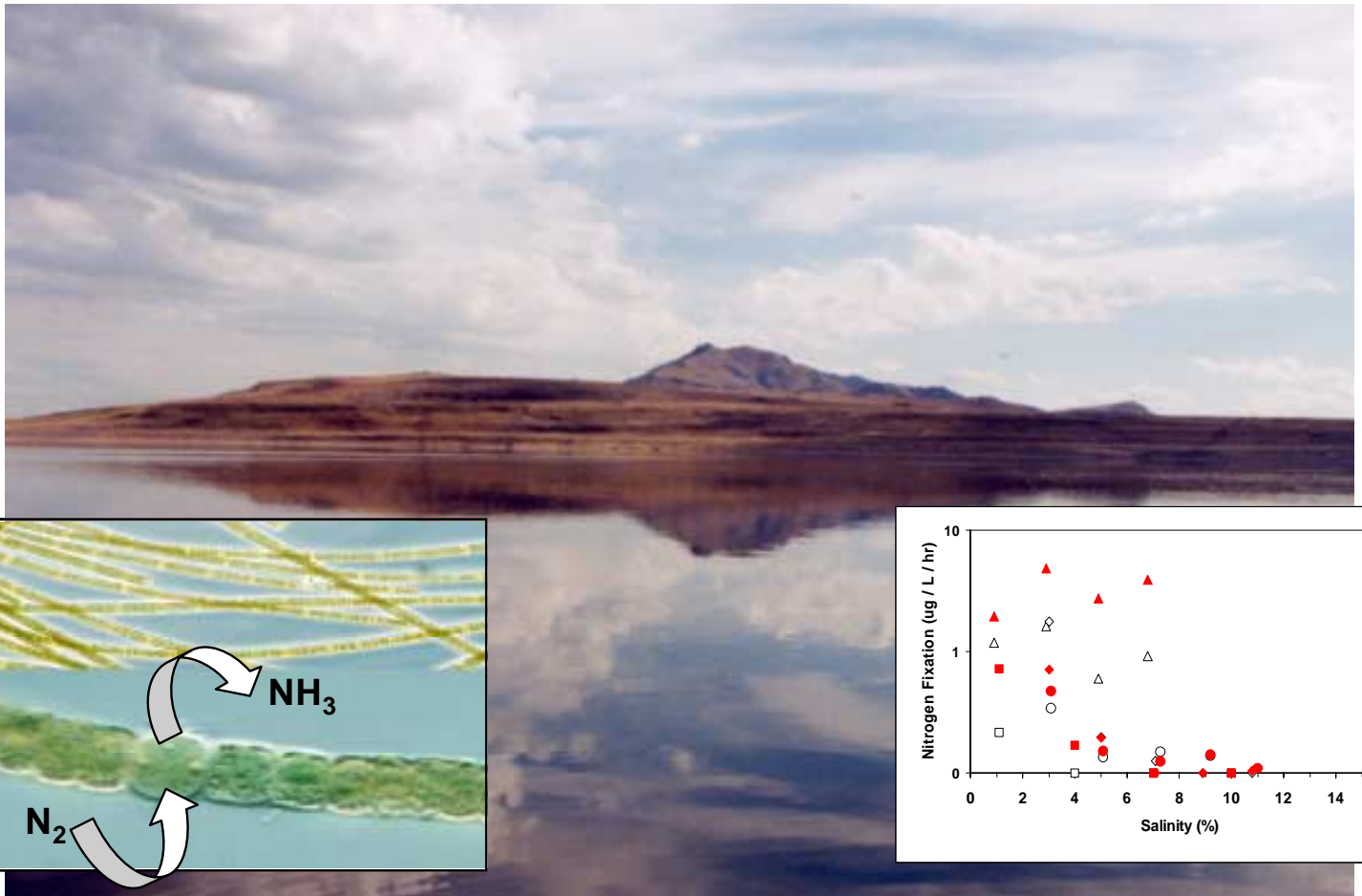


Report To  
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## Continuing Analysis of Phytoplankton Nutrient Limitation in Farmington Bay and the Great Salt Lake

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## Summary

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Farmington Bay is a nutrient-enriched, highly eutrophic embayment of the Great Salt Lake. The highly variable salinity of the bay influences what species of plankton can survive there. Previous analyses suggested that cyanobacteria (blue-green algae) may not be able to survive or fix atmospheric nitrogen at high salinities, thus maintaining the lake in a nitrogen-limited state. To determine the interacting influence of nutrients and salinity on the growth and nitrogen fixation of plankton we performed a 28-day bioassay with water from Farmington and Gilbert Bays in October 2004. We tested the response of the plankton to additions of nitrogen (N) and phosphorus (P) at salinities of 3%, 5%, 7%, 9% or 11%. Algal inocula from a variety of salinities were added to provide colonists for the cultures.

The plankton from Gilbert Bay were clearly N limited, and chlorophyll levels in N enriched treatments increased ca. 500% above control treatments in nearly all salinity treatments. Phosphorus additions had no effect or were slightly inhibitory to algal growth. In the Farmington Bay water, nitrogen additions also stimulated algal growth, but only at the 9 and 11% salinities. In all of the salinity treatments of Farmington Bay water, algal growth was very high at the start of the experiment, even in the control treatments, suggesting that nutrients were not limiting at the time the water was collected. In contrast to previous experiments, nitrogen fixation rates were low in most treatments. However, phosphorus stimulated N-fixation in Farmington Bay water when the salinity was 3%. Cyanobacterial biomass was highest at salinities less than 7%.

The different responses in this experiment than in previous bioassays was likely due to the limnological conditions in the lake immediately prior to collection of water used in the bioassay. High winds the night before mixed the water column of Farmington Bay and entrained hydrogen sulfide. The water column in Farmington Bay was anoxic when the water was collected, and hydrogen sulfide persisted in the experimental flasks for at least one week. Hydrogen sulfide is toxic to most aquatic organisms, including nitrogen-fixing cyanobacteria, but it can promote the growth of non-oxygenic purple-sulfur bacteria. Oxygen levels in Gilbert Bay were only 1 mg L<sup>-1</sup>, suggesting that hydrogen sulfide had also been entrained into the upper water column and stripped much of the oxygen from the water. The wind-induced mixing likely also entrained nutrients from the deep brine layer, and these may have provided sufficient nutrients to the microbes so that the response to added nutrients was muted.

Nevertheless, the results of the assay, when combined with those of three previous experiments, indicate that at salinities <7%, nitrogen-fixing cyanobacteria provide adequate nitrogen for algal growth, and the community will thus be limited by phosphorus or by light. At higher salinities such as those that occur in Gilbert Bay or in Farmington Bay during droughts, nitrogen fixation cannot occur, leading to persistent nitrogen limitation of those communities. 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. However, if nutrient controls are deemed to be warranted, field experiments that incorporate both pelagic (open water) and benthic (sediment) processes are needed to better understand nitrogen fixation and nutrient cycling under more natural conditions.

## Introduction

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 thought to be 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 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 limiting nutrient. Consequently, the question of nitrogen versus phosphorus limitation can 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; Wurtsbaugh and Marcarelli 2004a), but the factor(s) controlling nitrogen fixation are not understood. Experiments in our laboratory have indicated that salinity and nutrients interact to control nitrogen fixation, and thus maintain most of the lake in an N-limited state (Lester 2003, Wurtsbaugh and Marcarelli 2004a). At salinities less than 7%, cyanobacteria may become abundant and fix nitrogen so that phosphorus becomes the limiting nutrient. However, at salinities greater than 7%, nitrogen-fixing cyanobacteria do not become established and nitrogen remains the limiting nutrient (Wurtsbaugh and Marcarelli 2004a). If these results are confirmed, it would indicate that Great Salt Lake could be P-limited during low-salinity periods, and N-limited at other times or places.

These experiments suggest that nitrogen-fixing cyanobacteria may not function at higher salinities, although the mechanism behind this response remains unclear. 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. 2002a), 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  $\text{SO}_4^{2-}:\text{Mo}$  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 ocean. Despite these advances, the factor(s) controlling plankton growth and nitrogen fixation in hypersaline systems remains elusive.

Farmington Bay, which is located in the southeast corner of the Great Salt Lake, receives high nutrient loading and eutrophication is severe. The bay 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 2002). 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 the lake. Farmington Bay receives a large portion of the freshwater flowing into Great Salt Lake via the Jordan River and sewage canals, and therefore also receives a majority of the

nutrient loading to the lake. 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 ( $160 \text{ mg P m}^{-2} \text{ y}^{-1}$ ; Wetzel 2001). Chadwick et al. (1986) also estimated excessive phosphorus loading to the bay sufficient to promote extremely high algal populations. Because Farmington Bay is enclosed by Antelope Island on its western side and by an automobile causeway on the north (Figure 1), pollutants can accumulate in the bay. The bay is also shallow, so nutrients are concentrated in a relatively small volume of water and they may easily recycle between the sediments and water column. Chlorophyll levels (a measure of algal abundance) in the bay frequently exceed  $100 \mu\text{g L}^{-1}$  and Secchi depth transparencies are normally near 0.2 m (Wurtsbaugh and Marcarelli 2004b).

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). The impact of 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 eutrophication include toxic algal blooms, impaired recreational use, low oxygen levels, and odor. However, 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 in Gilbert Bay and, in turn, the brine shrimp that feed on the algae. Conversely, the anoxia and high hydrogen sulfide concentrations that develop in the deep brine layer of Gilbert Bay (Wurtsbaugh and Marcarelli 2004c) could be amplified by high nutrient loading from Farmington Bay. Furthermore, with increasing population growth, there is concern that impacts of eutrophication may extend from Farmington Bay into the main lake, where it could impact the brine shrimp harvest that contributes \$80 million to the Utah economy annually. Eutrophication likely also influences the birds that rely on the lake's brine shrimp and brine flies for food. The Utah Division of Water Quality may initiate Total Maximum Daily Load (TMDL) analyses of Farmington Bay 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.

The purpose of our study was to expand our knowledge of how salinity and nutrients interact to control phytoplankton growth and nitrogen fixation in Farmington Bay and the Great Salt Lake. This study builds on past experiments conducted by our research group and was designed to more finely determine the level at which nitrogen fixation is adversely affected by salinity in both Farmington and Gilbert Bays. Combined with the results of our previous experiment, these experiments should help determine which nutrient limits algal production at different salinities in Great Salt Lake, and therefore which nutrients should be the focus of management approaches for the lake.

## Methods

*Field sampling & background limnology*—Water for the experiments was collected between 10:00 and 14:00 on 18 Oct 2004 approximately 400 m south of the causeway in Farmington Bay and approximately 10 km NNW of Antelope Island in Gilbert Bay (N 41° 06.720 W 112° 27.986; Figure 1). The water was collected from a depth of 0.2 m using an 8-L horizontal Van Dorn sampler, placed in pre-washed 4-L polyethylene containers, and transported in coolers to the laboratory in Logan. Secchi depths were 0.22 m in Farmington Bay and 0.43 m in Gilbert Bay, while salinity was 3.5‰ and 17‰ respectively. The night and morning of 18 Oct 2004 was extremely stormy with winds reaching over 55 mph on Antelope Island and sustained at 20-25 mph for nearly a day prior to sampling. These winds likely entrained anoxic water rich in hydrogen sulfide and nutrients into the upper water column of Farmington Bay. Oxygen concentrations throughout the water column in Farmington Bay were nearly anoxic (Figure 2), and the collected water had a hydrogen sulfide smell. The chlorophyll level in the stock water collected from Farmington Bay was  $387 \mu\text{g L}^{-1}$ . In Gilbert Bay, surface oxygen concentrations were also low, with concentrations near  $1 \mu\text{g L}^{-1}$  throughout the water column (Figure 2), but there was no hydrogen sulfide odor. Chlorophyll concentration in the stock water from Gilbert Bay was  $11 \mu\text{g L}^{-1}$ .

Water was collected at each sample site for nutrient analyses. Total nitrogen (TN) was measured following persulfate digestion with the 2<sup>nd</sup> derivative method of Crumpton et al. (1992). Total phosphorus (TP) was measured after persulfate digestion with the malachite green spectrophotometric method of Linge and Oldham (2001, 2002), which corrects for interference from arsenate. In Farmington Bay, TN and TP concentrations were extremely high ( $13.9 \pm 0.2 \text{ mg N L}^{-1}$ ;  $0.93 \pm 0.05 \text{ mg P L}^{-1}$ ). Concentrations in Gilbert Bay were 30-40% of those in Farmington Bay, but still quite high ( $5.8 \pm 0.1 \text{ mg N L}^{-1}$ ;  $0.29 \pm 0.01 \text{ mg P L}^{-1}$ ).

*Experimental Design*—Factorial bioassays, where nutrient and salinity levels were simultaneously manipulated, were initiated with Gilbert Bay water on 19 Oct 2004 and with Farmington Bay water on 20 Oct 2004. Salinity treatments in the experiments ranged from 1‰, a low concentration where cyanobacterial nitrogen fixation is possible, to 11‰, a salinity where it was hypothesized that nitrogen fixation was impossible and well in excess of the previously suggested cutoff for nitrogen fixation at 7‰ (Table 1; Wurtsbaugh and Marcarelli 2004a). To obtain these salinities, an aliquot of water from the study bay was diluted with either distilled or saline water. The same aliquot volume of source water was used in all of the salinity treatments of the experiment to ensure that the same initial amount of algal biomass was present in each treatment. In both experiments, water was filtered in the lab through 153- $\mu\text{m}$  Nitex netting to remove macrozooplankton, and 141 mL (Gilbert Bay) or 686 mL (Farmington Bay) of water was added to 45 1-quart glass jars. Additionally, 2 mL of supplementary inocula water from three other sites (Table 2) was added to each jar to insure that a variety of phytoplankton with different salinity tolerances were present at the start of the experiment. Jars were randomly assigned to salinity treatments, then the study water was diluted to 800-mL using deionized water or different salinity mixtures (made with NaCl and MgSO<sub>4</sub> in a 7.8:1 ratio) 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. Concentrations for the nutrient treatments were  $1400 \mu\text{g L}^{-1}$  nitrogen (added as NH<sub>4</sub>NO<sub>3</sub>) and  $200 \mu\text{g L}^{-1}$  phosphorus (added as Na<sub>2</sub>HPO<sub>4</sub>). Nutrients were added to each non-control treatment from a stock solution and mixed immediately. After nutrient enrichment, the jars were placed randomly in a temperature controlled incubation room at 20°C, with light intensities of  $125 \mu\text{E m}^{-2} \text{ sec}^{-1}$  and an 18:6 light:dark photoperiod. The treatments were incubated for 28 days, and were sampled on days 0, 8, 16, 24 and 27 (Farmington) or 28 (Gilbert). Jars were agitated twice daily and randomized on the light table to ensure even irradiance.

*Sample analysis* – On sampling days, 50-mL aliquots of water were collected from each sample jar with a graduated cylinder. 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 bioassay incubation chamber. 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<sup>®</sup>. Ethylene and acetylene in each sample were measured at a later date using a SRI 8610C gas chromatograph equipped with a Poropak T column and a flame ionization detector (Capone 1993). The standards were used to construct a standard curve, which unknown samples were 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).

An index of algal biomass was measured weekly using chlorophyll *a* analyses. An aliquot (10 or 20 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, which was performed within 30 days of sample collection. To measure chlorophyll *a*, filters were extracted in 95% ethanol and chlorophyll *a* concentration was measured fluorometrically using a non-acidification technique with a Turner 10-AU fluorometer (Welschmeyer 1994).

Phytoplankton were collected from the initial water sample from each bay and from one replicate of each salinity/nutrient treatment combination at the end of the experiment. Approximately 40-mL of sample was preserved with 3% formalin. Phytoplankton cell density was determined by settling in Utermöhl chambers and counting on an inverted Olympus microscope at 1000X (Wetzel and Likens 2000). Phytoplankton were identified to the lowest taxonomic group possible (usually genus or species) using Felix and Rushforth (1979). 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:

$$\% \text{ of control} = [(\text{treatment value} - \text{control value}) / \text{control value}] * 100$$

Treatment effects were analyzed statistically within each bay using a three-way ANOVA with the PROC GLM statement in SAS version 8e with salinity, nutrient and day and all interactions of these treatments (salinity\*nutrient, salinity\*day, nutrient\*day, salinity\*nutrient\*day) as explanatory variables and chlorophyll *a* or nitrogen fixation as the response variables. Chlorophyll data were log transformed while nitrogen fixation data were cube root transformed to meet the ANOVA assumption that the residuals had a mean and standard deviation of zero and were normally distributed.

## Results

Very different results were obtained in the water from the two bays during the bioassay experiment. In Farmington Bay, there were few differences in chlorophyll *a* between the nutrient

treatments at most salinities (Figure 3). In the two highest salinities (9 & 11%), nitrogen stimulated the phytoplankton chlorophyll levels by day 24-28 of the experiment, but the differences were not large (Figure 3, Appendix 1). Overall, increasing salinity delayed peak values of chlorophyll in all nutrient treatments, with the peak occurring on day 8 at 3% and 5% salinities, on day 16 at 7%, and on day 24 at 9% and 11% salinities (Figure 3). The estimated chlorophyll concentrations were extremely high in the Farmington Bay water, reaching over  $1000 \mu\text{g L}^{-1}$  in many treatments, and were approximately ten times greater than those observed in previous bioassay experiments (Wurtsbaugh and Marcarelli 2004a). The three-way ANOVA indicated that there were significant interactions in chlorophyll response due to salinity, nutrient treatment, and the day of the experiment ( $F = 2.38$ ,  $p < 0.01$ ). For single factors, salinity treatment ( $F = 24.69$ ,  $p < 0.01$ ) and day of the experiment ( $F = 98.31$ ,  $p < 0.01$ ) were significant, but nutrient treatment was only marginally non-significant ( $F = 2.73$ ,  $p = 0.07$ ). This analysis supports the observations of delayed chlorophyll peaks with time in different salinities but no large response to either nitrogen or phosphorus additions (Figure 3).

In contrast, in Gilbert Bay nitrogen clearly stimulated chlorophyll levels at most salinities (Figure 4, Appendix 1). At 3% salinity, there was no difference noted between the different nutrient treatments. However, at the four higher salinities nitrogen stimulated chlorophyll levels 300-650% above controls. Phosphorus additions, in contrast, either had no effect or slightly depressed chlorophyll concentrations (Figure 4). In this experiment salinity also delayed the peak of chlorophyll, but only in the nitrogen treatments. Peak chlorophyll levels occurred on day 8 at 3% salinity, on day 16 at 5% and 7% and on day 24 at 9% and 11% salinities (Figure 4). The three-way ANOVA was highly significant ( $F = 78.2$ ,  $p < 0.01$ ) and all of the single factors and interactions were also highly significant ( $p \ll 0.01$ ).

In both bays, nitrogen fixation was very low for most of the experiment (Figure 3 and 4, right; Appendix 2). Significant nitrogen fixation was only observed in the 3% salinity in both bays. At this salinity, in the Farmington Bay water there was an initial increase in nitrogen fixation in both the control and phosphorus treatments on day 8, with a further increase on day 16 just in the control treatment, followed by a crash in both treatments by day 28 (Figure 3). The three-way ANOVA for nitrogen fixation in Farmington Bay was highly significant ( $F = 19.74$ ,  $p < 0.01$ ) and all of the single factors and interactions were highly significant ( $p \ll 0.01$ ). In Gilbert Bay, there was an initial increase in nitrogen fixation on day 8 in the 3% salinity, but only in the nitrogen treatment, which was unexpected, because adding nitrogen usually inhibits nitrogen fixation. However, the standard error of this value is greater than the mean, indicating that this rate may be driven by a single anomalous fixation measurement (Appendix 2). Other than that measurement, fixation rates were routinely low in all treatments in all measurement dates. The three-way ANOVA for nitrogen fixation in Gilbert Bay was highly significant ( $F = 2.97$ ,  $p \ll 0.01$ ) but only for the salinity, day and salinity\*day factors ( $p < 0.01$ , for all other factors  $p > 0.3$ ), indicating that nutrients did not significantly influence nitrogen fixation in this experiment.

Although nitrogen fixation rates were low in all of the treatments, cyanobacteria biomass and cell concentrations clearly responded to nutrient and salinity treatments. In Farmington Bay water, when salinity was adjusted to 3%, cyanobacteria comprised 20-39% of total algal biovolume at the end of the experiment in all nutrient treatments (Figure 5). At 5% salinity, cyanobacteria were abundant in the control and phosphorus treatment, but not in the nitrogen treatment. *Nodularia* sp., a heterocystous nitrogen-fixing cyanobacteria, was present in these treatments, along with non-heterocystous *Pseudoanabaena* sp. and *Spirulina* sp. (Appendix 3, 4). Heterocysts are specialized cells where nitrogen fixation occurs. It is interesting that despite the presence of heterocystous cyanobacteria, rates of nitrogen fixation were low in the experiment. In the Farmington Bay water, diatoms comprised a larger percent of the algal density and biovolume when salinities were increased. Green algae (Chlorophyte) density was

variable, but generally decreased with increasing salinity (Figure 5). In Gilbert Bay bioassay treatments, final algal densities and biovolumes were only about 10% of those in Farmington Bay. Cyanobacteria were only present in the 3% salinity treatment, and then only at very low densities. Green algae (primarily *Dunaliella viridis* and *Oocystis* sp.) dominated the algal community at the 3%, 5% and 7% salinities. Diatoms (Bacillariophyta) dominated the algal community at salinities of 9% and 11% (Figure 6; Appendices 3, 4).

## Discussion

Our factorial bioassay showed that the algal communities in Farmington and Gilbert bays may respond very differently to nutrient additions depending on the salinity of the bay and other environmental conditions. Algal communities from Gilbert Bay were clearly nitrogen limited at salinities  $\geq 5\%$ . 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 to 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%. 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. 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 algal production in most saline lakes is 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 effect was observed in the Gilbert Bay bioassay treatments of the current experiment, 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 metazoans, and complex responses to experiments may be influenced by these often ignored interactions.

In Farmington Bay, the lack of response to nutrient additions in our October experiment was likely a consequence of high winds immediately prior to sample collection that apparently mixed hydrogen sulfide rich water from the deep-brine layer into the surface water. Our research group has observed this phenomenon on two previous occasions (Wurtsbaugh et al. 2002, Wurtsbaugh and Marcarelli 2004c). When hydrogen sulfide mixes with oxygenated water, the sulfide is oxidized to sulfate, and this reaction requires two molecules of oxygen for every molecule of sulfide oxidized. The oxidation of sulfide can lead to prolonged anoxic events, such as those observed in Salton Sea (Watts et al. 2001) and the freshwater Onondaga Lake in New York (Effler et al. 1988) at hydrogen sulfide concentrations similar to those previously observed in Farmington Bay (Wurtsbaugh and Marcarelli 2004c). The near anoxia in Farmington Bay when we collected water for the bioassay was quite likely due to the entrainment of hydrogen sulfide from deeper water, and its reaction with oxygen. Likewise, when we collected bioassay water in Gilbert Bay, oxygen concentrations were near  $1 \text{ mg L}^{-1}$  throughout the water column, or about 75% lower than we observed in the fall in a previous analysis (Wurtsbaugh and Marcarelli



2004c). It is likely that the high winds the previous night had entrained some hydrogen sulfide into the upper mixed layer, and stripped some oxygen from the water. Sulfide inhibits oxygenic photosynthesis, but promotes anoxygenic photosynthesis by some cyanobacteria (Coen et al. 1986; Cohen 1989). It is also directly lethal to many organisms in the range of 1-5 mg L<sup>-1</sup>, and this toxicity has been linked to mass die-offs of phytoplankton, zooplankton and fish in the Salton Sea (Watts et al. 2001). In our experiment, the smell of hydrogen sulfide lingered in the Farmington Bay treatments for at least the first 8 days of the trial, indicating that the algae in these treatments may have been experiencing hydrogen sulfide toxicity for at least one-quarter of the experiment.

If the wind event mixed the deep brine layer into the surficial waters, it also likely brought up extremely high concentrations of dissolved N and P that would have been accumulating in the deep water. In Farmington Bay, TN and TP concentrations in the water collected for the bioassay were extremely high. Concentrations in Gilbert Bay were 30-40% of those in Farmington Bay, but still quite high. These very high nutrient levels in the stock water may have precluded the possibility of seeing responses to nutrient additions. This seems likely, as the chlorophyll levels in all of the control treatments of Farmington Bay water increased to 1000 µg L<sup>-1</sup> or more during the experiment. Nitrogen additions did boost chlorophyll levels even higher in some of the low salinity treatments near the end of the experiment, but it is clear that nutrient levels were very high, even in the controls. Furthermore, the algal biovolumes in the Farmington Bay treatments were approximately three times greater than those observed in previous factorial bioassay experiments (Wurtsbaugh and Marcarelli 2004a), again indicating that nutrients were high enough to promote excessive algal growth. An additional potential confounding factor could have come from bacterioplankton that thrive at the deep-brine layer interface, at least in Gilbert Bay (Wurtsbaugh and Berry 1990). These chemophototrophic bacteria could have been mixed into the water column along with the hydrogen sulfide. These bacteria contain photosynthetic pigments called bacteriochlorophyll that create a signal similar to chlorophyll *a* in our analytical technique. The collection of Farmington Bay and Gilbert Bay water immediately after the wind event was thus inopportune for the bioassay experiment, but it nevertheless provided insights into how this system functions.

Salinity exerted an important control on nitrogen fixation in the long-term experiments. In this experiment, nitrogen fixation rates were very limited, and were only observed in the 3% salinity in both bays. This data can be combined with results from similar factorial bioassay experiments conducted in Farmington Bay (Figure 7; Wurtsbaugh and Marcarelli 2004a). The combined data, although variable, indicates that some nitrogen fixation can occur up to salinities of 7%, and that generally phosphorus stimulates rates compared to control treatments. Dubinin et al. (1992) reported nitrogen fixation by a cyanobacterium community (*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 strain of *Anabaena* sp. was inhibited 50% at a low salinity of 0.8%, but a salt-tolerant species (*Anabaena tortulosa*) was inhibited 50% at 1.5%. However, *Anabaena* is not noted as a halotolerant genus. In a situation more closely related to Farmington Bay, Pinckney et al. (1995) found that decreasing salinity 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. Herbst (1998) found the nitrogen fixation by a benthic algal community from Mono Lake was decreased by nearly half between salinities of 5 and 10%, and rates were reduced by 90% at 15% salinity. Recent experiments by Robinson (2005) showed that when *Nodularia* from Farmington Bay were exposed to 10% and 17% salinity, nitrogen fixation dropped to zero within two hours. Additionally, fixation was decreased by half at

salinities of 5.8 % compared to the salinity of 3.9%. All of these results indicate that increasing salinity dramatically depresses nitrogen fixation rates in a variety of environments.

High salinity likely limits the survival of nitrogen-fixing organisms, specifically cyanobacteria. In the current experiment, cyanobacteria were only observed in the 3% and 5% treatments and only with Farmington Bay water. A monitoring study in Farmington Bay in 1971 (Carter et al. 1971) indicated 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 (Wurtsbaugh and Marcarelli 2004a). 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. A mesocosm study in Mono Lake examined the community composition of benthic algal mats in response to experimental manipulation of salinity at five levels between 5 and 15% salinity. They found that the filamentous cyanobacteria *Oscillatoria* sp. only occurred in salinity treatments between 5% and 10% and no other cyanobacteria species were present at any salinity (Herbst and Blinn 1998). The results of these two studies support the hypothesis that salinities greater than 7% may limit cyanobacterial survival in Farmington Bay.

The compiled results of all of the factorial bioassay experiments conducted in our lab suggest that given the salinities normally observed in Farmington Bay (<7%), phosphorus can control the rate of nitrogen fixation rates of cyanobacteria, leading to phosphorus limitation of the algal community (Table 3). This situation is similar to that suggested for freshwater lakes (Schindler 1977). However, during droughts, salinities rise above 7% in Farmington Bay, and in Gilbert Bay salinities are almost always above 7%. Our 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. This has clearly been illustrated in our experiments, where nitrogen limitation is constantly observed at salinities greater than or equal to 7%. 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 may need to be controlled. At higher salinities, nitrogen control would be appropriate.

It should also be noted from our results that phosphorus limitation of algal biomass is not always observed at low salinities, because nitrogen fixation is not always stimulated by phosphorus additions (Table 3). A suite of other factors as well as phosphorus can limit nitrogen fixation, including trace elements such as iron or molybdenum (Wurtsbaugh and Horne 1983; Howarth et al. 1988), grazing (Marino et al. 2002), turbulence (Mosiander et al. 2002b), temperature (McQueen and Lean 1987) and light (Lewis and Levine 1984). Light limitation of nitrogen fixation may be particularly important in Farmington Bay because light penetration is low due to eutrophic conditions (Wurtsbaugh and Marcarelli 2004b) and photosynthesis by cyanobacteria may be limited, leading to a lack of energy to carry out the energetically expensive nitrogen fixation reaction (Lewis and Levine 1984). It is likely that in the experiments where no nitrogen-fixation response was observed, some other factor may have been limiting nitrogen fixation, leading to the perpetuation of nitrogen limitation despite the low salinity.

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 in Farmington Bay after day 8-24 (depending on salinity) 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 nutrient control is an appropriate management strategy for Farmington Bay, field experiments in limnocorrals or shore-based mesocosms should be used to study nutrient limitation under more natural conditions.

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## Figures and Tables:

**Table 1.** Factorial bioassay experiments completed in 2004. The salinity of the source water, and those used in the experiments are shown. Nutrient treatments were controls (C), +nitrogen (N), and +phosphorus (P).

Bay	Experiment initiation date	Source Salinity (%)	Salinity Treatments (%)	Nutrient Treatments
Gilbert	19 Oct 2004	17	3, 5, 7, 9, 11	C, N, P
Farmington	20 Oct 2004	3.5	3, 5, 7, 9, 11	C, N, P

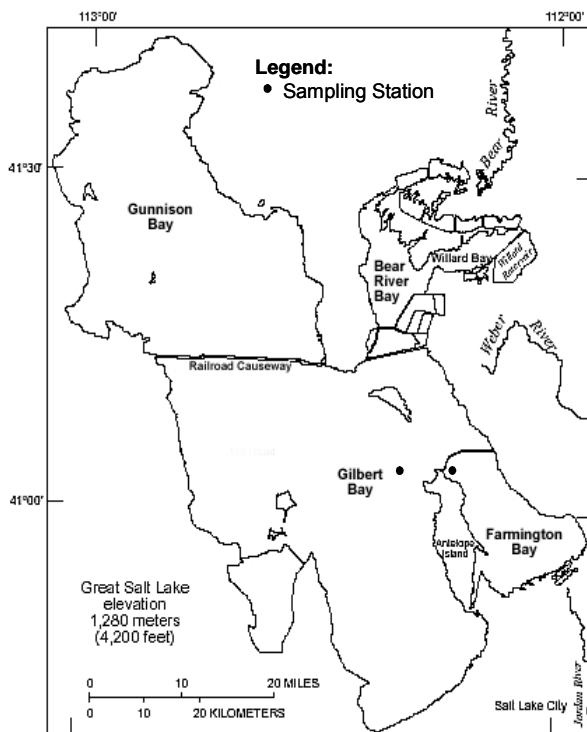
**Table 2.** Sources of supplementary inocula for the experiments. Salinities at each site are indicated.

Site	Salinity	Collection Date	Description
Thiokol Spring	0.7%	16 Oct 2004	Spring on SE edge of Thiokol that forms Blue Creek, ½ mile SE of Lampo Junction
Farmington Bay pond / channel	5.5%	18 Oct 2004	Pond / channel that passed under causeway approx. mid-way to Antelope Island, water likely originated from Farmington Bay and had a red tinge
Farmington / Gilbert mixing zone	12.0%	18 Oct 2004	Collected from boat on north side of Antelope Island bridge in mixing zone of water coming out of Farmington Bay and mixing into Gilbert Bay.

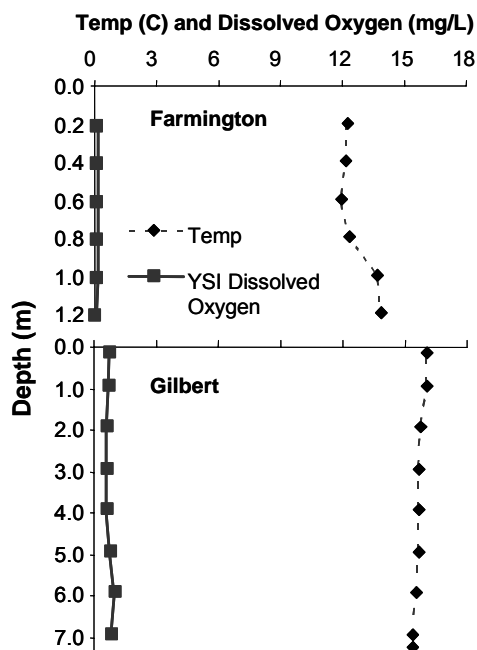
**Table 3.** Summary of results from all of the factorial bioassay experiments conducted by the USU Limnology Lab between 2002 and 2004. All experiments were conducted with water from Farmington Bay, except the 2004 experiment which was conducted with Gilbert Bay water. We omitted results from the 2004 Farmington Bay experiment because of suspected hydrogen sulfide toxicity. + indicates a positive response to nutrient addition, - indicates a negative response, and 0 indicates a negligible response between -25% and +25% of the control response. Each symbol represents a 100% increase above control rates (e.g + - 25 - 100%, ++ - 100-200, etc.). Note the dominance of chlorophyll responses to N additions, particularly at salinities greater than 5%. Also where a strong chlorophyll response to P was noted, there was a concurrent strong response of nitrogen fixation to P, indicating that a chlorophyll a P response is reliant on the occurrence of nitrogen fixation. Details on each experiment can be found as follows: 2002 (Lester 2003), 2003 A and B (Wurtsbaugh and Marcarelli 2004a), and 2004 (this report).

Salinity	Experiment	Chlorophyll response		Nitrogen-Fixation Response	
		N	P	N	P
1%	2003 A	-	+	-	+
	2003 B	++	0	-	0
3%	2002	++	+++	-	+++++++
	2003 A	0	+++++	-	+++++
	2004	0	0	0	+
4%	2003 B	++	+	0	0
5%	2003 A	0	++++	-	++++
	2004	++	0	-	+
6%	2002	+	0	0	0
7%	2003 A	0	0	-	0
	2003 B	++	0	0	0
	2004	+++++	0	++++*	-
9%	2004	+++++	0	-	-
10%	2003 B	+++	0	0	0
11%	2004	+++++	0	+	+
13%	2002	+++	0	0	0

\*Although this shows a very large response, the fixation rates here were actually very low and may be below the detection limit of the nitrogen fixation measurement technique.

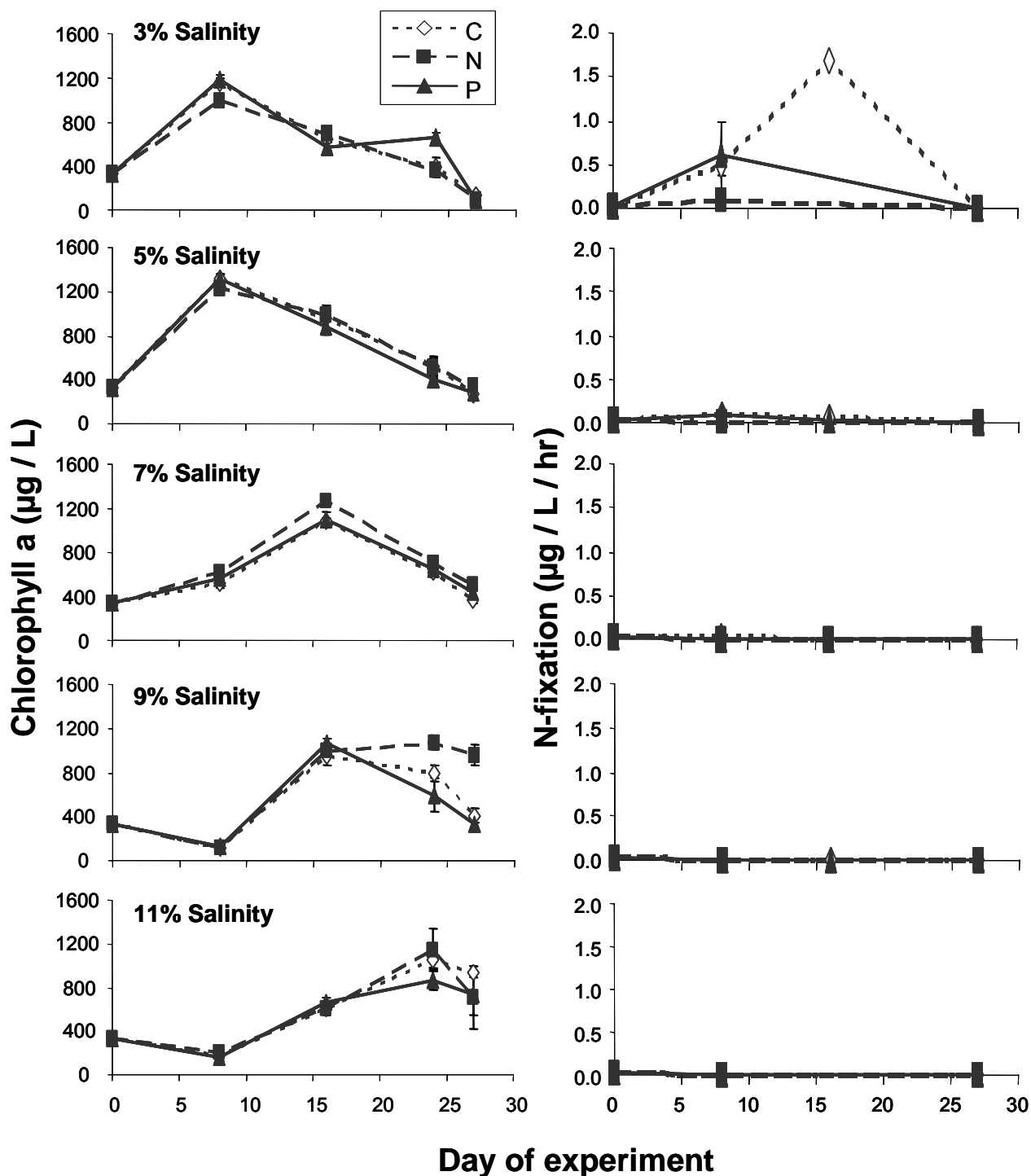


**Figure 1:** Map of Great Salt Lake, showing the locations of the Farmington Bay and the sites (solid dots) where water was collected for bioassay experiments in this study.

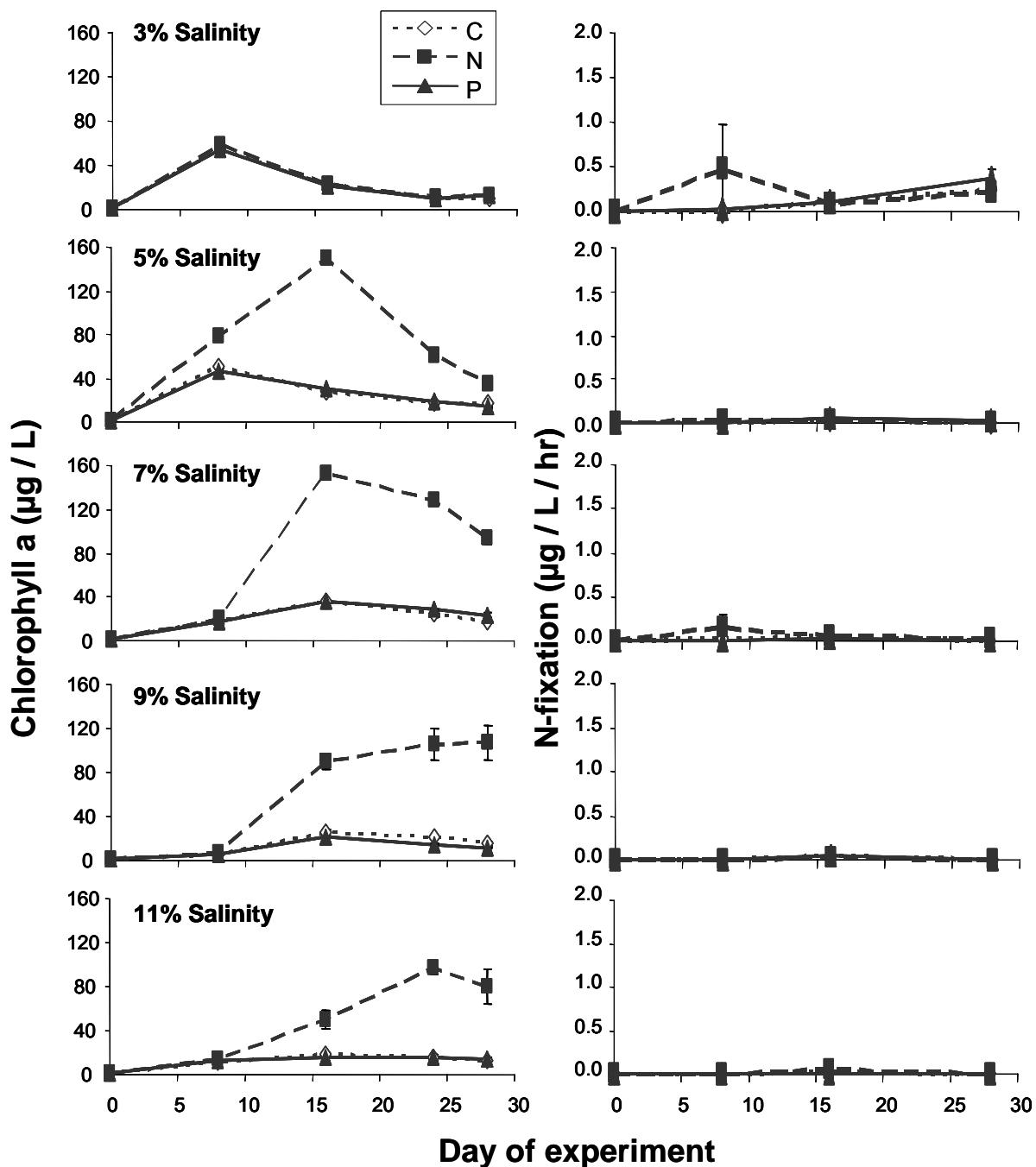


**Figure 2:** Temperature and oxygen profiles in Farmington and Gilbert Bays on 18 Oct 2004, when the water for the bioassays was collected. Note the different y-axis scales between the two panels. In Farmington Bay, there was inverse thermal stratification, indicating that the bay was underlain by a salt wedge. The entire water column was nearly anoxic. Oxygen concentrations were also low in Gilbert Bay, but there was no thermal or salinity stratification. Note that the hypersaline conditions in Gilbert Bay limit the amount of oxygen that can dissolve in the water. Oxygen and temperature were measured with a YSI Model 58 sensor, and the field concentrations were corrected for salinity and temperature.

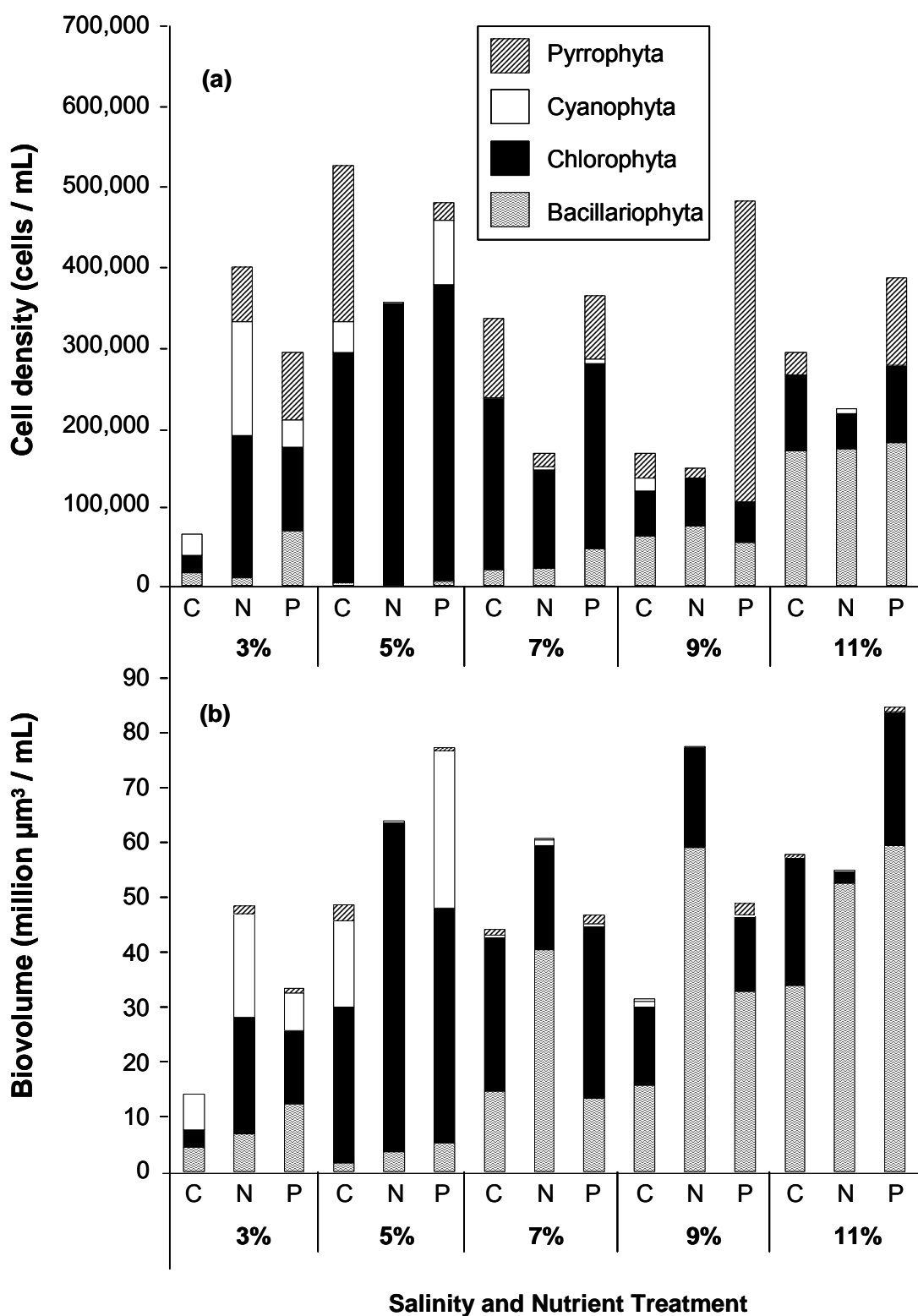




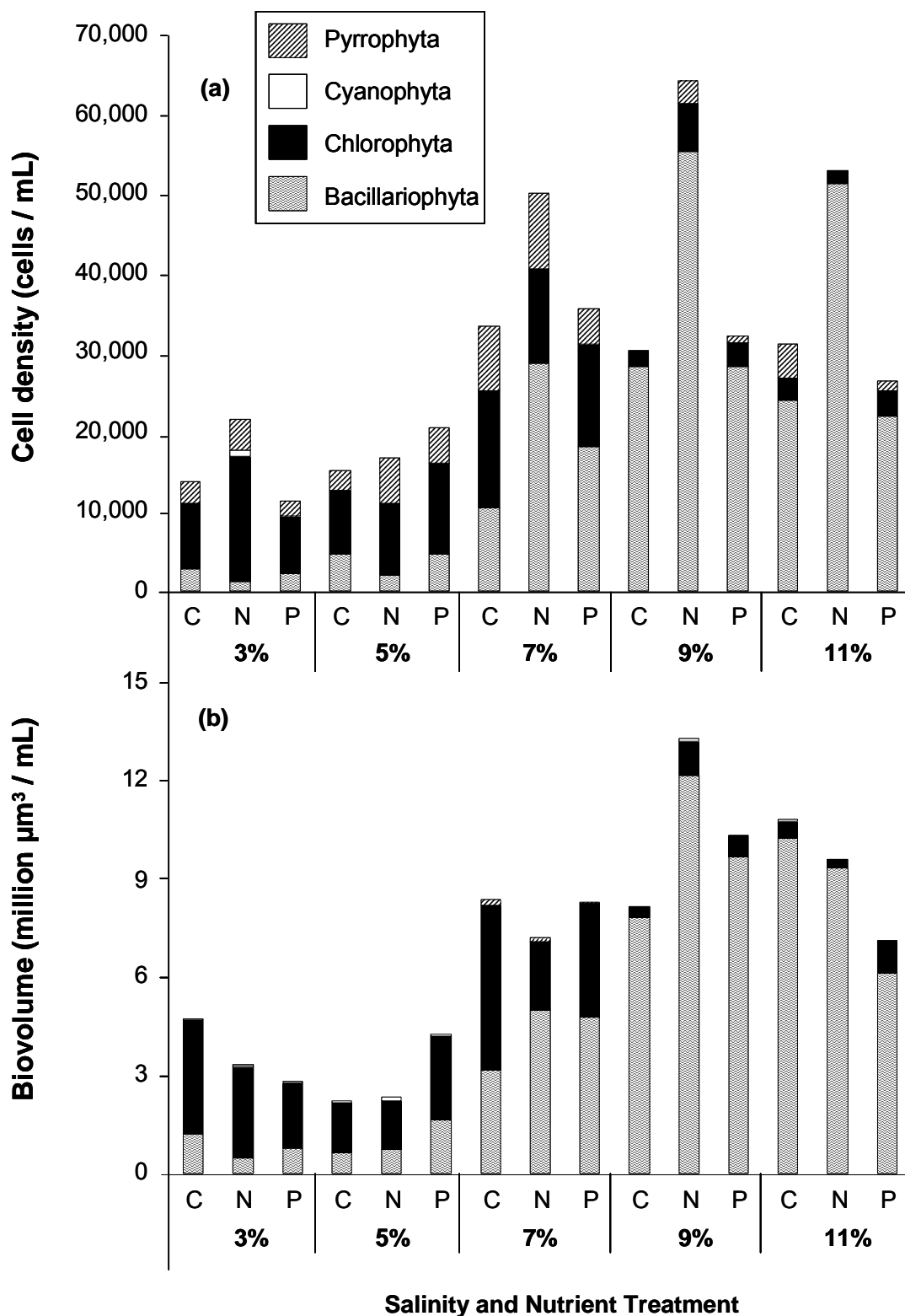
**Figure 3:** Responses of chlorophyll *a* concentrations (left) and nitrogen fixation (right) to nitrogen or phosphorus additions at five salinities with Farmington Bay water. Note the initial increase in chlorophyll in all treatments at low salinities, and delayed increases at higher salinities, with little difference between nutrient treatments except in the 9 and 11% salinities on days 24 and 28. Nitrogen fixation was only observed in the 3% treatment and was greatest in the control treatment. Day 24 nitrogen fixation data was omitted because sampling problems caused uncertainty in the results. Error bars  $\pm 1$  S.E. C = Controls; N = +nitrogen; P = +phosphorus.



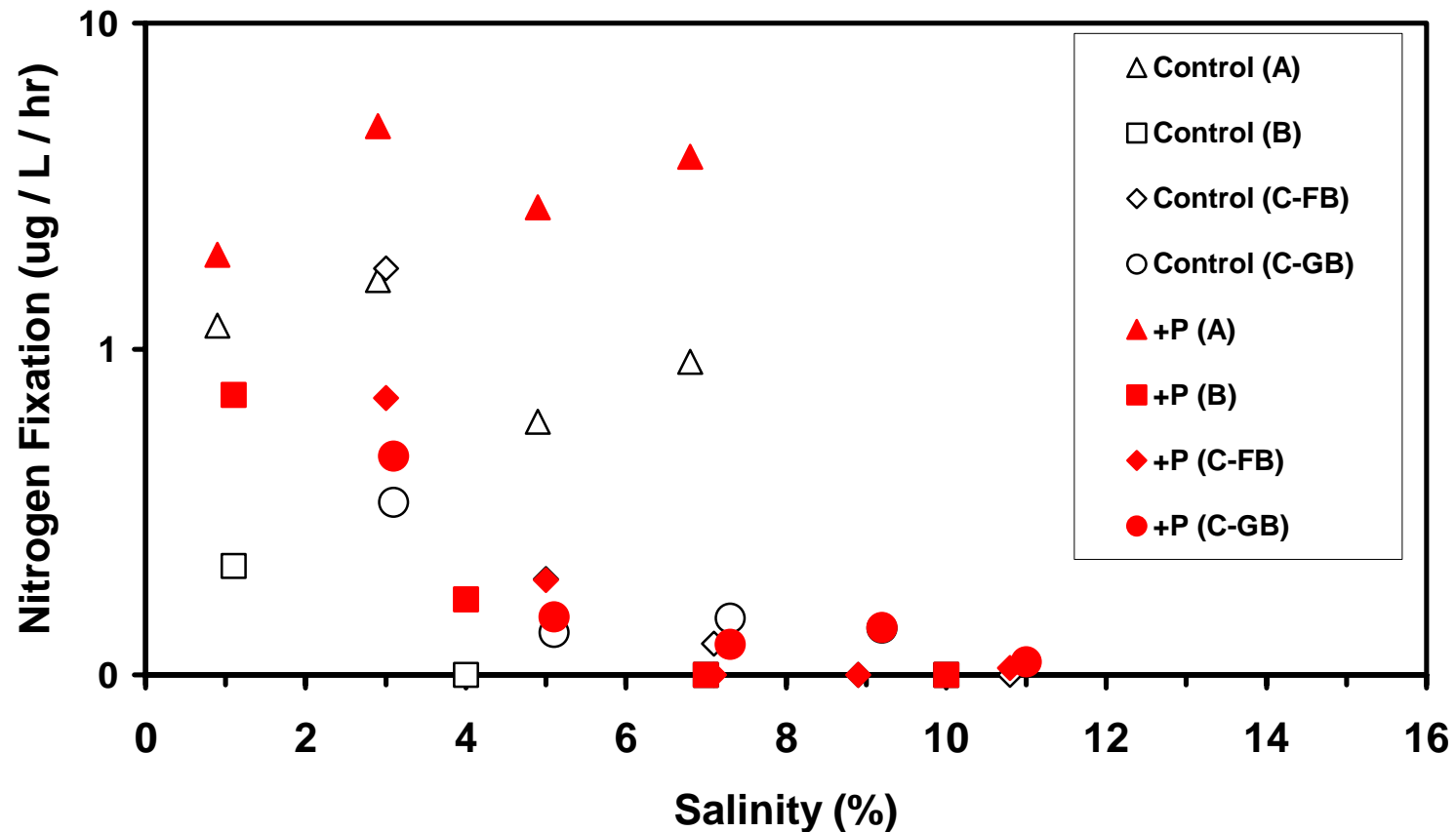
**Figure 4:** Responses of chlorophyll a concentrations (left) and nitrogen fixation (right) to nitrogen or phosphorus additions at five salinities with Gilbert Bay water. Note the initial increase in chlorophyll in all treatments, and the lack of nutrient response at 3%, while there was clear nitrogen stimulation of chlorophyll at all other salinities. Note the low levels of nitrogen fixation with the exception of some early fixation on day 8 in the 3% salinity. Day 24 nitrogen fixation data was omitted because sampling problems caused uncertainty in the results. Error bars  $\pm 1$  S.E. C = Controls; N = +nitrogen; P = +phosphorus.



**Figure 5:** Farmington Bay – (a) Cell density and (b) biovolume of algal cells on day 24 of the experiment at salinities of 3, 5, 7, 9 and 11%. Note non-consistent response of either algal density or biovolume to nutrient addition, but increasing importance of diatoms at increasing salinities. Cyanobacteria only showed noticeable biovolume and density at the two lowest salinities. 1 million  $\mu\text{m}^3/\text{mL} = 10^6 \mu\text{m}^3/\text{mL}$ . C = Controls; N = +nitrogen; P = +phosphorus.



**Figure 6:** Gilbert Bay – (a) Cell density and (b) biovolume of algal cells on day 24 of the experiment at salinities of 3, 5, 7, 9 and 11%. Note non-consistent response of either algal density or biovolume to nutrient addition, but increase in cell density and biovolume and increasing percentage of diatoms at increasing salinities. There was no noticeable cyanobacteria biovolume or density at any salinity. 1 million  $\mu\text{m}^3/\text{mL} = 10^6 \mu\text{m}^3/\text{mL}$ . C = Controls; N = +nitrogen; P = +phosphorus.



**Figure 7:** Maximum nitrogen fixation rates in relation to test salinities that were observed during all the factorial experiments in the control and +phosphorus treatments. The experiment described in this report is labeled as experiment C; data from experiments A and B can be found in Wurtsbaugh and Marcarelli 2004a. Fixation rates were higher in the first experiment (A) than in any other experiment. Maximum fixation rates showed no clear relationship with salinity from 1-7%, but declined to near zero at salinities greater than 7%. Maximum fixation rates occurred on different days in the different treatments. Some points are offset slightly on the X-axis to minimize overlap of multiple data. Note log scale on Y-axis.

**Appendix 1:** Average chlorophyll a responses in the October, 2004 bioassays utilizing water from Farmington and Gilbert Bays. Values are reported in  $\mu\text{g L}^{-1}$ , with standard errors following in parentheses. N = 3 for all measurements. Treatments are: C = control, N = + nitrogen and P = + phosphorus. Dashes indicate dates when samples weren't taken or missing data.

		Salinity and Nutrient Treatments														
		3%			5%			7%			9%			11%		
Bay	Experiment Day	C	N	P	C	N	P	C	N	P	C	N	P	C	N	P
Farmington	0	332 (8)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Farmington	8	1156 (34)	992 (14)	1176 (46)	1316 (44)	1233 (13)	1310 (44)	515 (38)	620 (40)	558 (44)	113 (15)	109 (11)	128 (13)	178 (8)	199 (22)	164 (31)
Farmington	16	639 (18)	688 (27)	572 (35)	958 (126)	982 (48)	881 (67)	1076 (27)	1260 (42)	1096 (72)	956 (85)	1001 (33)	1072 (40)	608 (59)	610 (48)	657 (46)
Farmington	24	388 (83)	359 (59)	660 (37)	516 (77)	515 (100)	410 (43)	618 (35)	698 (36)	639 (58)	792 (69)	1073 (61)	598 (157)	1050 (71)	1143 (198)	871 (95)
Farmington	27	139 (28)	84 (5)	88 (14)	272 (31)	342 (56)	281 (7)	359 (28)	508 (19)	442 (55)	408 (68)	957 (88)	334 (66)	942 (46)	710 (291)	740 (197)
		3%			5%			7%			9%			11%		
Bay	Experiment Day	C	N	P	C	N	P	C	N	P	C	N	P	C	N	P
Gilbert	0	-	-	-	2.0 (0.1)	-	-	-	-	-	-	-	-	-	-	-
Gilbert	8	54.9 (4.9)	59.0 (1.8)	54.2 (2.7)	50.4 (2.4)	78.7 (2.9)	47.0 (2.5)	18.7 (0.2)	20.3 (1.2)	16.8 (0.2)	6.3 (0.8)	6.6 (1.6)	5.1 (0.2)	11.8 (0.4)	13.8 (0.3)	13.6 (0.8)
Gilbert	16	22.5 (1.3)	23.0 (2.3)	20.9 (1.0)	27.4 (1.6)	150.3 (4.6)	30.2 (0.6)	35.8 (1.8)	152.1 (2.7)	36.7 (1.2)	25.3 (2.7)	89.8 (6.5)	21.3 (3.9)	18.8 (1.9)	50.2 (8.1)	15.1 (1.6)
Gilbert	24	9.8 (2.0)	11.1 (2.8)	10.3 (0.6)	17.5 (1.2)	60.7 (1.4)	19.2 (0.1)	24.1 (2.5)	127.8 (3.6)	28.7 (0.8)	22.0 (1.5)	106.0 (14.0)	14.8 (1.1)	15.6 (2.9)	97.7 (5.9)	15.7 (0.6)
Gilbert	28	10.3 (1.1)	12.7 (2.2)	13.3 (0.7)	17.2 (1.0)	35.4 (1.8)	14.0 (0.7)	18.0 (2.7)	93.5 (5.8)	23.6 (1.9)	16.0 (1.8)	107.1 (15.4)	12.1 (0.5)	12.2 (2.9)	80.0 (15.3)	14.2 (1.5)







Region	Experiment Day	Salinity	Nutrient	Chlorophyta (green algae)				Pyrrophyta (Chrysophytes)			Cyanophyta (cyanobacteria)			All Taxa
				Dunaliella viridis	Oocystis sp.	UNID Bi-flagellate	Sphaerellopsis sp.	Desmar-ella sp.	Gleno-dinium sp.	UNID Chrysophyte	Nodula-ria sp.	Pseudo-anabaena sp.	Spirulina sp.	
Inocula	0	5.5	-	85.6	0.0	25.0	0.0	0.0	0.0	99.5	0.0	0.0	0.0	214.0
		12	-	1.5	19.1	1.7	0.0	0.0	0.0	8.1	43.6	0.0	0.0	77.4
Farmington	24	3	C	1.0	22.9	0.0	0.0	0.0	0.0	0.0	13.4	8.7	2.2	64.3
			N	0.0	137.5	37.8	0.0	0.0	0.0	67.6	129.5	0.0	12.4	399.0
			P	15.7	76.6	10.8	0.0	0.0	0.0	86.5	0.0	30.2	4.3	292.7
		5	C	25.7	240.7	22.7	0.0	2.0	1.0	192.9	0.0	14.6	23.2	526.2
			N	26.2	324.6	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.7	354.0
			P	55.6	301.1	13.5	0.0	0.0	0.0	23.3	60.6	9.8	5.7	479.1
		7	C	68.1	131.6	14.1	0.0	0.0	0.0	98.5	0.0	0.0	1.6	326.2
			N	22.8	100.8	0.0	0.0	0.0	0.2	15.7	3.6	0.0	0.0	165.2
			P	37.5	192.4	0.0	0.0	0.0	0.0	77.8	6.5	0.0	0.0	359.5
		9	C	26.2	27.2	0.0	0.0	0.0	0.0	29.9	16.2	0.0	0.2	138.8
			N	47.3	11.6	0.4	0.0	0.0	0.0	11.9	0.0	0.0	0.0	113.6
			P	23.2	28.6	0.0	0.0	0.0	0.0	376.9	0.0	0.0	0.0	458.9
		11	C	94.1	2.3	0.0	0.0	2.8	0.0	24.5	0.0	0.0	0.0	208.4
			N	36.0	5.5	2.0	0.0	0.0	0.0	0.0	4.8	0.0	0.0	137.4
			P	45.1	14.9	37.8	0.0	1.5	0.0	105.8	0.0	0.0	0.0	306.0
Gilbert	24	3	C	6.4	1.8	0.0	0.0	0.0	0.0	2.5	0.0	0.0	0.0	13.3
			N	7.4	2.7	5.6	0.0	0.0	0.0	3.8	0.8	0.0	0.0	21.3
			P	5.3	1.7	0.0	0.0	0.0	0.0	1.9	0.0	0.0	0.0	10.5
		5	C	2.7	3.7	1.7	0.0	0.0	0.0	2.4	0.0	0.0	0.0	14.1
			N	2.1	7.0	0.0	0.0	0.0	0.0	5.5	0.0	0.0	0.0	15.8
			P	9.1	2.4	0.0	0.0	0.0	0.0	4.4	0.0	0.0	0.0	18.5
		7	C	12.2	2.5	0.0	0.0	0.0	0.0	8.0	0.0	0.0	0.0	31.3
			N	8.4	3.2	0.0	0.0	0.0	0.0	9.7	0.0	0.0	0.0	49.2
			P	8.8	4.0	0.0	0.0	0.0	0.0	4.4	0.0	0.0	0.0	33.7
		9	C	0.1	1.2	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	17.2
			N	1.1	1.1	3.4	0.0	0.0	0.0	2.9	0.0	0.0	0.0	37.2
			P	0.8	0.3	1.7	0.0	0.0	0.0	0.8	0.0	0.0	0.0	19.5
		11	C	2.4	0.1	0.5	0.0	0.0	0.0	4.2	0.0	0.0	0.0	19.2
			N	1.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	27.3
			P	0.5	0.0	2.6	0.0	0.0	0.0	1.2	0.0	0.0	0.0	15.5

**Appendix 4:** Biovolume responses in the bioassay. Values are reported as million  $\mu\text{m}^3$  / mL. All values are the results from a single replicate, so variance estimates were not possible.

Region	Experiment Day	Salinity	Nutrient	Bacillariophyta (Diatoms)									Chlorophyta			
				Amphora coffeaeformis	Amphora delicatissima	Chaetoceros sp.	Entomo-neis sp.	Navicula sp.	Nitzschia accicularis	Nitzschia epithetmoides	Nitzschia fonticola	Nitzschia palea	UNID Diatom	Carteria sp.	Dunaliella salina	
Inocula	0	5.5	-	0.87	0.01	0.00	0.00	0.19	0.00	0.00	0.10	0.00	0.00	1.21	0.00	
		12	-	0.28	0.00	0.00	0.00	0.14	0.03	0.00	0.00	0.04	0.00	0.00	0.00	
Farmington	24	3	C	0.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.06	0.00	0.00	
			N	6.49	0.00	0.00	0.00	0.09	0.06	0.00	0.03	0.00	0.00	0.00	0.00	0.00
			P	2.20	0.00	0.00	0.00	0.27	0.11	0.00	0.02	0.00	9.43	0.00	0.00	0.00
		5	C	0.64	0.00	0.00	0.00	0.38	0.09	0.00	0.00	0.00	0.00	0.00	2.75	0.00
			N	2.39	0.00	0.00	0.00	0.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.17
			P	3.31	0.00	0.00	0.00	0.98	0.00	0.00	0.00	0.00	0.00	0.00	0.39	0.00
		7	C	2.81	0.00	0.00	0.00	5.90	0.00	0.00	0.00	0.00	0.00	0.00	1.02	0.00
			N	37.01	0.00	1.69	0.00	0.45	0.00	0.00	0.01	0.76	0.00	0.00	0.73	0.23
			P	7.25	0.00	3.55	0.00	1.25	0.00	0.00	0.00	0.00	0.00	0.00	0.75	0.00
		9	C	5.65	0.00	0.00	0.00	5.00	0.00	0.00	0.00	0.00	0.00	0.00	1.61	0.00
			N	9.07	0.00	0.00	0.00	24.96	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00
			P	7.39	0.00	0.00	8.47	8.45	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		11	C	1.03	0.06	0.00	0.00	16.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
			N	3.00	0.00	0.00	0.00	24.36	0.20	0.66	0.02	0.00	0.00	0.00	1.28	0.00
			P	4.30	0.00	0.00	8.27	21.45	0.00	1.29	0.00	0.00	2.56	0.89	0.00	
Gilbert	24	3	C	0.51	0.00	0.00	0.31	0.09	0.21	0.00	0.00	0.00	0.00	0.00	0.26	
			N	0.21	0.00	0.00	0.00	0.07	0.13	0.00	0.00	0.00	0.00	0.00	0.00	
			P	0.17	0.00	0.00	0.00	0.25	0.07	0.06	0.00	0.00	0.00	0.00	0.00	
		5	C	0.07	0.01	0.03	0.00	0.24	0.04	0.00	0.00	0.00	0.00	0.11	0.00	
			N	0.23	0.00	0.00	0.00	0.26	0.00	0.00	0.00	0.00	0.00	0.10	0.00	
			P	0.44	0.00	0.00	0.00	0.59	0.03	0.00	0.00	0.00	0.00	0.00	0.00	
		7	C	0.85	0.00	1.00	0.00	0.49	0.00	0.08	0.01	0.28	0.00	0.39	0.00	
			N	0.21	0.00	3.77	0.00	0.52	0.00	0.00	0.00	0.00	0.00	0.24	0.00	
			P	0.04	0.00	3.60	0.00	0.46	0.01	0.09	0.00	0.14	0.00	0.00	0.00	
		9	C	0.00	0.00	0.26	0.00	3.78	0.00	0.00	0.00	0.00	0.00	0.10	0.00	
			N	0.05	0.00	0.23	0.00	5.93	0.00	0.00	0.00	0.00	0.00	0.59	0.00	
			P	0.24	0.00	0.26	0.00	4.50	0.00	0.21	0.00	0.00	0.00	0.39	0.00	
		11	C	0.00	0.01	0.00	0.00	5.12	0.00	0.00	0.00	0.00	0.00	0.04	0.00	
			N	0.18	0.00	0.00	0.00	4.59	0.00	0.00	0.00	0.00	0.00	0.13	0.00	
			P	0.00	0.01	0.00	0.00	3.07	0.00	0.00	0.00	0.00	0.00	0.26	0.60	

Region	Experiment Day	Salinity	Nutrient	Chlorophyta (green algae)				Pyrrophyta (Chrysophytes)			Cyanophyta (cyanobacteria)			10 <sup>6</sup> uL/mL
				Dunaliella viridis	Oocystis sp.	UNID Bi-flagellate	Sphaerellopsis sp.	Desmar-ella sp.	Glenodinium sp.	UNID Chrysophyte	Nodularia sp.	Pseudo-anabaena sp.	Spirulina sp.	All Taxa
Inocula	0	5.5	-	18.40	0.00	0.41	0.00	0.00	0.00	1.39	0.00	0.00	0.00	22.60
		12	-	0.28	2.41	0.02	0.00	0.00	0.00	0.17	2.86	0.00	0.00	6.61
Farmington	24	3	C	0.15	3.01	0.00	0.00	0.00	0.00	0.00	0.95	4.54	0.96	14.14
			N	0.00	20.76	0.50	0.00	0.00	0.00	1.31	11.15	0.00	7.51	48.21
			P	2.75	10.52	0.13	0.00	0.00	0.00	0.95	0.00	5.23	1.49	33.11
		5	C	4.26	21.30	0.24	0.00	0.02	0.00	3.02	0.00	13.55	2.04	48.28
			N	6.10	52.41	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.14	63.01
			P	9.71	32.47	0.15	0.00	0.00	0.00	0.50	17.58	8.35	2.03	76.15
		7	C	12.30	14.41	0.12	0.00	0.00	0.00	1.08	0.00	0.00	0.66	38.30
			N	4.81	13.25	0.00	0.00	0.00	0.03	0.15	0.92	0.00	0.00	60.04
			P	5.91	24.65	0.00	0.00	0.00	0.00	1.67	0.42	0.00	0.00	45.46
		9	C	7.72	4.93	0.00	0.00	0.00	0.00	0.58	0.92	0.00	0.14	26.59
			N	14.81	3.34	0.01	0.00	0.00	0.00	0.19	0.00	0.00	0.00	52.38
			P	7.04	6.58	0.00	0.00	0.00	0.00	2.40	0.00	0.00	0.00	40.34
		11	C	22.66	0.64	0.00	0.00	0.07	0.00	0.53	0.00	0.00	0.00	41.32
			N	0.00	0.76	0.00	0.00	0.00	0.00	0.00	0.24	0.00	0.00	30.53
			P	19.45	3.15	0.85	0.00	0.06	0.00	0.67	0.00	0.00	0.00	62.97
Gilbert	24	3	C	2.89	0.33	0.00	0.00	0.00	0.01	0.05	0.00	0.00	0.00	4.65
			N	2.23	0.48	0.06	0.00	0.00	0.00	0.05	0.05	0.00	0.00	3.28
			P	1.65	0.32	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	2.56
		5	C	0.80	0.61	0.01	0.00	0.00	0.00	0.04	0.00	0.00	0.00	1.96
			N	0.41	0.96	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.00	2.08
			P	2.17	0.39	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	3.68
		7	C	3.78	0.54	0.00	0.31	0.00	0.00	0.17	0.00	0.00	0.00	7.90
			N	1.44	0.41	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.00	6.70
			P	2.63	0.84	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	7.86
		9	C	0.02	0.23	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.39
			N	0.25	0.20	0.04	0.00	0.00	0.00	0.06	0.00	0.00	0.00	7.36
			P	0.14	0.07	0.03	0.00	0.00	0.00	0.01	0.00	0.00	0.00	5.84
		11	C	0.47	0.01	0.01	0.00	0.00	0.00	0.04	0.00	0.00	0.00	5.70
			N	0.12	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.02
			P	0.07	0.00	0.04	0.00	0.00	0.00	0.02	0.00	0.00	0.00	4.07