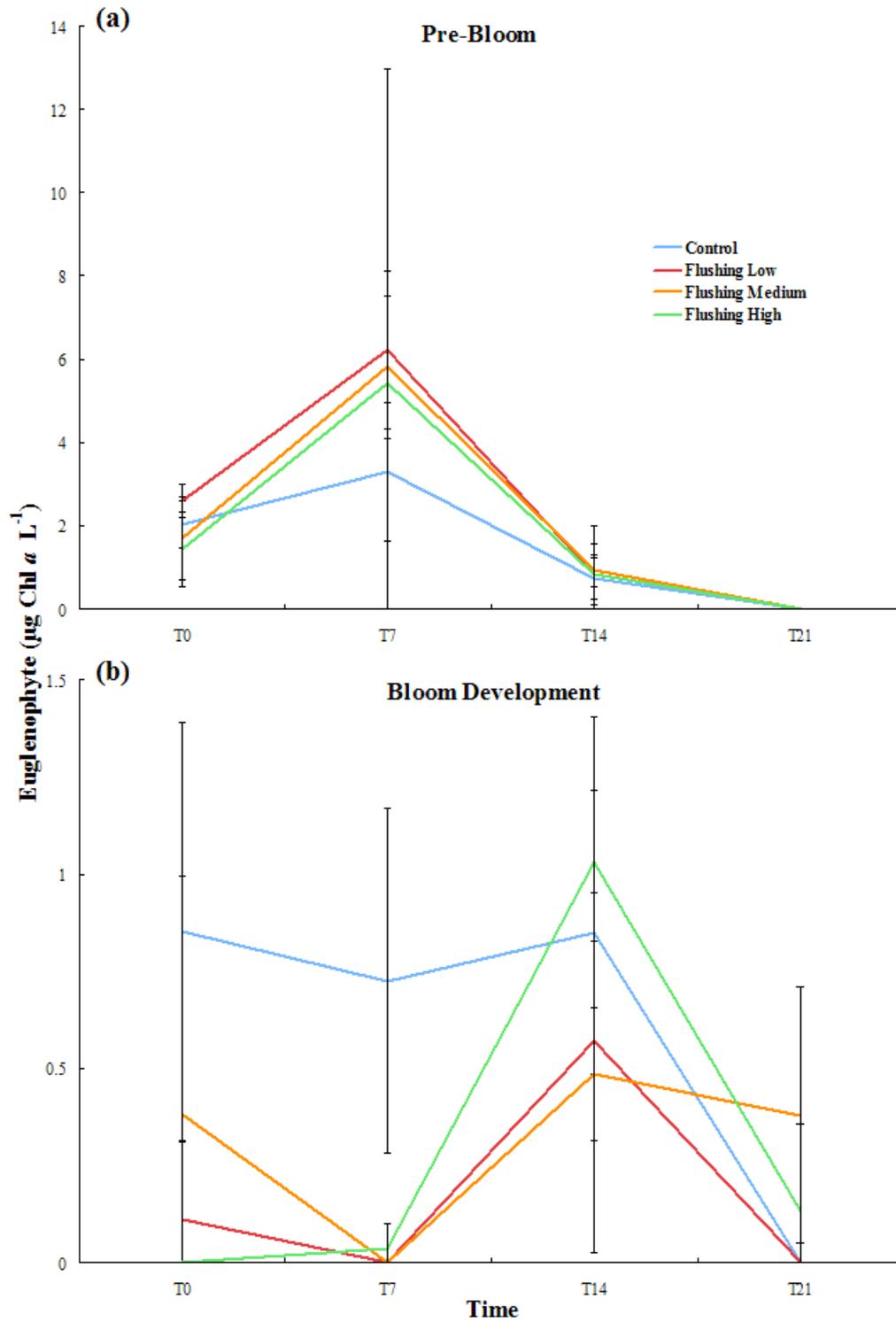


Figure 14: Chlorophyte pigment levels as represented by CHEMTAX analysis during pre-bloom (a) and bloom development (b) experiments.

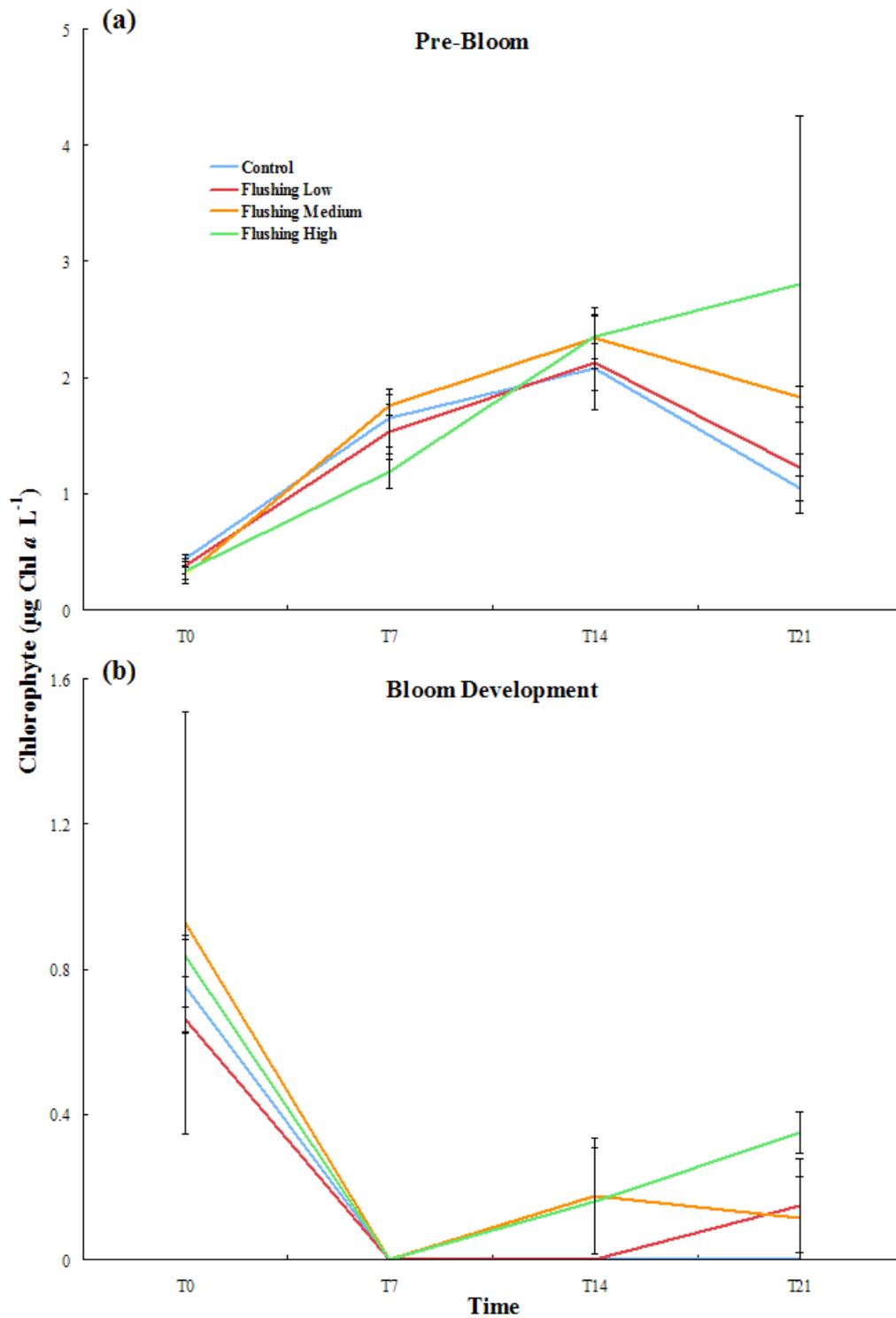


Figure 15: Prasinophyte pigment levels as represented by CHEMTAX analysis during pre-bloom (a) and bloom development (b) experiments.

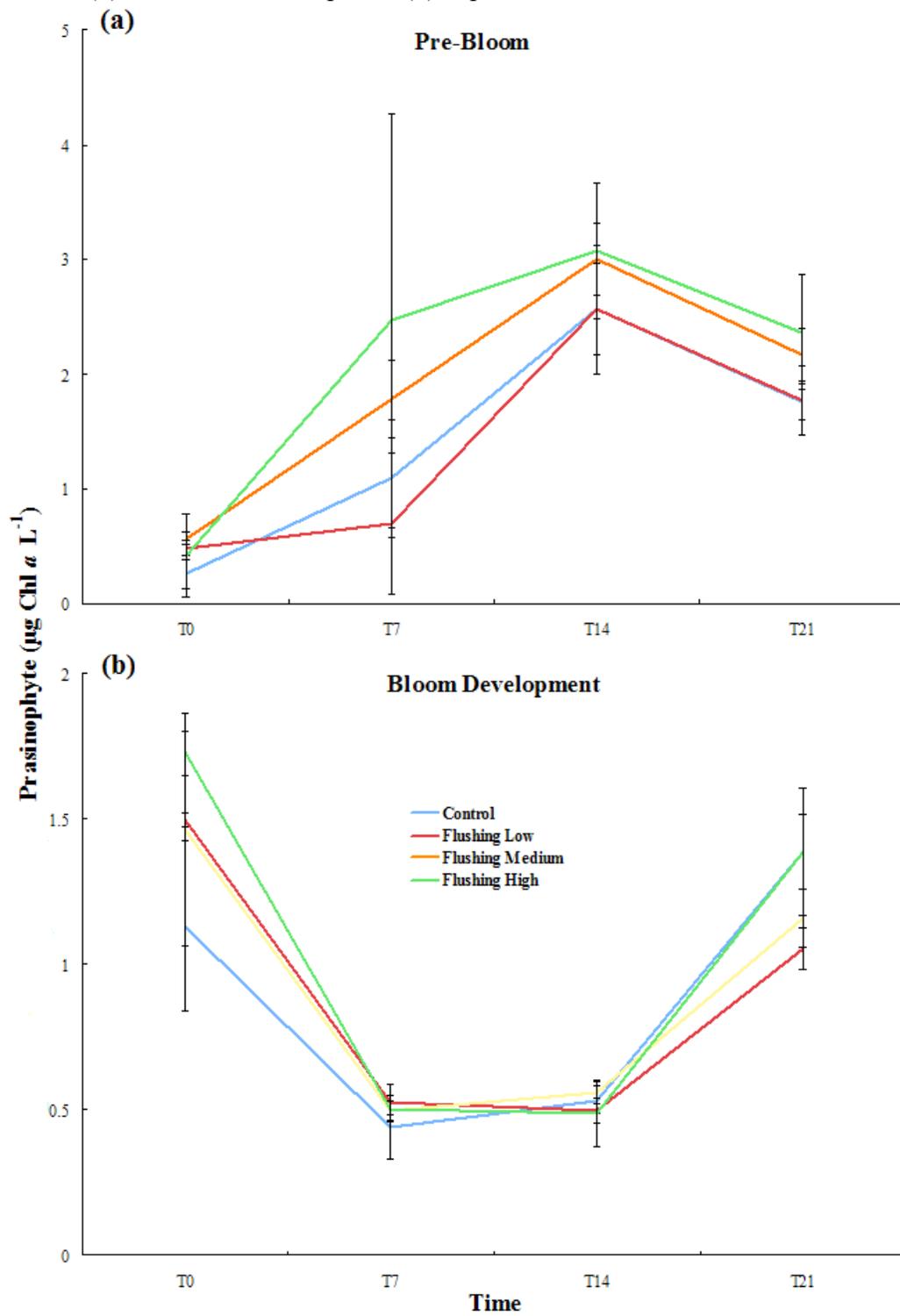


Figure 16: Dinoflagellate pigment levels as represented by CHEMTAX analysis during pre-bloom (a) and bloom development (b) experiments.

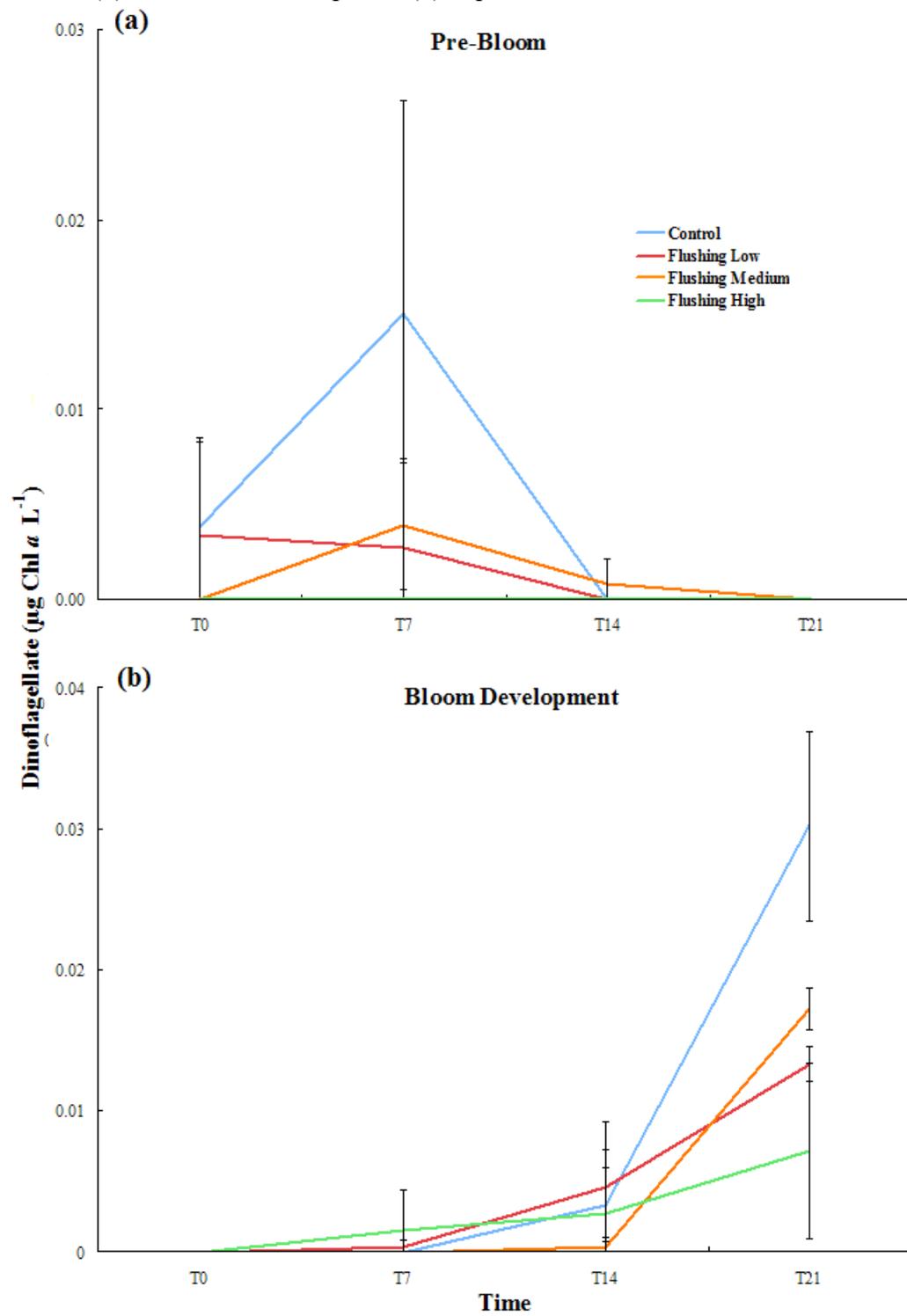


Figure 17: Haptophyte pigment levels as represented by CHEMTAX analysis during pre-bloom (a) and bloom development (b) experiments.

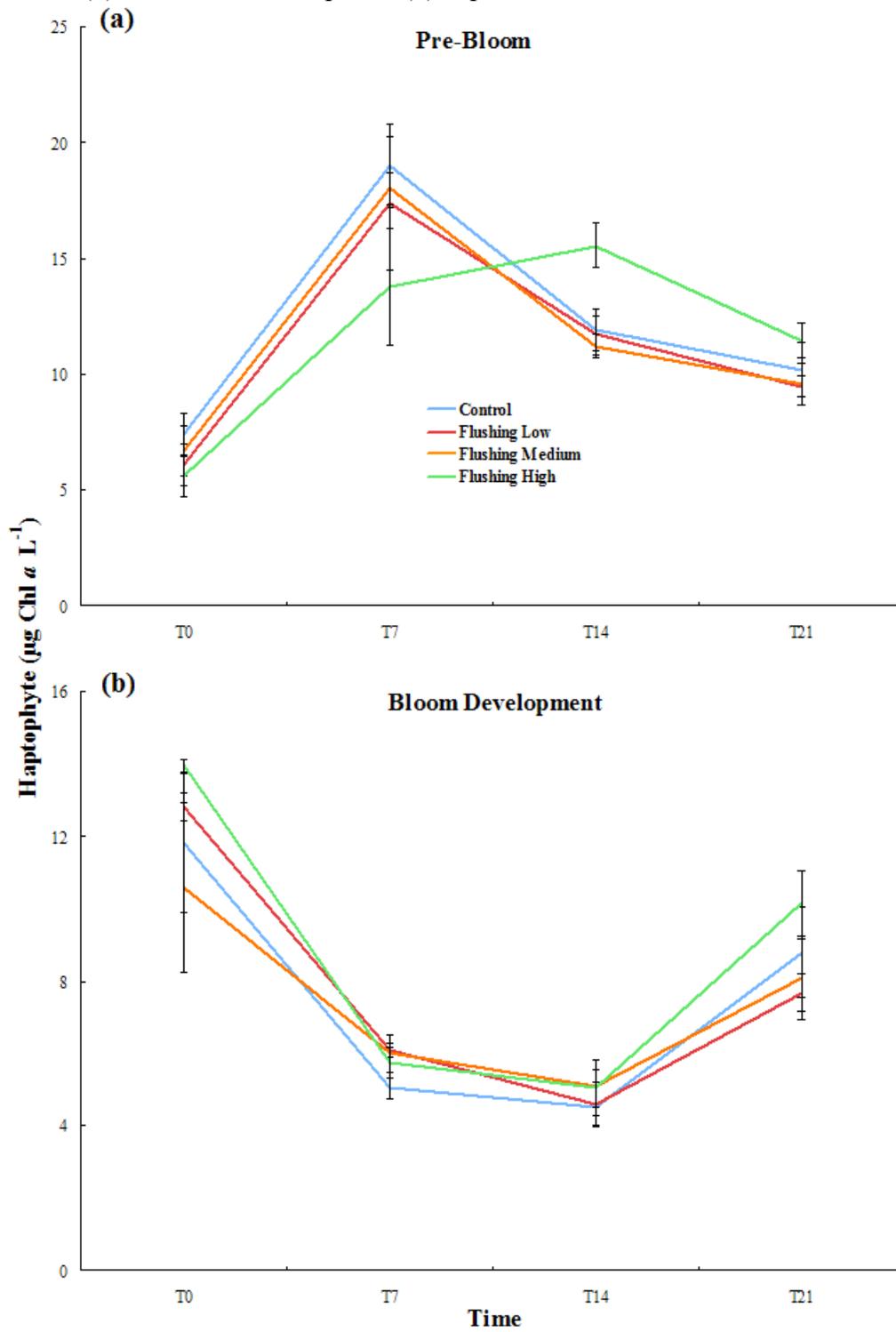


Figure 18: Chryptophyte pigment levels as represented by CHEMTAX analysis during pre-bloom (a) and bloom development (b) experiments.

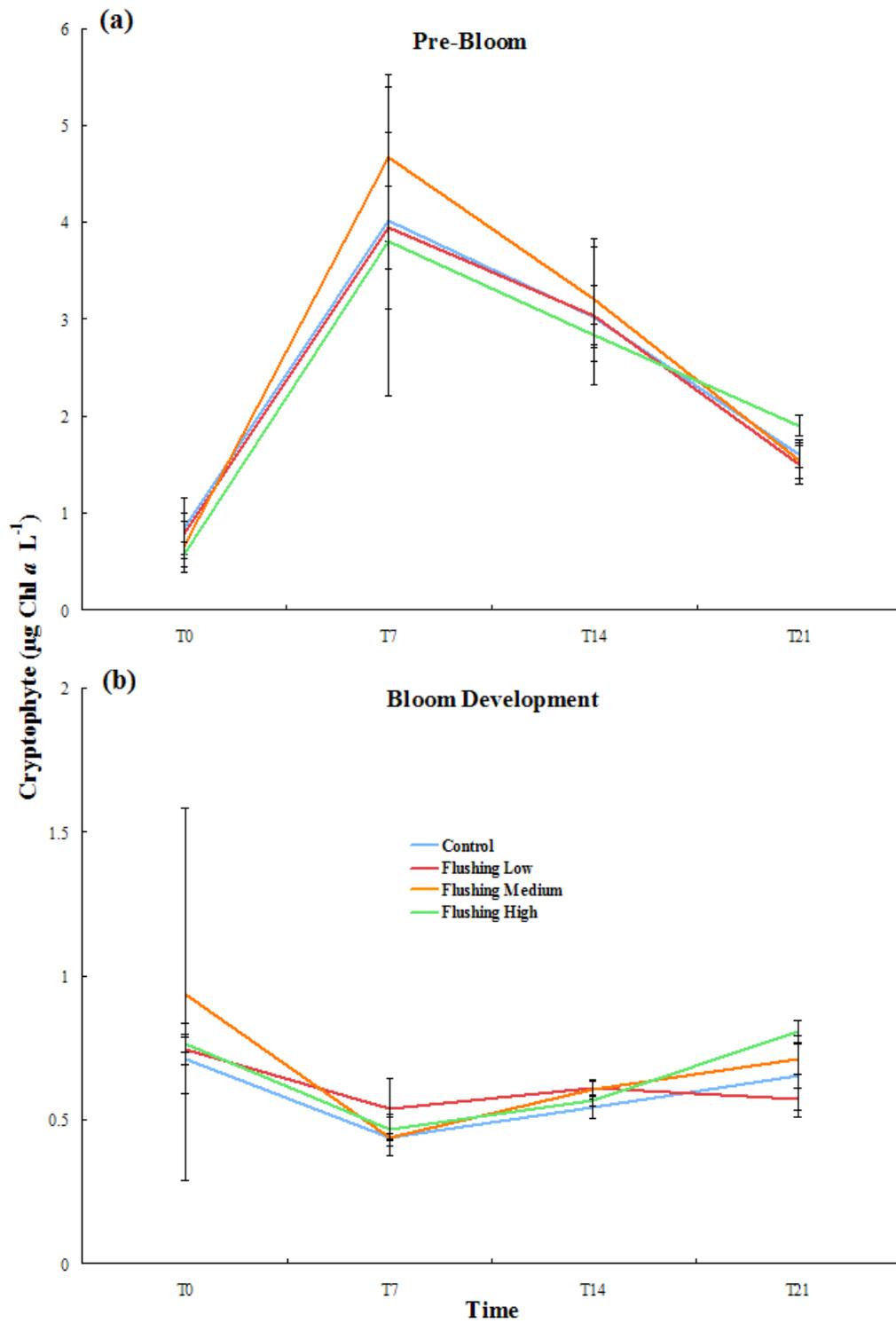
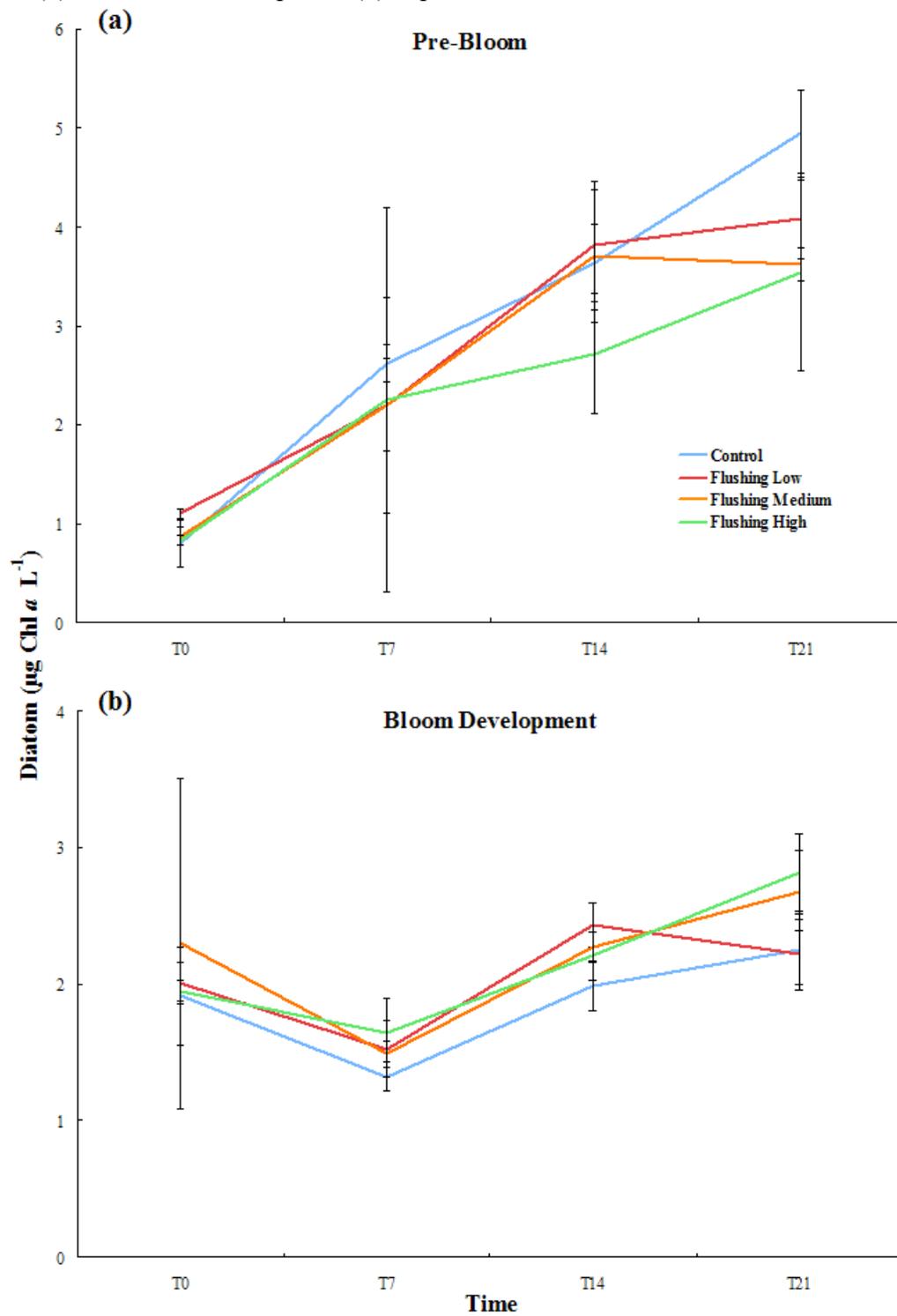
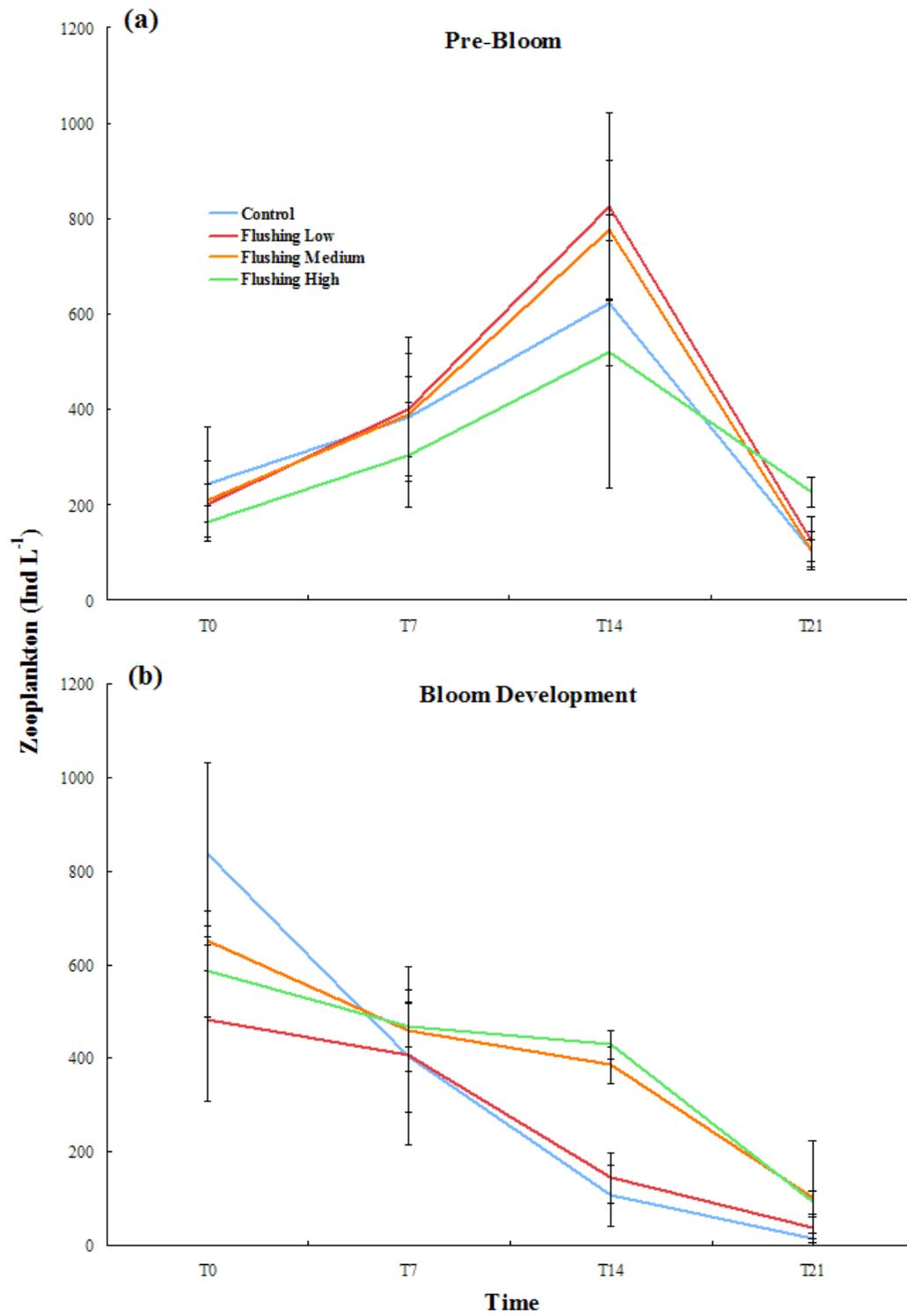


Figure 19: Diatom pigment levels as represented by CHEMTAX analysis during pre-bloom (a) and bloom development (b) experiments.



Zooplankton density followed a similar trend as zooplankton biovolume in both pre-bloom and bloom development experiments (Figure 9 *a, b*). Throughout the pre-bloom experiment total zooplankton density ranged from 480-840 individuals L^{-1} at T0, then peaked at T14 (520-830 individuals L^{-1}) and decreased to T21 (103-226 individuals L^{-1}). There was no significant trend caused by flushing treatments (Figure 20*a*). During the bloom development experiment, total density was decreasing to T21. This ranged from 480-840 individual L^{-1} at experiment initiation and dropped rapidly to 37-150 individuals L^{-1} by experiment conclusion (Figure 20*b*). Rotifers, copepod adults, and nauplii dominated both density and biovolume throughout both experiments.

Figure 20: Total zooplankton density during pre-bloom (a) and bloom development (b) experiments.



Taxonomic groups generally followed this trend, with copepod adults and nauplii being the exception. Copepod nauplii were the only group significantly increased by high and medium flushing treatments during bloom development, both in density and biovolume (P -value <0.001). However, no significant change was observed during pre-bloom conditions. Copepod nauplii density was decreasing through the pre-bloom experiment, initiation levels were between 63-94 individuals L^{-1} and decreased to T21 (7-21 individuals L^{-1} , Figure 21a). This trend was also observed in copepod nauplii biovolume, where levels started out at $0.57\text{-}0.81 \times 10^8 \mu\text{m}^3 L^{-1}$ and decreased to $0.04\text{-}0.18 \times 10^8 \mu\text{m}^3 L^{-1}$ (Figure 22a). During bloom development, copepod nauplii density increased in high and medium flushing compared to the control, which remained constant. Particularly during T14 when ambient toxicity was lower in high flushing mesocosms compared to the control. Density peaked at T14 (223 and 271 individuals L^{-1} for medium and high flushing), versus 42 individuals L^{-1} observed in the control (Figure 21b). Similarly, nauplii biovolume followed the same pattern and peaked at T14. Medium and high flushing had maximal levels of 1.4 and $1.8 \times 10^8 \mu\text{m}^3 L^{-1}$ compared to the control ($0.25 \times 10^8 \mu\text{m}^3 L^{-1}$; Figure 22b).

Figure 21: Copepod nauplii density during pre-bloom (a) and bloom development (b) experiments.

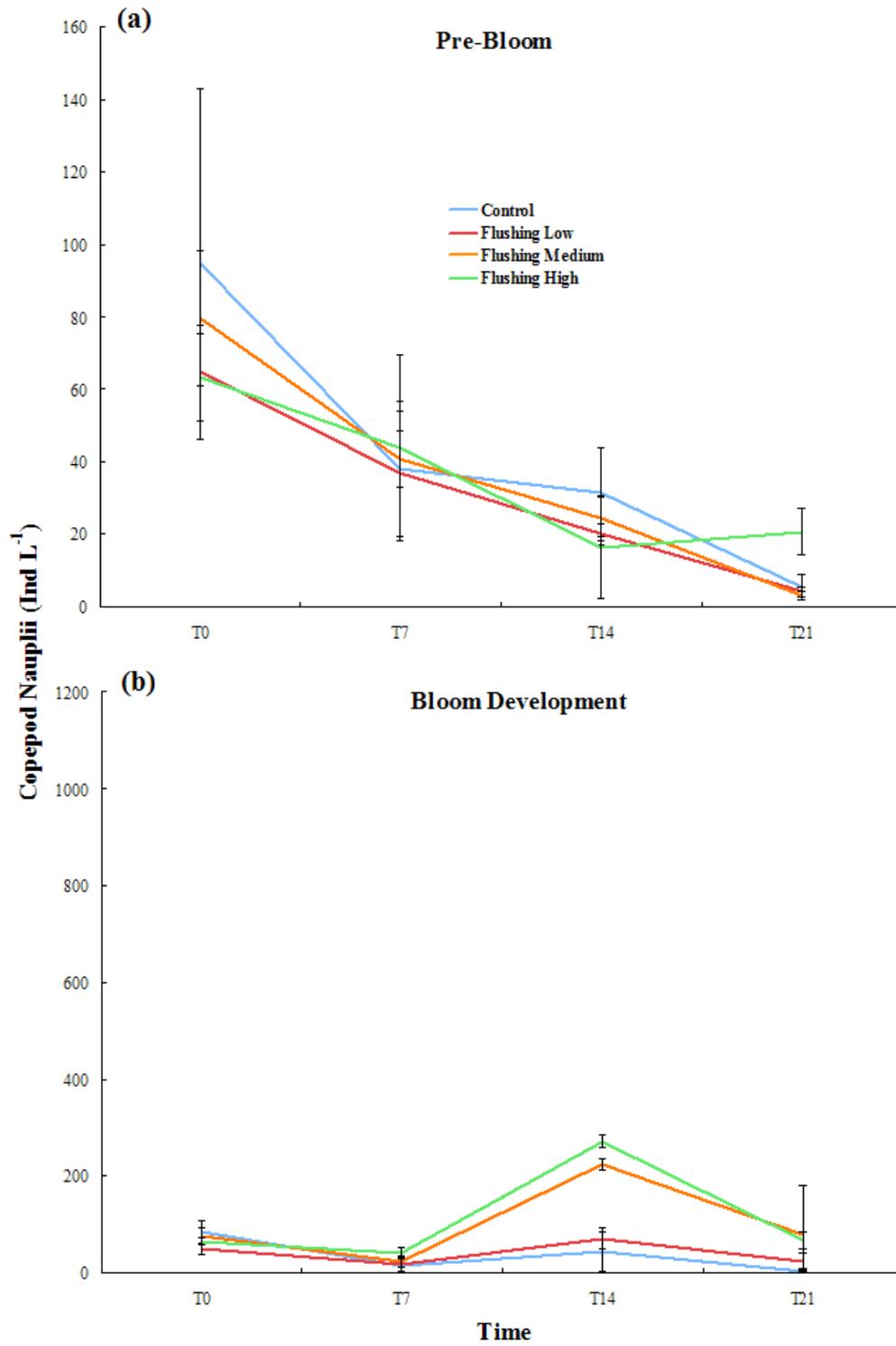
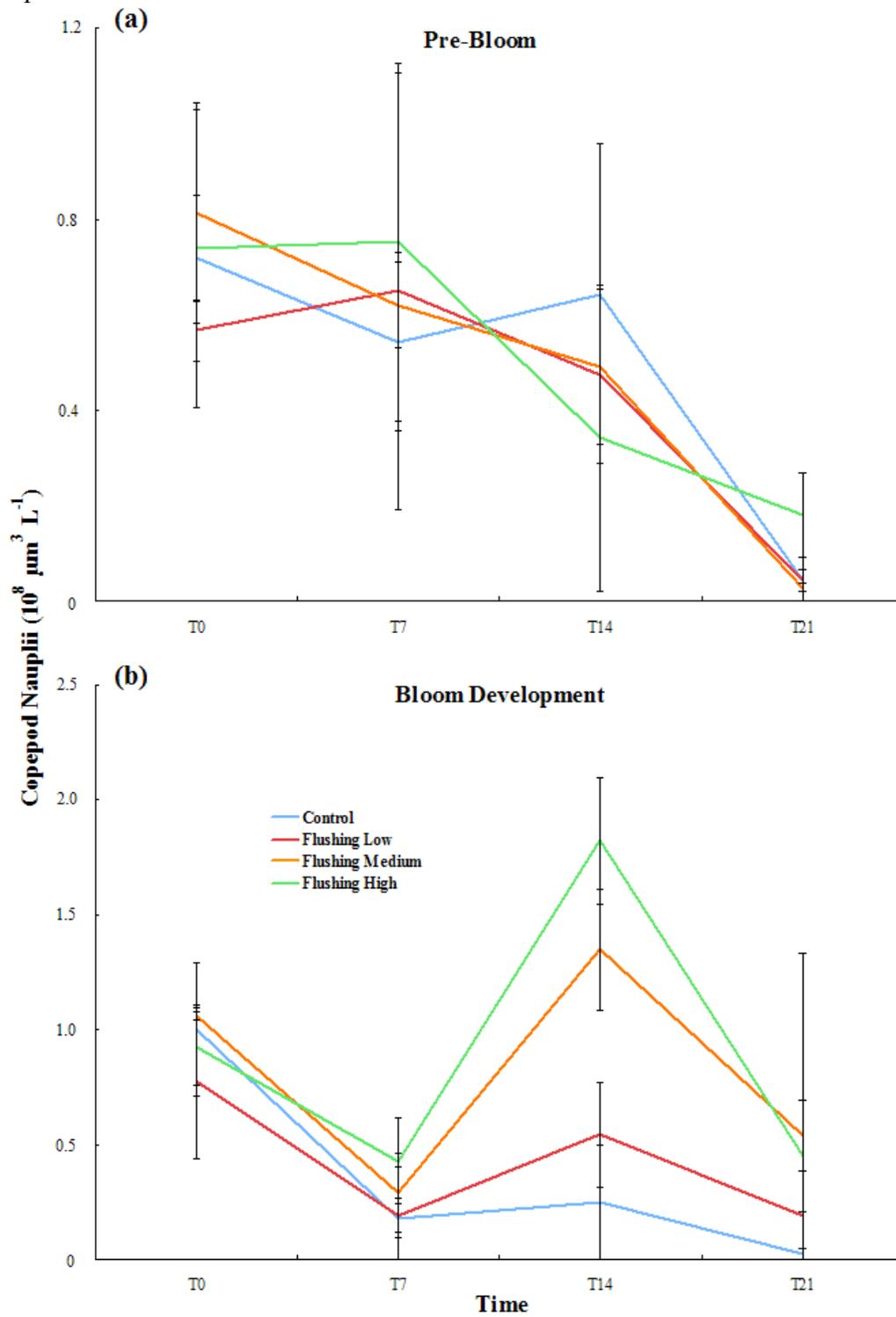


Figure 22: Copepod nauplii biovolume during pre-bloom (a) and bloom development (b) experiments.



Adult copepod density peaked at T14 for control, low, and medium flushing mesocosms in the pre-bloom experiment. High flushing experienced a modest dilution effect of mature copepods, although this was not significant (Figure 23a). Biovolume of adult copepods increased to T21 of the pre-bloom experiment and reached slightly higher levels in flushing mesocosms; particularly in medium flushing (Figure 24a). During the bloom development experiment, adult copepod density was low and consistent between treatments to experiment conclusion (Figure 23b). Biovolume peaked at T7 and declined to T21 in all treatments; maximal levels were reaching in low flushing (Figure 24b).

Figure 23: Adult copepod density during pre-bloom (a) and bloom development (b) experiments.

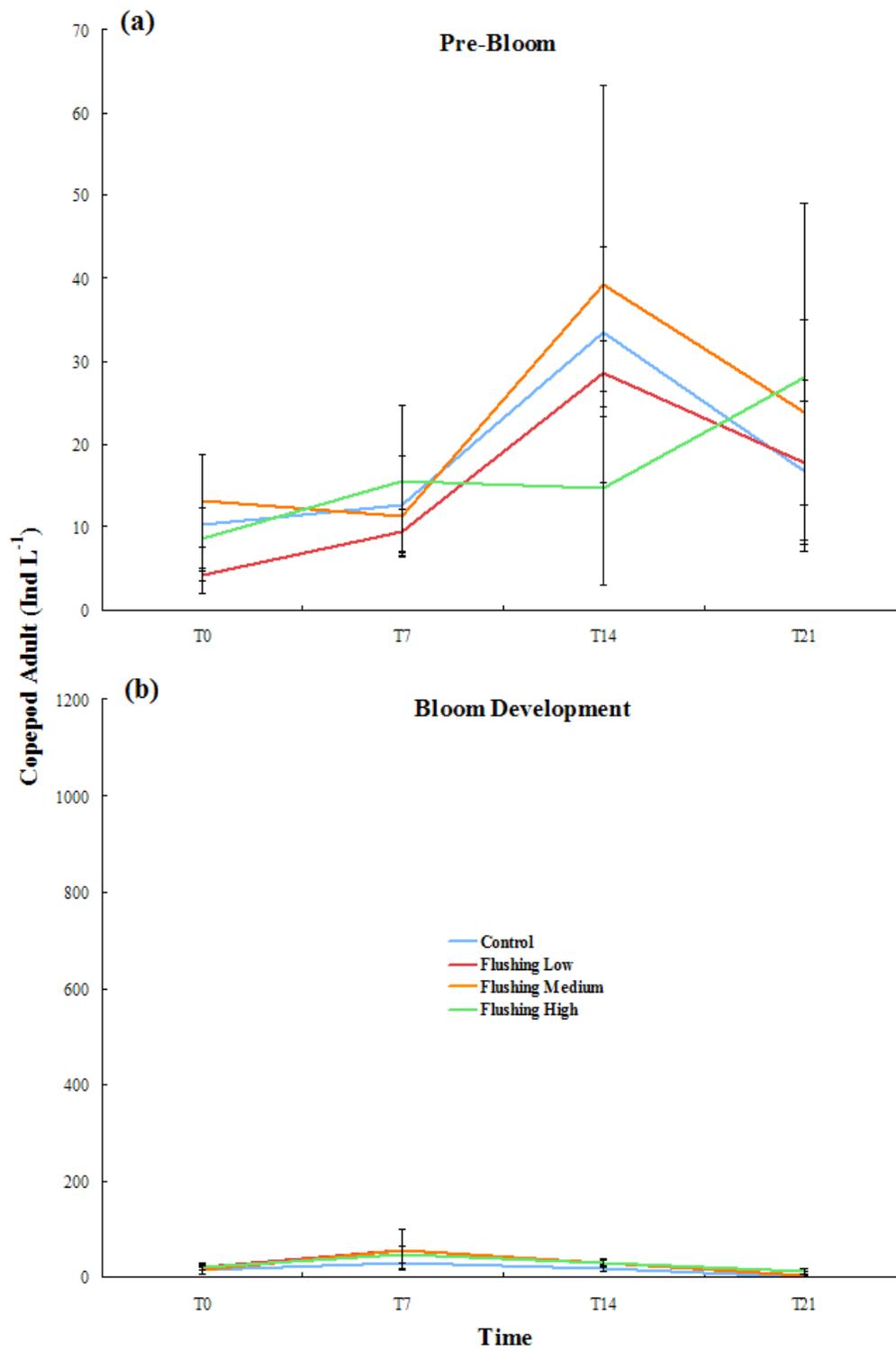
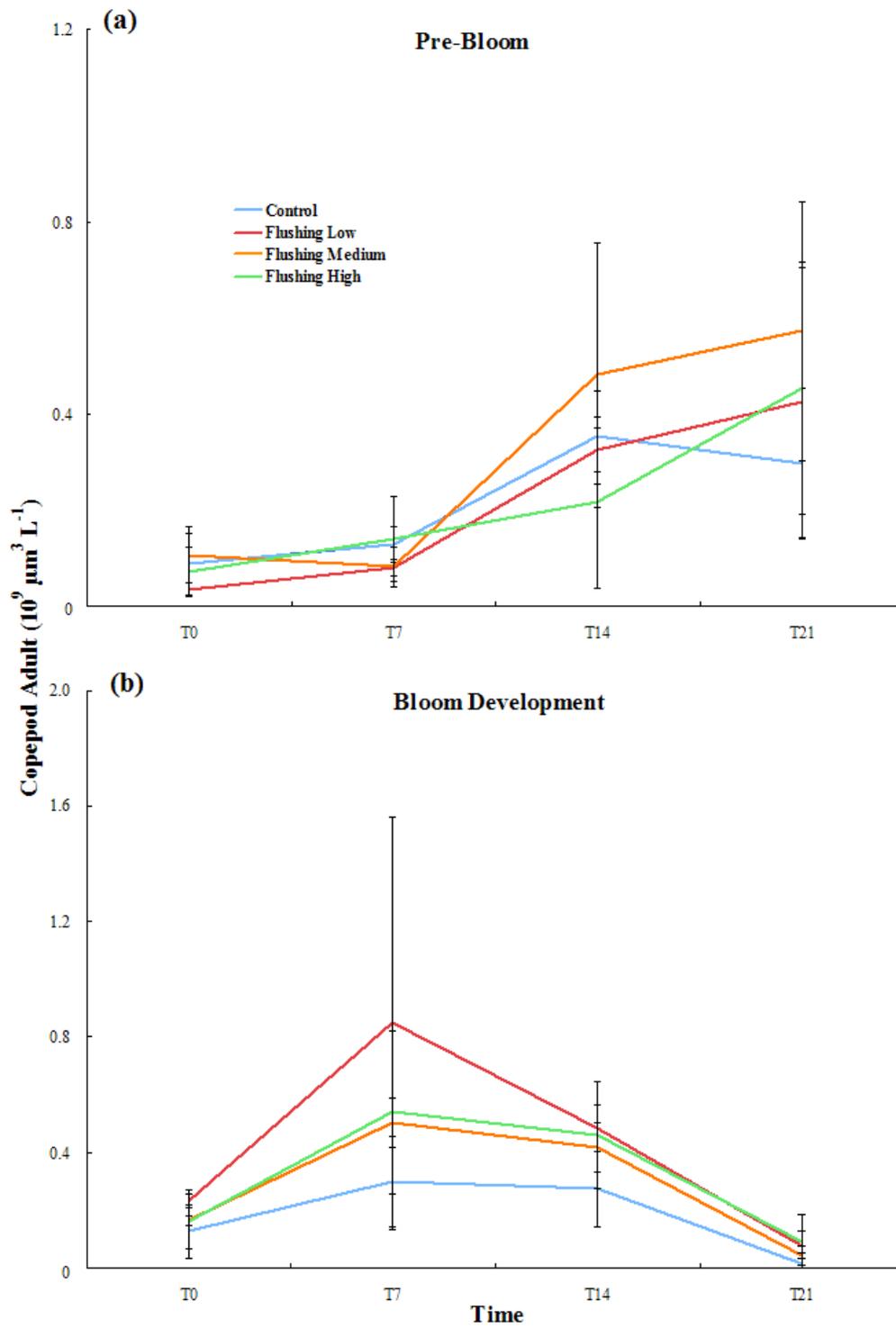


Figure 24: Adult copepod biovolume during pre-bloom (a) and bloom development (b) experiments.



Similar to the total zooplankton trend observed under pre-bloom conditions, rotifer density also peaked at T14, and then decreased to T21 with no significant difference between any treatment and the control (Figure 25a). This would be expected, since rotifers were dominant in community assemblage. Furthermore, biovolume followed this trend, with only high flushing diverging between T14 and T21 and maintaining constant levels (Figure 26a). During the bloom development experiment, rotifer density and biovolume decreased rapidly throughout the experiment and there was no trend between treatments (Figure 25b, 26b). Again, this closely followed total zooplankton observations.

Figure 25: Rotifer density during pre-bloom (a) and bloom development (b) experiments.

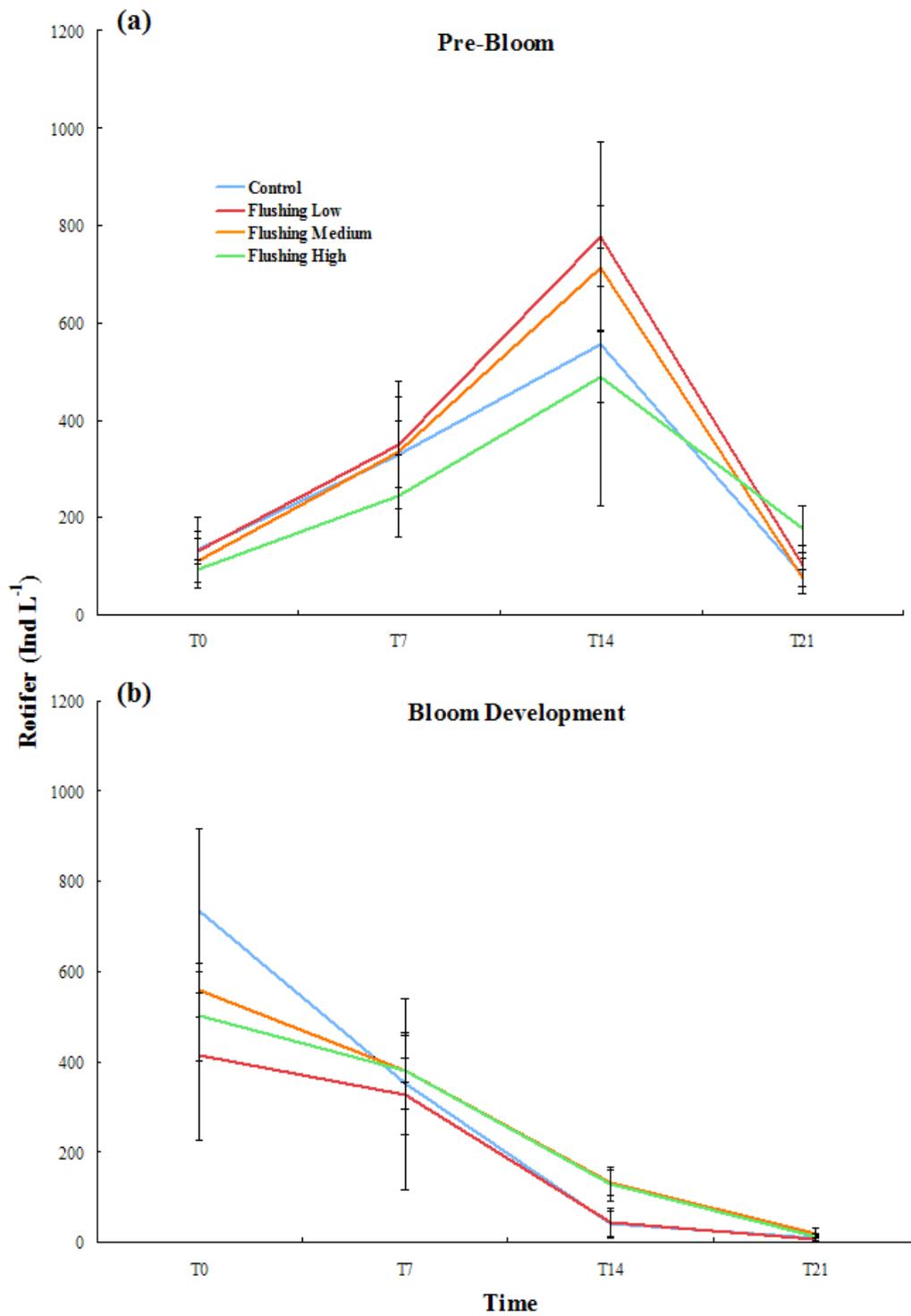
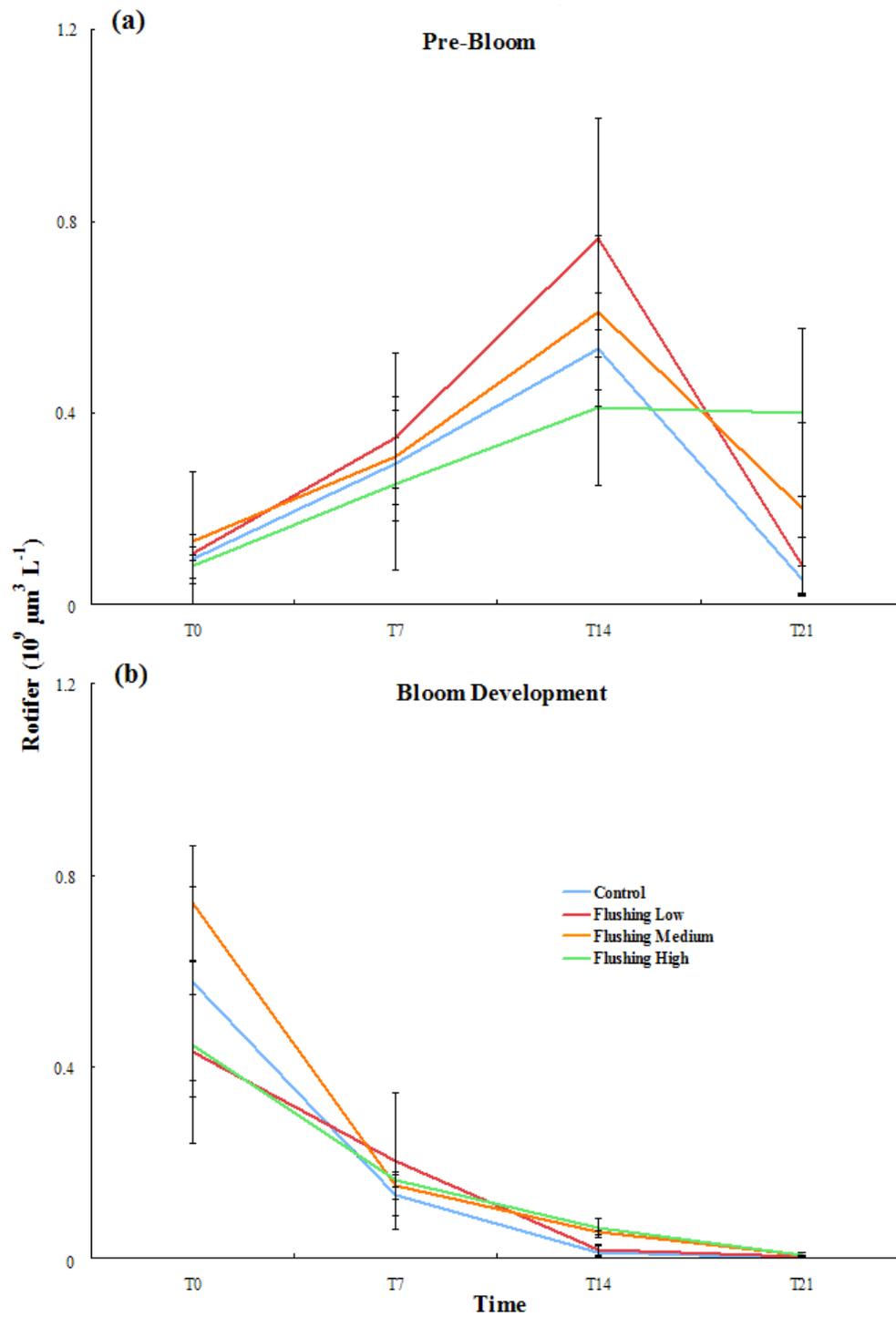


Figure 26: Rotifer biovolume during pre-bloom (a) and bloom development (b) experiments.



Protozoa and cladoceran levels were very low throughout both experiments, making it difficult to decipher trends. In the pre-bloom experiment, protozoa density and biovolume were extremely low and all values were close to zero by experiment end (Figure 27, 28). During the bloom development experiment, all sampling times resulted in zero density and biovolume (no data to show). Similarly, cladoceran density and biovolume started out quite low in the pre-bloom experiment and continued to decrease to T21, at experiment conclusion there were virtually no cladocerans present (Figure 29, 30). During the bloom development experiment, almost all sampling times were characterized by zero values for both density and biovolume, with no correlation to control versus flushing (data not shown).

Figure 27: Protozoa density during the pre-bloom experiment.

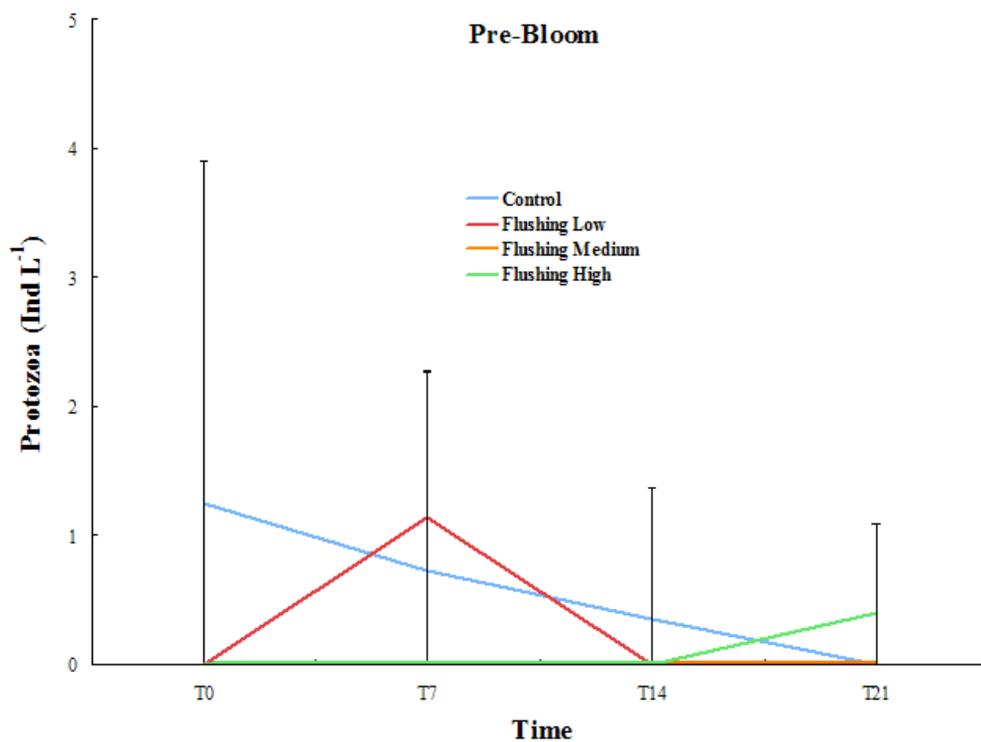


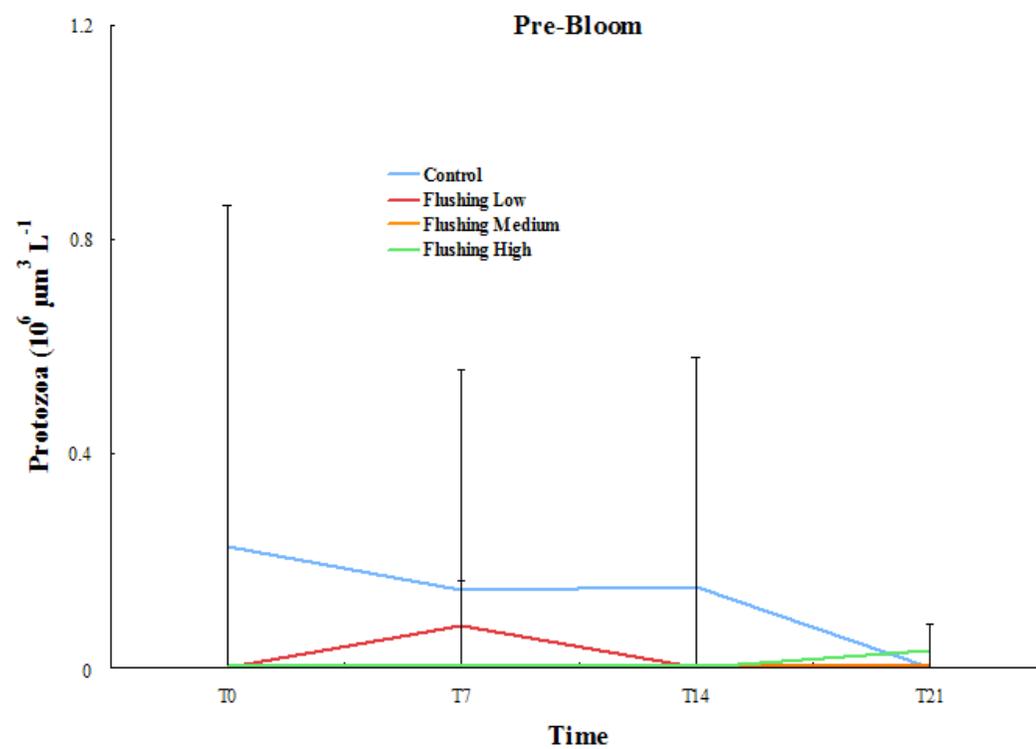
Figure 28: Protozoa biovolume during the pre-bloom experiment.

Figure 29: Cladoceran density during the pre-bloom experiment.

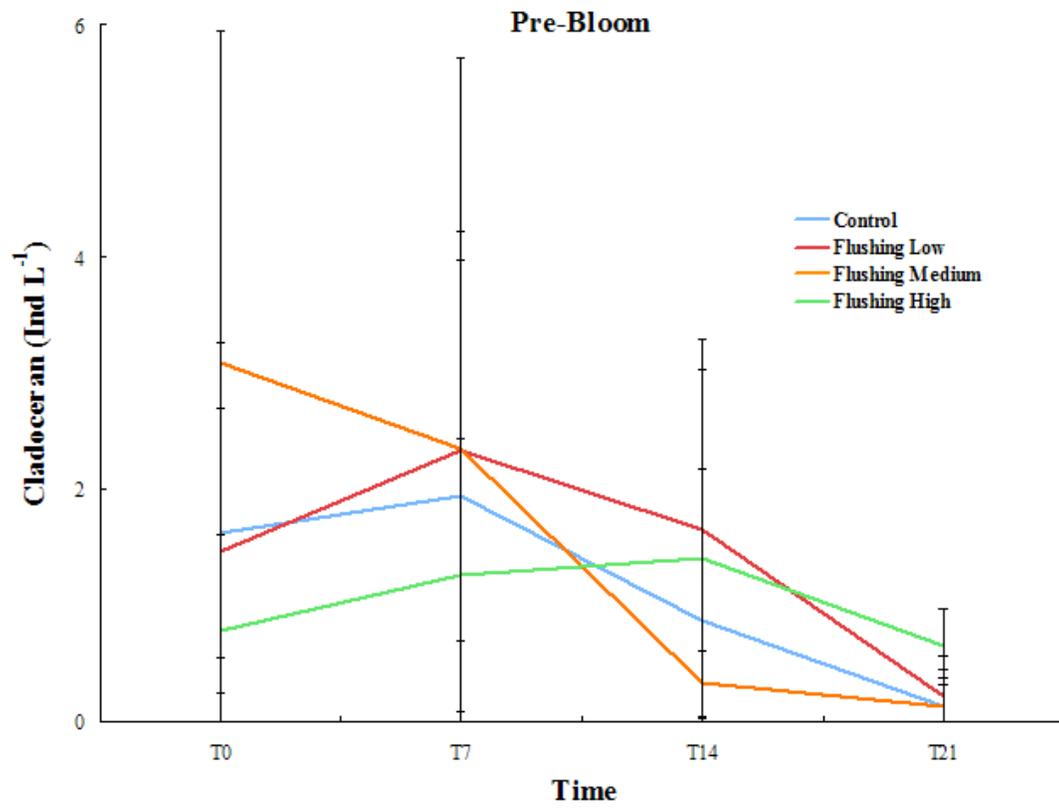
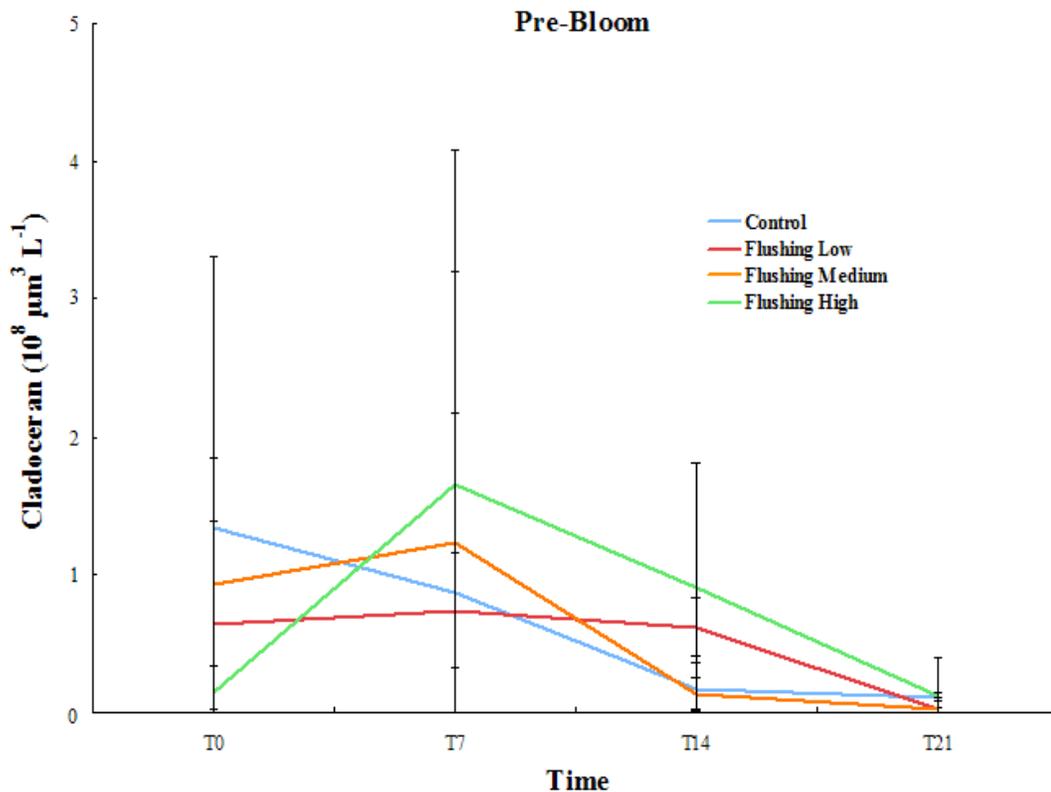
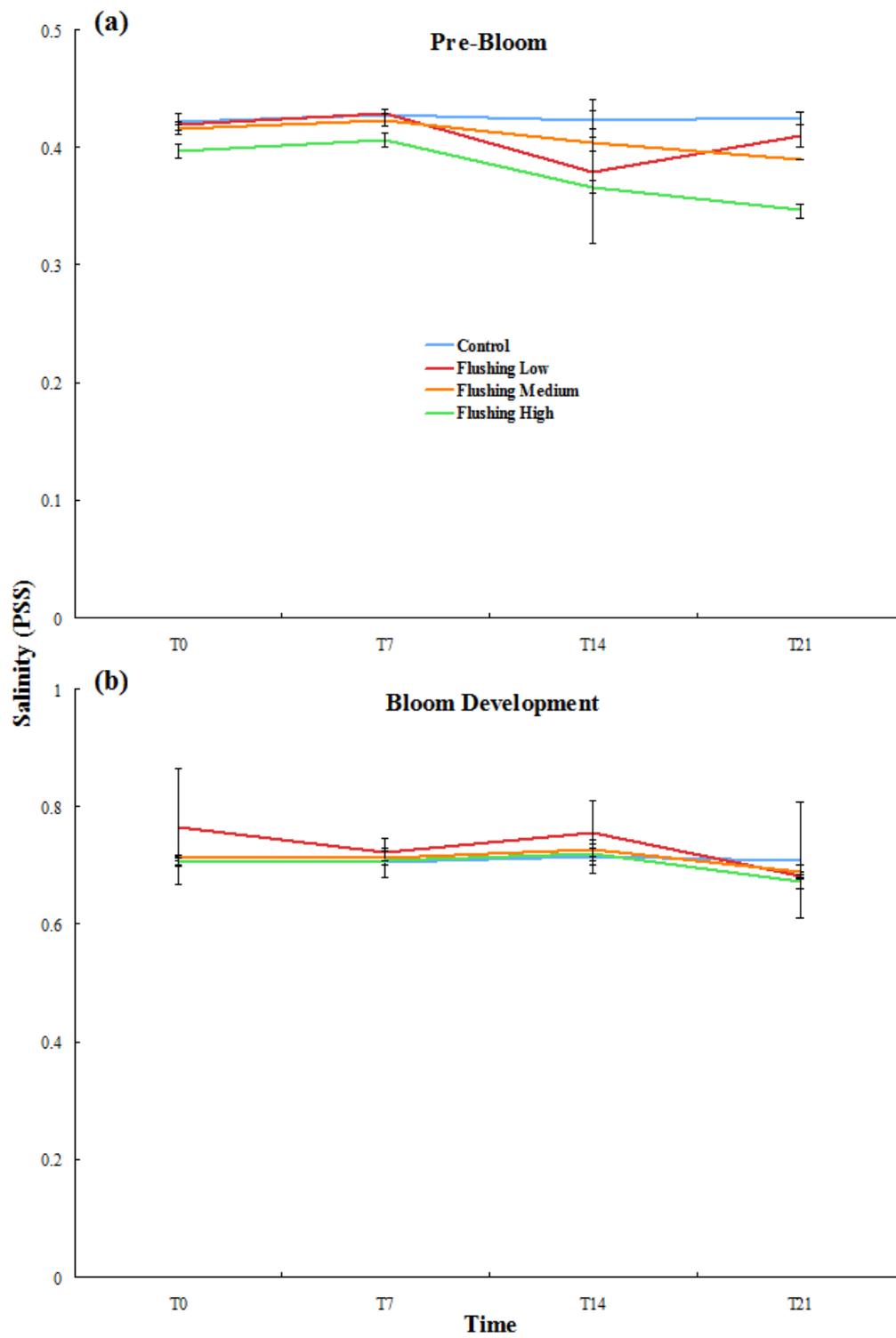


Figure 30: Cladoceran biovolume during the pre-bloom experiment.



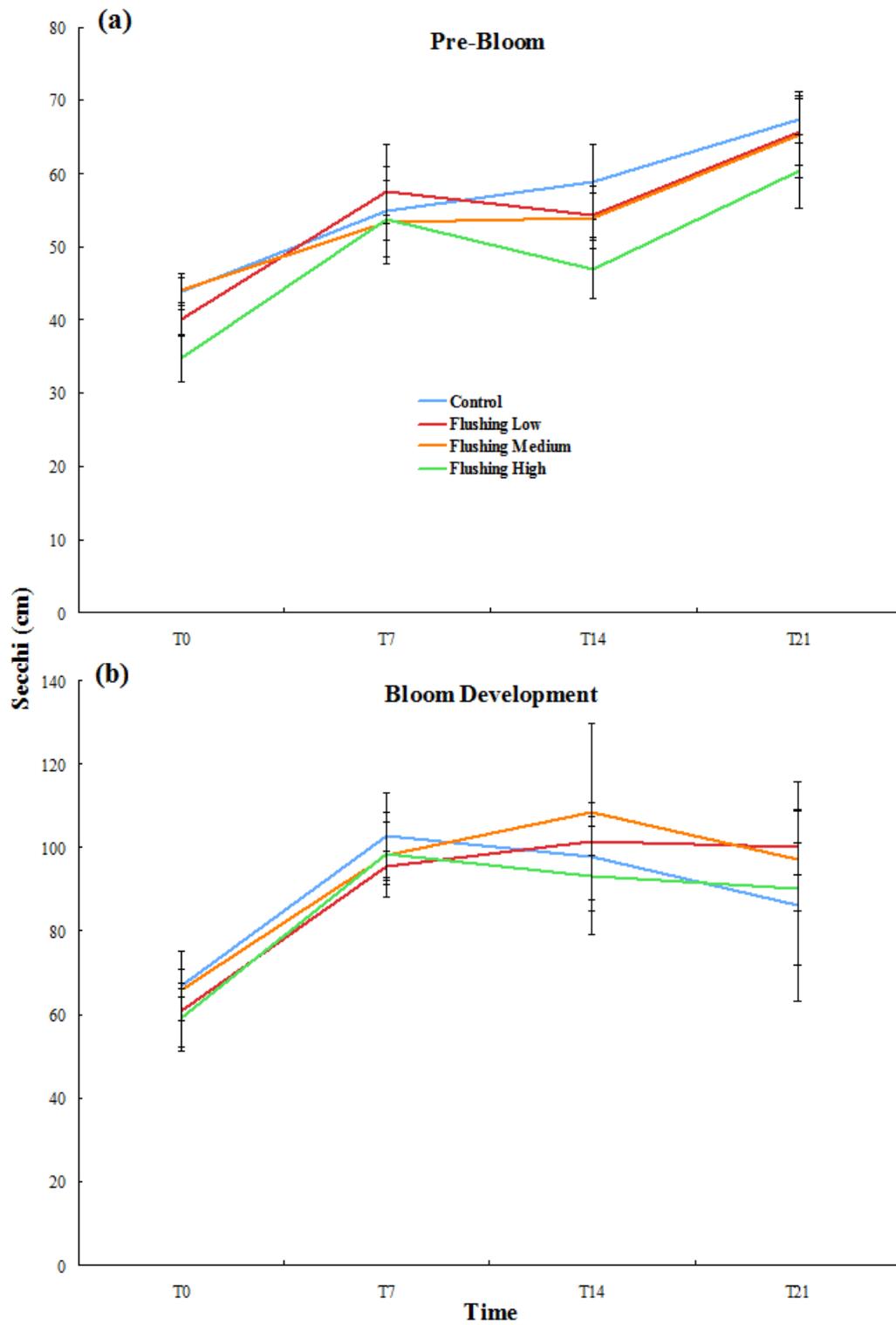
Analysis of abiotic water quality parameters showed little effect from experimental treatments. During the pre-bloom experiment, salinity was significantly lower in high and medium magnitude flushing versus the control (high P -value < 0.001, medium P -value = 0.035). Although, all salinity levels remained low throughout the experiment and high flushing had a salinity level of 0.34 PSS compared to the control, which was 0.43 at experiment completion (Figure 31a). There was no significant difference observed during bloom development and salinity levels remained consistent, ranging between 0.68 and 0.76 PSS (Figure 31b).

Figure 31: Salinity levels during pre-bloom (a) and bloom development (b) experiments.



Additionally, turbidity significantly increased with high flushing treatments (P – value < 0.001) in the pre-bloom experiment (Figure 32a). Secchi depth was consistently lower in high flushing compared to the control, but this could be in part due to sampling technique, which followed the flushing treatment and therefore, a great deal of mixing was occurring within the mesocosms, resulting in increased turbidity. This trend was not observed in the bloom development experiment; however, phytoplankton biomass was lower during this sampling period, resulting in decreased overall turbidity within the lake and mesocosms (Figure 32b).

Figure 32: Secchi depth during pre-bloom (a) and bloom development (b) experiments.



All other response variables, including temperature, pH, dissolved oxygen, and oxidation-reduction potential showed no significant deviation from the control. This indicates a benign effect of pulsed flushing on surface water quality. Similarly, there were no significant abiotic parameters throughout the bloom development experiment.

Water temperature did show a predictable seasonal warming trend during the pre-bloom experiment, ranging from 7-11°C (Figure 33*a*). Similarly, there was little fluctuation during the bloom development experiment; temperature remained between 15-19°C (Figure 33*b*). Throughout both experiments pH showed little fluctuation and remained between 8.0 and 9.1 (Figure 34*a, b*). Dissolve oxygen and oxidation-reduction potential were not effected by any level of flushing in either experiment (Figure 35*a, b* and 36*a, b*).

Figure 33: Ambient water temperature during pre-bloom (a) and bloom development (b) experiments.

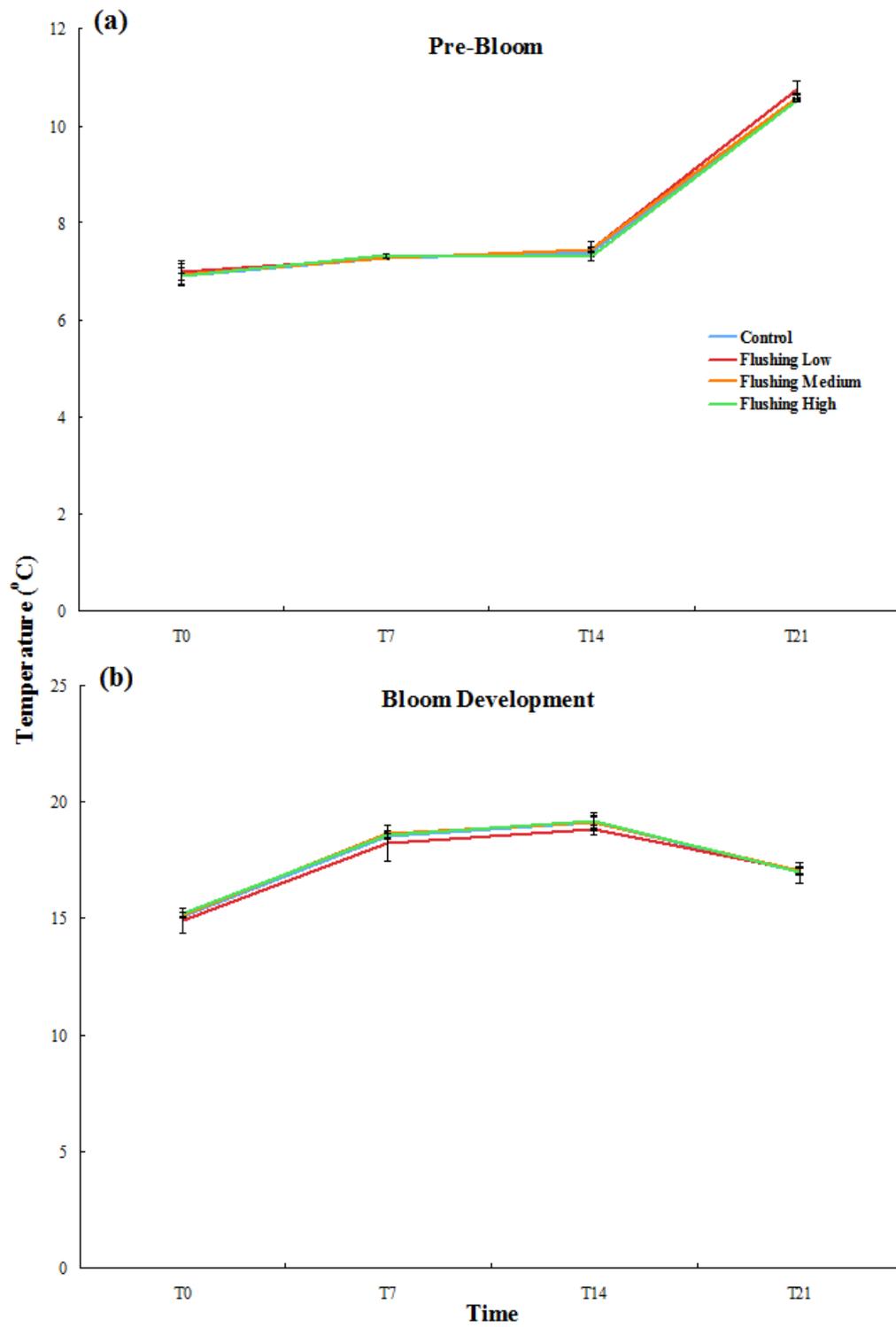


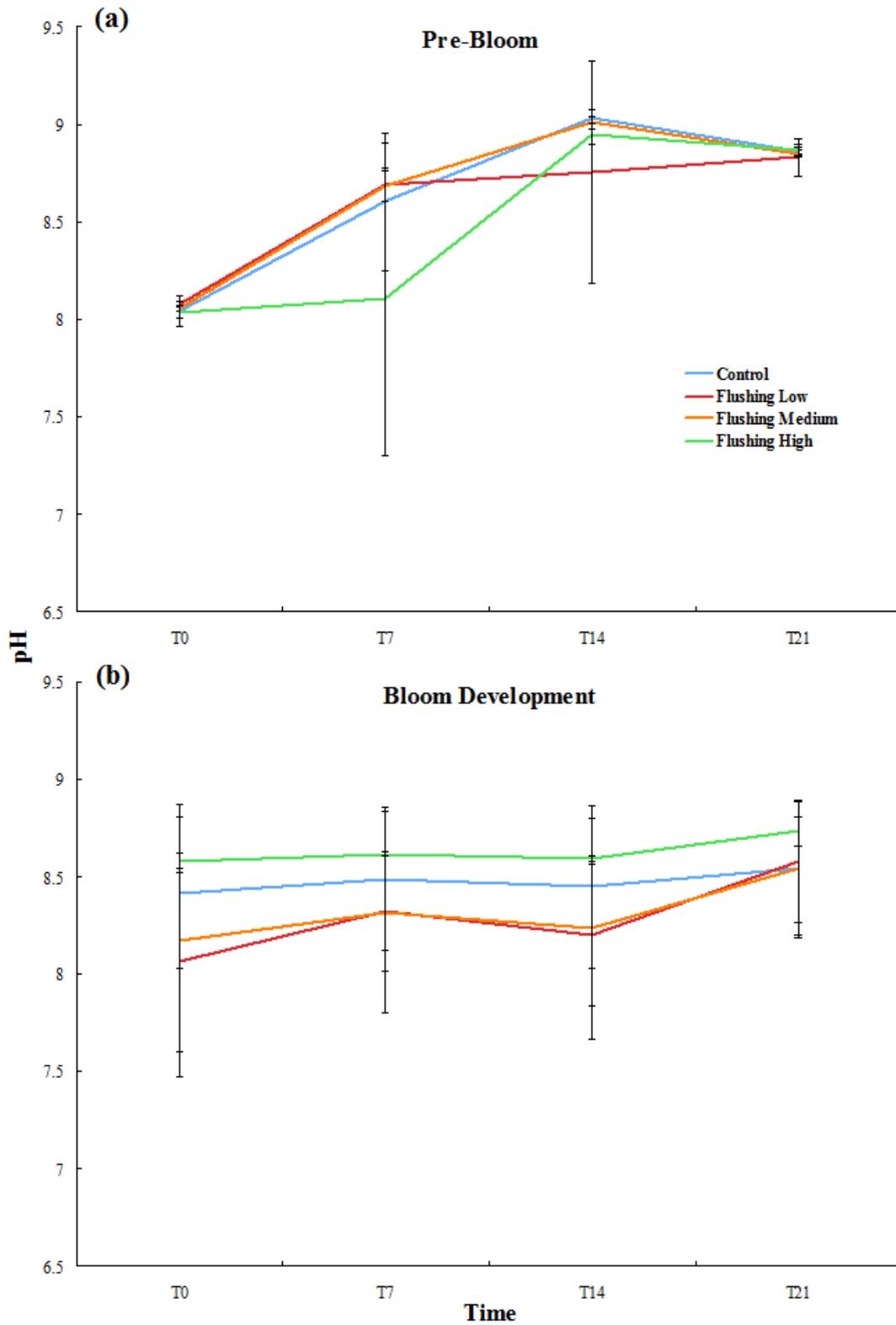
Figure 34: pH levels during pre-bloom (a) and bloom development (b) experiments.

Figure 35: Dissolved oxygen levels during pre-bloom (a) and bloom development (b) experiments.

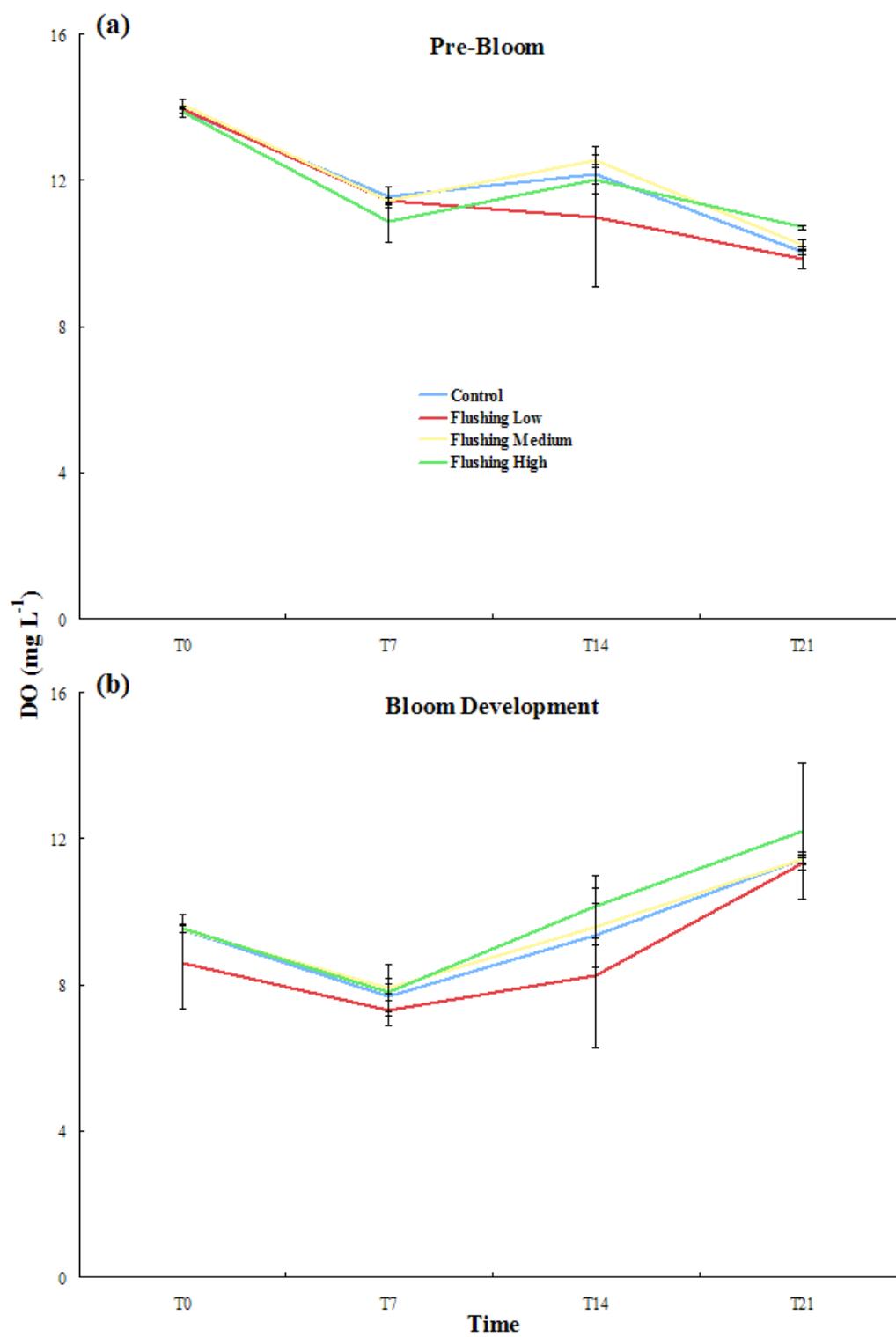
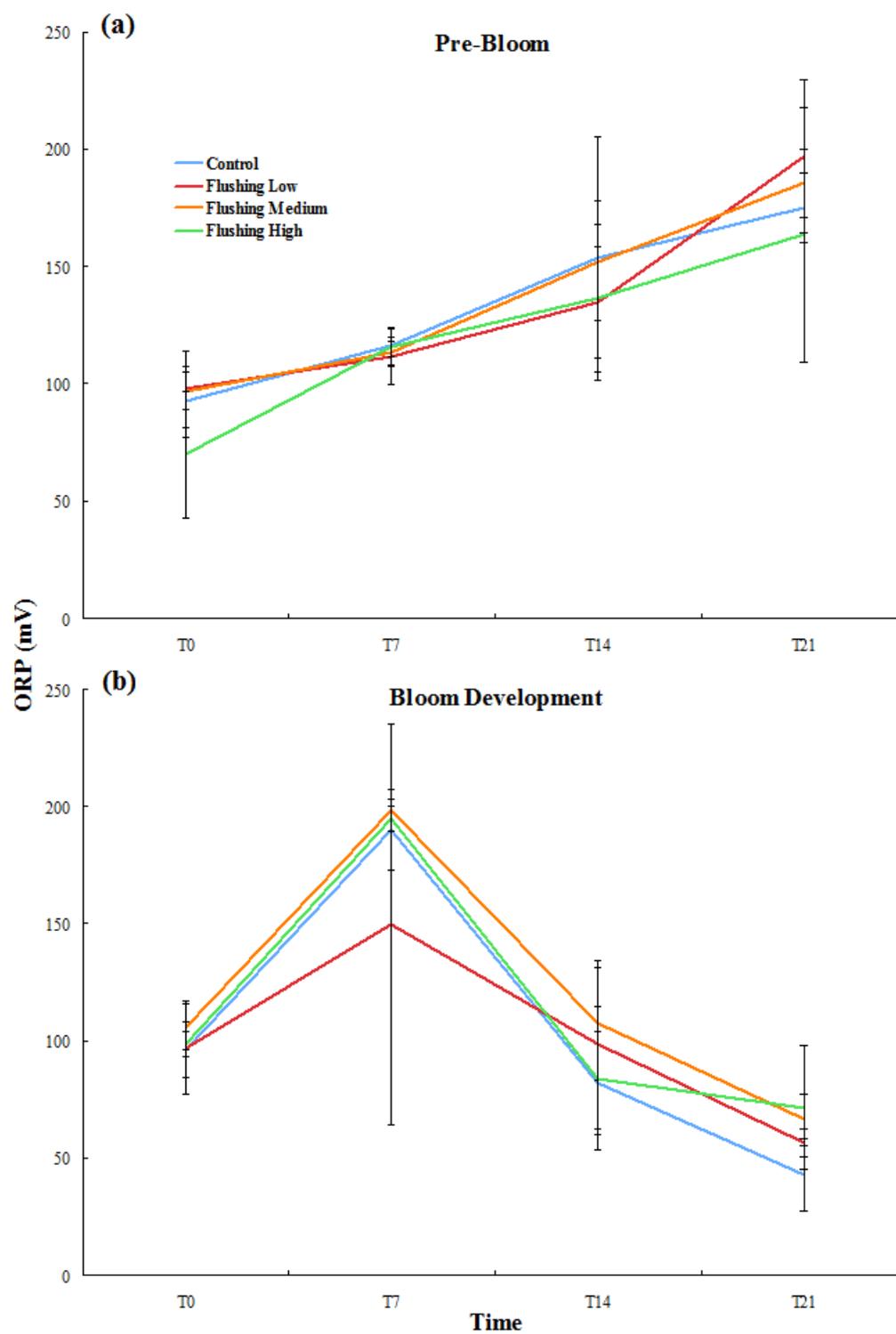


Figure 36: Oxidation-reduction potential during pre-bloom (a) and bloom development (b) experiments.



VITA

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