Report To

Central Davis Sewer Improvement District

2627 W Shepard Lane

Kaysville, UT 84039

(801) 451-2190

Analysis of Phytoplankton Nutrient Limitation in Farmington Bay and the Great Salt Lake

Dr. Wayne Wurtsbaugh

Ms. Amy Marcarelli

Department of Aquatic, Watershed & Earth Resources

Utah State University

Logan, UT 84322-5210

wurts@cc.usu.edu; 435-797-2584

amym@cc.usu.edu; 435-797-2517

June 18, 2004

Summary

Wurtsbaugh, W.A. and A. Marcarelli. 2004. Analysis of Phytoplankton Nutrient Limitation in Farmington Bay and the Great Salt Lake. Report to the Central Davis County Sewer Improvement District. 57 pp.

The Great Salt Lake is bordered to the south and east by a growing metropolitan area that contributes high nutrients to Farmington Bay. This large bay is eutrophic, and there is concern that continued increases in effluents from the Salt Lake City area could extend to impact the much larger, and currently less productive, Gilbert Bay. This study focused on determining how nutrient supplies might limit, and therefore control, algal populations in Farmington Bay and Gilbert Bay at different salinities. We tested both short and long-term responses of algal growth using laboratory nutrient addition bioassays in the summer and fall of 2003. Because some phytoplankton can alleviate nitrogen deficiency by fixing atmospheric nitrogen, we also determined how nutrients and salinity influenced nitrogen fixation.

Two types of assays were used in the analysis. To determine what nutrients currently control algal growth in Farmington Bay, four, week-long Simple Bioassays were used to measure how chlorophyll *a*, nitrogen fixation and algal biovolume responded to additions of nitrogen, phosphorus, or nitrogen+phosphorus. All four of these assays indicated that the algal population was stimulated by nitrogen and not by phosphorus. Additionally, nitrogen fixation rates by these N-limited populations were negligible. These results were consistent with earlier studies that showed nitrogen limitation of Great Salt Lake algae.

To understand how changes in nutrient loading and salinity might interact to control the algal population, particularly nitrogen-fixing cyanobacteria, two Factorial Bioassays were conducted. In these experiments salinities were varied from 1% to 10%, and nitrogen or phosphorus was added. Algal inocula from water bodies of varying salinity were introduced at the start of the experiments. In both of these assays, the algal populations were initially stimulated by nitrogen and not by phosphorus, as observed in the Simple Bioassays. However, at lower salinities, nitrogen-fixing cyanobacterial communities developed after 2-3 weeks, allowing the communities to overcome nitrogen limitation and become phosphorus limited. The two experiments differed, however, because in the first experiment, nitrogen-fixing communities developed in salinities up to 7%, whereas in the second experiment significant nitrogen fixation occurred only in the 1% salinity treatment. The upper limit for nitrogen fixation for the Great Salt Lake plankton community appears to be near 7%. Nutrients and salinity thus appear to interact to control whether nitrogen of phosphorus ultimately limits the abundance of phytoplankton in the lake. More experiments are needed to more precisely define the salinity range over which this interaction occurs, and to determine if these relationships hold under environmental conditions that more closely approximate those in the Great Salt Lake.

Introduction:

Eutrophication in Farmington Bay – The Great Salt Lake is bordered on its eastern and southeastern shores by the greater metropolitan area of Salt Lake City. The population within the watershed is currently 1.4 million, and it is expected to grow to five million by 2050 (Utah Governor's Office of Planning and Budget, 1992). Portions of the Great Salt Lake are receiving high nutrient loading and eutrophication is severe. Agricultural sources of nutrients are thought to be the leading factor degrading stream water quality in the basin (NAWQA; Baskin et al. 2002), but the domestic and industrial wastes of the entire Salt Lake metropolitan area also flow into this terminal basin.

Farmington Bay receives a large portion of this nutrient loading via the Jordan River and from sewage canals. Wetlands at the southern end of the bay intercept and process an undetermined portion of the nutrients, but nutrient loading rates to the bay remain high. In a preliminary estimate, Gross (2001) calculated that phosphorus loading to the bay was ten-times greater than that necessary to cause the bay to be eutrophic (160 mg P m^{-2} y^{-1}; Wetzel 2001). Chadwick et al. (1986) also predicted excessive phosphorus loading to the bay that would promote extremely high algal populations. Because Farmington Bay is enclosed by Antelope Island on its western side (Figure 1) and by an automobile causeway to the north, pollutants can concentrate in the bay. The bay is also shallow, so nutrients are not diluted into a large volume of water and may easily recycle between the sediments and water column. Chlorophyll levels (a measure of algal abundance) in the bay frequently exceed 100 µg L^{-1} and Secchi depth transparencies normally range from 0.1 - 0.2 m (Marcarelli et al. 2003).

The impact of this eutrophication on the Farmington Bay ecosystem is currently being addressed by the Farmington Bay Water Quality Working Group convened by the Utah Division of Water Quality. Potential impacts of the eutrophication include toxic algal blooms, impaired recreational use, low oxygen levels, and odor. Toxic algal blooms could impact brine shrimp populations directly. The high densities of algae could also indirectly affect biota by producing considerable organic matter. When this organic matter decomposes, oxygen is removed from the water, causing anoxia. Measurements of dissolved oxygen in the bay during the summer indicate that there are high oxygen levels during the day when algal photosynthesis is active, but oxygen concentrations decline to near zero at night when photosynthesis stops but algal and bacterial respiration continues. Low oxygen levels are exacerbated because a portion of Farmington Bay is underlain by a high-density salt wedge that enters from Gilbert Bay. This salt wedge mixes

infrequently and is not in contact with the atmosphere. Consequently, the decomposition of organic matter in this layer may cause prolonged anoxia there (Wurtsbaugh & Marcarelli 2004a).

Under anoxic conditions, the oxidation-reduction potential (redox) is lowered, allowing abundant sulfates in the water and sediments to be reduced to hydrogen sulfide (H_2S). This gas possesses an odor similar to rotten eggs and is likely a source of the odor problems affecting Salt Lake City (Israelsen et al. 1985). Hydrogen sulfide has been linked to odor problems in cities located near eutrophic estuaries and other bodies of saline water (e.g., Muezzinoglu 2000). Hydrogen sulfide is directly lethal to many organisms in the range of 1 - 5 mg / L (Watts et al. 2001), levels reached in the salt wedge of Farmington Bay (Wurtsbaugh and Marcarelli 2004a). Additionally, if hydrogen-sulfide containing water from a brine layer is mixed into the water column, the hydrogen sulfide can react with oxygen and deplete oxygen concentrations, causing complete, prolonged anoxia. In the Salton Sea, Watts et al. (2001) linked the combined effects of toxicity and anoxia caused by hydrogen sulfide oxidation to mass die-offs of phytoplankton, zooplankton, and fish. Wurtsbaugh and Marcarelli (2004) observed a storm-caused deoxygenation event of this type in Farmington Bay, but it is not known how plankton populations responded. It is possible that brine shrimp and brine flies could be impacted by frequent anoxia, even though these organisms are tolerant of low oxygen conditions (Bassett 2003; Kling 2003). If these brine shrimp and brine flies are negatively impacted by eutrophication, it could in turn impact bird populations that rely on these organisms for food during their annual migration.

Eutrophication is not restricted to Farmington Bay. NASA images show plumes of chlorophyll-rich water extending miles from the bay into the main lake (http://earth.jsc.nasa.gov/). The impact of this algal plume on the main lake is unknown, but due to high dilution rates, it is possible that the current nutrient loading enhances phytoplankton populations and, in turn, the brine shrimp that feed on the algae. However, with increasing population growth there is concern that potential negative impacts of eutrophication may extend from Farmington Bay and into the main lake, where it could impact the brine shrimp harvest that contributes $80 million to the Utah economy annually.

Water quality in the Great Salt Lake has received only limited attention during the past 30 years (e.g., Carter 1971; Coburn and Eckhoff 1972; Sorensen et al. 1988), but State and Federal agencies are increasingly addressing water quality concerns (Naftz et al. 2000). A USGS NAQWA study of water quality in the basin's rivers and groundwater was begun in 1997 (Baskin et al. 2002). In 2003, the USGS initiated a 10-year plan to study physical and chemical aspects of

the Great Salt Lake (Goddard et al. 2002). This project is currently using stable isotope analyses to determine nitrogen sources to the lake, and it will begin measuring nutrient loading to the lake in 2004. The Utah Division of Water Quality will soon begin measuring phosphorus and nitrogen loading to Farmington Bay, and TMDL development may proceed in 1-2 years if beneficial uses are found to be impaired. However, before a TMDL estimate can be made for the Great Salt Lake, it is critical that we understand what nutrient(s) limit algal production in Farmington Bay and the Great Salt Lake, as this important factor will dictate what management approaches should be used to improve water quality.

Control factors and nitrogen fixation--Nitrogen is believed to control primary production in estuaries, coastal oceans (Paerl 1996), and most saline lakes (Javor 1989), whereas algal growth in fresh waters is more frequently limited by phosphorus. However, many bioassays and whole-lake experiments have shown nitrogen to be limiting in lakes and streams as frequently as they are limited by phosphorus (Fee 1979; Elser et al. 1990; Francoeur 2001). Schindler (1977) argued that nitrogen should never limit production in lakes because nitrogen-fixing cyanobacteria should be able to make up nitrogen deficits so that phosphorus becomes the controlling nutrient (Figure 2). Consequently, the question of nitrogen versus phosphorus limitation could be restated to ask what factor(s) limit nitrogen fixation in aquatic systems. Despite its importance, the factor(s) that limit nitrogen fixation in both fresh and saline waters are poorly understood (Vitousek et al. 2002). In some saline systems, iron or molybdenum supplies (Wurtsbaugh & Horne 1983; Howarth and Cole 1985; Evans & Prepas 1997), or zooplankton grazing coupled with low cyanobacterial growth rates (Marino et al. 2002) may be important, but it is unclear how broadly applicable these control mechanisms are.

Previous bioassays have indicated that plankton in the main basin of the Great Salt Lake are nitrogen limited (Porcella and Holman 1972; Stephens & Gillespie 1976; Wurtsbaugh 1988), but the factor(s) controlling nitrogen fixation are not understood. A preliminary experiment in our laboratory indicates that salinity and nutrients interact to control nitrogen fixation, and thus maintain the lake in a N-limited state. At salinities of 3%, cyanobacteria became abundant and fixed nitrogen so that phosphorus was the limiting nutrient. However, at salinities of 6 and 13%, nitrogen-fixing cyanobacteria did not become established in the Great Salt Lake water, and nitrogen remained the limiting nutrient (Lester 2003). The experiment suggested that nitrogen-fixation cyanobacteria may not function at higher salinities, although the mechanism behind this remains unclear.

If these results are confirmed, it would indicate that Great Salt Lake could be P-limited during low-salinity periods in the estuary (Farmington Bay), and N-limited at other times or places. Salinity controls on nitrogen-fixing cyanobacteria have been reported by others (Potts 1980; Dubinin et al. 1992; Fernandes et al. 1993; Pinckney et al. 1995; but see Moisander et al. 2002), and some argue that increasing the sulfate content of the water inhibits molybdenum uptake, and consequently, nitrogen fixation of cyanobacteria (Howarth & Cole 1985; Stal et al. 1999; Marino et al. 2002). However, Wurtsbaugh (1988) found that lowering the $SO_4^=$:Mo ratio did not stimulate planktonic growth or nitrogen fixation in the Great Salt Lake. Evans and Prepas (1997) argue that high salinities (or alkalinities) inhibit iron uptake and thus restrict nitrogen fixation. Recently, Mills et al. (2004) performed bioassay experiments indicating that low iron and phosphorus supplies simultaneously limit nitrogen fixation in the oceans. Despite these advances, the factor(s) controlling plankton growth and nitrogen fixation in hypersaline systems remains elusive.

With the current high nutrient loading to Farmington Bay, it is possible that the phytoplankton there are not nutrient limited at all, but rather are limited by light. During much of the year, Secchi depths range from 0.1 to 0.2 m in Farmington Bay (Wurtsbaugh and Marcarelli 2004b), suggesting that there is sufficient light for primary production in only the top 0.2-0.4 m of the water column. However, if nutrient loading to the bay was reduced and algal populations decreased sufficiently to increase water clarity and light penetration, then the system would eventually become nutrient limited. Therefore, even though the bay may now be limited by light and not nutrients, it is important that we determine what nutrient(s) would limit algal production in the bay and in the Great Salt Lake. Consequently, the purpose of our study was to conduct experiments in the laboratory to determine whether nitrogen or phosphorus control phytoplankton growth and nitrogen fixation in Farmington Bay and the Great Salt Lake, and to examine how nutrient limitation is affected by different salinities found in Farmington Bay and Great Salt Lake during a year.

Methods

Two types of assays were used: *simple bioassays* to evaluate the extant nutrient status of the phytoplankton communities, and *factorial bioassays* that tested how the phytoplankton community responded to both different salinities and nutrients during month-long incubations (Table 1).

Simple bioassay design – Simple bioassays, where only nutrient levels were manipulated, were initiated on four dates: 6 Jun 03, 29 Aug 03, 9 Oct 03 and 4 Nov 03. Water was collected either from the mid-station in the central region of Farmington Bay (Station 3 or Station 2, depending on

water levels, Figure 1) or from the causeway when low water levels prevented boat passage through the causeway breach (4 Nov 03). Additionally, water was collected in Gilbert Bay (N 41º 03.363, W 112º 19.201) on 29 Aug 03 to conduct a comparison of nutrient limitation between the two bays. Water was collected with an 8-L horizontal Van Dorn bottle from 0.5-m depth and transported to the laboratory facility in Logan in 10-L polyethylene containers.

In the laboratory, macrozooplankton were removed by filtering through 153-µm Nitex netting, and twelve, 800-mL aliquots of water were randomly distributed to 1-quart glass jars. Jars were then randomly assigned to four treatments: control, +nitrogen, +phosphorus, +nitrogen+phosphorus. Concentrations for the treatments were 1400 µg/L nitrogen (added as NH_4NO_3) and 200 µg/L phosphorus (added as Na_2HPO_4). Nutrients were added to each non-control treatment from a stock solution and mixed immediately. Jars were then placed randomly in a temperature controlled incubation room at 20ºC, with light intensities of approximately 150 µE / m^2 / sec and an 18:6 light:dark photoperiod. Experiments were run for 6 days, except for the 12 Oct 03 experiment, where the experiment was lengthened to 26 days to determine long-term algal biomass responses to nutrient enrichment. Jars were agitated twice daily and re-randomized to ensure even light distribution once daily. Each jar was sampled after 3 and 6 days to examine algal responses to enrichment.

Factorial bioassay design – Factorial bioassays, where nutrient and salinity levels were manipulated were initiated on two dates: 3 Jul 03 and 9 Oct 03. Water was collected as above from the mid-station in the central region of Farmington Bay (Station 2 or 3, Figure 1). Salinities used in the experiments ranged from 1%, a low concentration where cyanobacterial nitrogen fixation is possible, to the concentration in Farmington Bay at the time of each experiment, where it was hypothesized that nitrogen fixation was impossible (Table 1).

In the factorial experiments, an aliquot of water collected from Farmington Bay was diluted with either distilled water or with saline water to provide the desired salinities. The same aliquot volume of source water was used in all of the salinity treatments of the experiment. In both experiments, water collected in Farmington Bay was filtered in the lab through 153µm Nitex netting and 111-mL (Experiment A) or 80-mL (Experiment B) of Farmington Bay water was added to 36 1-quart glass jars. Additionally, 2.3 mL of supplementary inocula water from low-salinity sites in Great Salt Lake and surrounding wetlands and from a high-salinity site in Gilbert Bay was added to each jar to insure that a variety of phytoplankton with different salinity

tolerances were present at the start of the experiment (Table 2). Jars were randomly assigned to salinity treatments, then the Farmington Bay water was diluted to 800-mL using dionized water or different salinity mixtures (made with $MgSO_4$ and NaCl in dionized water) to reach the desired end salinity. The nine jars within each salinity treatment were then randomly assigned to three nutrient treatments: control, +nitrogen and +phosphorus. Nutrients were added to the non-control treatments as described above. After nutrient enrichment, the 27 jars were placed randomly in a temperature controlled incubation room at 20ºC, with light intensities of approximately 150 µE / m^2 / sec and an 18:6 light:dark photoperiod. Experiments were incubated for 28-30 days, and were sampled approximately every 7 days. Jars were agitated and randomized as described above.

Sample analysis – On sampling days for both types of experiments, 50-mL aliquots of water were collected from each sample jar using a 60-mL polyethylene syringe with a large tip opening. This aliquot was transferred to a 62-mL glass serum vial and sealed with a septum for nitrogen fixation analysis. N-fixation was measured using an acetylene reduction assay (Stewart et al. 1967; Flett et al. 1976). This is an indirect method for estimating nitrogen fixation where the biota is saturated with acetylene gas, which is converted to ethylene gas at a rate related to the potential nitrogen fixation rate. Once in the serum vial, samples were injected with acetylene and incubated for 2-hours in the incubation chamber where the bioassay was conducted. Standards containing known concentrations of ethylene were also run. At the end of the incubation, gas samples were collected in cleaned and re-evacuated 3-mL Vacutainers©. Ethylene and acetylene in each sample were measured at a later date using a SRI 8610 gas chromatograph equipped with a Poropak T column and a flame ionization detector (Capone 1993). Standards were used to construct a standard curve, which unknown samples were then compared against to determine the amount of ethylene in each sample. Ethylene concentration was converted to amount of nitrogen gas fixed using an assumed 3:1 molar ratio (Capone 1993).

Overall algal biomass was estimated using chlorophyll *a* analyses. An aliquot (usually 10-mL) was removed from the serum vial after termination of the acetylene reduction assay and filtered through a 25-mm Millipore AP 40 glass fiber filter. The filter was wrapped in tin foil and immediately frozen to prevent sample degradation until analysis (less than 30 days). To measure chlorophyll *a,* filters were extracted in 95% ethanol and chlorophyll *a* concentration was measured fluorometrically using a non-acidification technique (Welschmeyer 1994).

Phytoplankton were collected from one replicate of each treatment at the beginning and end of each experiment. Approximately 40-mL of sample was preserved with Bouin's solution (80% formaldehyde, saturated with picric acid; 20% glacial acetic acid)**.** Phytoplankton cell density was determined by settling and counting samples in Utermöhl chambers on an inverted microscope at 400 or 1000X (Wetzel and Likens 2000). Phytoplankton were identified to the lowest taxonomic group possible using Felix and Rushforth (1979), usually genus or species. Because algal volumes can vary immensely between species, and because many ecological processes are more dependent on biovolumes than on densities, we also estimated the volume of each taxon. Length and width measurements were made on 10 individuals of each taxa and biovolumes were calculated using equations in Hillebrand et al. (1999).

Results were analyzed graphically both as simple treatment responses and as responses relative to control treatments. Percent of control responses were calculated using the following equation:

% of control = [(treatment value – control value) / control value] *100

Treatment effects were analyzed statistically using one way ANOVAS for the simple bioassays, and two way (treatment x salinity) ANOVAS using SAS v. 8e. Responses to bioassay were analyzed graphically. Differences due to treatments were determined using post-hoc Tukey tests. Results were analyzed separately on each day of the experiment. When transformations were necessary to meet the assumptions of ANOVA, a logarithmic transformation was used for chlorophyll *a* data, and a cube root transformation was used for nitrogen fixation data. These transformations were the most appropriate transformations selected from a range of transformations that allowed the assumptions of the statistical analyses to be met.

Results:

Simple Bioassays — Similar responses to nutrient additions were obtained in all four simple nutrient addition experiments. For simplicity, the results of Experiment 2, where nutrient limitation was measured in both Farmington and Gilbert Bays, will first be discussed, and then related to results observed in all of the nutrient addition bioassays.

In Experiment 2, initial chlorophyll *a* concentrations were extremely high (>350 µg/L) in water collected in Farmington Bay, indicating that algal levels were elevated prior to the initiation of the experiment. Nevertheless, a significant increase in chlorophyll *a* was observed within three days when N or N+P were added to the cultures ($F = 9.32$, $p = 0.005$), indicating strong N-limitation of the algal community (Figure 3). However, by day 6 of the experiment the response had subsided, and there was no significant treatment effect on this data ($F = 1.86$, $p = 0.21$). The subsidence of chlorophyll levels after six days in Farmington Bay was unusual among all of the nutrient-limitation bioassays: in all other experiments, N limitation was strongly indicated by chlorophyll *a* values on day 6 (see below).

In Experiment 2, initial chlorophyll levels in Gilbert Bay were much lower, but a response to nitrogen (or N+P) was also evident after 6 days (Figure 3). There was no significant difference between treatment observed on day 3 ($F = 0.67$, $p = 0.60$), but there was significantly higher chlorophyll *a* in the N and N+P treatments on day 6 ($F = 14.41$, $p =0.001$). Additionally, chlorophyll levels on days 3 and 6 in the *control* treatments were also significantly higher than at the start of the experiment, suggesting that some factor other than nutrients was suppressing the algal population in the Lake. Brine shrimp biomass at this time in Gilbert Bay was high (Wurtsbaugh and Marcarelli 2004b), so it is possible that grazing pressure was controlling algal populations in the lake, and this effect was eliminated in our experiment, resulting in an increase in algal populations in the control treatments.

Responses of algal cell density and biovolume to nutrients were not as striking as were the responses of chlorophyll *a* during Experiment 2. In treatments utilizing Farmington Bay water, cell densities were greatest in the P treatment, almost entirely due to the abundance of a chrysophyte species (Figure 4a; Appendix 3). However, greatest biovolume was observed in the NP treatments, where biovolume was dominated by the chlorophyte *Carteria* sp., with additional chlorophyte biovolume contributed by *Dunaliella salina, Dunaliella viridis*, and *Oocystis* sp. (Figure 4b; Appendix 4). In contrast, little difference in cell density or biovolume was observed between the treatments in Gilbert Bay, all of which were dominated by the small *Dunaliella viridis*. The different density and biovolume responses observed may indicate that when Great Salt Lake algae are presented with excess nutrients, they increase productivity by increasing the productivity of individual cells, rather than by cellular reproduction or growth. Therefore, responses in chlorophyll *a* were observed, but no differences in cell density or size.

Similarly, chlorophyll *a* and biovolume responses to nutrients were observed in all of the simple bioassays, and in the initial responses of the factorial assays (Figure 5; Appendix 1). Nitrogen and N+P treatments routinely stimulated chlorophyll *a*, with the strongest responses (100 – 250% greater than control treatments) observed in +N treatments alone (Figure 5a). Low, negative responses were usually observed in phosphorus treatments. Generally, algal biovolume decreased relative to control treatments in both the nitrogen and phosphorus treatments, and had small positive or negative responses in the NP treatments (Figure 5b; Appendix 4). Nitrogen fixation showed very small and inconsistent responses to nutrient additions in the simple bioassay experiments, and fixation rates were routinely near or below the level of detection of the acetylene reduction assay used (Figure 5c; Appendix 2). The lack of nitrogen fixation responses in these short-term assays was expected, given that source salinities were high, and few, if any, nitrogen-fixing taxa were present. In Experiment 3 we tracked chlorophyll concentrations for 26 days to see if the response to the nutrients would change through time. Chlorophyll levels increased in controls, +N, +P, and N+P treatments through day 16-13, and then declined thereafter. Chlorophyll was stimulated most throughout the experiment by the addition of nitrogen or nitrogen plus phosphorus (Appendix 10).

Factorial Bioassays – Very different results were obtained in the two long-term factorial bioassay experiments. In experiment A at the lowest salinity (1%), there were only small responses of chlorophyll *a* in the nutrient treatments (Figure 6; Appendix 5). Nitrogen significantly stimulated chlorophyll *a* production after 9 days (Two-way ANOVA, $F = 14.19$, $p < 0.0001$), similar to the simple nutrient bioassays (Figure 5), and phosphorus stimulated chlorophyll levels significantly after 23 days ($F = 5.09$, $p = 0.0004$). A switch between nitrogen and phosphorus limitation occurred somewhere between these two dates in all of the salinity treatments. Statistical analysis of the chlorophyll data on day 16 of the experiment was marginally non-significant ($F = 2.00$, $p = 0.076$), with no salinity response observed and no difference between nitrogen and phosphorus treatments. This ambiguity represents a switch between initial nitrogen limitation and ultimate phosphorus limitation where both nutrients appear to be important and not different statistically.

In Experiment A, nitrogen fixation was initially below limits of detection, but rates increased both in control treatments and particularly in phosphorus treatments during the long incubation (Figure 6, right; Appendix 6). In the control treatments, nitrogen fixation rates increased most in the 1% and 3% treatments, with limited increases at 5% and 7% salinities. In the phosphorus

treatments, fixation rates were markedly and significantly greater than in control treatments on all sample dates, as indicated by two-way ANOVA analyses (Day 9: $F = 27.99$, $p < 0.0001$, Day 16: $F = 19.11$, $p < 0.0001$, Day 23: $F = 9.27$, $p < 0.0001$, Day 30: $F = 10.29$, $p < 0.0001$). At 1% salinity, peak fixation rates were observed by day 15, but at higher salinities the peak was delayed to day 23 (3% and 5%), or day 30 (7%). This suggests that cyanobacterial dominance was delayed at higher salinities (Figure 6). The relatively high fixation rate in the +P, 7% salinity treatment was due to a high rate in only one of the three replicates—the remaining two replicates had fixation rates near zero (Appendix 6). The increasing rate of nitrogen fixation with time in different nutrient treatments is further supported by algal density and biovolume measured on day 30 of the experiment, where cyanobacteria comprised 60-80% of the algal biovolume in P treatments at all salinities (Figure 7 a, b). *Nodularia* sp. was the dominant heterocystous cyanobacteria present (Appendix 7, 8), but the non-heterocystous nitrogen fixing *Microcoleus* sp. also became abundant by the end of the experiment, particularly in the +P treatment. Heterocysts are specialized cells where nitrogen fixation occurs. Nitrogen additions to the cultures suppressed nitrogen fixation (Figure 6) and cyanobacterial abundances (Figure 7). This response was expected because an abundant nitrogen source allows other, non-fixing species of algae, to thrive, thus out competing cyanobacteria for phosphorus or other nutrients.

Phytoplankton in Factorial Experiment B also showed initial and significant chlorophyll *a* responses to N additions compared to other nutrient treatments in all salinities ($F = 6.92$, $p < 0.001$; Figures 5, 8). In the nitrogen treatment, chlorophyll increased from 18 µg/L to over 80 µg/L by day 7. In contrast to Experiment A, this N response remained significantly greater than in the other treatments for the duration of the experiment (Day 14: $F = 8.17$, $p < 0.0001$, Day 21: $F = 13.66$, $p < 0.0001$, Day 28: $F = 12.2$, $p < 0.0001$), with chlorophyll *a* decreasing after day 7. Chlorophyll *a* concentrations also responded somewhat to phosphorus additions, but only in the 1% and 4% salinity treatments. Responses of nitrogen fixation to phosphorus additions were limited in the 1% salinity treatment (day 14, 21). The low nitrogen fixation response was consistent with the plankton species analyses on day 28, where cyanobacteria were present but did not dominate the algal community as observed in Experiment A (Figure 9). The cyanobacteria present at the end of this experiment were primarily *Microcoleus* sp. (Appendix 7, 8).

The different chlorophyll responses to nutrient additions in the factorial experiments appear to be driven by the presence or absence of nitrogen fixation in the treatments (Figure 10). In

Experiment A, the greatest stimulation in chlorophyll *a* occurred in phosphorus treatments at low salinities, and nitrogen fixation rates were also consistently high at the intermediate salinities. In contrast, nitrogen fixation rates in Experiment B were only weekly stimulated above control treatments by phosphorus additions, and nitrogen limitation of chlorophyll levels was maintained for the duration of the experiment at all salinities (Figure 10).

Nitrogen fixation rates in the factorial experiments appear to have been limited to salinities of 7% or less (Figure 11). When maximum fixation rate measurements from the control and +phosphorus treatments for the two experiments are combined, it is clear that: (1) fixation rates in phosphorus treatments were always higher than controls; (2) that rates were much higher in Experiment A than in Experiment B, and; (3) that maximum fixation rates were relatively independent of salinity between 1 and 7%, but that no fixation was observed at 10%.

Discussion:

The short-term bioassays demonstrated that the natural phytoplankton populations in both Farmington Bay and Gilbert Bay were nitrogen limited. The phytoplankton always responded to nitrogen additions within 3–8 days by incrementing chlorophyll levels, often as much as 150–250% above controls. Algal density and biovolume, did not, however, respond consistently to the nutrient additions in the short-term bioassays. The increment in chlorophyll is often described as a “greening-effect”, and the net result is that the phytoplankton and cyanobacteria are better able to capture sunlight, and thus increase photosynthesis. Plankton in our month-long factorial experiments did increment biovolumes in response to nitrogen additions, at least in the higher salinities. The biovolume response to nitrogen might have been higher had we counted algal samples when chlorophyll levels peaked (usually on day 7 or 9), rather than at the end of the experiment when the chlorophyll data indicated that algae in the nitrogen treatments had usually declined to control levels.

Nitrogen limitation has been found in previous studies of Great Salt Lake phytoplankton. Stephens and Gillespie (1976) found that densities of *Dunaliella* sp. increased in response to nitrogen, but not phosphorus additions in laboratory cultures of Gilbert Bay water (salinity 13.5%). Porcella and Holman (1972) also found a positive response of *Dunaliella* to nitrogen and not to phosphorus when salinity in Gilbert Bay was near 16%. This study used EPA Algal Bottle Bioassay tests, but it is not clear from their report whether they were reporting turbidity

measurements (surrogate for algal density) or chlorophyll levels. Wurtsbaugh (1988) tested Gilbert Bay water during high water years (1985 – 1986) when salinities were 5% and found that chlorophyll concentrations responded significantly to nitrogen additions, but only marginally to phosphorus additions in 8-day bioassays similar to those described here. Post and Stube (1988) found that the microbial community in the north basin of the lake (Gunnison Bay), where salinities were >30%, was also nitrogen limited. Moreover, Javor's review (1989) of the literature on saline lakes indicates that most saline lakes are nitrogen limited.

In most of the short-term bioassay experiments that have been done with Great Salt Lake water, phosphorus additions actually *decrease* algal abundances. This occurred in our short-term bioassays, and it was also reported in those of Stephens and Gillespie (1976) and Porcella and Holman (1972), but not in the assays of Wurtsbaugh (1988). This decrease could be due to competition between phytoplankton and heterotrophic bacteria for phosphorus, since the latter are superior competitors for this nutrient (Brussaard and Riegman 1998). The increased bacterial populations might then compete with algal populations for some other limiting nutrient (i.e. nitrogen). Although this mechanism has not been demonstrated in the Great Salt Lake, the potential that it may occur reminds us that the algal open water community is a diverse, interacting assemblage of microbes and metozoans, and complex responses to experiments may be driven by these often ignored interactions.

Although phosphorus additions either had no effect or inhibited algal growth in short-term bioassays, they stimulated phytoplankton and cyanobacteria after 16 days during our first long-term experiment. Chlorophyll levels in the phosphorus treatments increased from approximately 10 µg/L to over 100 µg/L in the 3% and 5% salinity treatments receiving phosphorus additions in Experiment A, and these increases were coincident with large increases in nitrogen fixation and cyanobacteria biovolume. Increases in nitrogen fixation, chlorophyll and cyanobacterial biovolume were noted by Lester (2003) in long-term assays of Gilbert Bay water (Appendix 9) when the salinity was adjusted to 3%, but not in 6% or 13% salinities. Wurtsbaugh (1988) also found that phosphorus stimulated cyanobacterial nitrogen fixation of Gilbert Bay water when salinities were near 5%. In these experiments, it appears that increased phosphorus promotes cyanobacterial nitrogen fixation, which in turn, allows the plankton community to overcome its nitrogen deficit and increase algal production.

In our second long-term experiment, however, phosphorus additions did not promote nitrogen fixation except in the 1% salinity treatment, and only slightly promoted chlorophyll levels in the 3% salinity treatment. Nitrogen remained the limiting nutrient through at least day 21 in all four of the salinity treatments. It is not clear why nitrogen fixation was more limited by salinity in the second experiment than in the first. One possibility is that in the second experiment (B), *Nodularia* sp. added in the inocula were taken from a canal near Willard Bay where salinities were only 0.4%. Consequently, it is possible that this strain was poorly adapted to higher salinities, and was quickly out competed in our salinity treatments. It is also possible that the availability of some other nutrient, such as iron (Evans & Prepas 1997), restricted nitrogen fixation in this experiment.

Different cyanobacteria species were observed in the two different factorial experiments. In Experiment A, the dominant cyanobacteria observed was the heterocystous *Nodularia* sp. This species has been observed during low salinity periods in Farmington Bay (Carter 1971; Sorensen et al. 1988), and dominance of this species in our experiments indicates that this species may be fixing nitrogen when intermediate salinities dominate in Farmington Bay. In contrast, in Experiment B the main cyanobacteria observed was the non-heterocystous filamentous *Microcoleous* sp. This taxa was also observed by Lester (2003) in 6% salinity treatments in a similar bioassay experiment. This species is associated with nitrogen fixation in microbial mats in hypersaline systems (Dubinin et al. 1992), and Camacho and de Wit (2003) found that it was stimulated by phosphorus additions in a saline Spanish lake. However, the dominant cyanobacteria in the treatment (3% salinity, +P) with the highest fixation rate in Lester's study, was the unicellular *Coccochlorus* sp. Another unicellular cyanobacteria, *Synechococcus* sp., has been found to be responsible for very high rates of nitrogen fixation in the ocean (Zehr et al. 2001), so it is possible that *Coccochlorus* also fixes nitrogen.

Salinity exerted an important control on nitrogen fixation in the long-term experiments. In Experiment A, nitrogen fixation responded earlier in the lower salinity treatments in both controls and particularly in phosphorus treatments. With the exception of one replicate, nitrogen fixation rates were low in the 7% salinity treatment. In Experiment B, nitrogen fixation reached moderate rates in the 1% salinity treatment, but was negligible at salinities of 4-10%. The combined data (Figure 11) indicates that some nitrogen fixation can occur up to salinities of 7%. This is relatively similar to the finding of Lester (2003) who found high nitrogen fixation rates at 3%, but none at 6% and 12%. Dubinin et al. (1992) reported nitrogen fixation by the

cyanobacterium *Microcoleus chthonoplastes* up to salinities of 15%, but in those experiments the organism was grown at 6% salinity and only exposed to the higher test salinities for 6 hours. Fernandes et al. (1993) found that nitrogen fixation in a salt-sensitive stain of *Anabaena* sp. was inhibited 50% at a salinity of 0.8%, but a salt-tolerant species (*Anabaena tortulosa*) was inhibited 50% at 1.5%. However, this genus is not noted as a halotolerant species. In a situation more closely related to Farmington Bay, Pinckney et al. (1995) found that decreasing salinity from ambient salinity levels of 9% to 4.5% significantly increased nitrogen fixation rates in a microbial mat dominated by non-heterocystous *Microcoleus chthonoplastes*, and rates were increased approximately 75% by phosphorus addition in cultures held in the dark.

A monitoring study in Farmington Bay in 1971 (Carter 1971) provided results consistent with our work suggesting that nitrogen-fixing cyanobacteria should not be abundant at salinities < 6-8%. In the 1971 study, plankton samples were collected at 13 stations along salinity gradients in Farmington Bay. An analysis of this work (Figure 12) indicates that the heterocystous nitrogen-fixing cyanobacteria, *Nodularia* sp. was usually not abundant in areas where salinities were greater than 7%, but was abundant at lower salinities. Exact concordance between abundances and salinity would not be expected in the bay, because wind mixing could easily transport *Nodularia* from an area where it was actively growing to another area where salinities would not support continued growth or nitrogen fixation.

Our results reported here suggest that given the salinities normally observed in Farmington Bay (<7%), phosphorus controls nitrogen fixation rates of cyanobacteria, thus the supply of this nutrient results in nitrogen also limiting the phytoplankton community. This situation is similar to that suggested for freshwater lakes (Schindler 1977). However, during droughts salinities rise well above 7% in both Farmington Bay and in Gilbert Bay salinities are almost always above 7%. Our initial results suggest that nitrogen-fixing species would be inhibited at those salinities, and thus the community would remain nitrogen-limited, regardless of the phosphorus concentrations. If nutrients were to be controlled to reduce eutrophication in Farmington Bay, the expected salinity levels would thus need to be incorporated into the decision process. At low salinities (approximately <7%) phosphorus would need to be controlled. At higher salinities, nitrogen control would be appropriate.

Our initial experiments are unable to resolve how nutrients control algal growth in the varying salinities of Farmington Bay and the Great Salt Lake. In Experiment A, salinities were

insufficient to test the high salinity limit of the cyanobacteria, as they fixed nitrogen and grew well to the highest salinity tested (7%). Salinity was much more restrictive in Experiment B, and appreciable fixation only occurred at 1% salinity. Additional laboratory assays will be needed to clearly delimit the salinity where nitrogen fixation can occur and determine when and where nitrogen or phosphorus is likely to limit algal growth. Future experiments should have more rigorously-controlled inocula to ensure that a full range of halo-tolerant species are present.

Laboratory bioassays also have limitations, and field experiments are needed to unequivocally determine nutrient limitation in the lake. Laboratory assays impart controlled conditions on the microbial communities, thus simplifying environmental variables and the interpretation of results. However, these assays also modify the environment so that experimental artifacts could occur. For example, we removed macrozooplankton (primarily brine shrimp) from the assays, but we did not remove microzooplankton. Consequently, grazing and nutrient recycling by zooplankton in the assays was not the same as in the lake, and this could alter the response to nutrients. It is possible that the decline in the algal populations after day 14-21 was due to algal senescence and/or grazing by protozoans (Gliwicz et al. 1995). Additionally, the flask experiments we used do not evaluate nutrient cycling between the benthic sediments and the water column, which can have important implications for the relative balance of nitrogen and phosphorus limitation in lakes (Levine and Schindler 1992). Consequently, to determine whether eutrophication control is appropriate for Farmington Bay, field experiments in limnocorrals or shore-based mesocosms should be used to study nutrient limitation under more natural conditions.

Literature Cited:

Baskin, RL, KM Waddell, SA Thiros, EM Giddings, HK Hadley, DW Stephens & SJ Gerner. 2002. Water-Quality Assessment of the Great Salt Lake Basins, Utah, Idaho, and Wyoming—Environmental Setting and Study Design. US. Geological Survey Water-Resources Investigations Report 02-4115 (NAQWA Program). Salt Lake City.

Bassett J. 2003. Oxygen tolerance of brine shrimp and their vertical distribution during the day in the water column at Farmington Bay. *In* Marcarelli AM et al. Continuing studies of water quality in Farmington Bay and the Great Salt Lake, Utah. Aquatic Ecology Practicum Class Project 2002. College of Natural Resources, Utah State University. pp. 27 – 37.

Brussaard, CPD and R. Riegman. 1998. Influence of bacteria on phytoplankton cell mortality with phosphorus or nitrogen as the algal-growth-limiting nutrient. Aquatic Microbial Ecology 14:271280.

Capone DG. 1993. Determination of nitrogenase activity in aquatic samples using the acetylene reduction procedure. Pp. 621-631 *In* PF Kemp, BF Sherr, EB Sherr and JJ Cole (eds.). Handbook of Methods in Microbial Ecology. Lewis Publishers, Boca Raton, FL.

Camacho A. and R. de Wit. 2003. Effect of nitrogen and phosphorus additions on a benthic microbial mat from a hypersaline lake. Aquatic microbial ecology. 32:261-273.

Carter, C. K., Ed. (1971). Some ecological considerations of the Farmington Bay estuary and adjacent Great Salt Lake Park. Salt Lake City, University of Utah. 153 pp.

Chadwick, D.G. Jr., J.P. Riley, A.J. Seirstad, D.L. Sorensen, and N.E. Stauffer, Jr. 1986. Expected effects of in-lake dikes on water levels and quality in the Farmington Bay and the east shore areas of the Great Salt Lake, Utah. Utah Water Research laboratory, Logan, Utah. 91 p.

Coburn, A. and D.W. Eckhoff. 1972. Pollution input from the lower Jordan basin to Antelope Island estuary in the Great Salt Lake and Utah's water resources. Am. Wat. Res. Assoc., Utah Section, Annual Conf., 1st, Proceedings. Pp. 12-140.

Dubinin, AV, LM Gerasimenko & GA Zavarzin. 1992. Nitrogen-fixation by cyanobacterium *Microcoleus chthonoplastes* from hypersaline lagoons of Lake Sivash. Microbiol. 61:593-597.

Elser JJ, ER Marzolf and CR Goldman. 1990. Phosphorus and nitrogen limitation in the freshwaters of North America: a review and critique of experimental enrichments. Can. J. Fish. Aquat. Sci 47: 1468-1477.

Evans JC and EE Preppas. 1997. Relative importance of iron and molybdenum in restricting phytoplankton biomass in high phosphorus saline lakes. Limnol. Oceanogr. 42: 461 – 472.

Fee, EJ. 1979. A relation between lake morphometry and primary productivity and its use in interpreting whole-lake eutrophication experiments. Limnol. Oceanogr. 24: 401 – 416.

Felix EA and SR Rushforth. 1979. The algal flora of the Great Salt Lake, Utah, U.S.A. Nova Hedwigia 31: 163 – 195.

Fernandes, TA, V Iyer & K Apte. 1993. Differential responses of nitrogen-fixing cyano-bacteria to salinity and osmotic stresses. App. Environ. Microbiol. 59:899-904.

Flett RJ, RD Hamilton and NER Campbell. 1976. Aquatic acetylene-reduction techniques: solutions to several problems. Can. J. Microbiol. 22: 43 – 51.

Francoeur SN. 2001. Meta-analysis of lotic nutrient amendment experiments: detecting and quantifying subtle differences. J. N. Am. Benthol. Soc. 20: 358-368.

Gliwicz, Z.M., W.A. Wurtsbaugh and A. Ward. 1995. Brine shrimp ecology in the Great Salt Lake, Utah. 1994-1995 Performance Report to the Utah Division of Wildlife Resources, Salt Lake City, Utah. 83 p.

Goddard KE, DL Naftz and RL Baskin. 2002. Proposal UT02C. An integrated approach to understanding the physical and chemical characteristics of Great Salt Lake, Utah. USGS proposal.

Gross, D. 2002. Nutrient loading to Farmington Bay. AWER 4510 Final Project, Utah State University, Logan UT. 12 p.

Hillebrand H, C-D Durselen, D Kirschtel, U Pollingher and T Zohary. 1999. Biovolume calculation for pelagic and benthic microalgae. J. Phycol. 35: 403 – 424.

Howarth RW and JJ Cole. 1985. Molybdenum availability, nitrogen limitation, and phytoplankton growth in natural waters. Science 229: 653-655.

Israelsen, C.E., D. Sorenson, A. Seirstad and C. Brennand. 1985. Preliminary identification, analysis, and classification of odor-causing mechanisms influenced by decreasing salinity of the Great Salt Lake. Utah Water Research Laboratory, USU, Logan, UT.

Javor, B. 1989. Hypersaline environments. Springer-Verlag, Berlin. 328 p.

Kling, Jason. 2003. A study of oxygen tolerance of *Ephydra cinerea* and *E. hian* in Farmington Bay and the Great Salt Lake, Utah. *In* Marcarelli AM et al. Continuing studies of water quality in Farmington Bay and the Great Salt Lake, Utah. Aquatic Ecology Practicum Class Project 2002. College of Natural Resources, Utah State University. pp. 60 – 69.

Levine, S. N. and D. W. Schindler. 1992. Modification of the N-P Ratio in lakes by in situ processes. Limnology and Oceanography 37: 917-935.

Lester, O. 2003. The effect of salinity and nutrient on community composition, growth and nitrogen fixation rates of phytoplankton in the Great Salt Lake, Utah. *In* Marcarelli AM et al. Continuing studies of water quality in Farmington Bay and the Great Salt Lake, Utah. Aquatic Ecology Practicum Class Project 2002. College of Natural Resources, Utah State University. pp. 5 – 18.

Marcarelli AM, WA Wurtsbaugh, B Albrecht, E Archer, J Bassett, M Beckstrand, M Hadley, J Kling, O Lester, P MacKinnon and T-h Thing. 2003. Continuing studies of water quality in Farmington Bay and the Great Salt Lake, Utah. Aquatic Ecology Practicum Class Project 2002. College of Natural Resources, Utah State University. 91 p.

Marino R, Chan F, Howarth RW, Pace M and Likens GE. 2002. Ecological and biogeochemical intereactions constrain planktonic nitrogen fixation in estuaries. Ecosystems 5: 719 – 725.

Mills MM, Ridame C, Davey M, La Roche J and Geider RJ. 2004. Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. Nature 429: 292 – 294.

Moisander, P.H. E McClinton III, and H.W. Paerl. 2002. Salinity effects on growth, photosynthetic parameters, and nitrogenase activity in estuarine planktonic cyanobacteria. Microb. Ecol. 43: 432-442.

Muezzinoglu A., D. Sponza, I. Koken, N, Alparslan, A. Akyarli and N. Ozture. 2000. Hydrogen sulfide and odor control in Izmir Bay. Water, Air and Soil Pollution 123: 245-257.

Naftz DL, DW Stephens, E Callender and PC Van Metre. 2000. Reconstructed historical changes in the environmental health and watersheds by using sediment cores from lakes and reservoirs in Salt Lake Valley, Utah. USGS Fact Sheet FS-164-00. NAWQA Program, Salt Lake City, Utah. 6 p.

Paerl HW. 1996. A comparison of cyanobacterial bloom dynamics in freshwater, estuarine and marine environments. Phycologia 35: 25 – 35.

Pinckney, J, HW Paerl & BM Bebout. 1995. Salinity control of benthic microbial mat community production in a Bahamian hypersaline lagoon. J. Exp. Mar. Biol. Ecol. 187:223-237.

Porcella, DB and JA Holman. 1972. Nutrients, algal growth, and culture of brine shrimp in the southern Great Salt Lake. Pp. 142-155, In The Great Salt lake and Utah's water resources. Proceedings First Annual Conference of the Utah Section of the American Water Resources Association. Salt Lake City, Utah.

Potts, M. 1980. Blue-green algae (Cyanophyta) in marine coastal environments of the Sinai Peninsula; distribution, zonation, stratification and taxonomic diversity. Phycologia 19: 60 – 73,

Post, F.J. and J.C. Stube. 1988. A microcosm study of nitrogen utilization in the Great Salt Lake, Utah. Hydrobiologia 158:89-100.

Schindler, D. 1977. Evolution of phosphorus limitation in lakes. Science 195: 260 – 262.

Sorensen, D.L., J.P. Riley, W. A. Wurtsbaugh and 11 others. 1988. Great Salt Lake inter-island diking: water quality considerations. Utah Water Research Laboratory, Utah State University, Logan, UT. 261 pp.

Stal LJ, M Staal and M. Billbrandt. 1999. Nutrient control of cyanobacterial blooms in the Baltic Sea. Aquat. Microb. Ecol. 18: 165 – 173.

Stephens, DW & DM Gillespie. 1976. Phytoplankton production in the Great Salt Lake, Utah, and a laboratory study of algal response to enrichment. Limnol. Oceanogr. 21: 74-87.

Stewart WDP, GP Fitzgerald and HR Burris. 1967. *In situ* studies on N-fixation using the acetylene reduction technique. Proc. Nat. Acad. Sci. U.S.A. 58: 2071 – 2078.

Sturm, P.A. 1980. The Great Salt Lake brine system. Pp. 147-162 *In* J.W. Gwynn, Great Salt Lake: A scientific, historical and economic overview. Utah Department of Natural Resources Bulletin 116.

Vitousek PM, K Cassman, C Cleveland, T Crews, CB Field, NB Grimm, RW Howarth, R Marino, L Martinelli, EB Rastetter and JI Sprent. 2002. Towards an ecological understanding of biological nitrogen fixation. Biogeochemistry 57/58: 1-45.

Watts J.M., B.K. Swan, M.A. Tiffany and S.H. Hurlbert. 2001. Thermal, mixing, and oxygen regimes of the Salton Sea, California, 1997-1999. Hydrobiologia 466: 159-176.

Welschmeyer NA. 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and phaeopigments. Limnol. Oceanoogr. 39: 1985 – 1992.

Wetzel RG. 2001. Limnology. Academic Science, San Diego.

Wetzel RG and GE Likens. 2000. Limnological Analyses, 3rd ed. Springer, New York. 429 pp.

Wurtsbaugh, W. 1988. Iron, molybdenum and phosphorus limitation of N2 fixation maintains nitrogen deficiency of plankton in the Great Salt Lake drainage (Utah, USA). Verh. Int. Ver. Limnol. 23:121-130.

Wurtsbaugh WA and AJ Horne. 1983. Iron in eutrophic Clear Lake, California: its importance for algal nitrogen fixation and growth. Can. J. Fish. Aquat. Sci. 40: 1419-1429.

Wurtsbaugh, WA and A Marcarelli. 2004a. Hydrogen sulfide in Farmington Bay and the Great Salt Lake: A potential odor-causing agent. Report to the Central Davis Sewer Improvement District, Utah. 29 p.

Wurtsbaugh, W. and A. Marcarelli. 2004b. Analysis of phytoplankton nutrient limitation in Farmington Bay and the Great Salt Lake. Report to Central Davis Sewer Improvement District. 57 pp.

Zehr JP, JB Waterbury , PJ Turner, JP Montoya, E Omoregie, GF Steward, A Hansen and DM Karl. 2001. Unicellular cyanobacteria fix N_2 in the subtropical North Pacific Ocean. Nature 412: 635-638.

Figures and Tables:

Table 1. Nutrient bioassay experiments completed in 2003, using source water from either Farmington Bay (FB) or Gilbert Bay (GB). The salinity of the source water, and those used in the experiments are shown. Nutrient treatments were controls (C), +nitrogen (N), +phosphorus (P), and a combination of N+P.

Experiment	Type	Source	Date	Source Salinity (%)	Salinity Treatments (%)	Nutrient Treatments
1	Simple	FB	6 Jun 03	5.3		C, N, P, N+P
2	Simple	FB, GB	29 Aug 03	10.3, 15.4		C, N, P, N+P
3	Simple	FB	9 Oct 03	10.4		C, N, P, N+P
4	Simple	FB	4 Nov 03	8.6		C, N, P, N+P
A	Factorial	FB	3 Jul 03	7.2	1, 3, 5, 7	C, N, P
B	Factorial	FB	9 Oct 03	10.0	1, 4, 7, 10	C, N, P

Table 2. Sources of supplementary inocula for the Factorial Bioassay Experiments. Salinities at each site are indicated.

Experiment	Sources and salinities (%)
A	Hull Lake, public shooting grounds (1.5%), Bear River Refuge, canal by D-line dyke (0.5%), Great Salt Lake station 14 (13.5%),
B	Canal north of Willard Bay (0.4%), sheet water north of Antelope Island Causeway (3.6% - 5.5%), Great Salt Lake, off northern tip of Antelope Island (16.0%)

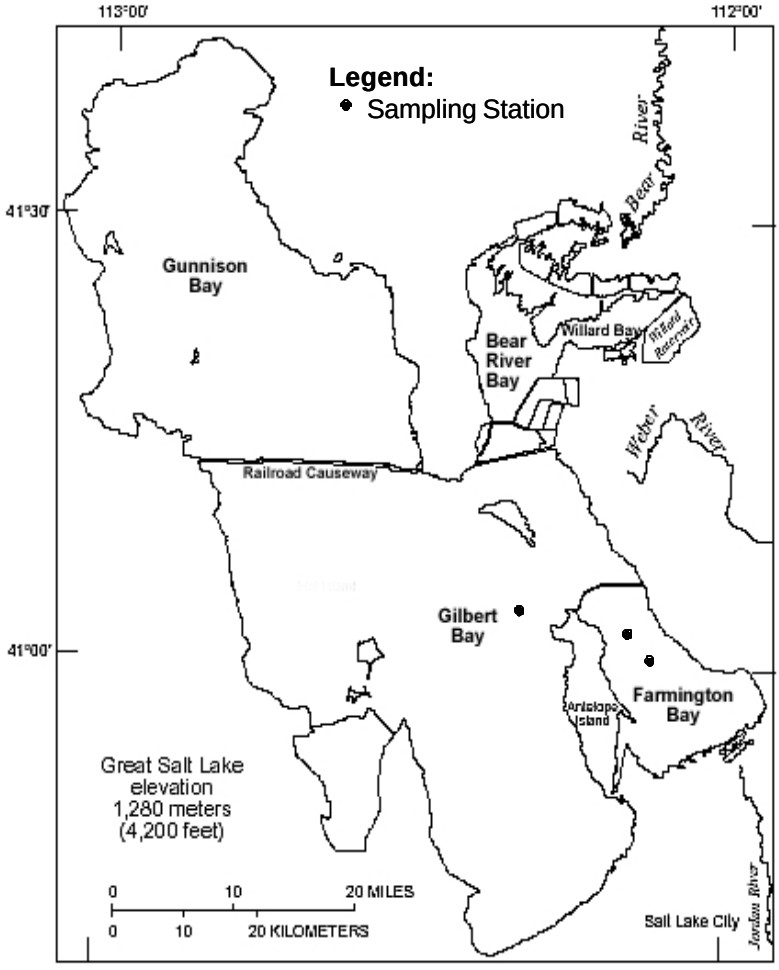


Figure 1: Map of Great Salt Lake, showing the locations of the Farmington Bay and railroad causeways and the sites where was collected for bioassay experiments in this study.

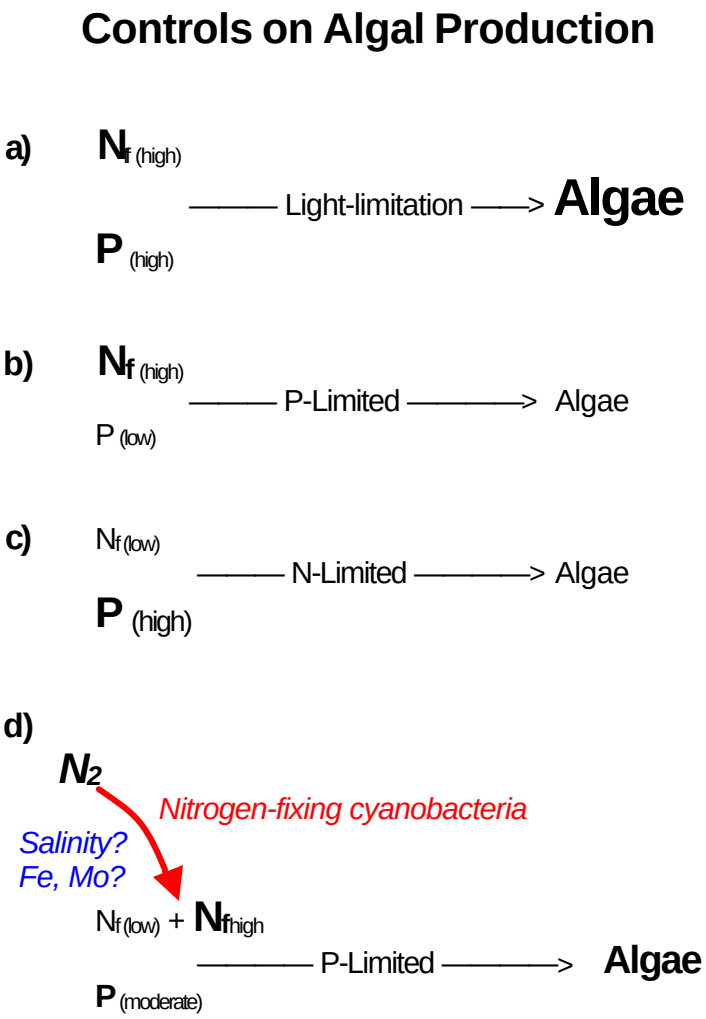


Figure 2. Light and nutrient controls on algal growth in different environments. The size of text indicates the relative concentrations of nutrients or algal abundance.

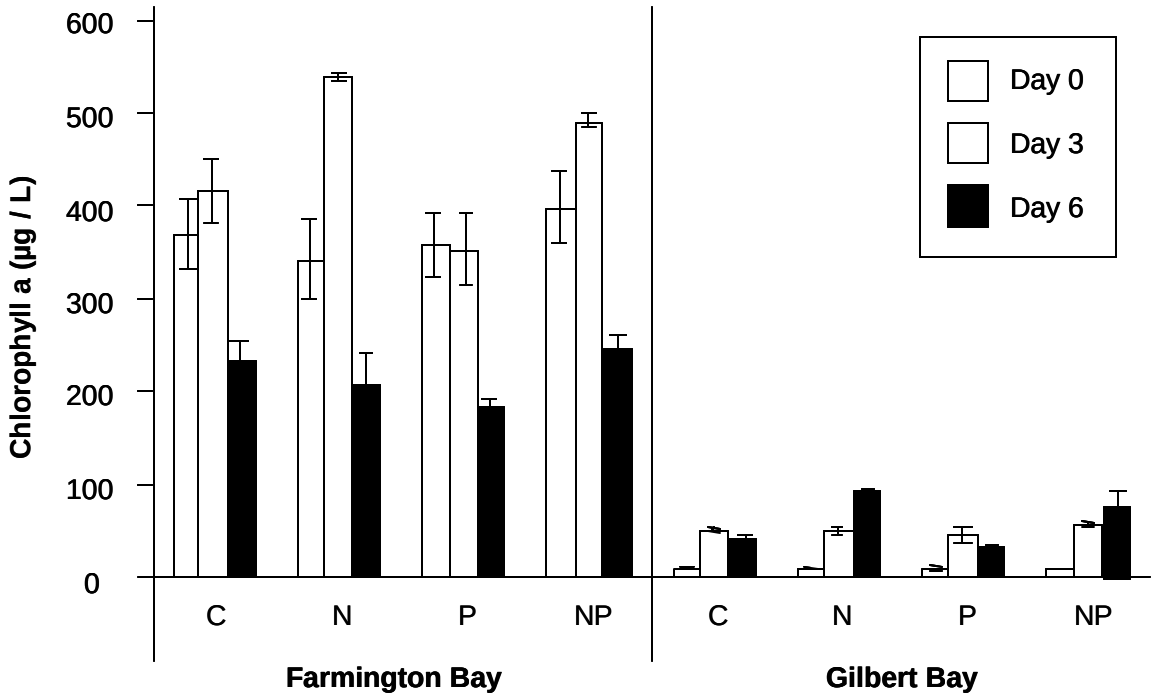


Figure 3: Chlorophyll *a* concentrations on day 0, 3 and 6 in SIMPLE BIOASSAY experiment 2. Treatments were: controls (C), +Nitrogen (N), +Phosphorus (P), and +Nitrogen and Phosphorus (NP) In this experiment, we tested both Farmington Bay water (left) and Gilbert Bay water (right). Error bars are ± 1 S.E. Note the extremely high initial chlorophyll level of the phytoplankton from Farmington Bay that was used in this study (ca. 350 µg/L).

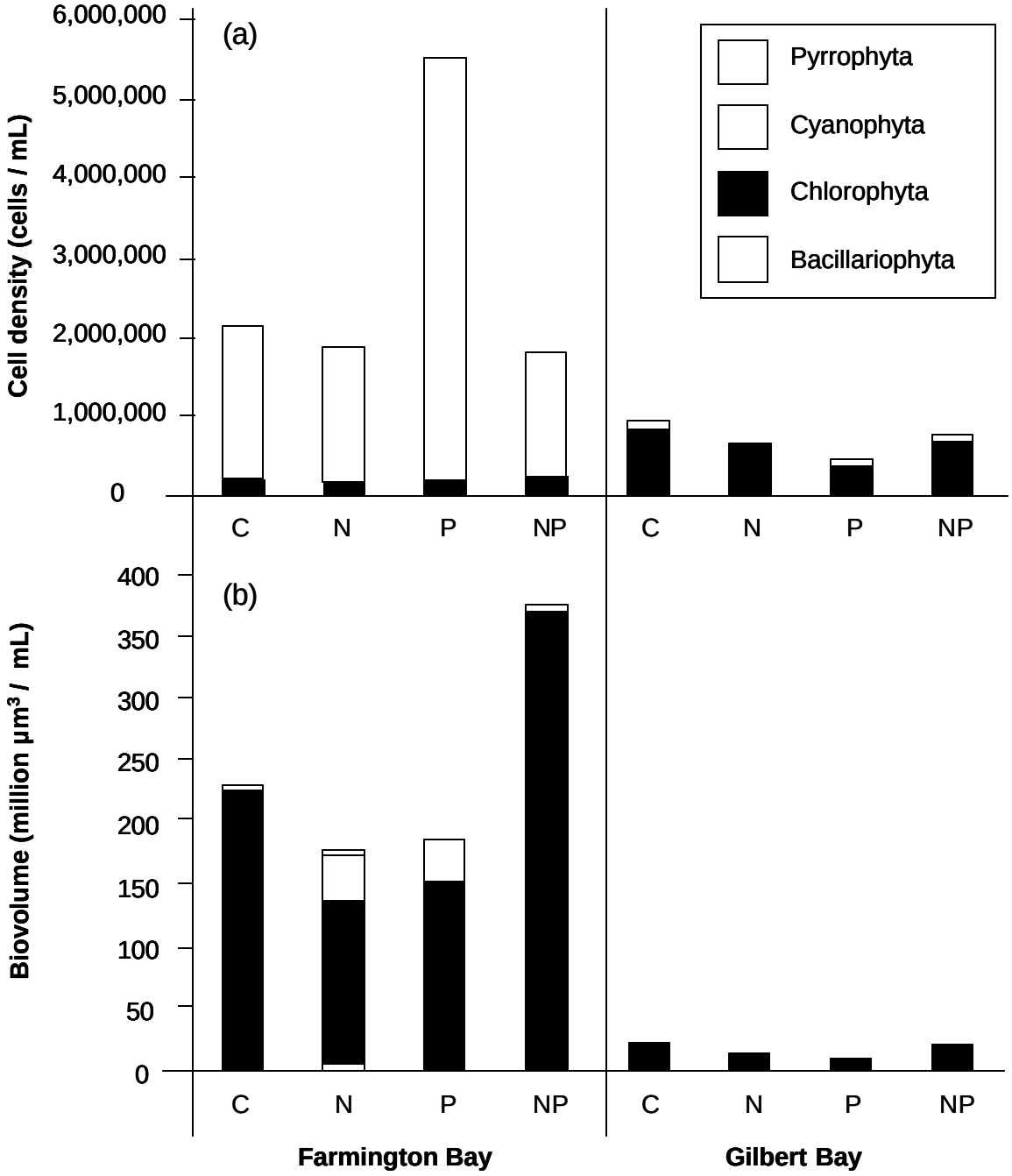


Figure 4: Densities (above) and biovolumes (below) of different phytoplankton taxa in SIMPLE BIOASSAY Experiment 2. Data are from the final day of the experiment (day 6). Biovolume is expressed as million μm^3 / mL (1 million μm^3 / mL = 10^6 μm^3 / mL).

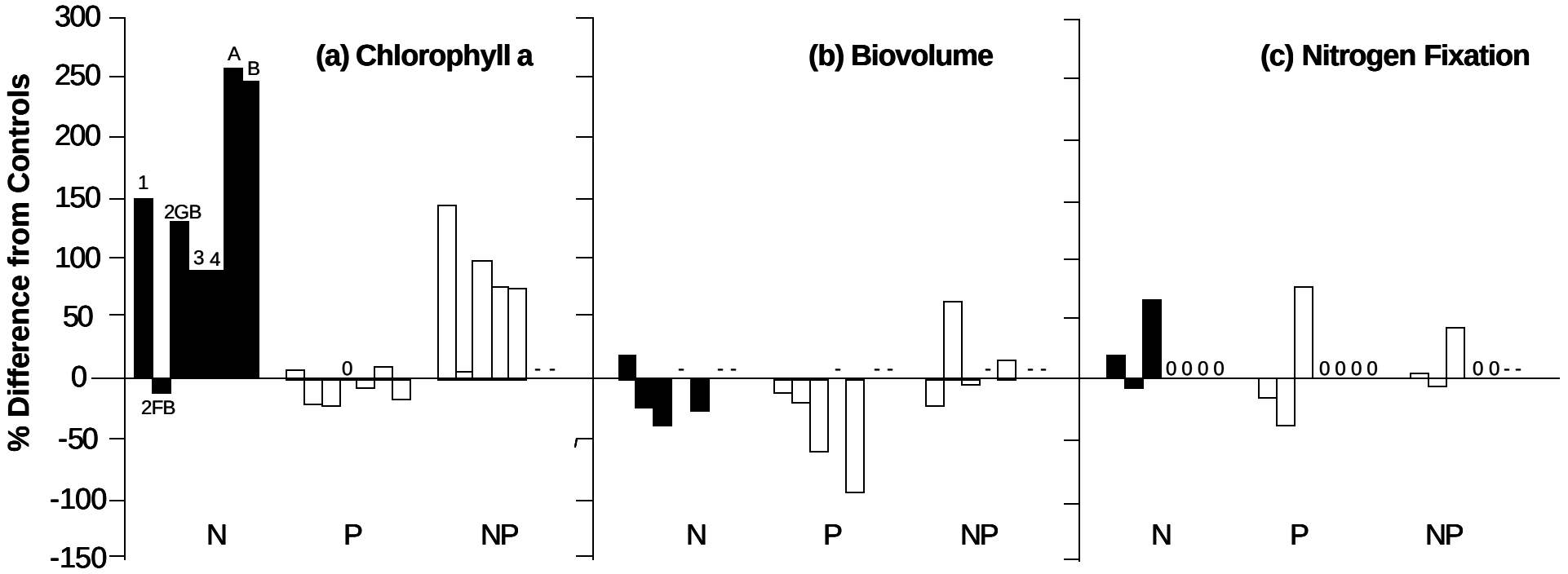


Figure 5: Summary of short-term responses of phytoplankton to nutrient additions in all experiments on day 6 (Simple Bioassays), or days 7-9 (Factorial Bioassays). Responses are reported as % responses above mean control levels for (a) chlorophyll *a,* (b) total algal biovolume and (c) nitrogen fixation. 0 indicates no difference from response, - indicates no comparison possible because of lack of either control or nutrient treatment. Treatment labels are: N = +Nitrogen, P = +Phosphorus, NP = +Nitrogen+Phosphorus. Experiment IDs, from left to right on each treatment response, are: 1 = Simple Bioassay #1, 2FB = Simple Bioassay #2 Farmington Bay, 2GB = Simple Bioassay #2 Gilbert Bay, 3 = Simple Bioassay #3, 4 = Simple Bioassay #4, A = first Factorial Bioassay, B = second Factorial Bioassay. Data for the factorial bioassays is for the highest salinity treatments (7%, Exp. A; 10%, Exp. B).

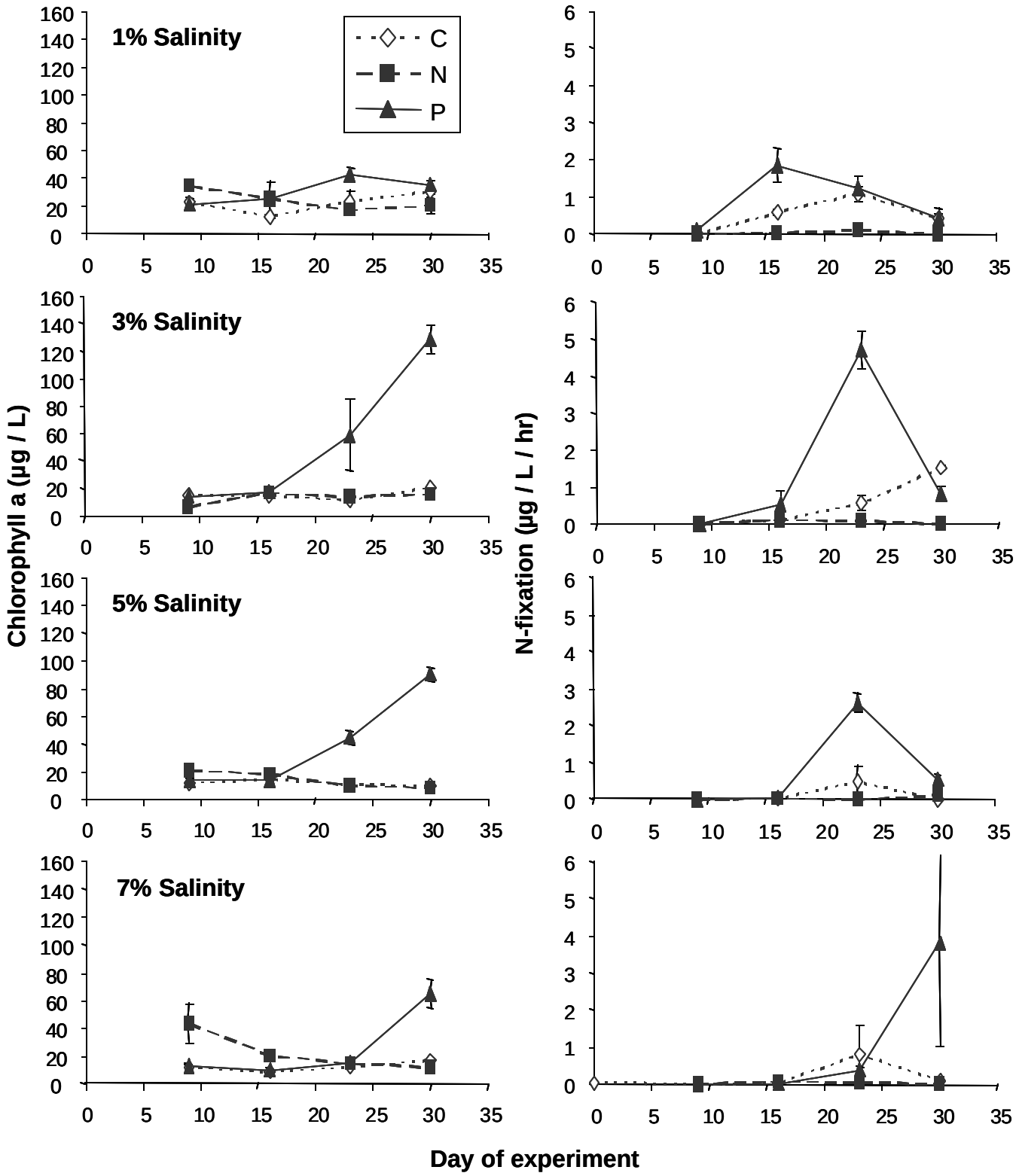


Figure 6: Responses of chlorophyll *a* concentrations (left) and nitrogen fixation (right) to nitrogen or phosphorus additions at four salinities in Factorial Experiment A. Note the delays in peak nitrogen fixation rates with increasing salinities. Error bars ± 1 S.E. C = Controls; N = +nitrogen; P = +phosphorus.

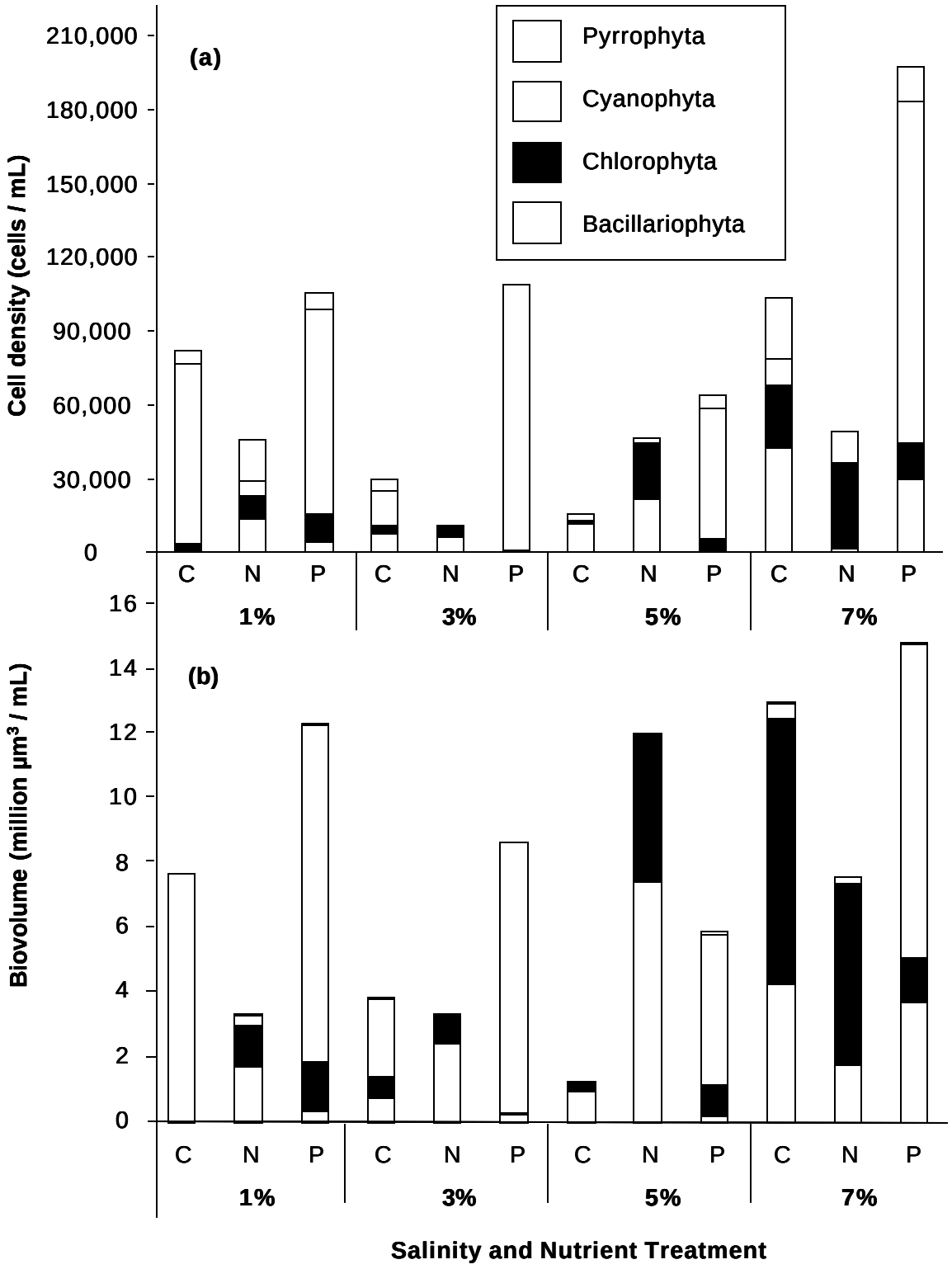


Figure 7: FACTORIAL EXPERIMENT A – (a) Cell density and (b) biovolume of algal cells on day 30 of the experiment at salinities of 1, 3, 5 and 7%. Note higher density and biovolume of cyanobacteria in the control and P treatments, particularly at low salinities. 1 million μm^3/mL = 10^6 um^3/mL. C = Controls; N = +nitrogen; P = +phosphorus.

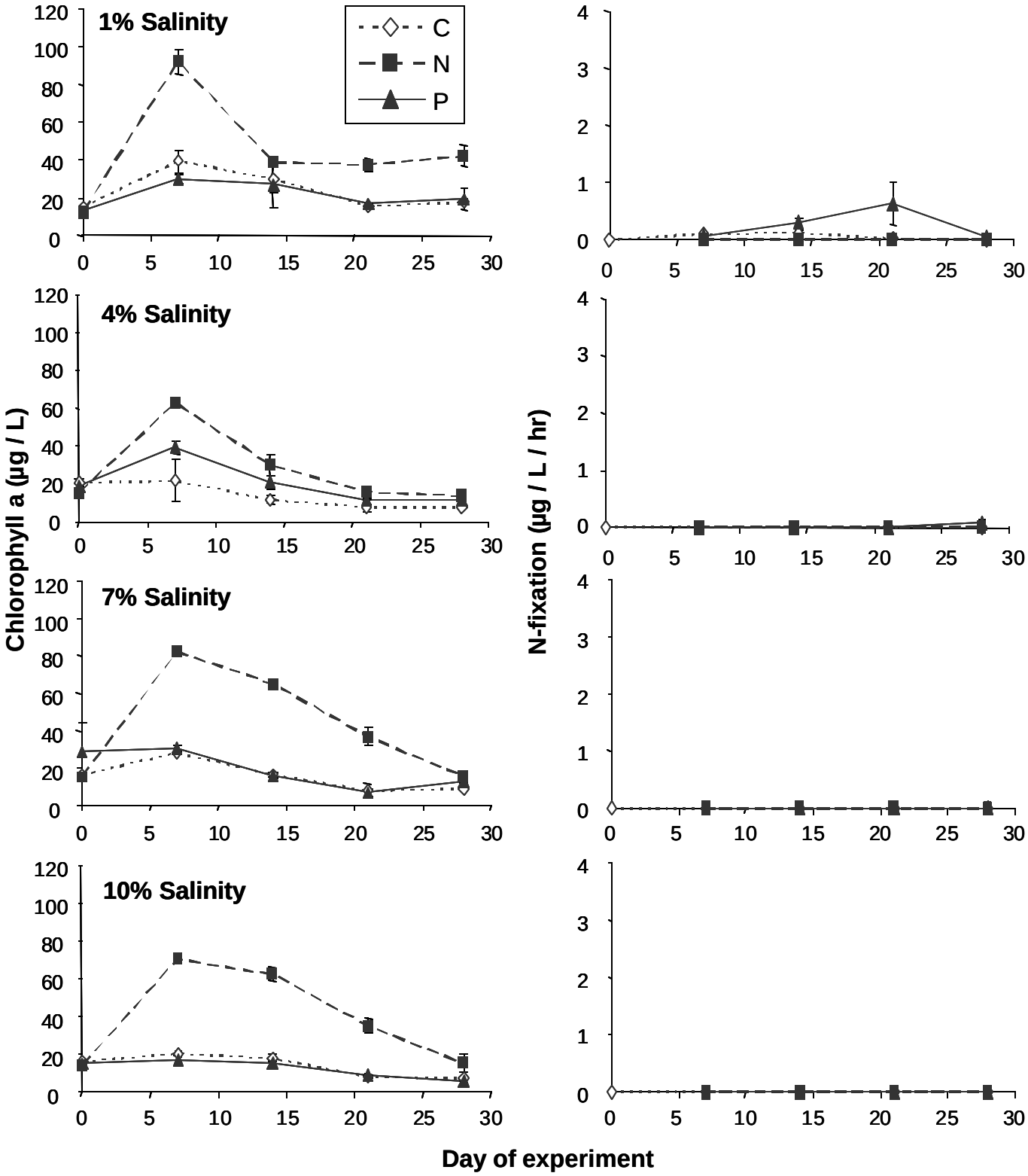


Figure 8: Factorial Experiment B. Changes in chlorophyll *a* and nitrogen fixation rates during the 28-day experiment. Note the low levels of nitrogen fixation in most treatments and lack of chlorophyll *a* response in the P treatments at all salinities.. Error bars ± 1 S.E. C = Controls; N = +nitrogen; P = +phosphorus.

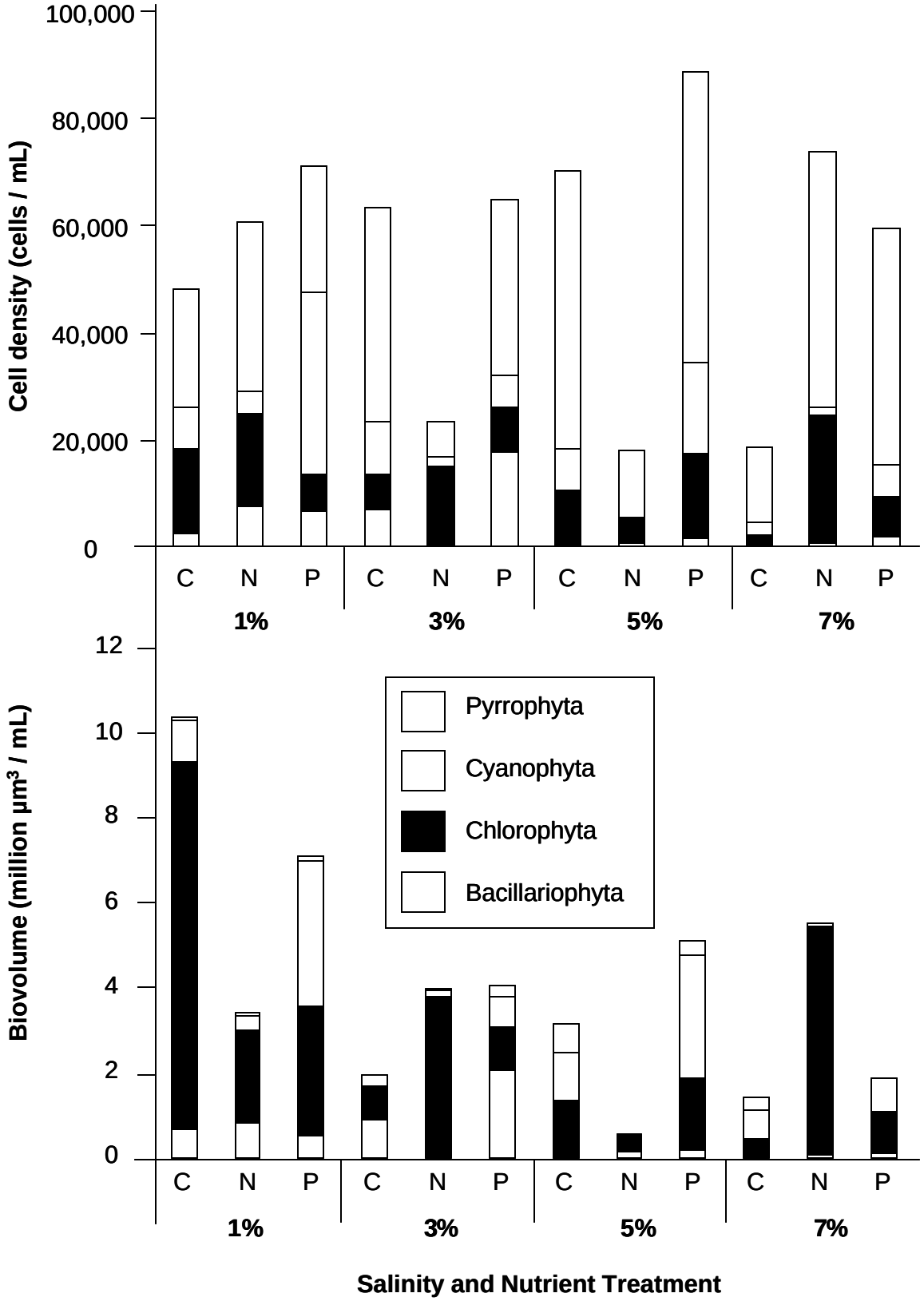


Figure 9: FACTORIAL EXPERIMENT B – (a) Cell density and (b) biovolume of algal cells on day 28 of the experiment at salinities of 1, 3, 5 and 7%. Note the relatively low densities and biovolumes of cyanobacteria compared to Experiment A. C = Controls; N = +nitrogen; P = +phosphorus.

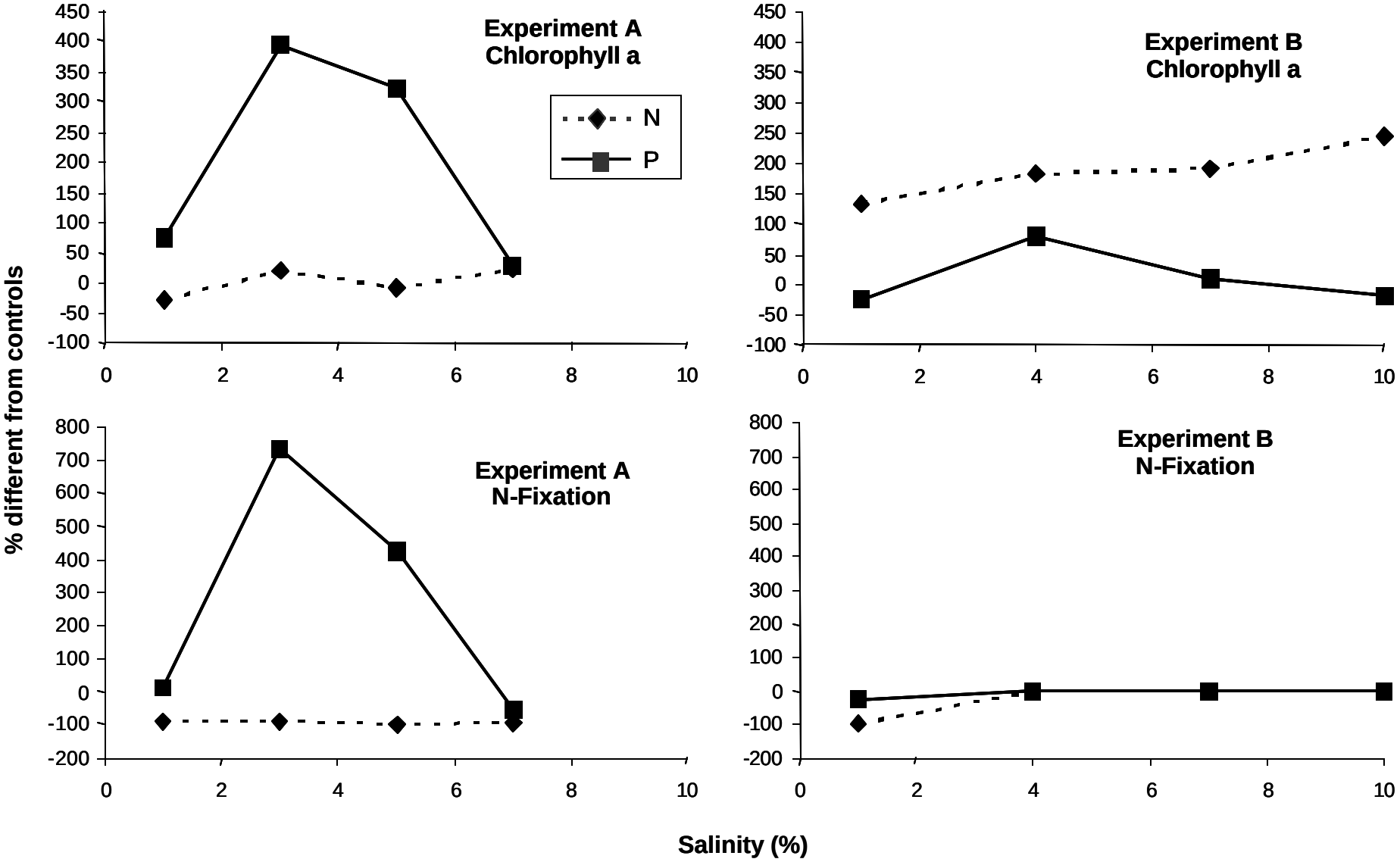


Figure 10: Chlorophyll *a* (above) and nitrogen fixation (below) responses to salinity and nutrient treatment for Experiment A on day 23 and Experiment B on day 6. These dates are when maximum response to nutrient enrichments was observed in each experiment. Note high nitrogen fixation response, and consequent high chlorophyll response in +P treatments in Experiment A. In contrast, note lack of n-fixation response in Experiment B, and greater chlorophyll *a* response in +N treatments.

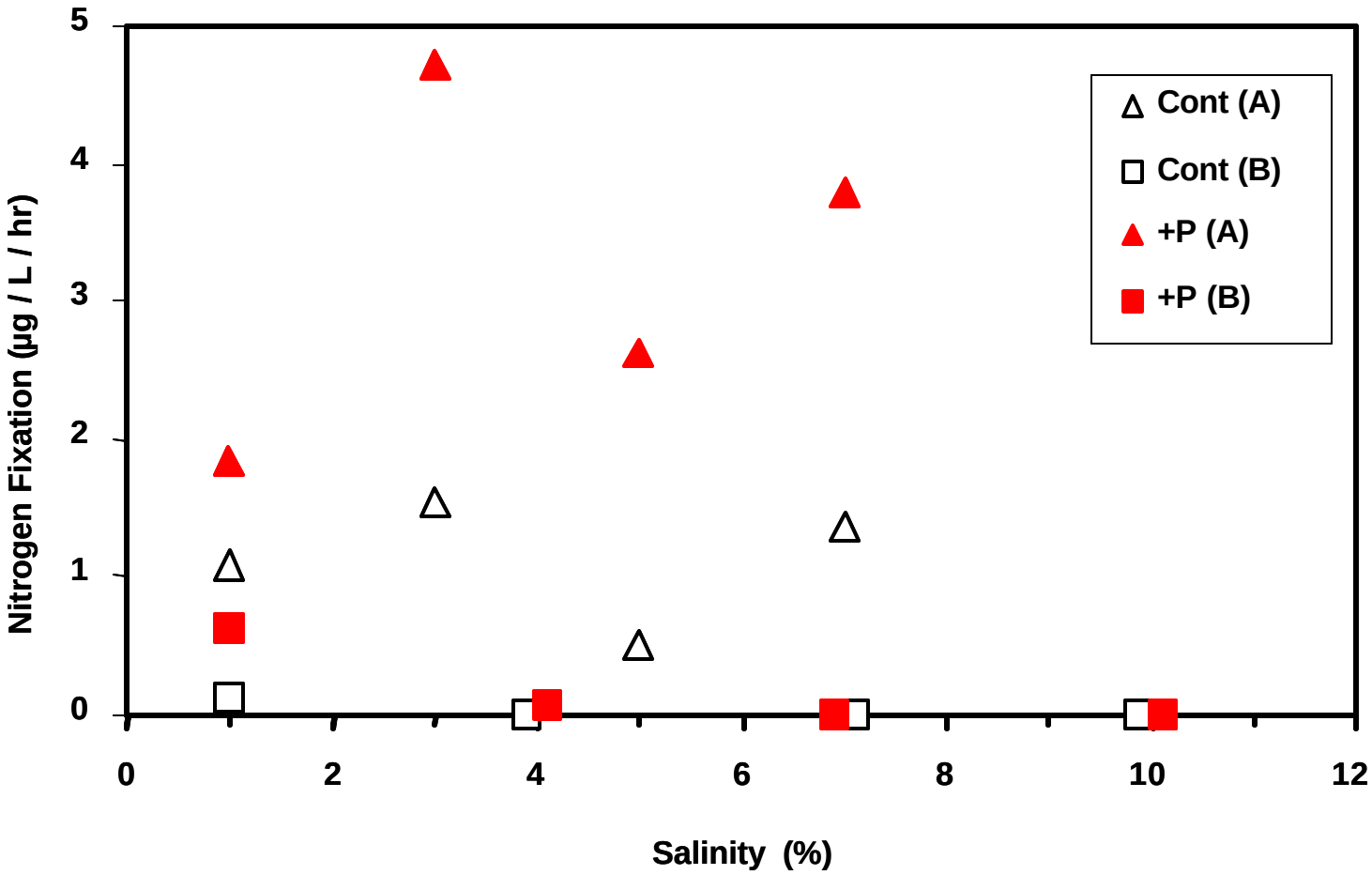


Figure 11: Maximum nitrogen fixation rates in relation to test salinities that were observed during the 4-week long factorial experiments in the control and +phosphorus treatments. Fixation rates were higher in the first experiment (A) than in the second experiment (B), and phosphorus treatments (+P) were significantly higher than controls (Cont). Maximum fixation rates showed no clear relationship with salinity from 1-7%, but declined to zero at a salinity of 10%. Maximum fixation rates occurred on different days in the different treatments.

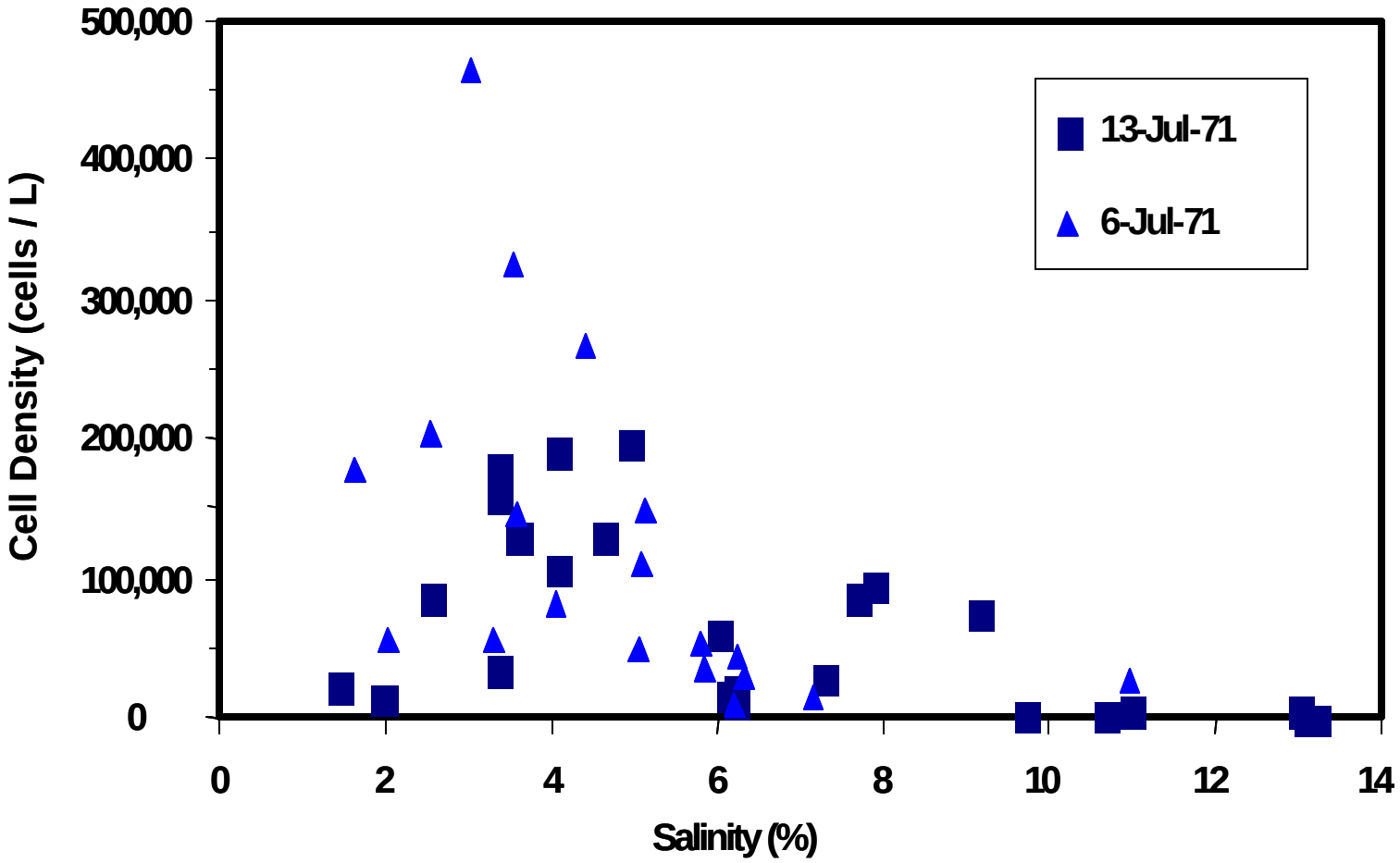


Figure 12. Abundances of *Nodularia* sp. along salinity gradients in Farmington Bay during July 1971. Data of C.R. Hayes, and C. Bott, in Carter (1971). Salinity was estimated using the NaCl concentrations given by Bott, and assuming that this salt comprised 86.4% of the total salt in the bay (Sturm 1980).

Appendix 1: Average chlorophyll *a* response in simple bioassays. Values are reported in µg / L, with standard errors following in parentheses. N = 3 for all measurements. Treatments are: C = control, N = + nitrogen, P = + phosphorus and NP = + nitrogen + phosphorus. Dashes indicate dates when samples weren't taken or missing data.

				TREATMENT			
Experiment ID	**Lake Bay**	**Experiment Day**	**Initial**	**C**	**N**	**P**	**NP**
1	Farmington	0	52.18 (2.0)	–	–	–	–
1	Farmington	3	–	52.7 (0.9)	174.4 (5.9)	51.4 (4.3)	174.4 (24.2)
1	Farmington	6	–	50.2 (2.7)	125.4 (5.6)	54.3 (1.0)	122.3 (2.3)
2	Farmington	0	–	366.0 (37.1)	338.0 (42.3)	356.0 (35.8)	393.5 (38.2)
2	Farmington	3	–	413.5 (35.0)	537.5 (5.6)	349 (40.3)	487 (6.55)
2	Farmington	6	–	232.0 (16.0)	207.0 (33.4)	184.0 (7.9)	245.0 (11.6)
2	Gilbert	0	–	8.9 (0.7)	7.7 (0.5)	8.4 (2.3)	8.3 (0.1)
2	Gilbert	3	–	48.8 (1.7)	49.2 (5.3)	44.9 (8.6)	55.4 (2.0)
2	Gilbert	6	–	39.2 (7.1)	90.6 (4.5)	30.2 (2.6)	77.5 (13.5)
3	Farmington	0	114.5 (57.3)	–	–	–	–
3	Farmington	3	–	176.0 (3.6)	346.5 (23.0)	178.0 (2.6)	302.5 (5.1)
3	Farmington	6	–	251.0 (6.3)	477.5 (12.1)	249.0 (5.2)	443.5 (17.0)
3	Farmington	13	–	272.5 (3.6)	501.0 (16.5)	334.0 (9.5)	351.0 (49.0)
3	Farmington	20	–	287.5 (10.1)	302.5 (16.3)	316.0 (7.9)	265.0 (7.1)
3	Farmington	26	–	261.5 (7.4)	285.0 (5.4)	275.0 (9.7)	253.0 (13.8)
4	Farmington	0	166.5 (6.1)	–	–	–	–
4	Farmington	3	–	256.5 (5.4)	487.5 (133.2)	275.0 (5.8)	599.5 (9.1)
4	Farmington	6	–	338.0 (6.1)	639.0 (31.2)	317.0 (21.0)	595.0 (15.1)

Appendix 2: Average nitrogen fixation responses in simple bioassays. Values are reported in µg / L / hr, with standard errors following in parentheses. N = 3 for all measurements. Treatments are: C = control, N = + nitrogen, P = + phosphorus and NP = + nitrogen + phosphorus. Dashes indicate dates when samples weren't taken or missing data.

			TREATMENT				
Experiment ID	**Lake Bay**	**Experiment Day**	**Initial**	**C**	**N**	**P**	**NP**
1	Farmington	0	0.08 (0.079)	–	–	–	–
1	Farmington	6	–	0.08 (0.005)	0.09 (0.004)	0.06 (0.001)	0.08 (0.003)
2	Farmington	0	–	0.10 (0.012)	0.07 (0.012)	0.07 (0.008)	0.07 (0.004)
2	Farmington	3	–	0.14 (0.002)	0.14 (0.004)	0.14 (0.001)	0.15 (0.002)
2	Farmington	6	–	0.17 (0.012)	0.16 (0.009)	0.11 (0.052)	0.16 (0.001)
2	Gilbert	0	–	0.04 (0.010)	0.04 (0.004)	0.04 (0.005)	0.04 (0.013)
2	Gilbert	3	–	0.12 (0.003)	0.14 (0.005)	0.12 (0.007)	0.13 (0.004)
2	Gilbert	6	–	0.09 (0.042)	0.14 (0.004)	0.15 (0.008)	0.12 (0.014)
3	Farmington	0	0.00 (0.000)	–	–	–	–
3	Farmington	3	–	0.00 (0.000)	0.00 (0.000)	0.00 (0.000)	0.00 (0.000)
3	Farmington	6	–	0.00 (0.000)	0.00 (0.000)	0.00 (0.000)	0.00 (0.000)
4	Farmington	0	0.00 (0.000)	–	–	–	–
4	Farmington	3	–	0.01 (0.011)	7.84 (7.384)	0.00 (0.000)	0.00 (0.000)
4	Farmington	6	–	0.00 (0.000)	0.00 (0.000)	0.02 (0.015)	0.00 (0.000)

Appendix 3: Cell density responses in simple bioassays. Values are reported in cells / mL. All values are the results from a single replicate, so variance estimates were not possible.

Experiment ID	Lake Bay	Experiment Day	Treatment	*Amphora coffeaeformis*	*Amphora delicatissima*	*Amphora* sp.	*Carteria* sp.	*Chaetocerous* sp.	*Cyclotella* sp.	*Dunaliella salina*	*Dunaliella viridis*
1	Farmington	6	C	243	0	8011	0	0	0	0	13133
1	Farmington	6	N	324	437	24469	0	0	0	0	5936
1	Farmington	6	NP	228	0	0	971	0	0	228	5067
1	Farmington	6	P	162	728	14565	0	0	0	728	682
2	Farmington	0	C	0	0	0	61173	0	0	0	52434
2	Farmington	0	N	0	0	0	0	0	0	3953	10542
2	Farmington	0	NP	0	0	0	1339	0	0	0	3213
2	Farmington	0	P	0	0	1785	0	0	0	297	11007
2	Farmington	6	C	0	0	0	90120	0	0	0	108554
2	Farmington	6	N	2185	0	0	41510	0	0	0	87390
2	Farmington	6	NP	0	0	0	120843	0	0	4096	104458
2	Farmington	6	P	0	0	0	47518	0	0	0	127807
2	Gilbert	0	C	0	0	0	0	0	0	0	25780
2	Gilbert	0	N	24	0	0	48	0	0	0	17229
2	Gilbert	0	NP	0	0	23	0	0	0	0	18274
2	Gilbert	0	P	0	0	0	44	0	0	0	14599
2	Gilbert	6	C	0	0	0	0	0	0	0	828215
2	Gilbert	6	N	0	0	0	0	0	0	0	646246
2	Gilbert	6	NP	0	0	0	0	0	0	0	677542
2	Gilbert	6	P	0	0	0	0	0	0	0	365725
3	Farmington	6	N	0	0	0	18934	0	0	0	313874
3	Farmington	6	NP	0	0	0	26217	0	0	0	19663
3	Farmington	6	P	0	0	0	402	0	0	0	25703
4	Farmington	0	initial	410	0	0	0	1311	0	1475	327711
4	Farmington	6	C	0	0	0	0	0	0	0	799614
4	Farmington	6	N	0	0	0	6554	0	0	0	216289
4	Farmington	6	NP	0	0	0	2185	0	0	0	380145
4	Farmington	6	P	0	0	0	0	99	0	297	36095

Experiment ID	Lake Bay	Experiment Day	Treatment	*Glenodinium* sp.	*Microcoleus* sp.	*Navicula graciloides*	*Navicula lanceolata*	*Navicula* sp.	*Navicula tripuctata*	*Nitzschia accicularis*	*Nitzschia epithemoides*
1	Farmington	6	C	121	0	0	0	243	0	36971	0
1	Farmington	6	N	0	0	0	0	810	0	53281	0
1	Farmington	6	NP	68	228	455	0	607	0	28053	0
1	Farmington	6	P	0	0	0	0	688	0	28604	0
2	Farmington	0	C	0	0	0	0	0	0	8739	0
2	Farmington	0	N	0	565	0	0	0	0	188	0
2	Farmington	0	NP	0	669	0	0	0	0	0	0
2	Farmington	0	P	0	1487	0	0	0	0	0	0
2	Farmington	6	C	0	0	0	0	0	0	0	0
2	Farmington	6	N	0	2185	0	0	0	0	6554	0
2	Farmington	6	NP	0	14337	0	0	0	0	2048	0
2	Farmington	6	P	0	0	0	0	0	0	0	0
2	Gilbert	0	C	0	0	0	0	0	0	0	0
2	Gilbert	0	N	0	0	0	0	0	0	0	0
2	Gilbert	0	NP	0	0	0	0	0	0	0	0
2	Gilbert	0	P	0	0	0	0	0	0	0	22
2	Gilbert	6	C	0	0	0	0	0	0	0	0
2	Gilbert	6	N	0	0	0	0	0	0	0	0
2	Gilbert	6	NP	0	0	0	0	0	0	0	0
2	Gilbert	6	P	0	0	0	0	0	0	0	0
3	Farmington	6	N	0	19663	0	0	0	0	24032	0
3	Farmington	6	NP	1639	6554	0	0	0	0	16386	0
3	Farmington	6	P	0	1104	0	0	0	0	2610	0
4	Farmington	0	initial	655	41292	0	0	0	410	52434	0
4	Farmington	6	C	0	22940	0	0	0	0	127807	0
4	Farmington	6	N	0	30149	0	0	0	0	144193	0
4	Farmington	6	NP	0	30586	0	0	0	0	222843	0
4	Farmington	6	P	99	1587	0	0	0	0	10809	0

Experiment ID	Lake Bay	Experiment Day	Treatment	*Nitzschia fonticola*	*Nitzschia palea*	*Nodularia* sp.	*Oocystis* sp.	*Phaedactylum* sp.	*Pseudoanabaena* sp.	*Rhopalodia musculus*	*Spermatozopsis* sp.
1	Farmington	6	C	0	0	0	414299	0	0	0	0
1	Farmington	6	N	437	0	0	441941	0	0	0	0
1	Farmington	6	NP	0	0	0	400877	0	243	0	0
1	Farmington	6	P	364	0	0	284590	0	0	0	0
2	Farmington	0	C	0	0	0	0	0	0	0	0
2	Farmington	0	N	188	0	0	1694	0	0	0	0
2	Farmington	0	NP	0	268	535	0	0	0	0	0
2	Farmington	0	P	0	0	0	595	0	297	0	0
2	Farmington	6	C	0	0	0	0	0	0	0	0
2	Farmington	6	N	0	2185	0	34956	0	0	0	0
2	Farmington	6	NP	0	0	0	6145	0	0	0	0
2	Farmington	6	P	0	3277	0	11470	0	0	0	0
2	Gilbert	0	C	0	0	0	0	0	0	0	0
2	Gilbert	0	N	0	0	0	0	0	0	0	0
2	Gilbert	0	NP	0	0	0	0	0	0	0	0
2	Gilbert	0	P	0	0	0	0	0	0	0	0
2	Gilbert	6	C	0	0	0	0	0	0	0	0
2	Gilbert	6	N	0	0	0	0	0	0	0	0
2	Gilbert	6	NP	0	0	0	0	0	0	0	0
2	Gilbert	6	P	328	0	0	0	0	0	0	0
3	Farmington	6	N	0	0	0	0	0	0	0	0
3	Farmington	6	NP	0	0	0	0	0	0	0	0
3	Farmington	6	P	0	0	0	402	0	0	0	0
4	Farmington	0	initial	0	0	0	0	0	0	0	0
4	Farmington	6	C	0	1639	0	3277	0	0	0	0
4	Farmington	6	N	0	0	0	17041	0	0	0	0
4	Farmington	6	NP	0	0	0	0	0	0	0	0
4	Farmington	6	P	0	0	0	0	0	0	0	0

Experiment ID	Lake Bay	Experiment Day	Treatment	*Sphaerellopsis* sp.	*Spirulina* sp.	*Treubaria* sp.	UNID Bacteria	UNID Biglagellate	UNID Chryso-phyte	UNID Green Oval	ALL TAXA
1	Farmington	6	C	0	0	0	0	0	175799	0	648819
1	Farmington	6	N	0	0	0	0	0	152292	207113	887040
1	Farmington	6	NP	0	0	0	0	0	74129	0	511153
1	Farmington	6	P	0	0	0	0	0	136235	225028	692375
2	Farmington	0	C	0	0	0	0	0	1114217	0	1236562
2	Farmington	0	N	0	0	0	0	0	172816	0	189947
2	Farmington	0	NP	0	0	0	0	0	110308	0	116332
2	Farmington	0	P	0	0	0	0	0	257028	0	272497
2	Farmington	6	C	0	0	0	0	0	1929398	0	2128072
2	Farmington	6	N	0	0	0	0	0	1690988	0	1867952
2	Farmington	6	NP	0	0	0	0	0	1593494	0	1845422
2	Farmington	6	P	0	0	0	0	0	5302361	0	5492434
2	Gilbert	0	C	0	0	0	0	0	4260	0	30040
2	Gilbert	0	N	0	0	0	0	0	1711	0	19012
2	Gilbert	0	NP	0	0	0	0	0	3596	0	21893
2	Gilbert	0	P	0	0	0	0	0	4559	0	19224
2	Gilbert	6	C	0	0	0	0	0	104272	0	932486
2	Gilbert	6	N	0	0	0	0	0	10487	0	656733
2	Gilbert	6	NP	0	0	0	0	0	76193	0	753735
2	Gilbert	6	P	0	0	0	0	0	88810	0	454863
3	Farmington	6	N	0	0	0	0	0	434763	0	811266
3	Farmington	6	NP	0	0	0	0	514506	1101108	0	1686072
3	Farmington	6	P	0	0	0	0	0	61446	0	91667
4	Farmington	0	initial	0	0	0	0	0	768810	0	1194506
4	Farmington	6	C	0	0	0	0	0	698024	0	1653301
4	Farmington	6	N	0	0	0	0	0	1094554	0	1508781
4	Farmington	6	NP	0	0	0	0	0	1284627	0	1920386
4	Farmington	6	P	0	0	0	0	0	36492	0	85478

Appendix 4: Biovolume response in simple bioassays. Values are reported in μm^3 / mL. All values are the results from a single replicate, so variance estimates were not possible.

Experiment ID	Lake Bay	Experiment Day	Treatment	*Amphora coffeaeformis*	*Amphora delicatissima*	*Amphora* sp.	*Carteria* sp.	*Chaetocerous* sp.	*Cyclotella* sp.	*Dunaliella salina*	*Dunaliella viridis*
1	Farmington	6	C	103063	0	982886	0	0	0	0	745258
1	Farmington	6	N	521931	16441	1578187	0	0	0	0	609554
1	Farmington	6	NP	199802	0	0	1318008	0	0	398153	197730
1	Farmington	6	P	97427	271839	2882781	0	0	0	2856194	43666
2	Farmington	0	C	0	0	0	125286949	0	0	0	5714391
2	Farmington	0	N	0	0	0	0	0	0	11609277	1658589
2	Farmington	0	NP	0	0	0	5802867	0	0	0	564977
2	Farmington	0	P	0	0	219983	0	0	0	429968	743287
2	Farmington	6	C	0	0	0	216348262	0	0	0	9037285
2	Farmington	6	N	2430893	0	0	116620225	0	0	0	10860337
2	Farmington	6	NP	0	0	0	328721043	0	0	32419652	6246025
2	Farmington	6	P	0	0	0	134982795	0	0	0	14294918
2	Gilbert	0	C	0	0	0	0	0	0	0	868060
2	Gilbert	0	N	16579	0	0	81206	0	0	0	507690
2	Gilbert	0	NP	0	0	979	0	0	0	0	383431
2	Gilbert	0	P	0	0	0	83107	0	0	0	508504
2	Gilbert	6	C	0	0	0	0	0	0	0	21631950
2	Gilbert	6	N	0	0	0	0	0	0	0	13915427
2	Gilbert	6	NP	0	0	0	0	0	0	0	21080332
2	Gilbert	6	P	0	0	0	0	0	0	0	8704795
3	Farmington	6	N	0	0	0	43806239	0	0	0	18628291
3	Farmington	6	NP	0	0	0	73880729	0	0	0	1912533
3	Farmington	6	P	0	0	0	2033356	0	0	0	1307529
4	Farmington	0	initial	263738	0	0	0	281158	0	6940375	23679041
4	Farmington	6	C	0	0	0	0	0	0	0	61222779
4	Farmington	6	N	0	0	0	18090164	0	0	0	10338536
4	Farmington	6	NP	0	0	0	4509482	0	0	0	40702504
4	Farmington	6	P	0	0	0	0	21321	0	1007846	3649271

Experiment ID	Lake Bay	Experiment Day	Treatment	*Glenodinium* sp.	*Microcoleus* sp.	*Navicula graciloides*	*Navicula lanceolata*	*Navicula* sp.	*Navicula tripuctata*	*Nitzschia accicularis*	*Nitzschia epithemoides*
1	Farmington	6	C	97427	0	0	0	2407551	0	6620109	0
1	Farmington	6	N	0	0	0	0	5947001	0	18261161	0
1	Farmington	6	NP	54803	32077	118930	0	3433163	0	4620263	0
1	Farmington	6	P	0	0	0	0	3706796	0	4247385	0
2	Farmington	0	C	0	0	0	0	0	0	1969955	0
2	Farmington	0	N	0	82751	0	0	0	0	84748	0
2	Farmington	0	NP	0	51649	0	0	0	0	0	0
2	Farmington	0	P	0	53657	0	0	0	0	0	0
2	Farmington	6	C	0	0	0	0	0	0	0	0
2	Farmington	6	N	0	34781470	0	0	0	0	1569983	0
2	Farmington	6	NP	0	1137646	0	0	0	0	385487	0
2	Farmington	6	P	0	0	0	0	0	0	0	0
2	Gilbert	0	C	0	0	0	0	0	0	0	0
2	Gilbert	0	N	0	0	0	0	0	0	0	0
2	Gilbert	0	NP	0	0	0	0	0	0	0	0
2	Gilbert	0	P	0	0	0	0	0	0	0	175834
2	Gilbert	6	C	0	0	0	0	0	0	0	0
2	Gilbert	6	N	0	0	0	0	0	0	0	0
2	Gilbert	6	NP	0	0	0	0	0	0	0	0
2	Gilbert	6	P	0	0	0	0	0	0	0	0
3	Farmington	6	N	0	3274072	0	0	0	0	6167789	0
3	Farmington	6	NP	1908310	532369	0	0	0	0	4998177	0
3	Farmington	6	P	0	182844	0	0	0	0	671750	0
4	Farmington	0	initial	488527	4815360	0	0	0	594512	15055126	0
4	Farmington	6	C	0	2682594	0	0	0	0	39134190	0
4	Farmington	6	N	0	2438095	0	0	0	0	34687648	0
4	Farmington	6	NP	0	3226054	0	0	0	0	55209566	0
4	Farmington	6	P	38377	210364	0	0	0	0	3010735	0

Experiment ID	Lake Bay	Experiment Day	Treatment	*Nitzschia fonticola*	*Nitzschia palea*	*Nodularia* sp.	*Oocystis* sp.	*Phaedactylum* sp.	*Pseudoanabaena* sp.	*Rhopalodia musculus*	*Spermatozopsis* sp.
1	Farmington	6	C	0	0	0	51167926	0	0	0	0
1	Farmington	6	N	10467	0	0	43344959	0	0	0	0
1	Farmington	6	NP	0	0	0	38947335	0	17978	0	0
1	Farmington	6	P	53163	0	0	38468486	0	0	0	0
2	Farmington	0	C	0	0	0	0	0	0	0	0
2	Farmington	0	N	20614	0	0	236110	0	0	0	0
2	Farmington	0	NP	0	40198	104628	0	0	0	0	0
2	Farmington	0	P	0	0	0	100326	0	62541	0	0
2	Farmington	6	C	0	0	0	0	0	0	0	0
2	Farmington	6	N	0	519174	0	5494665	0	0	0	0
2	Farmington	6	NP	0	0	0	888007	0	0	0	0
2	Farmington	6	P	0	700885	0	1999528	0	0	0	0
2	Gilbert	0	C	0	0	0	0	0	0	0	0
2	Gilbert	0	N	0	0	0	0	0	0	0	0
2	Gilbert	0	NP	0	0	0	0	0	0	0	0
2	Gilbert	0	P	0	0	0	0	0	0	0	0
2	Gilbert	6	C	0	0	0	0	0	0	0	0
2	Gilbert	6	N	0	0	0	0	0	0	0	0
2	Gilbert	6	NP	0	0	0	0	0	0	0	0
2	Gilbert	6	P	25917	0	0	0	0	0	0	0
3	Farmington	6	N	0	0	0	0	0	0	0	0
3	Farmington	6	NP	0	0	0	0	0	0	0	0
3	Farmington	6	P	0	0	0	51969	0	0	0	0
4	Farmington	0	initial	0	0	0	0	0	0	0	0
4	Farmington	6	C	0	342655	0	751580	0	0	0	0
4	Farmington	6	N	0	0	0	1856756	0	0	0	0
4	Farmington	6	NP	0	0	0	0	0	0	0	0
4	Farmington	6	P	0	0	0	0	0	0	0	0

Experiment ID	Lake Bay	Experiment Day	Treatment	*Sphaerellopsis* sp.	*Spirulina* sp.	*Treubaria* sp.	UNID Bacteria	UNID Biglagellate	UNID Chryso-phyte	UNID Green Oval	ALL TAXA
1	Farmington	6	C	0	0	0	0	0	1079421	0	63203641
1	Farmington	6	N	0	0	0	0	0	682570	5301514	76273786
1	Farmington	6	NP	0	0	0	0	0	228637	0	49566878
1	Farmington	6	P	0	0	0	0	0	574632	2948854	56151223
2	Farmington	0	C	0	0	0	0	0	3634309	0	136605603
2	Farmington	0	N	0	0	0	0	0	377625	0	14069714
2	Farmington	0	NP	0	0	0	0	0	702730	0	7267050
2	Farmington	0	P	0	0	0	0	0	272427	0	1882189
2	Farmington	6	C	0	0	0	0	0	3375545	0	228761092
2	Farmington	6	N	0	0	0	0	0	4544713	0	176821459
2	Farmington	6	NP	0	0	0	0	0	6234323	0	376032183
2	Farmington	6	P	0	0	0	0	0	33779363	0	185757489
2	Gilbert	0	C	0	0	0	0	0	27140	0	895200
2	Gilbert	0	N	0	0	0	0	0	14507	0	619982
2	Gilbert	0	NP	0	0	0	0	0	34839	0	419249
2	Gilbert	0	P	0	0	0	0	0	24904	0	792350
2	Gilbert	6	C	0	0	0	0	0	664276	0	22296226
2	Gilbert	6	N	0	0	0	0	0	22915	0	13938342
2	Gilbert	6	NP	0	0	0	0	0	416166	0	21496498
2	Gilbert	6	P	0	0	0	0	0	485080	0	9215792
3	Farmington	6	N	0	0	0	0	0	3686488	0	75562879
3	Farmington	6	NP	0	0	0	0	9856531	10668558	0	103757207
3	Farmington	6	P	0	0	0	0	0	595344	0	4842791
4	Farmington	0	initial	0	0	0	0	0	9228075	0	61345912
4	Farmington	6	C	0	0	0	0	0	1876015	0	106009814
4	Farmington	6	N	0	0	0	0	0	12049337	0	79460537
4	Farmington	6	NP	0	0	0	0	0	20135402	0	123783009
4	Farmington	6	P	0	0	0	0	0	63843	0	8001758

Appendix 5: Chlorophyll *a* responses in factorial bioassays. Values are reported in µg / L, with standard errors following in parentheses. N = 3 for all measurements. Treatments are: C = control, N = + nitrogen, P = + phosphorus. Salinities are reported and were different for Experiment A and Experiment B. ** indicates that the initial sample for Experiment A was measured on initial Farmington Bay water, not on any of the experimental units. Dashes indicate dates when samples weren't taken or missing data.

		Salinity and Nutrient Treatments											
		1 %			**3 %**			**5 %**			**7 %**		
Experiment ID	**Experiment Day**	**C**	**N**	**P**	**C**	**N**	**P**	**C**	**N**	**P**	**C**	**N**	**P**
A	Initial	**11.3 (0.1)	–	–	–	–	–	–	–	–	–	–	–
A	9	23.3 (2.1)	34.4 (3.8)	21.1 (0.3)	15.0 (1.0)	6.3 (0.7)	14.2 (0.2)	11.4 (1.7)	20.9 (0.4)	14.5 (0.9)	12.1 (0.1)	43.3 (13.8)	13.3 (0.2)
A	16	12.7 (1.4)	25.1 (13.0)	25.1 (4.5)	14.8 (0.9)	16.9 (1.7)	17.7 (2.9)	14.9 (0.3)	17.9 (0.8)	14.3 (0.5)	9.4 (0.2)	20.6 (1.0)	10.1 (0.7)
A	23	24.2 (6.6)	17.1 (3.2)	42.5 (5.5)	11.9 (1.7)	14.3 (0.6)	59.0 (26.6)	10.4 (0.3)	9.6 (0.5)	44.4 (4.6)	12.1 (0.7)	15.0 (0.1)	15.6 (0.8)
A	30	30.7 (2.2)	20.2 (5.2)	35.4 (2.8)	21.1 (2.9)	16.1 (1.1)	128.6 (10.2)	10.1 (0.1)	8.3 (1.1)	90.1 (4.9)	17.0 (1.6)	12.2 0.3)	64.7 (10.4)
		1 %			**4%**			**7%**			**10%**		
		C	**N**	**P**	**C**	**N**	**P**	**C**	**N**	**P**	**C**	**N**	**P**
B	0	15.0 (0.4)	12.9 (1.4)	12.7 (1.0)	21.0 (1.6)	15.4 (0.6)	18.7 (3.4)	16.8 (1.0)	15.3 (0.3)	28.9 (15.5)	16.4 (0.6)	14.1 (0.6)	15.1 (0.8)
B	7	39.5 (5.9)	91.8 (6.3)	29.9 (1.9)	22.0 (10.9)	62.6 (1.1)	39.4 (3.9)	28.2 (0.2)	82.2 (1.7)	30.7 (1.5)	20.5 (1.0)	70.8 (2.5)	16.9 (1.9)
B	14	29.9 (7.1)	38.4 (2.3)	26.7 (9.7)	11.9 (2.4)	29.9 (5.6)	20.5 (4.1)	16.6 (0.8)	64.8 (0.5)	16.3 (1.0)	18.2 (1.7)	62.5 (3.6)	15.3 (1.8)
B	21	15.5 (1.3)	37.6 (3.3)	16.7 (0.4)	7.6 (1.8)	15.7 (1.9)	11.8 (0.2)	8.2 (3.4)	37.0 (4.8)	7.3 (2.0)	7.9 (0.6)	35.3 (3.7)	8.9 (0.5)
B	28	17.7 (1.7)	42.3 (5.4)	19.5 (4.5)	7.7 (1.0)	13.5 (1.6)	11.3 (1.8)	9.1 (0.8)	15.8 (2.9)	12.4 (0.9)	7.5 (0.4)	15.3 (4.6)	6.0 (0.7)

Appendix 6: Nitrogen fixation responses in factorial bioassays. Values are reported in µg / L / hr, with standard errors and number of replicates following in parentheses. Treatments are: C = control, N = + nitrogen, P = + phosphorus and NP = + nitrogen + phosphorus. Salinities are reported and were different for Experiment A and Experiment B. Dashes indicate dates when samples weren't taken or missing data.

		Salinity and Nutrient Treatments					
		1 %			**3 %**		
Experiment ID	Experiment Day	**C**	**N**	**P**	**C**	**N**	**P**
A	0	–	–	–	–	–	–
A	9	0.01 (0.008,3)	0.00 (0.004,3)	0.10 (0.012,3)	0.00 (0.000,2)	0.00 (0.000,2)	0.00 (0.000,2)
A	16	0.58 (0.059,3)	0.04 (0.008,3)	1.84 (0.447,3)	0.10 (0.019,3)	0.08 (0.007,3)	0.53 (0.334,3)
A	23	1.08 (0.197,3)	0.10 (0.030,3)	1.21 (0.356,3)	0.56 (0.217,3)	0.05 (0.006,3)	4.72 (0.501,3)
A	30	0.39 (0.139,3)	0.01 (0.006,3)	0.39 (0.303,3)	1.52 (0.039,3)	0.00 (0.000,3)	0.82 (0.189,3)
		5 %			**7 %**		
		C	**N**	**P**	**C**	**N**	**P**
A	0	–	–	–	0.04 (0.025,3)	–	–
A	9	0.00 (0.000,2)	0.00 (0.000,2)	0.00 (0.000,2)	0.00 (0.000,3)	0.00 (0.000,3)	0.00 (0.000,3)
A	16	0.03 (0.013,3)	0.03 (0.005,3)	0.06 (0.007,3)	0.03 (0.002,3)	0.06 (0.017,3)	0.02 (0.008,3)
A	23	0.49 (0.422,3)	0.00 (0.005,3)	2.62 (0.223,3)	0.81 (0.786,3)	0.04 (0.014,3)	0.38 (0.111,3)
A	30	0.00 (0.001,3)	0.12 (0.129,3)	0.54 (0.107,3)	0.09 (0.035,3)	0.00 (0.000,3)	3.79 (2.757,3)
		1 %			**4 %**		
		C	**N**	**P**	**C**	**N**	**P**
B	0	0.00 (– , 1)	–	–	0.00 (– , 1)	–	–
B	7	0.08 (0.010,3)	0.00 (0.000,3)	0.06 (0.013,3)	0.00 (0.000,3)	0.00 (0.000,3)	0.00 (0.000,3)
B	14	0.11 (0.037,3)	0.00 (0.000,3)	0.28 (0.092,3)	0.00 (0.000,3)	0.00 (0.000,3)	0.00 (0.002,3)
B	21	0.02 (0.013,3)	0.00 (0.000,3)	0.62 (0.373,3)	0.00 (0.000,3)	0.00 (0.000,3)	0.00 (0.000,2)
B	28	0.00 (0.003,3)	0.00 (0.000,2)	0.04 (0.026,3)	0.00 (0.000,3)	0.00 (0.001,2)	0.07 (0.070,3)
		7 %			**10 %**		
		C	**N**	**P**	**C**	**N**	**P**
B	0	0.00 (–,1)	–	–	0.00 (– , 1)	–	–
B	7	0.00 (0.000,3)	0.00 (0.000,3)	0.00 (0.000,3)	0.00 (0.000,3)	0.00 (0.000,3)	0.00 (0.000,3)
B	14	0.00 (0.000,3)	0.00 (0.000,3)	0.00 (0.000,3)	0.00 (0.000,3)	0.00 (0.000,3)	0.00 (0.000,3)
B	21	0.00 (0.000,3)	0.00 (0.000,3)	0.00 (0.00,3)	0.00 (0.000,3)	0.00 (0.000,3)	0.00 (0.000,3)
B	28	0.00 (0.000,2)	0.00 (0.000,3)	0.00 (0.000,3)	0.00 (0.000,3)	0.00 (0.000,3)	0.00 (0.000,3)

Appendix 7: Cell density responses in factorial bioassays. Values are reported in cells / mL. All values are the results from a single replicate, so variance estimates were not possible.

Experiment ID	Experiment Day	Salinity (g / L)	Treatment	*Amphora coffeaeformis*	*Amphora delicatissima*	*Amphora* sp.	*Carteria* sp.	*Chaetoceros* sp.	*Cyclotella* sp.	*Dunaliella salina*	*Dunaliella viridis*
A	0	70	initial	0	0	8725	0	860	0	0	860
A	30	10	C	0	0	0	0	0	0	0	410
A	30	10	N	0	0	5462	0	7647	0	0	4895
A	30	10	P	0	0	3059	0	655	0	0	874
A	30	30	C	19	0	6072	0	1523	0	58	2564
A	30	30	N	2813	246	1830	0	55	0	0	1775
A	30	30	P	243	0	0	0	0	0	0	0
A	30	50	C	0	392	8764	0	2499	0	37	1044
A	30	50	N	7282	0	9030	0	4175	0	0	3010
A	30	50	P	205	0	0	0	273	0	0	1229
A	30	70	C	0	0	33857	0	8691	0	905	17381
A	30	70	N	1386	66	0	0	0	0	0	7458
A	30	70	P	0	0	26035	0	3277	0	0	6008
B	0	10	C	0	0	0	0	0	0	0	9831
B	0	40	C	0	0	0	0	0	0	936	1404
B	0	100	C	0	0	0	0	0	0	0	1639
B	7	70	P	0	0	0	1990	0	0	0	16971
B	14	40	N	0	0	1536	0	20	0	0	287
B	28	10	C	364	0	0	0	1238	0	1529	11652
B	28	10	N	55	0	7483	0	0	0	0	4479
B	28	10	P	0	0	0	468	6554	0	0	312
B	28	40	C	0	0	6937	0	0	0	0	1147
B	28	40	N	27	0	0	0	27	0	1202	10323
B	28	40	P	0	0	4038	0	13770	0	0	2516
B	28	70	C	0	0	0	0	0	0	164	3496
B	28	70	N	0	0	0	36	0	0	0	2895
B	28	70	P	0	0	0	0	1490	0	199	11122
B	28	100	C	0	0	0	22	0	0	0	1408
B	28	100	N	0	0	0	1966	0	0	328	19171
B	28	100	P	0	0	0	0	1806	0	0	6554

Experiment ID	Experiment Day	Salinity (g / L)	Treatment	*Glenodinium* sp.	*Microcoleus* sp.	*Navicula graciloides*	*Navicula lanceolata*	*Navicula* sp.	*Navicula tripuctata*	*Nitzschia accicularis*	*Nitzschia epithemoides*
A	0	70	initial	0	0	0	0	0	0	6759	0
A	30	10	C	0	307	0	0	0	0	0	0
A	30	10	N	0	0	0	0	0	0	485	0
A	30	10	P	0	0	0	0	0	0	0	0
A	30	30	C	19	0	0	0	0	19	0	0
A	30	30	N	0	0	0	0	0	0	27	0
A	30	30	P	0	0	0	0	0	0	0	0
A	30	50	C	19	0	0	0	0	0	0	0
A	30	50	N	0	0	0	0	0	0	0	0
A	30	50	P	0	0	0	0	0	0	0	0
A	30	70	C	0	0	0	0	0	0	0	0
A	30	70	N	0	0	0	0	66	0	0	0
A	30	70	P	0	0	0	0	0	0	0	0
B	0	10	C	0	0	0	0	0	0	6554	0
B	0	40	C	0	0	0	0	0	0	7022	0
B	0	100	C	0	2458	0	0	0	0	4096	0
B	7	70	P	0	8310	0	0	0	0	0	0
B	14	40	N	0	3625	0	0	0	0	0	0
B	28	10	C	0	5316	0	0	0	0	655	0
B	28	10	N	0	710	0	0	0	0	0	0
B	28	10	P	0	12016	0	0	0	0	0	0
B	28	40	C	0	9667	0	0	0	0	0	0
B	28	40	N	0	2021	27	0	0	0	0	0
B	28	40	P	0	6223	0	0	0	0	0	0
B	28	70	C	55	7428	0	0	0	0	0	0
B	28	70	N	0	0	55	0	0	0	0	0
B	28	70	P	0	17180	0	0	0	0	0	0
B	28	100	C	0	2297	0	0	0	0	0	0
B	28	100	N	0	1311	0	0	0	0	0	0
B	28	100	P	0	5819	0	0	0	0	0	0

Experiment ID	Experiment Day	Salinity (g / L)	Treatment	*Nitzschia fonticola*	*Nitzschia palea*	*Nodularia* sp.	*Oocystis* sp.	*Phaedactylum* sp.	*Pseudoanabaena* sp.	*Rhopalodia musculus*	*Spermatozopsis* sp.
A	0	70	initial	0	0	0	4383	0	0	0	0
A	30	10	C	0	0	819	3175	0	0	0	0
A	30	10	N	0	0	5583	4612	0	0	0	0
A	30	10	P	0	0	83020	11142	0	0	0	0
A	30	30	C	0	0	13320	733	0	0	0	0
A	30	30	N	164	683	0	3414	0	0	0	0
A	30	30	P	0	0	107902	485	0	0	0	0
A	30	50	C	0	0	205	0	0	0	0	0
A	30	50	N	0	1020	0	19614	0	0	0	0
A	30	50	P	0	0	53048	3482	0	0	0	0
A	30	70	C	0	0	10592	7061	0	0	0	0
A	30	70	N	0	66	0	27260	0	0	0	0
A	30	70	P	182	0	139095	8921	0	0	0	0
B	0	10	C	0	0	0	0	0	0	0	0
B	0	40	C	468	0	0	0	0	0	0	0
B	0	100	C	0	0	0	0	0	0	0	0
B	7	70	P	0	0	0	702	0	0	0	0
B	14	40	N	0	20	0	3502	0	0	0	0
B	28	10	C	36	0	0	2695	0	0	0	0
B	28	10	N	0	0	0	12781	0	0	0	0
B	28	10	P	0	0	0	6008	0	0	0	0
B	28	40	C	0	0	0	5571	0	0	0	0
B	28	40	N	55	137	0	3004	0	0	0	0
B	28	40	P	0	0	0	5627	0	0	0	0
B	28	70	C	0	0	0	7046	0	0	0	0
B	28	70	N	109	328	0	1912	0	0	0	0
B	28	70	P	0	0	0	4369	0	0	0	0
B	28	100	C	0	0	0	715	0	0	0	0
B	28	100	N	410	164	0	2458	0	0	0	0
B	28	100	P	67	0	0	936	0	0	0	0

Experiment ID	Experiment Day	Salinity (g / L)	Treatment	*Sphaerellopsis* sp.	*Spirulina* sp.	*Treubaria* sp.	UNID Bacteria	UNID Biglagellate	UNID Chrysophyte	UNID Green Oval	ALL TAXA
A	0	70	initial	41	0	0	0	0	15935	0	37564
A	30	10	C	0	71892	0	0	0	5325	0	81928
A	30	10	N	0	0	0	0	0	16992	0	45677
A	30	10	P	0	0	0	0	0	6336	0	105086
A	30	30	C	0	0	0	0	0	5012	0	29340
A	30	30	N	0	0	0	0	0	0	0	11006
A	30	30	P	0	0	0	0	0	0	0	108630
A	30	50	C	19	0	0	0	0	2163	0	15141
A	30	50	N	0	0	0	0	0	2039	0	46171
A	30	50	P	0	0	0	0	0	4916	0	63153
A	30	70	C	0	0	0	0	0	25348	0	103835
A	30	70	N	0	0	0	0	0	12673	0	48975
A	30	70	P	0	0	0	0	0	14019	0	197537
B	0	10	C	0	0	0	0	13108	1068337	0	1097831
B	0	40	C	0	0	0	0	19663	396998	0	426492
B	0	100	C	0	0	0	0	14747	592337	0	615277
B	7	70	P	0	0	0	0	878	25573	0	54423
B	14	40	N	0	0	0	0	0	8971	0	17963
B	28	10	C	0	2622	0	0	0	22066	0	48173
B	28	10	N	0	3605	0	0	0	31351	0	60463
B	28	10	P	0	22159	0	0	0	20053	0	67571
B	28	40	C	0	0	0	0	0	39981	0	63303
B	28	40	N	0	0	0	0	0	6063	0	22885
B	28	40	P	0	0	0	0	0	32771	0	64946
B	28	70	C	0	0	0	0	0	52051	0	70239
B	28	70	N	0	0	0	0	0	12562	0	17897
B	28	70	P	0	0	0	0	0	54519	0	88879
B	28	100	C	0	0	0	0	0	14192	0	18633
B	28	100	N	0	0	0	0	0	48010	0	73817
B	28	100	P	0	0	0	0	0	44542	0	59724

Appendix 8: Biovolume responses in factorial bioassays. Values are reported in μm^3 / mL. All values are the results from a single replicate, so variance estimates were not possible.

Experiment ID	Experiment Day	Salinity (g / L)	Treatment	*Amphora coffeaeformis*	*Amphora delicatissima*	*Amphora* sp.	*Carteria* sp.	*Chaetoceros* sp.	*Cyclotella* sp.	*Dunaliella salina*	*Dunaliella viridis*
A	0	70	initial	0	0	1124999	0	105583	0	0	39388
A	30	10	C	0	0	0	0	0	0	0	0
A	30	10	N	0	0	706486	0	855252	0	0	232405
A	30	10	P	0	0	307032	0	61095	0	0	51497
A	30	30	C	8635	0	594862	0	138570	0	304417	186047
A	30	30	N	1902661	20413	273199	0	3963	0	0	106929
A	30	30	P	259806	0	0	0	0	0	0	0
A	30	50	C	0	10818	773739	0	204184	0	62643	52832
A	30	50	N	4493824	0	2167389	0	472997	0	0	152297
A	30	50	P	151306	0	0	0	52563	0	0	115963
A	30	70	C	0	0	3215456	0	1094733	0	5016614	2232069
A	30	70	N	1555233	788	0	0	0	0	0	1266564
A	30	70	P	0	0	3437693	0	293293	0	0	266623
B	0	10	C	0	0	0	0	0	0	0	682333
B	0	40	C	0	0	0	0	0	0	4452722	177084
B	0	100	C	0	0	0	0	0	0	0	79921
B	7	70	P	0	0	0	2721026	0	0	0	1174432
B	14	40	N	0	0	160805	0	3083	0	0	23872
B	28	10	C	357533	0	0	0	122500	0	7748997	443293
B	28	10	N	98678	0	705884	0	0	0	0	174760
B	28	10	P	0	0	0	2252220	492598	0	0	15223
B	28	40	C	0	0	905141	0	0	0	0	60670
B	28	40	N	15984	0	0	0	1566	0	2925735	498551
B	28	40	P	0	0	508153	0	1534864	0	0	243410
B	28	70	C	0	0	0	0	0	0	565797	196959
B	28	70	N	0	0	0	0	0	0	0	135700
B	28	70	P	0	0	0	0	166613	0	189793	1224393
B	28	100	C	0	0	0	92757	0	0	0	255064
B	28	100	N	0	0	0	3772407	0	0	0	1359571
B	28	100	P	0	0	0	0	112162	0	0	800950

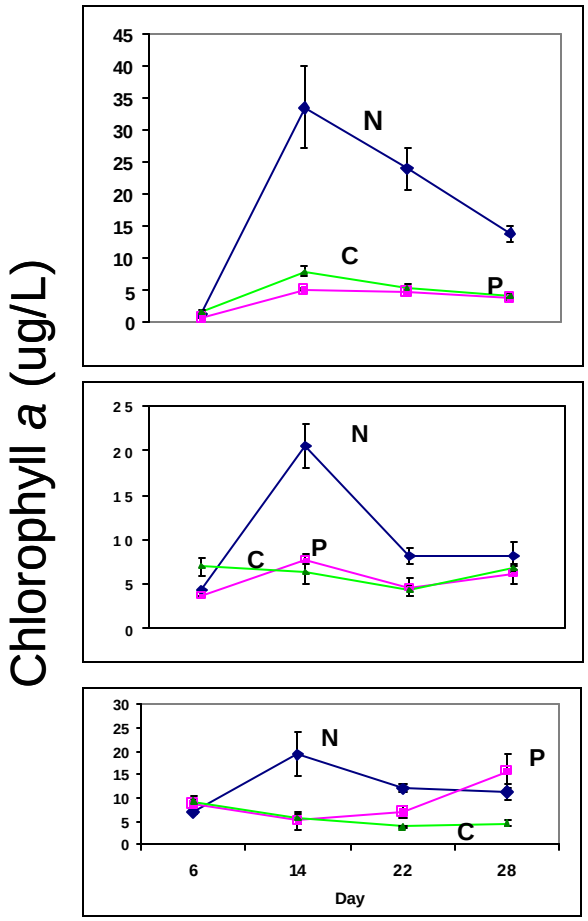
Experiment ID	Experiment Day	Salinity (g / L)	Treatment	*Glenodinium* sp.	*Microcoleus* sp.	*Navicula graciloides*	*Navicula lanceolata*	*Navicula* sp.	*Navicula tripuctata*	*Nitzschia accicularis*	*Nitzschia epithemoides*
A	0	70	initial	0	0	0	0	0	0	1573568	0
A	30	10	C	0	27401	0	0	0	0	0	0
A	30	10	N	0	0	0	0	0	0	204681	0
A	30	10	P	0	0	0	0	0	0	0	0
A	30	30	C	22566	0	0	0	0	25790	0	0
A	30	30	N	0	0	0	0	0	0	6261	0
A	30	30	P	0	0	0	0	0	0	0	0
A	30	50	C	19244	0	0	0	0	0	0	0
A	30	50	N	0	0	0	0	0	0	0	0
A	30	50	P	0	0	0	0	0	0	0	0
A	30	70	C	0	0	0	0	0	0	0	0
A	30	70	N	0	0	0	0	238417	0	0	0
A	30	70	P	0	0	0	0	0	0	0	0
B	0	10	C	0	0	0	0	0	0	869605	0
B	0	40	C	0	0	0	0	0	0	1938491	0
B	0	100	C	0	313158	0	0	0	0	1040114	0
B	7	70	P	0	861577	0	0	0	0	0	0
B	14	40	N	0	441267	0	0	0	0	0	0
B	28	10	C	0	487692	0	0	0	0	181947	0
B	28	10	N	0	65222	0	0	0	0	0	0
B	28	10	P	0	925775	0	0	0	0	0	0
B	28	40	C	0	0	0	0	0	0	0	0
B	28	40	N	0	193356	4958	0	0	0	0	0
B	28	40	P	0	706932	0	0	0	0	0	0
B	28	70	C	36840	1156129	0	0	0	0	0	0
B	28	70	N	0	0	14679	0	0	0	0	0
B	28	70	P	0	2918139	0	0	0	0	0	0
B	28	100	C	0	679808	0	0	0	0	0	0
B	28	100	N	0	72809	0	0	0	0	0	0
B	28	100	P	0	818734	0	0	0	0	0	0

Experiment ID	Experiment Day	Salinity (g / L)	Treatment	*Nitzschia fonticola*	*Nitzschia palea*	*Nodularia* sp.	*Oocystis* sp.	*Phaedactylum* sp.	*Pseudoanabaena* sp.	*Rhopalodia musculus*	*Spermatozopsis* sp.
A	0	70	initial	0	0	0	561314	0	0	0	0
A	30	10	C	0	0	35838	0	0	0	0	0
A	30	10	N	0	0	310447	1033264	0	0	0	0
A	30	10	P	0	0	10442270	1491256	0	0	0	0
A	30	30	C	0	0	2416516	193200	0	0	0	0
A	30	30	N	14994	251135	0	803716	0	0	0	0
A	30	30	P	0	0	8375137	71965	0	0	0	0
A	30	50	C	0	0	3795	0	0	0	0	0
A	30	50	N	0	331858	0	4441338	0	0	0	0
A	30	50	P	0	0	4626200	882852	0	0	0	0
A	30	70	C	0	0	474176	979247	0	0	0	0
A	30	70	N	0	25297	0	4351264	0	0	0	0
A	30	70	P	6853	0	9689750	1127414	0	0	0	0
B	0	10	C	0	0	0	0	0	0	0	0
B	0	40	C	662882	0	0	0	0	0	0	0
B	0	100	C	0	0	0	0	0	0	0	0
B	7	70	P	0	0	0	93987	0	0	0	0
B	14	40	N	0	8130	0	304262	0	0	0	0
B	28	10	C	3613	0	0	466693	0	0	0	0
B	28	10	N	0	0	0	2011877	0	0	0	0
B	28	10	P	0	0	0	789420	0	0	0	0
B	28	40	C	0	0	0	713446	0	0	0	0
B	28	40	N	5677	17763	0	295494	0	0	0	0
B	28	40	P	0	0	0	787620	0	0	0	0
B	28	70	C	0	0	0	564150	0	0	0	0
B	28	70	N	11829	97875	0	245481	0	0	0	0
B	28	70	P	0	0	0	285788	0	0	0	0
B	28	100	C	0	0	0	111029	0	0	0	0
B	28	100	N	27452	38634	0	256774	0	0	0	0
B	28	100	P	1831	0	0	146610	0	0	0	0

Experi-ment ID	Experiment Day	Salinity (g / L)	Treat-ment	*Sphaerel-lopsis* sp.	*Spirulina* sp.	*Treubaria* sp.	UNID Bacteria	UNID Biglagellate	UNID Chryso-phyte	UNID Green Oval	ALL TAXA
A	0	70	initial	450948	0	0	0	0	42827	0	3898627
A	30	10	C	0	7694304	0	0	0	0	0	7757544
A	30	10	N	0	0	0	0	0	66480	0	3409015
A	30	10	P	0	0	0	0	0	40363	0	12393513
A	30	30	C	0	0	0	0	0	0	0	3890603
A	30	30	N	0	0	0	0	0	0	0	3383272
A	30	30	P	0	0	0	0	0	0	0	8706907
A	30	50	C	157099	0	0	0	0	33903	0	1318256
A	30	50	N	0	0	0	0	0	12990	0	12072692
A	30	50	P	0	0	0	0	0	85931	0	5914813
A	30	70	C	0	0	0	0	0	44347	0	13056643
A	30	70	N	0	0	0	0	0	157683	0	7595247
A	30	70	P	0	0	0	0	0	54846	0	14876472
B	0	10	C	0	0	0	0	218752	7878770	0	9649460
B	0	40	C	0	0	0	0	283800	2168411	0	9683389
B	0	100	C	0	0	0	0	180156	6520712	0	8134060
B	7	70	P	0	0	0	0	9507	162917	0	5023445
B	14	40	N	0	0	0	0	0	41663	0	983083
B	28	10	C	0	494634	0	0	0	59304	0	10366208
B	28	10	N	0	273444	0	0	0	68506	0	3398370
B	28	10	P	0	2536214	0	0	0	93129	0	7104579
B	28	40	C	0	0	0	0	0	294850	0	1974107
B	28	40	N	0	0	0	0	0	16294	0	3975380
B	28	40	P	0	0	0	0	0	277876	0	4058854
B	28	70	C	0	0	0	0	0	647656	0	3167531
B	28	70	N	0	0	0	0	0	68615	0	574179
B	28	70	P	0	0	0	0	0	347321	0	5132047
B	28	100	C	0	0	0	0	0	275628	0	1414285
B	28	100	N	0	0	0	0	0	0	0	5527649
B	28	100	P	0	0	0	0	0	0	0	1880287

Appendix 9: Results from factorial bioassay of Olivia Lester, using Great Salt Lake water (Marcarelli et al. 2003). (A) Chlorophyll *a* (above), (B) nitrogen fixation (as acetylene reduction), and (C) algal biomass responses of Gilbert Bay plankton to salinity and nutrients during an October, 2002 experiment. The experiment was conducted nearly identically to the factorial bioassays described in the text. Note the strong initial chlorophyll response to nitrogen additions in all salinity treatments. In contrast, nitrogen fixation responded to phosphorus additions only at the lowest salinity (3%), and chlorophyll levels increased significantly in this treatment by day 28 of the experiment. Significant differences between nitrogen fixation treatments are indicated if histograms do not share a common letter.

A.

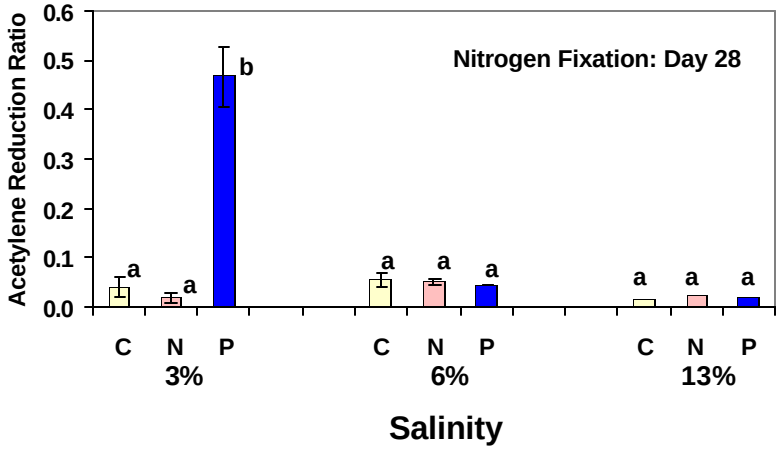


13%: N clearly limiting
No response to P

6% : N clearly limiting
No response to P

3%: N initially limiting
Large response to P by 28 d

B.



C.

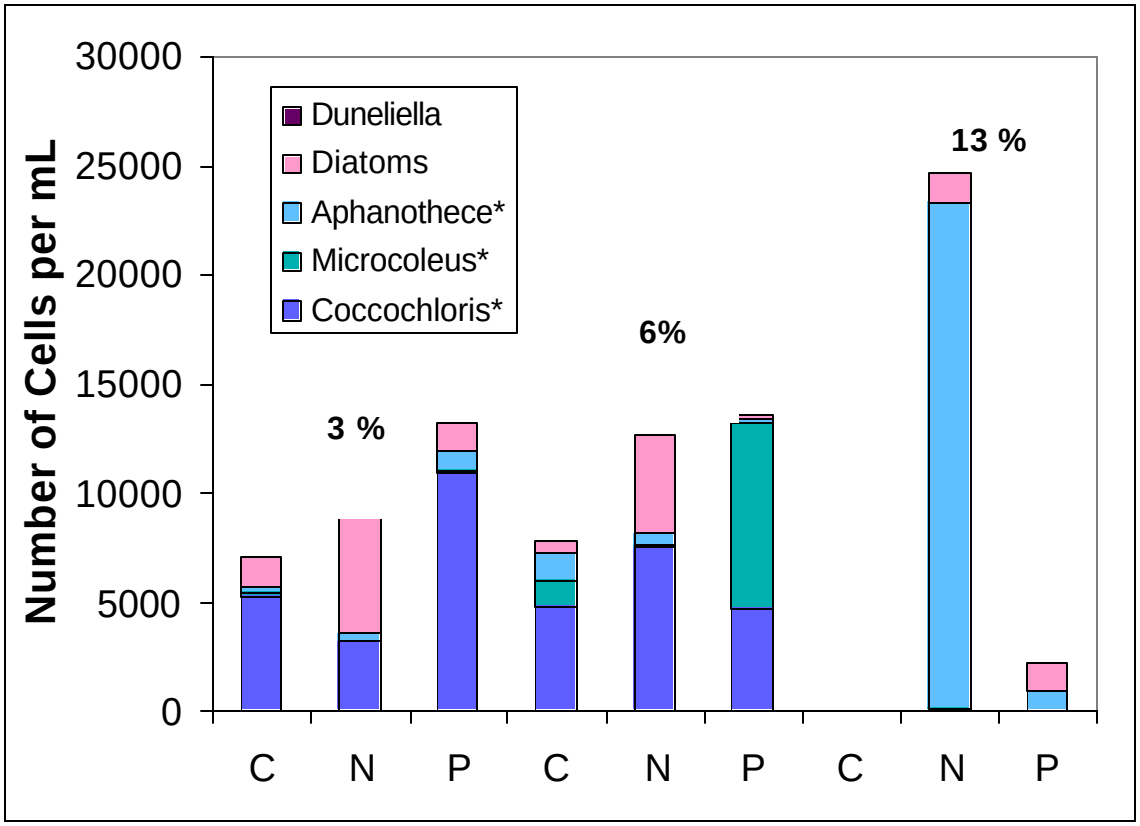


Figure 9.14. Algal densities in the nutrient treatments of O. Lester's experiment. Note dominance of cyanobacteria in all treatments, but particularly high percent of *Microcoleus* in the 3% P treatment and other low salinity treatments. In contrast, note absence of this taxa in the 13% salinity treatments. No data available for the control treatment at 13% salinity.

Table 9.3. Two-way Analysis of Variance for chlorophyll levels on the last day of the experiment (Day 26). Note that salinity, nutrient type, and particularly the interaction between these two treatments were highly significant. The analysis was done in Excel (2-way ANOVA with replication).

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Salinity	4041	2	2020.4	11.35	0.0006	3.55
Nutrient	1735	2	867.6	4.88	0.0203	3.55
Interaction	5821	4	1455.2	8.18	0.0006	2.93
Within	3203	18	177.9			
Total	14800	26				

Appendix 10. Long-term results of Simple Bioassay 3 that was run for 26 days.