

**BIOLÒGICAL EFFECTS OF ATMOSPHERIC DEPOSITION
ON ALGAL ASSEMBLAGES**

Draft Final Report

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EXECUTIVE SUMMARY

Approximately 27% of the nitrogen load to Sarasota Bay occurs via atmospheric deposition. Nutrient loads from all sources have increased algal productivity and thus decreased light available to seagrass meadows. Thus, there is a strong need to evaluate the relationship between atmospheric deposition and nonpoint source concentrations, nitrogen loading to bay and gulf waters, and the biological impact of atmospheric deposition.

The quantitative ramifications of growth stimulation due to nutrient loading from atmospheric deposition are currently unknown. The potential linkage of enhanced atmospheric nitrogen deposition to accelerated eutrophication in coastal waters appears to play a role in many urbanized and industrialized regions. The development, proliferation and persistence of the toxic dinoflagellate *Gymnodinium breve* within coastal waters of southwest Florida could be one key trophic response to nitrogen enrichment.

The primary objective was to determine the effects of atmospheric nitrogen deposition on the population dynamics of two distinct algal assemblages: a nearshore, nutrient enriched community (Bay station), and an offshore, nutrient poor community (Gulf station) during both the dry (winter) and wet (summer) seasons. Short-term bioassays were designed to measure phytoplankton responses to additions of atmospherically-derived nitrogen (=rainfall) as well as reagent-grade nitrate nitrogen (=nutrients).

During the summer bioassays all added nutrients were essentially depleted by the end of the first day. There was also a rapid decline in chlorophyll *a*, which was a measure of the growth response of the algal assemblages. This short-term phytoplankton response was likely due to photoacclimation during the bioassays. These findings suggest that new nitrogen derived solely from atmospheric deposition may not be significant enough to stimulate primary production in Sarasota estuarine waters, but that additional sources of nitrogen (e.g., sediment resuspension and terrestrial runoff), which are also associated with storm events, may also be necessary to increase production.

The positive growth response to rain and nutrient treatments in the Gulf bioassays during the winter was directly related to added nutrient concentration. Additionally, the growth response was similar between rainfall and nutrient treatments, strongly suggesting that the response was due primarily to nitrogen, and not some other component of the rainwater. The positive growth response was primarily due to the diatom fraction of the phytoplankton. The effect of atmospherically-derived nitrogen on the potential to elicit a bloom response of the toxic dinoflagellate *Gymnodinium breve* could not be determined from these results.

Although there was no consistent growth response to the addition of rainfall for all seasonal bioassays in the Gulf and Bay, there was a significant positive response under specific conditions. The fact that any significant increase in phytoplankton population growth resulting from rainfall additions was detected demonstrates that atmospherically derived nitrogen can be a significant nutrient supply for phytoplankton growth.

INTRODUCTION

Atmospheric Deposition and Nutrient Loading: Nitrogen

The atmosphere has been shown to be an important source of exogenous nitrogen to coastal and estuarine surface waters along the east coast of North America (Paerl 1985, Ryther and Dunstan 1971, Fisher *et al.* 1988, GESAMP 1989). The atmosphere can deliver exogenous nitrogen to the system in one of two ways: directly or indirectly. Direct deposition occurs as either wet or dry deposition that falls directly on the receiving body of water. Indirect deposition occurs through nitrogen being deposited within the watershed and reaching the estuary through runoff. Within the Chesapeake Bay watershed, atmospheric nitrate forms 23% to 33% of the total sources (direct and indirect) of nitrogen input (Tyler 1988). Transportation and utilities emissions are considered the major sources of atmospheric nitrogen oxides (NO_x). These compounds can be transported over long distances and deposited through either wet or dry precipitation (Hicks *et al.* 1992).

Investigations in other systems worldwide indicate that the proportion of direct input of nitrogen from the atmosphere to an estuary can range from 13% to 50% (Duce 1986, Hicks *et al.* 1992), and is in part the product of the ratio of catchment area to water body surface area. It is also of interest that few deposition programs monitor for organic nitrogen species, although some estimates indicate that organic nitrogen contributions could be substantial (Pedulla 1988).

Initial estimates for nitrogen loadings to Tampa Bay indicated that direct atmospheric inputs were on the order of 26-27% of total loads (Zarbock *et al.* 1994), with deposition to the watershed and eventual transport to the Bay increasing the atmospherically derived nitrogen loading by some unknown fraction. Direct atmospheric loadings of phosphorus and toxins were less dramatic in total tonnage and percentage of total loads (Zarbock *et al.* 1994, Frithsen *et al.* 1995).

Dixon *et al.* (1996) estimated direct atmospheric loadings of nitrogen to Tampa Bay to be 32%, which was second only to nonpoint source loadings (46%). When they compared atmospheric loadings of nitrogen to nonpoint source loadings, Dixon *et al.* (1996) estimated that, over the watershed as a whole, estimated nonpoint source runoff is mathematically equal to 25% of the total atmospheric deposition of nitrogen to the watershed, or a watershed retention rate of 75%. If the majority of nonpoint source loadings are assumed to represent atmospheric loadings, then the total atmospherically derived nitrogen loading to Tampa Bay could be as high as 70-85% of total loadings.

Biological Effects of Atmospheric Nitrogen Deposition

Atmospheric deposition, chiefly as precipitation, constitutes a significant yet frequently overlooked source of plant nutrients in estuaries and coastal ocean waters (Ryther and Dunstan 1971, Paerl 1985, Duce 1986). Precipitation enriched in biologically available forms of nitrogen

(NO_x, NH₄, organic N) is of particular concern, because coastal receiving waters are often nitrogen limited and hence sensitive to even small increases in these nutrients (Ryther and Dunstan 1971, Nixon 1981, Mann 1982, Carpenter and Capone 1983).

Using bioassays, Paerl (1985) demonstrated that North Carolina Atlantic coastal waters enriched with relatively large quantities (10 and 20% by volume) of natural rainfall exhibited stimulation of phytoplankton growth, and that the most likely nutritive component responsible was nitrogen. In a subsequent study, Paerl *et al.* (1990) used *in situ* bioassays to measure phytoplankton growth responses to rain additions by mimicking more natural input levels. Rainfall at naturally occurring dilutions (0.5 to 5%) stimulated both ¹⁴CO₂ assimilation and chlorophyll *a* production, in most cases in a significant way. The increase in phytoplankton production was linked to higher levels of nitrogen species present in the rainwater. At the start of this study it was unknown whether similar growth responses to nitrogen enriched rainfall could be expected from algal assemblages found in the Sarasota Bay region and local Gulf waters of Southwestern Florida.

The qualitative ramifications of growth stimulation due to nutrient loading from atmospheric deposition are currently unknown. The potential linkage of enhanced atmospheric nitrogen deposition to accelerated eutrophication in coastal waters appears to play a role in many urbanized and industrialized regions (Paerl *et al.* 1990). The development, proliferation and persistence of the toxic dinoflagellate *Gymnodinium breve* within coastal waters of southwest Florida could be one key trophic response to nitrogen enrichment.

BACKGROUND AND OBJECTIVES

Background

In characterizing the state of Sarasota Bay, three major problem areas were reported in the Sarasota Bay National Estuary Program's (SBNEP) Framework for Action (FFA). These three major problem areas were determined to be stormwater, wastewater, and habitat loss. Nutrient loads in Sarasota Bay are approximately 300% of that expected from a pristine, undeveloped watershed (Alderson 1992). Associated with these nutrient loads is the concern over metals contamination, low levels of dissolved oxygen and loss of tidal wetlands and seagrass coverage throughout the bay system.

Previous studies, as documented in SBNEP's Comprehensive Conservation and Management Plan (CCMP) and FFA, indicated that approximately 27% of the nitrogen load input to Sarasota Bay occurs via direct atmospheric deposition. Nutrient loads from all sources have increased algal productivity and thus decreased light available to seagrass meadows. Thus, there is a strong need to evaluate the relationship between atmospheric deposition and nonpoint source concentrations, nitrogen loading to bay and gulf waters, and the biological impact of atmospheric deposition.

The Tampa Bay Estuary Program (TBEP), in conjunction with the U.S. E.P.A. Great Waters Program has implemented a program to begin deposition measurements and air mass circulation modeling in upper Tampa Bay. The SBNEP is coordinating ongoing monitoring and modeling efforts with those of TBEP, with additional efforts focusing on the issue of identifying potential biological and ecological impacts (this project).

Objectives

The primary objective of this project was to determine the effects of atmospheric nitrogen deposition on the population dynamics of two distinct algal assemblages: a nearshore, nutrient enriched community, and an offshore, nutrient poor community. This project investigated these responses during both the dry (winter) and wet (summer) seasons.

PROJECT PLAN

The overall project plan integrated the following elements: 1) direct collection and measurement of atmospheric inorganic nitrogen (nitrate-nitrite and ammonium) deposition from individual storm events (once during the dry and wet season, respectively), 2) *in situ* bioassay experiments using resident phytoplankton from a nutrient enriched estuarine community and a nutrient poor offshore community and 3) measurement of algal growth rate and changes in algal species composition over a 3 day period as a response to nitrogen enrichment.

MATERIALS AND METHODS

Atmospherically-Derived Nitrogen (ADN) Rainfall Collection

Rainfall for each seasonal sampling event was collected by one of two methods. The first method consisted of precleaned acid-washed polyethylene buckets at Mote Marine Laboratory (MML: R1) (**Figure 1**) exposed only during selected storm events. The second source of rainwater came from the National Atmospheric Deposition Program (NADP) collecting site on south Lido Key (R2). Both sites are coastal and met NADP criteria for being free of extraneous sources of particulate matter, including trees, power lines and poles. At the termination of each storm event, the samples were transferred to polyethylene beakers and analyzed for pH within 24 hours. The remaining sample was placed in Ziplock™ freezer bags and frozen at -20° C.

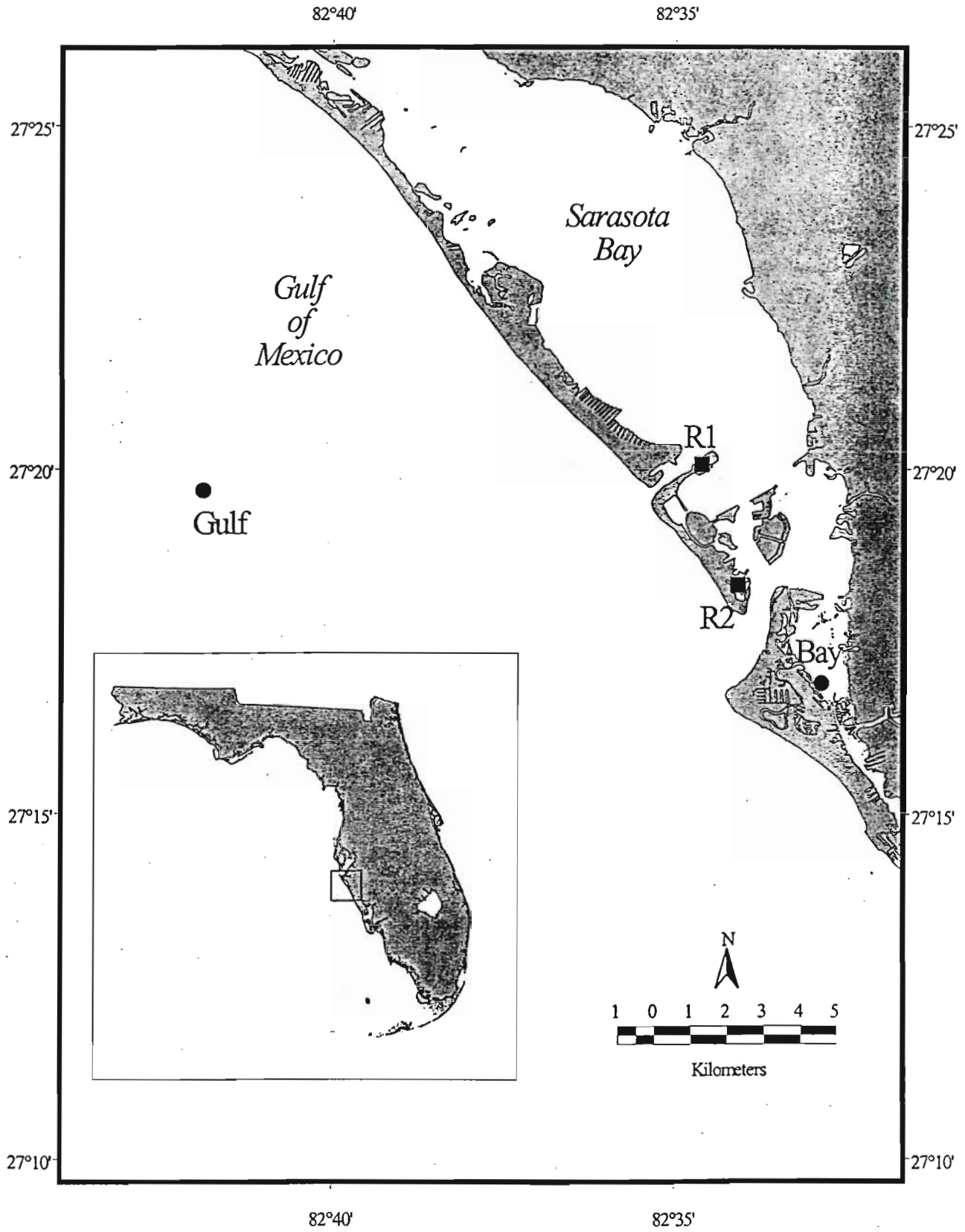


Figure 1. Area map showing locations of rainfall collection sites (R1 and R2) and rainfall bioassay sites (Gulf and Bay).

The dates of the individual storm events composited for each season were as follows:

<u>Winter</u>	<u>Date</u>	<u>Amount</u>	<u>Method</u>
	12/12/98	0.41 cm	NADP
	12/13/98	1.42 cm	MML
	12/14/98	2.08 cm	NADP
	01/03/99	2.34 cm.	NADP
<u>Summer</u>	<u>Date</u>	<u>Amount</u>	<u>Method</u>
	06/14/99	1.17 cm	NADP/MML
	06/18/99	2.03 cm	NADP
	06/19/99	1.60 cm	NADP/MML
	06/20/99	1.85 cm	NADP/MML
	06/21/99	1.06 cm	NADP
	07/01/99	4.57 cm	NADP/MML

Prior to each seasonal bioassay experiment, individual rainfall samples were allowed to thaw at room temperature and then combined into one composite sample. Combining the individual rainfall samples into one composite sample enabled the characterization of “typical” seasonal values for the constituent nutrient species. Subsamples were then analyzed for ammonium ($\text{NH}_4\text{-N}$), combined nitrate and nitrite ($\text{NO}_{2+3}\text{-N}$) and orthophosphate ($\text{PO}_4\text{-P}$).

***In situ* Bioassay Experiments**

Short-term bioassays were designed to measure phytoplankton responses to additions of atmospherically-derived nitrogen as well as reagent-grade nitrate nitrogen. Bioassay experiments utilized 10-L polyethylene Cubitainers as bioassay vessels. These flexible, semi-transparent (85% transparent to photosynthetically active radiation [PAR], 400 to 700 μm) containers have previously proven useful for determining nutrient limitation in North Carolina estuarine and coastal waters (Paerl *et al.*, 1990). Cubitainers are chemically inert, exhibiting neither toxic nor detectable phytoplankton community altering effects (“bottle effects”) in short term (2 to 5 day) *in situ* bioassay experiments (Paerl 1983, 1987). Because they are flexible and easily deployed off floating incubation rigs, natural surface and subsurface turbulence can to some extent be transferred to enclosed phytoplankton communities (Paerl *et al.*, 1990).

Incubations of 48 - 72 h duration provide sufficient time to measure taxa-specific responses before other (secondary) factors become limiting. Short-term incubations minimize container artifacts and provide acceptable resolution to document changes in phytoplankton community structure and function.

Treatments

Bioassays were designed to approximate natural rainfall dilutions in local estuarine and

coastal waters. Median weekly rainfall amounts for the NADP Verna Wellfield site during the period 1994-1997 were 4.47 cm and 0.12 cm, respectively, for summer and winter quarters. Following the assumptions of Paerl (1990), we calculated how much commonly encountered rainfall amounts would dilute a 2m well-mixed water column. A 1 cm rainfall event corresponds to a 0.5% dilution level, while a 2 cm event corresponds to a 1% dilution level, and so on. Based on a 10-L Cubitainer filled with 9.5L seawater, the addition of 295 ml of 100% rainfall equates to a 3% dilution, or a 6 cm (2 5/16") rainfall event. Likewise, the addition of 295 ml of 33% rainfall (rainfall diluted 1:3 with laboratory deionized water) equates to a 2 cm (13/16") event. Rainwater was diluted rather than simply adding less to keep dilution factors for all treatments the same. Similarly controls were prepared by adding 295ml of deionized water to the 9.5L of situ water.

The following five treatments were applied to each of the two seasonal experiments:

<u>Treatment</u>	<u>Conditions</u>
1	Rainfall 1 (100% dilution) – 6 cm rainfall event
2	Rainfall 2 (33% dilution) – 2 cm rainfall event
3	Nutrient 1 (Combined nitrate and nitrite equal to their concentration in rainfall 1 treatment)
4	Nutrient 2 (Combined nitrate and nitrite equal to their concentration in rainfall 2 treatment)
5	Control (Analyte-free water)

By designing treatments 3 and 4 to replicate the nitrogen concentration of respective rainwater treatments 1 and 2, any differences in the algal response to these treatments would be due to other substances in the rainwater besides nitrogen. If the responses between the treatments are similar, then nitrogen must be the causative agent. The control treatment (equal volumes of analyte-free water) was incubated in parallel with the other treatments to account for the actual dilution of resident phytoplankton. Each treatment was run in triplicate.

Station Selection/Location

The estuarine station (Bay, Figure 1; Latitude 27° 16.889'; Longitude 82° 32.827') was located in lower Roberts Bay approximately ½ mile north of the mouth of Phillippi Creek. Water depth at this station was approximately 2 meters. Nutrient concentration and chlorophyll *a* levels in Roberts Bay were ranked among the highest for the Sarasota Bay estuarine system (Lowry 1992). Mean dissolved inorganic nitrogen (DIN) (= ammonium plus nitrate-nitrite-nitrogen) in Roberts Bay from 1995-1998 was 0.038 mg/L (see **Table 1** for a summary of historical water quality for both sampling stations as well as each seasonal composite rainfall for the present study). While water for the Bay bioassays was collected in Roberts Bay, incubations were carried out near rainfall collection site R1 (MML) in waters of equivalent depth, so as to minimize the potential for vandalism or tampering. The Gulf station (Gulf, Figure 1; Latitude 27° 19.685'; Longitude 82° 41.880') was located approximately 10 kilometers due west of New Pass in 40 feet of water. This location coincides with a sampling station for monthly nutrient analysis from an ongoing red tide study being conducted by Mote Marine Laboratory. The mean value for DIN from the MML red tide sampling program at this location (1996-1998) was 0.006 mg/l.

Table 1. Mean concentration of nutrients, Chl *a*, and N:P ratios for Sarasota Bay and adjacent Gulf waters and rainfall. Historical Roberts Bay data from Sarasota County ambient monitoring program. Gulf of Mexico data from station RTT5, MML Red Tide Studies.

Parameter	units	<u>Roberts Bay</u>	<u>Gulf of Mexico</u>	<u>Rainfall (composite)</u>		<u>Rainfall (NADP: 1994-1996) Median for weekly values</u>	
		(1995-1998)	(1996-1998)	Winter	Summer	Winter (Jan-Mar)	Summer (Jun-Aug)
DNH ₄ -N	mg/l	0.017	<0.005	0.095	0.121	0.078	0.101
DNO ₂₊₃ -N	mg/l	0.021	<0.005	0.060	0.133	0.108	0.208
DIN	mg/l	0.038	0.006	0.155	0.254	0.186	0.309
Total N	mg/l	0.545	0.205	0.210	0.370	–	–
DPO ₄ -P	mg/l	0.055	0.012	0.009	0.020	–	–
Total P	mg/l	0.154	0.026	<0.005	0.060	–	–
IN:IP	mg/mg	0.9	0.6	17.2	12.7	–	–
TN:TP	mg/mg	5.2	8.2	8.4	6.2	–	–
Chl <i>a</i>	μg/l	5.3	–	–	–	–	–

Sampling Schedule

Winter (dry) season sampling was conducted during the week of January 18, 1999. Bioassays were initiated on January 18th and ran for 72 hours, terminating on January 21st. Summer (wet) season bioassays were initiated on July 5th and ran for 72 hours, terminating on July 8th.

Sampling Procedures

At each site (Bay and Gulf), a 150-liter acid-washed polyethylene compositing container was filled by repeated casts of a 5-liter Nisken water sampler from approximately 0.5 m depth. Fifteen cubitainers (five treatments by three replicates) were each filled with 9.5 liters of water, leaving a head space of 0.5 liters in each. Care was taken to keep the source water well mixed throughout this process. After all cubitainers were filled, 295 ml of the appropriate treatment (100% rainfall, 33% rainfall, 100% nutrient, 33% nutrient and analyte free water) was added to the respective Cubitainer. After the treatments were added, subsamples for chemotaxonomic photosynthetic pigments, particle size distribution, and nutrients were immediately withdrawn and kept at 4° C until analysis. These same subsamples were withdrawn at daily (~24 h) intervals until conclusion of the incubation (~72 h). Nutrient samples were passed through a 0.45µ filter during sample collection.

Laboratory Analyses

Nutrients. After filtration, nutrient samples were preserved with sulfuric acid and held at 4° C until processed. Laboratory analysis of all nutrient parameters was performed according to standard EPA-approved methodologies, as specified in the Project Quality Assurance Plan (1998). Minimum detection limit for all nutrient species (N, P) was 0.005 mg/l.

Particle Size Distributions: Particle size distributions for each replicate of each treatment on each day were determined with a Coulter Multisizer IIe, electronic particle sizer. The Multisizer was fitted with a 100 µm orifice which yielded an effective particle size range of 2 to 63 µm. Utilizing seawater as the electrolyte in whole water samples often yielded count overflows in the size range from 2 to 5 µm. Therefore, the lower size threshold was limited to 5 µm. Triplicate 0.5 ml aliquots were counted and averaged for each sample.

Photopigments: Water samples from each cubitainer were filtered on GF/F glassfiber filters using low vacuum (<100 mm Hg). The filters were stored in liquid nitrogen until processed for HPLC analysis. Samples filters were extracted in approximately 1.5 ml of 100% methanol, sonicated in an ice bath and placed in the freezer. After one hour the extract was isolated and passed through a 0.2 µm syringe filter. HPLC pigment analysis was performed on an HP 1100 HPLC fitted with 3 columns: a C18 ODS-Hypersil and then 2 C18 Polymeric columns. The solvent gradient was as described in Pinckney *et al.* (1996). Immediately prior to injection a mixture of 70% sample and 30% 1M ammonium acetate was prepared by the auto-injector. The analytical columns were maintained at 38 degrees C.

Pigment data derived from HPLC analyses were imported into ChemTax[®], a PC-based software package utilizing factor analysis and a steepest descent algorithm, and were used to assess phylogenetic group-contribution to the total chlorophyll *a* biomass (Mackey *et al*, 1996). ChemTax[®] optimizes pigment ratios within subsets of data (based on pigment ratios within the representative phylogenetic groups) and as such, it is generally regarded to provide more realistic estimations than regression models (e.g., Tester *et al*, 1995). Relative group contributions, expressed as a percentage of the total chlorophyll *a* biomass, were determined by ChemTax[®] using the initial pigment ratios suggested by Mackey *et al*. (1996).

RESULTS

Nutrient Concentration: Rainfall and Surface Waters

Table 1 summarizes information on the concentration of primary nutrients in both rainfall and local waters from the current study as well as previous studies from Sarasota Bay. **Table 2** compares *in situ* physical parameters from the present study to recent historical data from the study area. The data from Roberts Bay came from the Sarasota County Ambient Water Quality Monitoring Program. The data from the Gulf of Mexico was obtained from an ongoing MML red tide study in the vicinity of the Gulf station from the present study. Unless stated otherwise, values are mean concentrations over the period indicated. Median values for the weekly concentration of nutrients in rainfall were obtained from the National Atmospheric Deposition Project (NADP) Verna Wellfield site in Sarasota County. NADP data were arranged by season over the most recent three year period (1994-1997) and the median values obtained for comparison with rainfall data from the present study. Dissolved inorganic nitrogen (DIN) in the composite rainfall for each season from the current study closely approximated (within 80%) the seasonal DIN concentrations from the NADP site. The concentration of DIN in the composite winter rainfall was 0.155 mg/L, while the NADP site had a winter DIN median value of 0.186 mg/L. The concentration of DIN in the composite summer rainfall was 0.254 mg/L, while the NADP site had a DIN median value of 0.309 mg/L. A comparison of nitrogen concentrations from the current study and the NADP site shows that the concentrations in rainfall used in the bioassays are well within the range of expected values during both the winter (dry) and summer (wet) seasons for the Sarasota Bay region. Moreover, nitrogen concentrations are typically higher during the summer. It is likely, therefore, that the observed responses of resident algal populations to the addition of atmospheric nitrogen in the current study are reflective of a normal response and are not the result of some unusual rainfall conditions.

In order to better understand the response of resident algal populations to nutrient additions, it was necessary to determine the relative percent increase in nutrient concentrations in each of the bioassay treatments over the ambient DIN for each of the source waters. The concentration of DIN in the source water (both Bay and Gulf) was not directly measured prior to bioassay treatment additions. However, ambient source water concentrations of DIN was calculated by taking the mean DIN concentration from each of the control treatments and correcting for the amount of analyte free water that was added prior to nutrient analysis. The results of these calculations are presented in **Table 3**. (The relative increase in DIN concentrations for the two nutrient addition treatments are identical to the increases reported for the rainfall treatments since the quantity of DIN added to these treatments was the same as in their respective rainfall treatments).

Table 2. Comparison of *in situ* physical parameters from the present study and recent historical data. Historical Roberts Bay data from Sarasota County Ambient Monitoring Program. Gulf of Mexico data from station RTT5, MML Red Tide Studies. Historical values are mean values for the periods indicated. Data from the present study are single measurements made on Day 0. All values were obtained from 0.5 meters below the water surface.

<u>Parameter</u>	<u>Units</u>	<u>Roberts Bay (1995-1998)</u>		<u>Gulf of Mexico (1996-1998)</u>	
		<u>Dec-Feb</u>	<u>Jun-Aug</u>	<u>Dec-Feb</u>	<u>Jun-Aug</u>
Temperature	°C	17.9	29.7	19.4	31.4
Salinity	PSU	25.3	25.4	33.3	33.5

<u>Parameter</u>	<u>Units</u>	<u>Present Study</u>			
		<u>Roberts Bay</u>		<u>Gulf of Mexico</u>	
		<u>Winter</u>	<u>Summer</u>	<u>Winter</u>	<u>Summer</u>
Temperature	°C	20.2	29.8	18.0	28.6
Salinity	PSU	27.8	23.9	31.8	36.2

Table 3. DIN concentration (mg/l) in source water, rainfall and bioassays, including percent increase over ambient (source water) following the addition of treatments to source water.

	Bay-Summer	Bay-Winter	Gulf-Summer	Gulf-Winter
Source Water	0.068	0.024	0.022	0.011
Rain Water	0.254	0.155	0.254	0.155
Treatment 1, 3 (100% rain, nutrients)	0.074	0.028	0.029	0.016
Increase over ambient (mg/l)	0.006	0.004	0.007	0.005
Percent increase over ambient	8.1%	14.3%	24.1%	31.3%
Treatments 2, 4 (33% rain)	0.069	0.025	0.023	0.012
Increase over ambient (mg/l)	0.001	0.001	0.002	0.001
Percent increase over ambient	1.4%	4.0%	8.7%	8.3%

The largest relative increases in DIN due to the addition of rainwater treatments occurred during the winter bioassays. Moreover, these increases were higher in the Gulf bioassays than in the Bay bioassays. Although summer rainfall had a higher DIN concentration than the winter rainfall, the source waters at both locations had a much lower DIN concentration in the winter, resulting in a higher percent increase after the addition of rainwater to the winter bioassays. During the winter bioassays, the addition of 100% rainwater (treatment 1) to ambient Gulf source water resulted in a 31.3% increase in available DIN. During the summer, the addition of 100% rainwater to the Bay bioassay resulted in an 8.1% increase in available DIN. For both seasons, additions of 100% rainfall or nutrients resulted in concentration increases between 0.004 and 0.007 mg/l. The addition of 100% rainwater to all bioassays represented a 6 cm rainfall event.

By comparison, treatment 2 (33% rainwater) bioassays represented a much smaller increase in available DIN to all bioassays. Once again, the effect of this treatment was greater in the Gulf bioassays, but resulted in a less than 10% increase in DIN for both winter and summer bioassays. The addition of treatment 2 to Bay bioassays only increased the available DIN 4.0 and 1.4% for the winter and summer bioassays, respectively. For both seasons, additions of 33% rainfall or nutrients resulted in concentration increases between 0.001 and 0.002 mg/l. The addition of 33% rainwater to all bioassays represented a 2 cm rainfall event.

Nutrient Uptake: Bioassays

Dissolved Inorganic Nitrogen (DIN): **Figure 2** shows the temporal pattern of DIN for all treatments for both the Bay and Gulf stations during the winter bioassays. At the Bay station, DIN in all treatments decreased in half from their original concentration within the first day. For the final two days, DIN showed variable changes among the treatments. Treatment 1 (100% rainfall) increased from day 1 to day 2, and then declined on day 3. Treatment 3 (100% nutrients) continued to increase slightly after day 1. Treatments 2 and 4 (33% rainfall and nutrients, respectively) remained relatively stable after day 1, while Treatment 5 (control), decreased slightly after day 1. For the Bay bioassays, there were no significant differences (one-way ANOVA; $p > 0.05$) in mean DIN among treatments on any day.

The Gulf station showed slightly different temporal changes among treatments during the winter bioassays, in that there was a slight increase in DIN between day 2 and day 3 in all treatments. Once again, there was a large decline in DIN concentrations from day 0 to day 1 in Treatments 1 and 3 (100% rain and 100% nutrients). Treatments 2 and 4 (33% rain and 33% nutrients) showed little variation in DIN throughout the experiment, and after day 0, there was little difference in DIN among any of the treatments. One-way ANOVA showed significant differences among treatments only on Day 0 ($p = 0.025$). Tukey's multiple comparison test revealed significant differences between treatments 1 vs 5, 1 vs 2, 1 vs 4 and 3 vs 5. In this instance, the 100% rainfall treatment (and the 100% nutrient treatment) resulted in significantly different concentrations of DIN than the more dilute treatments and control. After day 0, however, these differences were no longer significant.

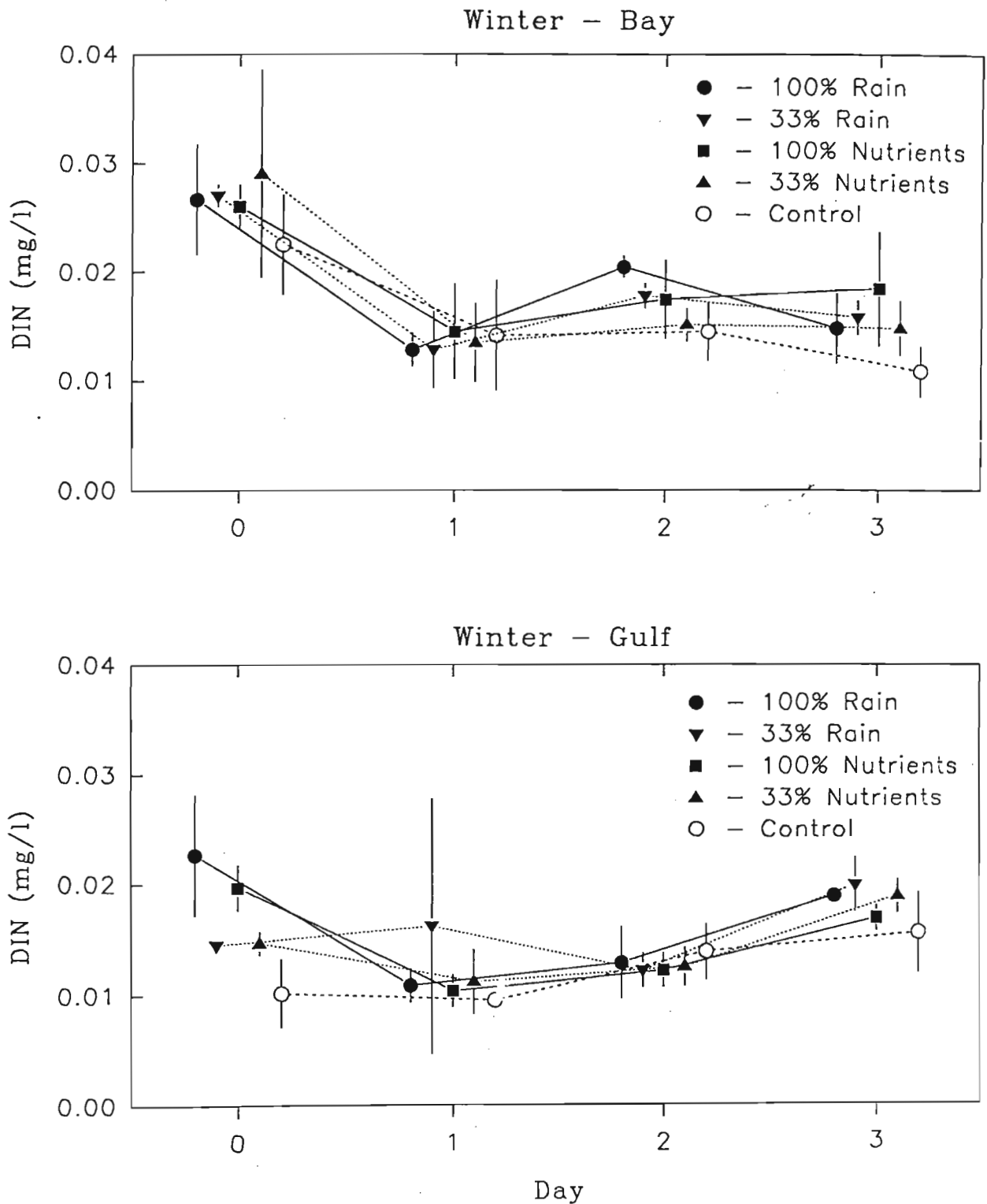


Figure 2. Rainfall and nutrient bioassay experiments with Sarasota Bay (top) and Gulf of Mexico (bottom) waters during the winter (Jan 18-21, 1999). Changes in dissolved inorganic nitrogen (DIN) for each treatment. Treatment legends are shown with each graph. Error bars are SEM (n=3). When error bars are not visible, they are smaller than symbol.

Figure 3 shows the temporal pattern of DIN for all treatments for both the Bay and Gulf stations during the summer bioassays. DIN was approximately three times higher in Bay samples compared to Gulf samples at the initiation of the bioassays. Furthermore, DIN from Bay bioassays was over two times greater in the summer than in the winter (see Table 2 for a summary of DIN concentrations for this study). However, after day 1, DIN in all treatments from both the Bay and the Gulf dropped to similarly low levels (ca. 0.01 mg/l) and remained low throughout the remainder of the bioassay. In essence, during the summer bioassays, all available nutrients within the cubitainers were utilized within one day.

Dissolved Ammonium Nitrogen (NH₄-N): **Figures 4 and 5** show temporal changes in the concentration of DNH₄-N for all bioassays treatments for winter and summer, respectively. During both seasons, the temporal changes in DNH₄-N reflected changes in DIN. In other words, after Day 1, dissolved nitrogen available to phytoplankton during bioassays was predominately in the form of DNH₄-N.

Dissolved Nitrate and Nitrite (NO₂₊₃-N): **Figures 6 and 7** show temporal changes in the concentration of DNO₂₊₃-N for all bioassay treatments for the winter and summer sampling events, respectively. In every situation except two (the 100% nutrient treatment for the Gulf during the winter, day 1; and the control for the Bay during summer, day 3), DNO₂₊₃-N was reduced to below detectable levels by the end of the first day. Values plotted are one-half of the method detection limit, or 0.0025mg/l for illustration only.

Particle Size Distribution

Particle size distributions in natural waters exhibit an exponential decay in particle number with increasing particle size. The size limit of detection for this project was established as the size at which the average number of particles in a size bin was greater than 3 x standard deviation of the average number in that bin. This criteria yielded an effective size range for the Bay bioassays of 5 to 15 μm. The Gulf water bioassays exhibited significantly fewer particles in all size ranges resulting in an effective size range of 5 to 10 μm.

Changes in particle numbers, expressed as growth, in each size bin of the Coulter Multisizer output for each treatment were tested against changes in particle numbers of the respective size bin of the control. Changes in particle numbers were evaluated using the growth equation:

$$k = \log_2(n_2/n_1)/(t_2-t_1)$$

where k is the growth rate in doublings per unit time, log₂ is the logarithm base 2, n₁ is the number of particles in a size bin at time 1, and t₁ is the time of sampling 1. Growth rates were determined for the time periods; day 0 to day 1, day 1 to day 2, day 2 to day 3, day 0 to day 2 and day 0 to day 3. Each size-bin growth rate for each treatment was tested against the control growth rates using a Student's T-test (p = 0.05) and the null hypothesis that the growth rates were equal.

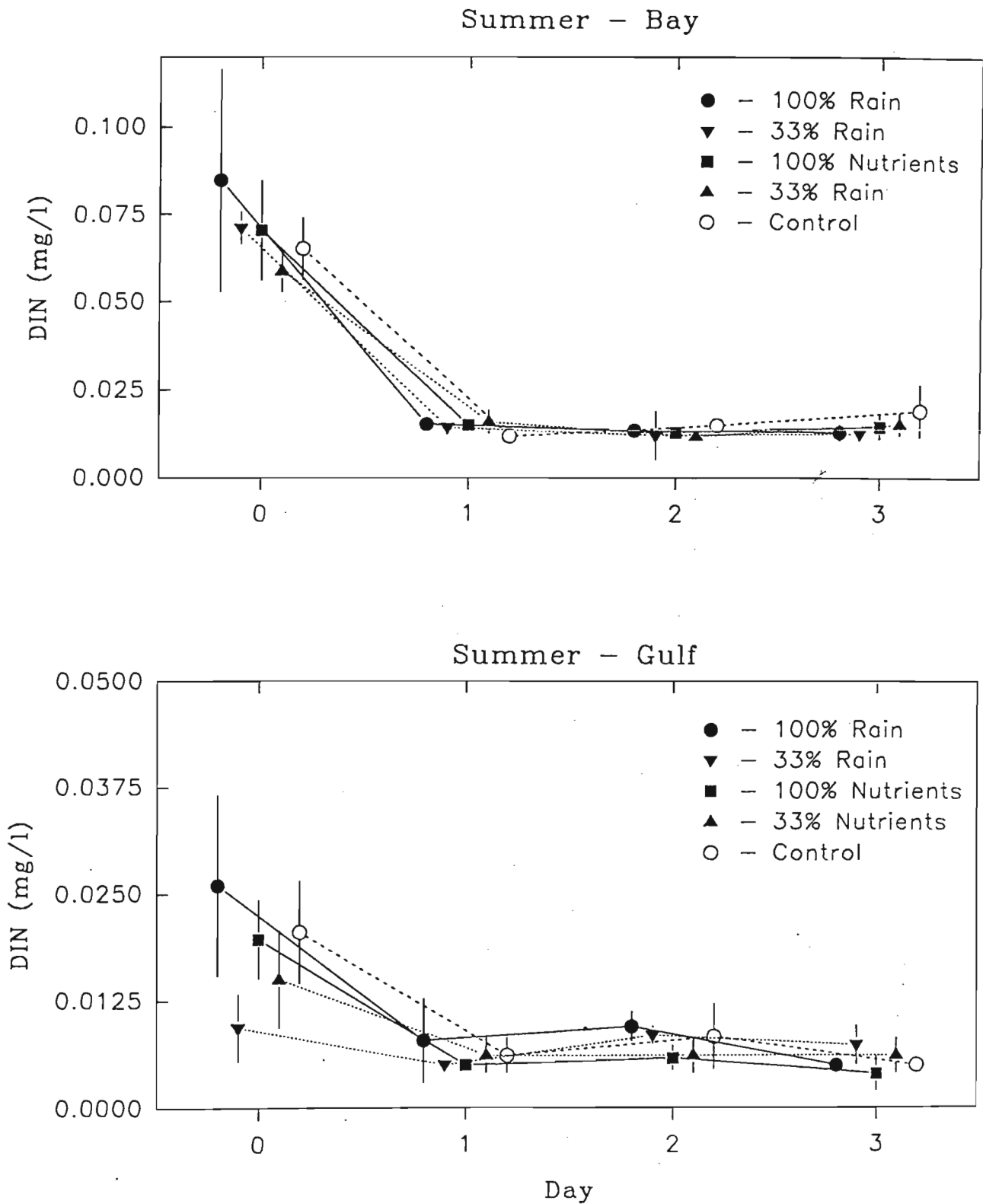


Figure 3. Rainfall and nutrient bioassay experiments with Sarasota Bay (top) and Gulf of Mexico (bottom) waters during the summer (July 5-8, 1999). Changes in dissolved inorganic nitrogen (DIN) for each treatment. Treatment legends are shown with each graph. Error bars are SEM (n=3). When error bars are not visible, they are smaller than symbol. Note different vertical scales.

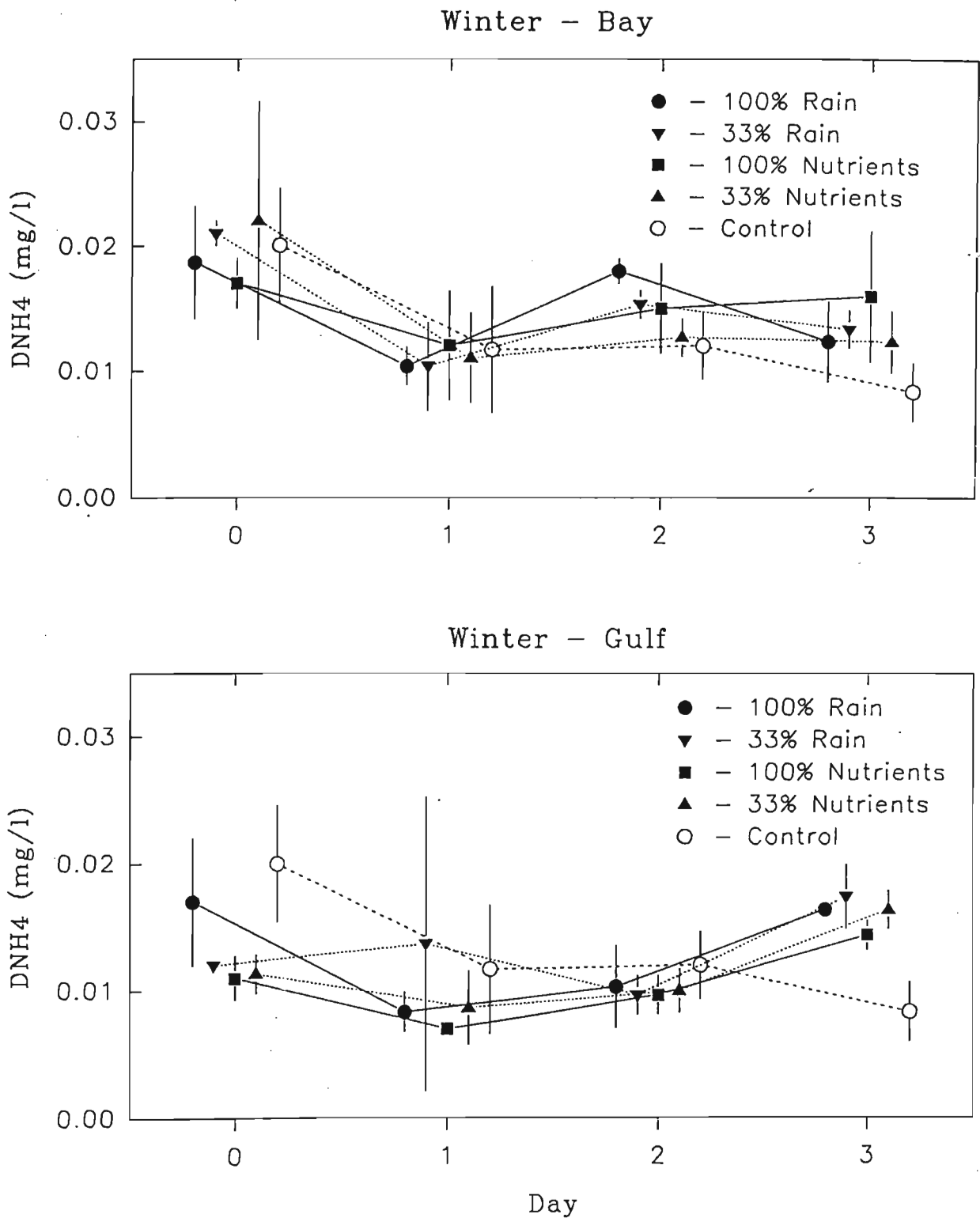


Figure 4. Rainfall and nutrient bioassay experiments with Sarasota Bay (top) and Gulf of Mexico (bottom) waters during the winter (Jan 18-21, 1999). Changes in dissolved ammonium ($\text{NH}_4\text{-N}$) for each treatment. Treatment legends are shown with each graph. Error bars are SEM ($n=3$). When error bars are not visible, they are smaller than symbol.

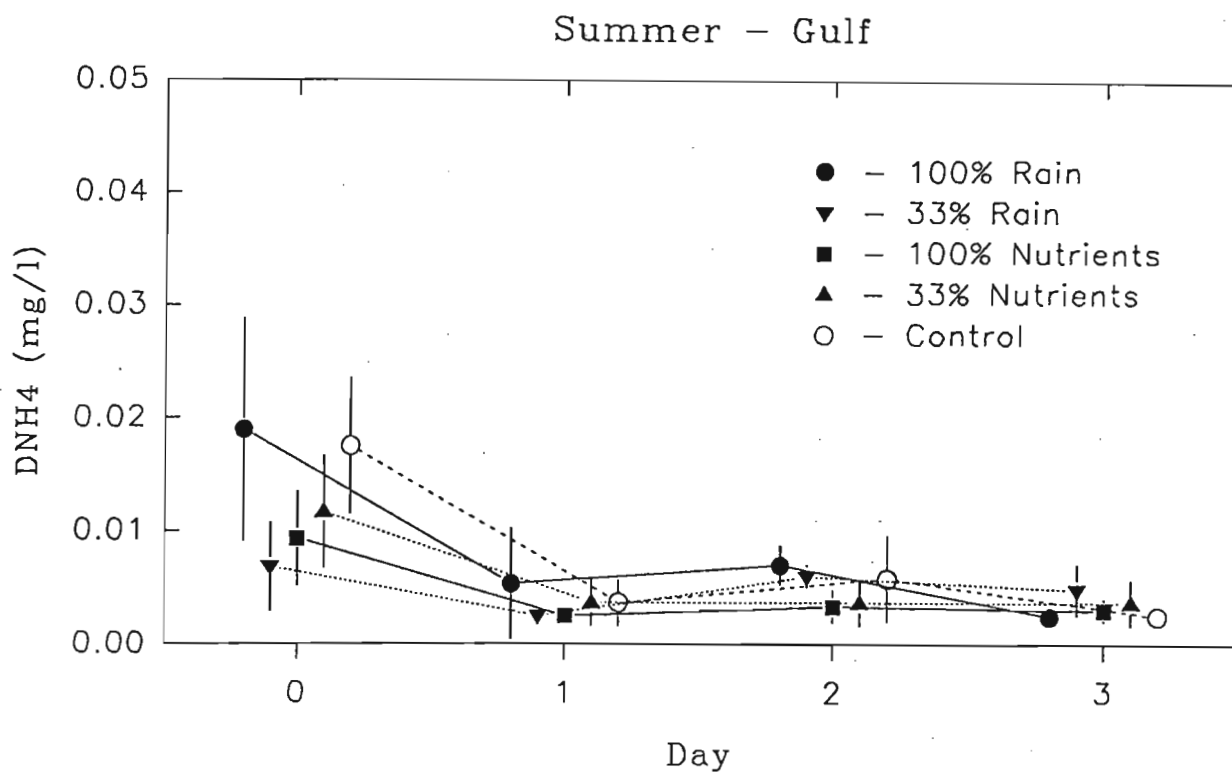
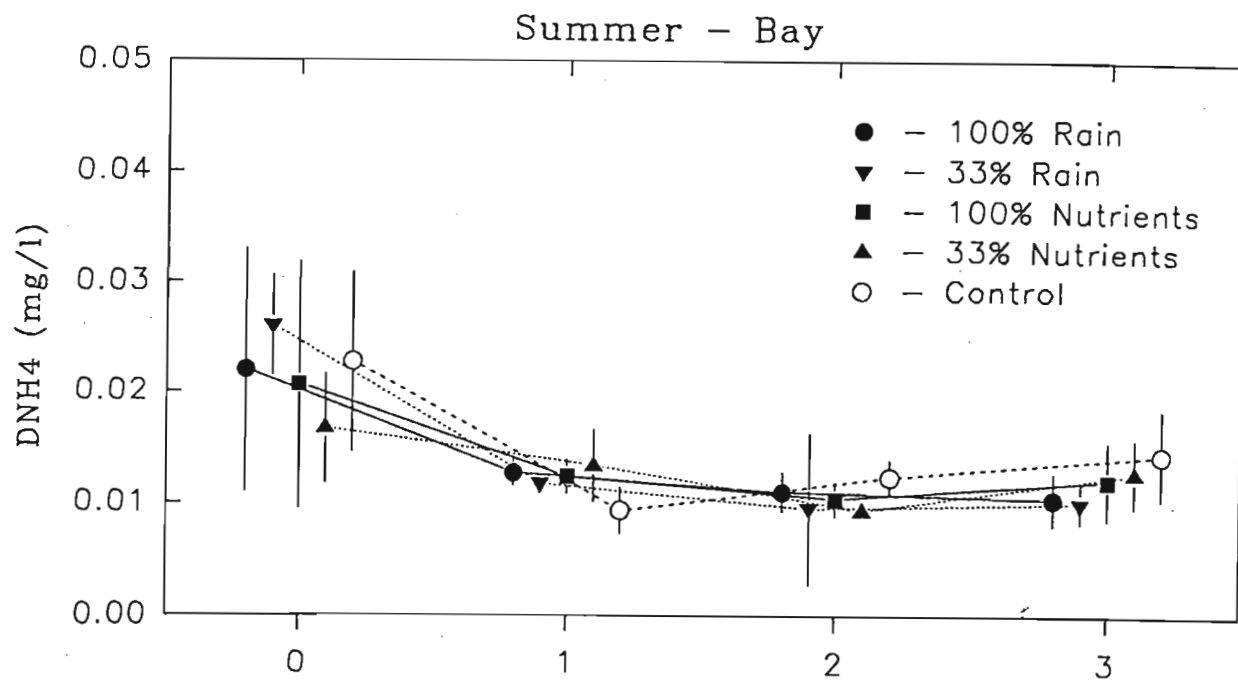


Figure 5. Rainfall and nutrient bioassay experiments with Sarasota Bay (top) and Gulf of Mexico (bottom) waters during the summer (July 5-8, 1999). Changes in dissolved ammonium ($\text{NH}_4\text{-N}$) for each treatment. Treatment legends are shown with each graph. Error bars are SEM ($n=3$). When error bars are not visible, they are smaller than symbol.

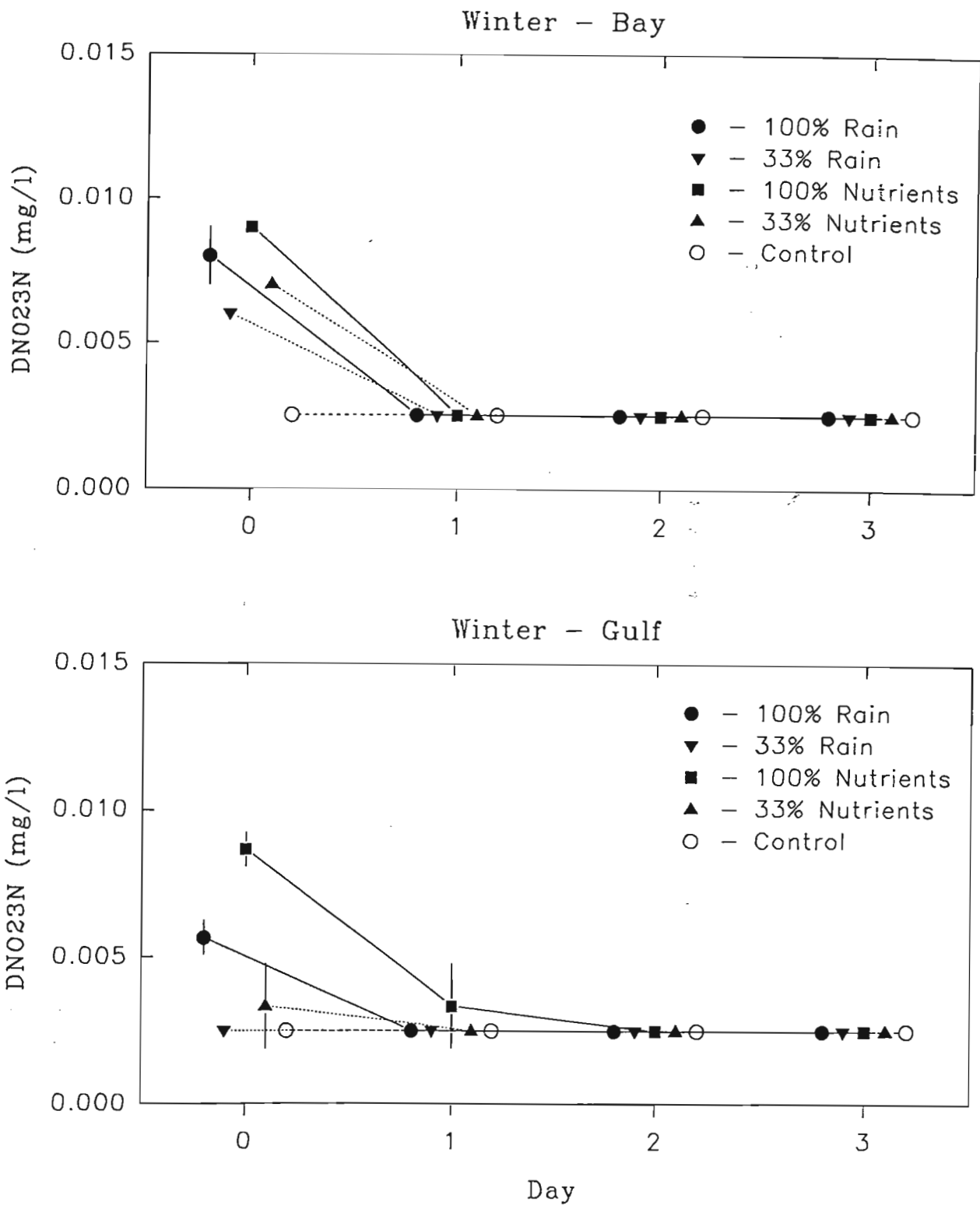


Figure 6. Rainfall and nutrient bioassay experiments with Sarasota Bay (top) and Gulf of Mexico (bottom) waters during the winter (Jan 18-21, 1999). Changes in dissolved nitrate and nitrite ($\text{DNO}_{2,3}\text{-N}$) for each treatment. Treatment legends are shown with each graph. Error bars are SEM ($n=3$). When error bars are not visible, they are smaller than symbol.

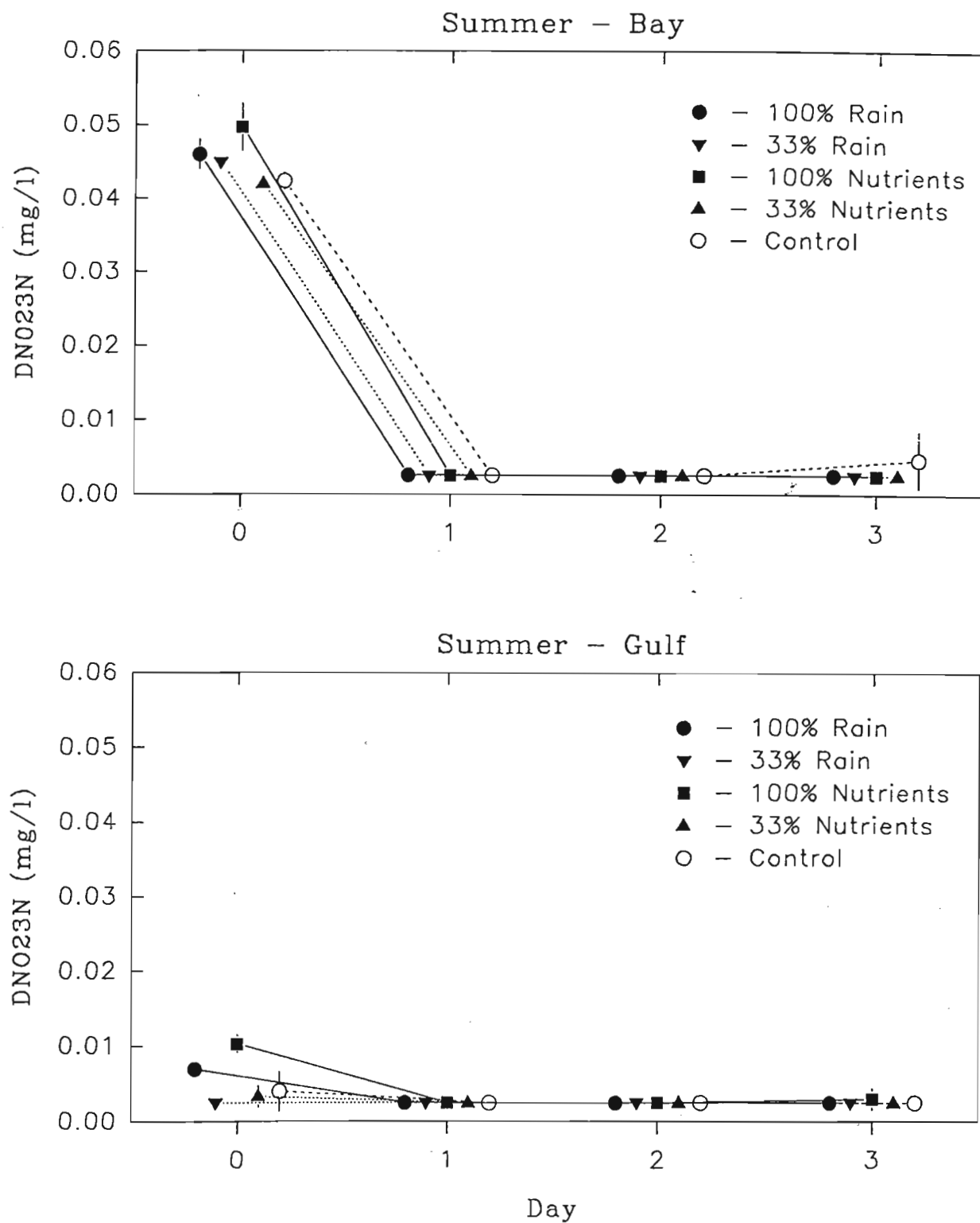


Figure 7. Rainfall and nutrient bioassay experiments with Sarasota Bay (top) and Gulf of Mexico (bottom) waters during the summer (July 5-8, 1999). Changes in dissolved nitrate and nitrite ($\text{DNO}_{2+3}\text{-N}$) for each treatment. Treatment legends are shown with each graph. Error bars are SEM ($n=3$). When error bars are not visible, they are smaller than symbol.

Table 4 lists combinations of treatments, days and locations that showed significant differences in growth in specific size ranges between the treatment and the control. It should be noted that non-significant differences do not mean the phytoplankton populations were not growing; it means they were not growing significantly different than the control.

During the first day (day 0 to day 1) of the Bay bioassays in the winter, all four treatments showed a significantly lower growth rate of particles in the 12 to 13 μm size range (Table 4; first column). However, the following day showed higher growth rates in three of the treatments (100% Rain, 100% Nutrients and 33% Rain). One explanation is that the added nutrients of the treatments in the first day delayed the growth cycle of the phytoplankton populations that were already utilizing ambient nutrients. The control population did not experience the nutrient spike and therefore continued to grow at the initial rate. After the nutrient additions were taken up during the first day (see Figure 2) in the treatment cubitainers, those populations began to grow faster than the control. No further significant growth was detected in Bay bioassays during the winter.

The Gulf bioassays during the winter showed no significant growth in particle size or number during the first day (Table 4; third column). During the second day the 100% Rain and 100% Nutrient treatments showed significant growth in the 6 to 7 μm size range. This growth continued to be significant in the third day for the 100% Nutrient treatment. These growth events in the second and third days contributed to significant growth being indicated for the cumulative growth periods of day 0-to-day 2 and day 0-to-day 3.

During first day of the summer Bay bioassays (Table 4; second column), the 33% Rain and 33% Nutrient treatments exhibited significant particle growth relative to the control, but the 100% enrichment treatments did not (**Figure 8**). It should be noted that the 100% Nutrient bioassay showed differences in growth rates relative to the control, but the growth was not statistically significant ($p > 0.05$). However, it is likely that additional treatment replicates would have reduced the variance to a level where the differences would have been significant. During days 2 and 3 significant increases were detected in particle counts in both rain treatments relative to the control, but only the 33% Nutrient treatment showed significant increase relative to the control during day 2.

Chemotaxonomic Photopigments

Individual pigment concentrations displayed a wide variety of responses. Some of these responses were consistent across treatments and controls and some were due to the treatments. **Figure 9** shows the response of chlorophyll *a* in each season and each treatment. The rapid decline in chlorophyll *a* concentration in all treatments (including the control) in the Bay bioassays in both winter and summer suggests there was a photoacclimation response to holding the cubitainers at the surface of the water column. Clearly, the chlorophyll *a* concentration in 100% Rain and 100% Nutrients treatment in the Gulf bioassays during the winter showed a strong positive growth response. **Table 5** shows the results of the statistical analyses (Student's T-test, $p = 0.05$) of the chlorophyll *a* growth data for the Gulf bioassays during winter. Changes in chlorophyll *a* in the 100% Rain treatment from day 1 to day 2 was significantly higher than in the control. The increase in chlorophyll *a* in the 100% Nutrient treatment from day 1 - day 2 compared to the control was

nearly significant and would most likely have been if more replicates had been conducted. This is supported by the significant increase in the 100% Nutrient treatment from day 0-to-day 2 and day 0-to-day 3. The variability in chlorophyll *a* concentration in the Gulf bioassays during summer suggests there was no consistent handling response nor treatment response.

Table 4. Range of particle sizes (in micrometers) in treatments that show some size bins with significant growth (+ or -) relative to the control over the specified time period. If a single size bin is significant within a band of not-significant size bins it is considered not significant. Two or more adjacent size bins must be significant to be listed. The layout of treatments in each major block is as indicated in the block below.

	Bay - Winter		Bay - Summer		Gulf - Winter		Gulf - Summer	
Day 0 – Day 1	- 12 - 13	- 11 - 13	NS	NS	NS	NS	+	+
	- 12 - 13	- 12 - 13	+	+	NS	NS	NS	NS
Day 1 – Day 2	+	+	+	NS	+	+	NS	NS
	12 - 13	12 - 14	7 - 11		6 - 7	6 - 6.5		
	+	NS	+	+	NS	NS	NS	NS
	12.5 - 13		7 - 10	6 - 11				
Day 2 – Day 3	NS	NS	+	NS	NS	+	NS	NS
	NS	NS	11 - 13		NS	5 - 5.5		
			+	NS	NS	NS	NS	NS
			5.5 - 6					
Day 0 – Day 2	NS	NS	-	+	+	NS	+	+
	NS	NS	5 - 6	9 - 10	9 - 9.5		6.5 - 10	8 - 10
			+	+	NS	NS	NS	NS
			7.4 - 13	8 - 10				
Day 0 – Day 3	NS	NS	NS	NS	+	+	+	+
	NS	NS			7 - 10	6 - 10	9 - 10	9 - 9.5
			+	NS	NS	NS	NS	NS
			6 - 11, 12 - 13					

100% Rain	100% Nutrient
33% Rain	33% Nutrient

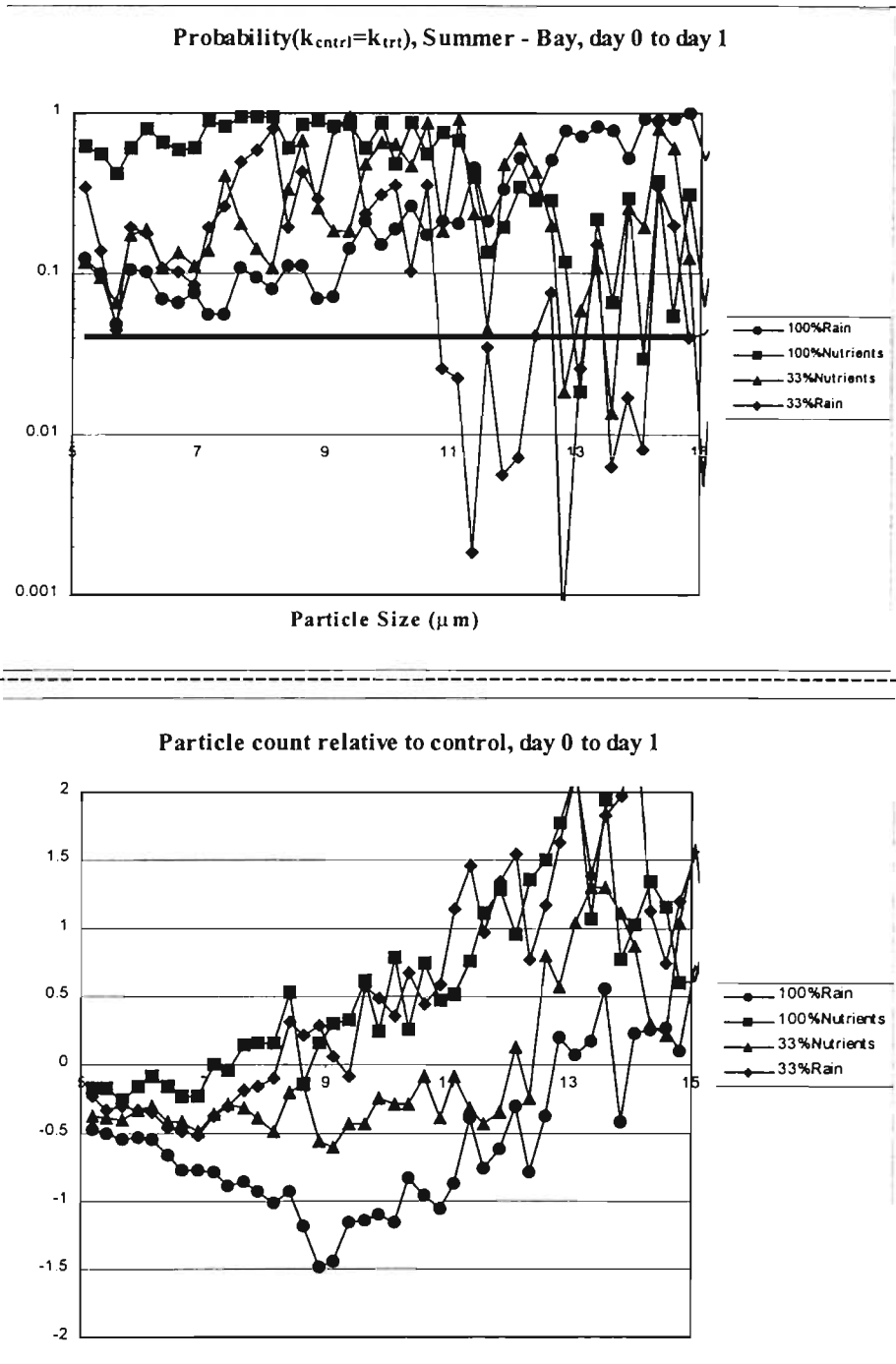


Figure 8. Particle size distribution results for the Summer – Bay experiment. These typical plots are included to illustrate the type of data analyzed. See Table 4 (Range of particle sizes) for the complete listing of results from the particle size analysis. Upper panel - Probability that particle growth of treatment equals control. Lower panel – Treatment particle count relative to control. Positive indicates treatment increased more than control.

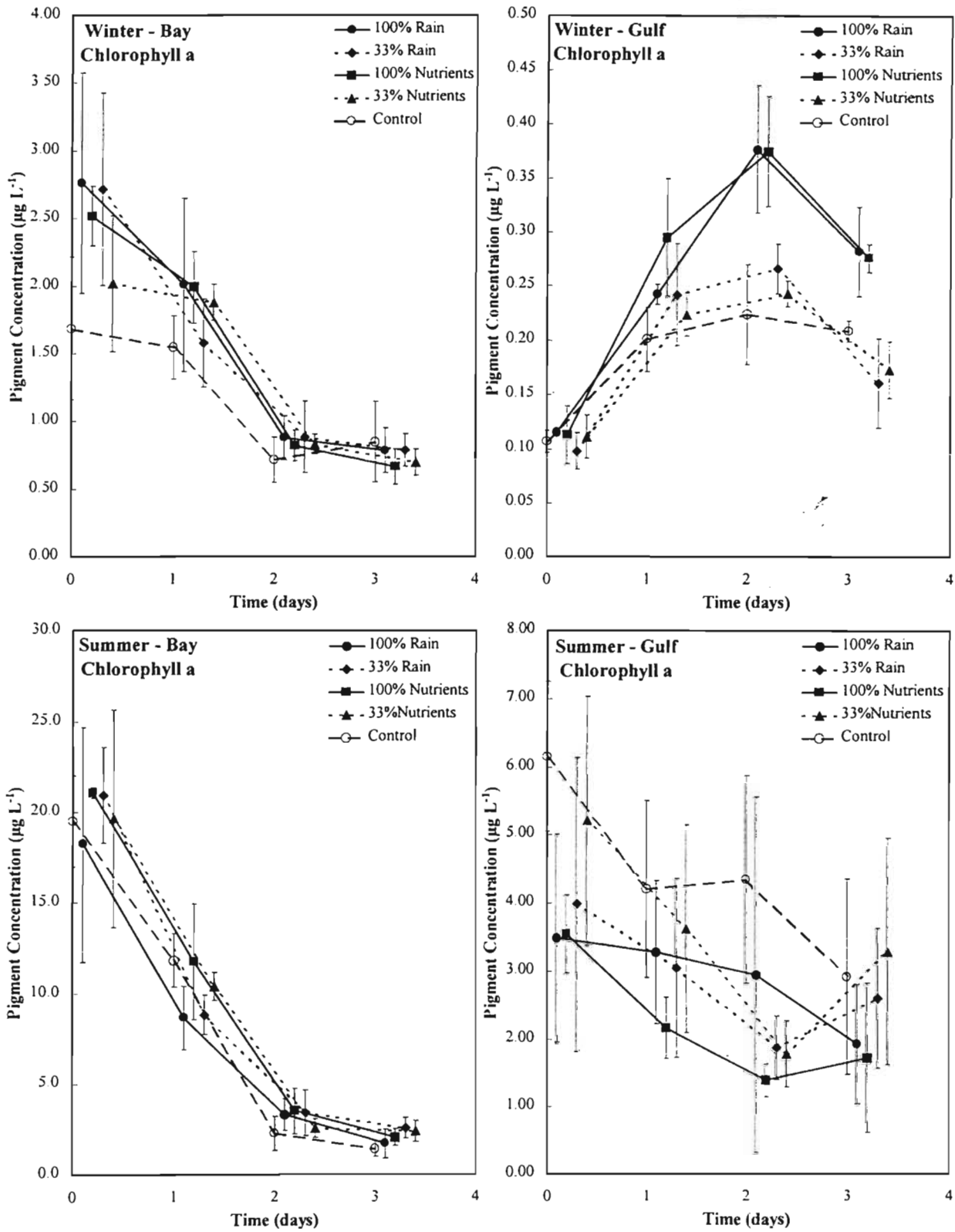


Figure 9. Chlorophyll *a* concentrations ($\mu\text{g/l}$) for the winter (top) and summer (bottom) bioassays. Treatment legends are shown with each graph. Error bars are SEM ($n=3$). When error bars are not visible, they are smaller than symbol. Note different vertical scales.

Table 5. Comparisons of Winter – Gulf chlorophyll *a* changes in the treatments versus the control over the specified time periods. Only results from the Gulf bioassays during winter are shown because it was the only bioassay in which detectable increases in chlorophyll *a* were found. Significance: NS = not significant, * = $p < 0.05$, ** = $p < 0.01$.

Winter – Gulf	100% rain	33% Rain	100% Nutrients	33% Nutrients
Day 0 – Day 1	NS	NS	NS	NS
Day 1 – Day 2	+ *	NS	NS	NS
Day 2 – Day 3	NS	NS	NS	NS
Day 0 – Day 2	+ *	NS	+ *	NS
Day 0 – Day 3	NS	NS	+ **	NS

Figures 10-16 illustrate changes in specific pigment concentrations for each series of bioassays. The relevance of particular pigment responses to the overall phytoplankton growth response is discussed in the section on photoacclimation.

Chemotaxonomy

ChemTax[®], which accounts for the relative shifts of photosynthetic and photoprotective pigments to reduce the impacts of photoacclimation on pigment-determined taxonomic shifts, was used to analyze shifts in phytoplankton populations during each of the bioassays. **Tables 6-9** summarize the statistical analysis (Student's T-test, $p = 0.05$) of shifts in phylogenetic groups of phytoplankton in each treatment relative to the control. Few significant changes in phytoplankton populations were noted.

Bay-Winter Bioassays. There were significant increases in the diatom fraction of the population during day 1 in the 100% Rain and 100% Nutrient treatments (**Table 6**). There was a concomitant significant decrease in the cryptophyte fraction on day 1 in the 100% Rain treatment (**Table 7**) and a significant decrease in the chlorophyte fraction in the 100% Rain treatment during the cumulative day 0 to day 2 time period (**Table 8**). Diatoms are known to respond rapidly to nutrient availability.

Bay- Summer Bioassays. Significant phylogenetic shifts only occurred in the cryptophyte fraction in the 33% Nutrient treatment during the cumulative day 0 to day 3 time period. Lack of significant shifts in phylogenetic groups does not imply there was not growth and possibly significantly different growth in the treatments, it only means the phylogenetic makeup of phytoplankton community did not change significantly (Table 7).

Gulf-Winter Bioassays. The diatom fraction was the only phylogenetic group that showed significant changes during this series of bioassays. The diatom fraction significantly increased in the 100% Rain treatment during day 2 and significant decrease in the 33% Rain treatment during day 3. Associated with all of the listed significant taxonomic shifts are non-significant counter shifts in the other taxa. They are not detected as significant because they are too small relative to the variance of the data.

Gulf-Summer Bioassays. There was a significant increase in the diatom fraction in the 100% Rain treatment from cumulative day 0 to day 2. There was also an increase in the cyanobacteria (**Table 9**) fraction in the 100% Nutrient treatment during day 2 and a decrease in the chlorophyte fraction (Table 8) from day 0 to day 3 in the 33% Nutrient treatment.

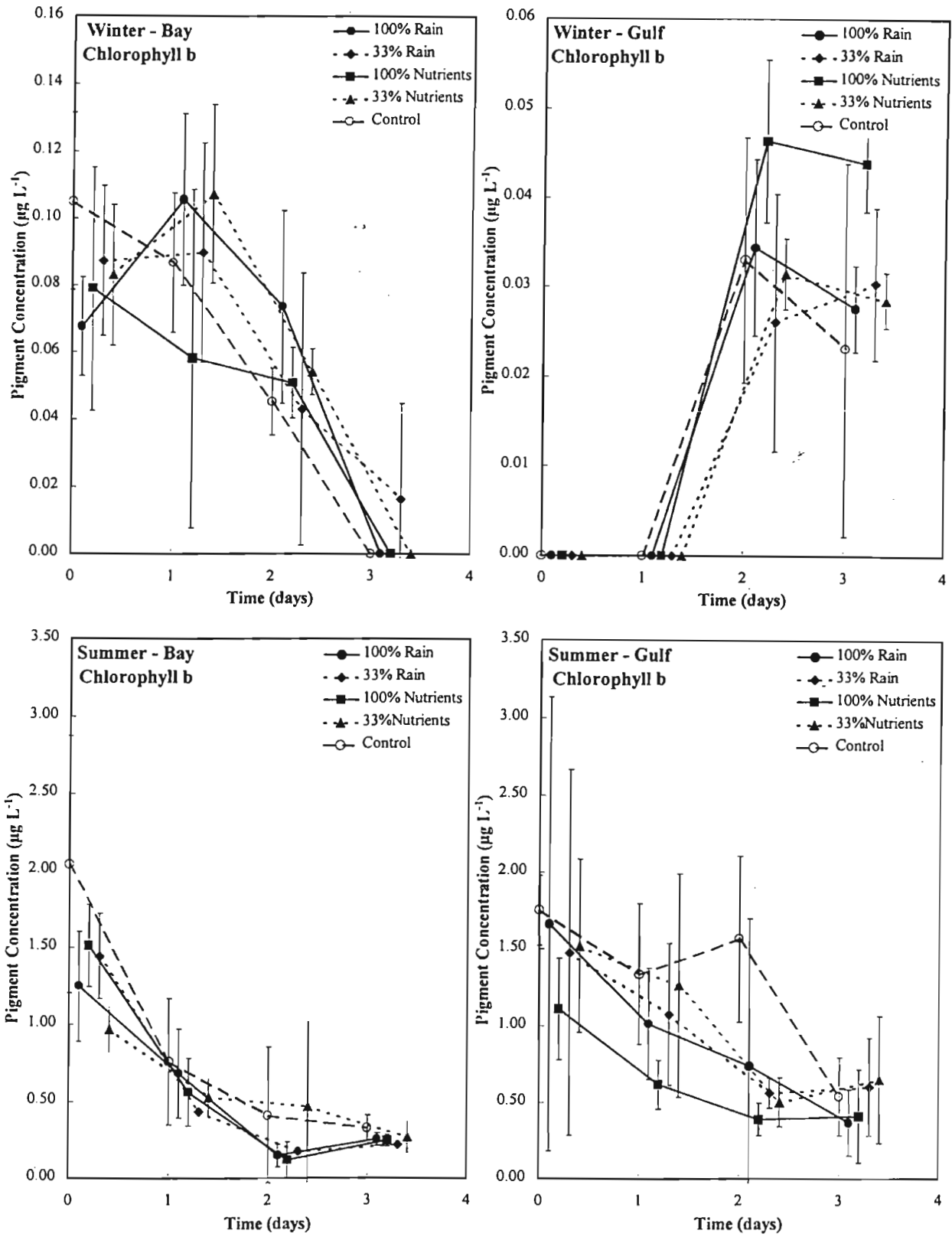


Figure 10. Chlorophyll *b* concentrations ($\mu\text{g/l}$) for the winter (top) and summer (bottom) bioassays. Treatment legends are shown with each graph. Error bars are SEM ($n=3$). Note different vertical scales.

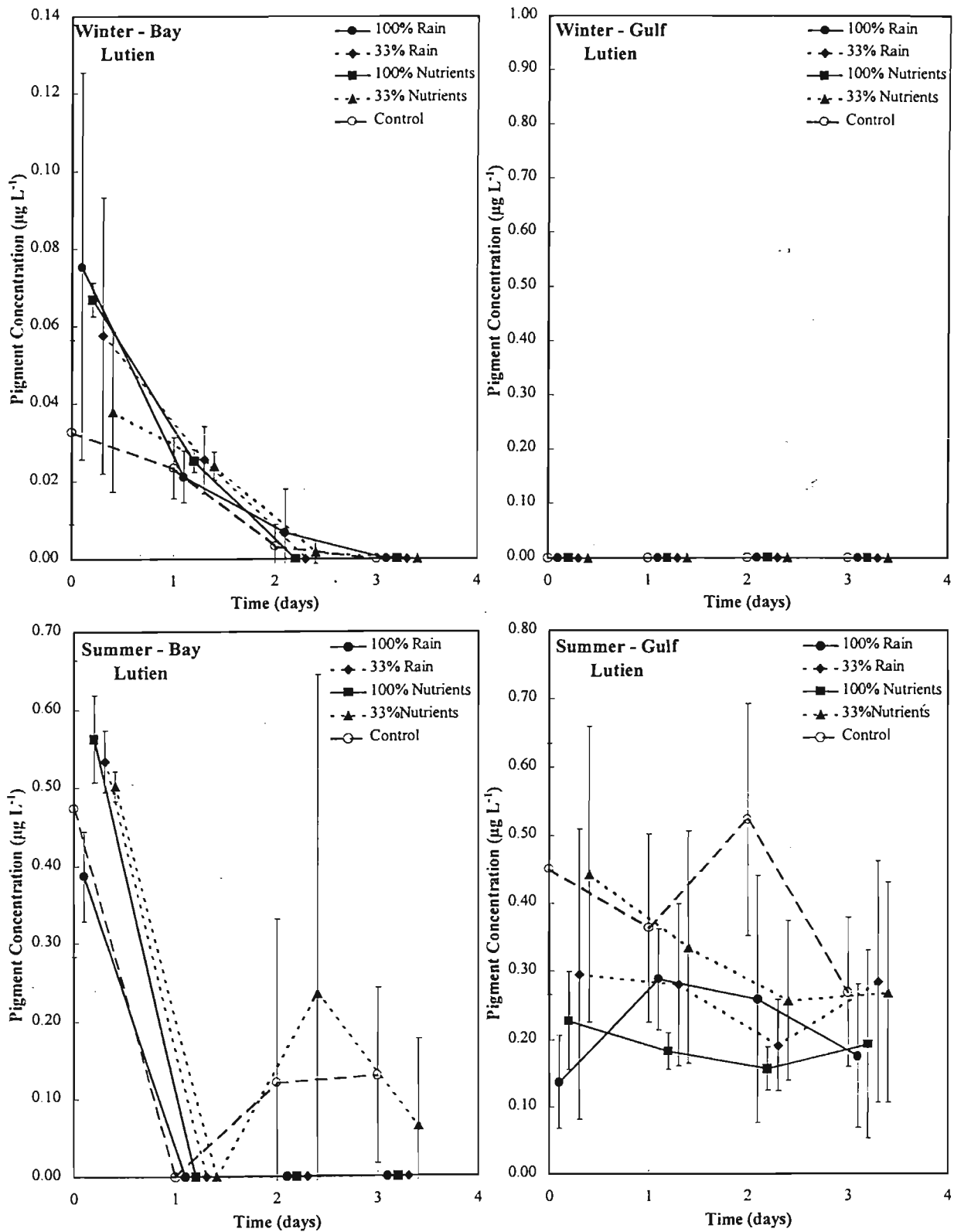


Figure 11. Lutien concentrations ($\mu\text{g/l}$) for the winter (top) and summer (bottom) bioassays. Treatment legends are shown with each graph. Error bars are SEM ($n=3$). Note different vertical scales.

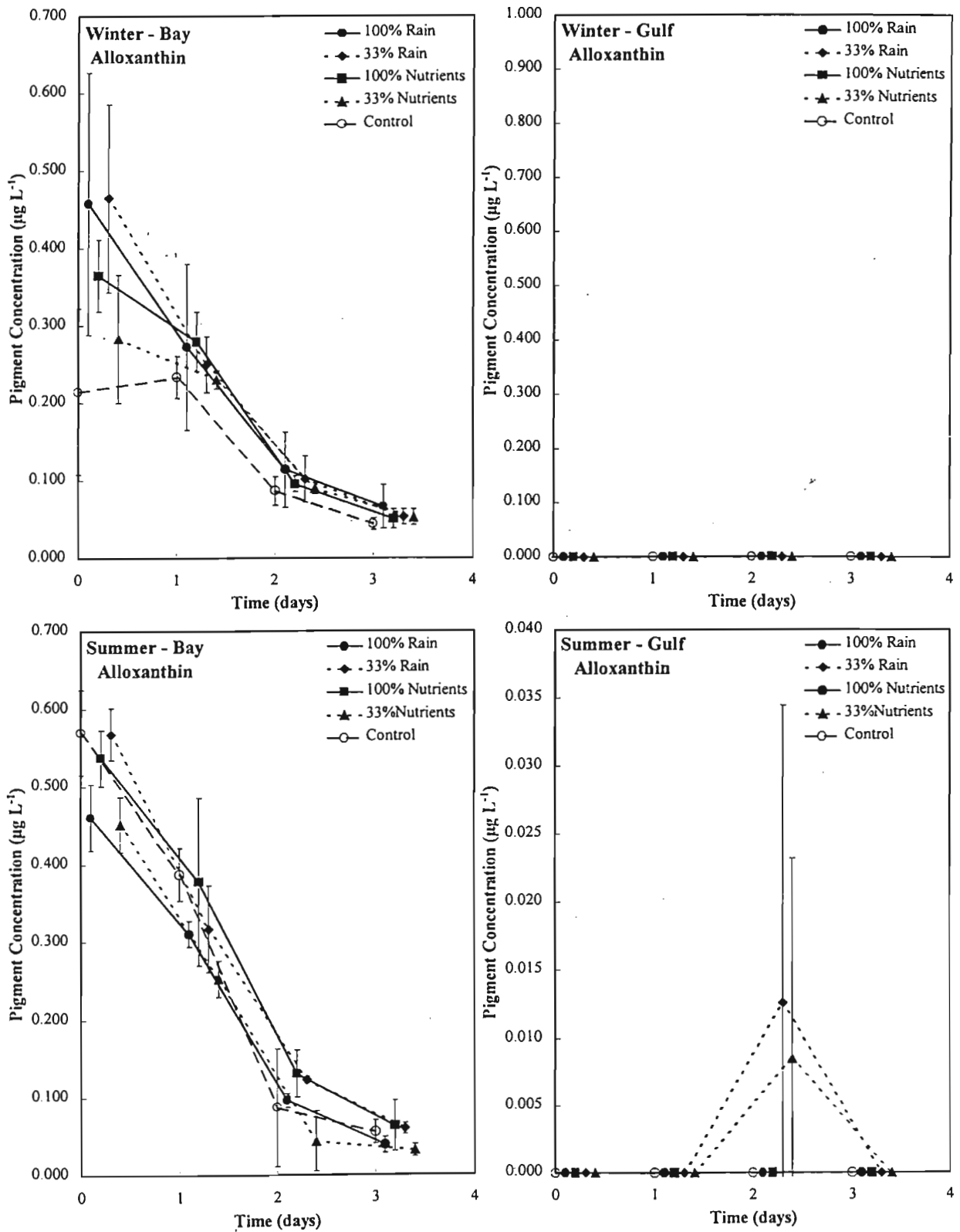


Figure 12. Alloxanthin concentrations ($\mu\text{g/l}$) for the winter (top) and summer (bottom) bioassays. Treatment legends are shown with each graph. Error bars are SEM ($n=3$). Note different vertical scales.

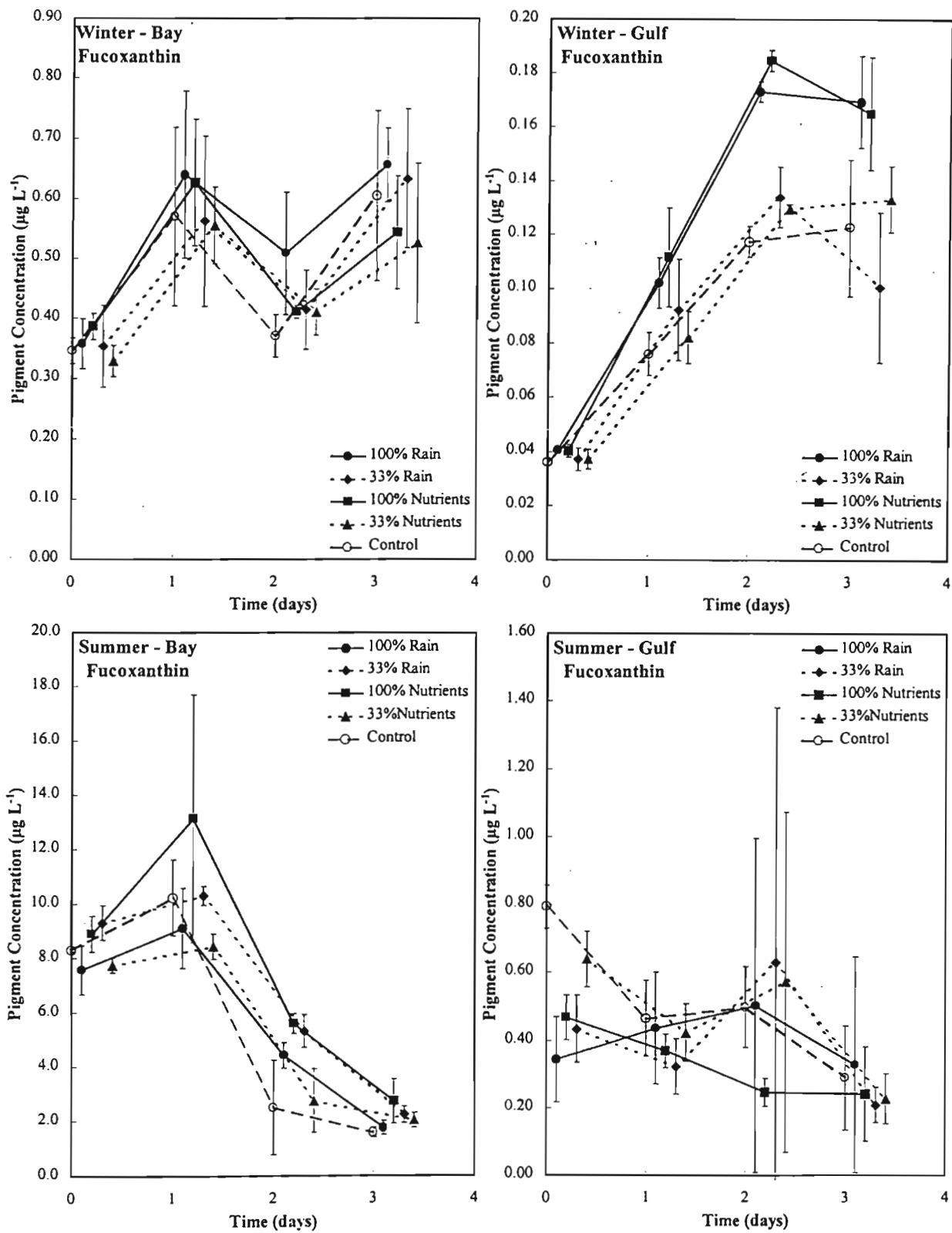


Figure 13. Fucoxanthin concentrations ($\mu\text{g/l}$) for the winter (top) and summer (bottom) bioassays. Treatment legends are shown with each graph. Error bars are SEM (n=3). Note different vertical scales.

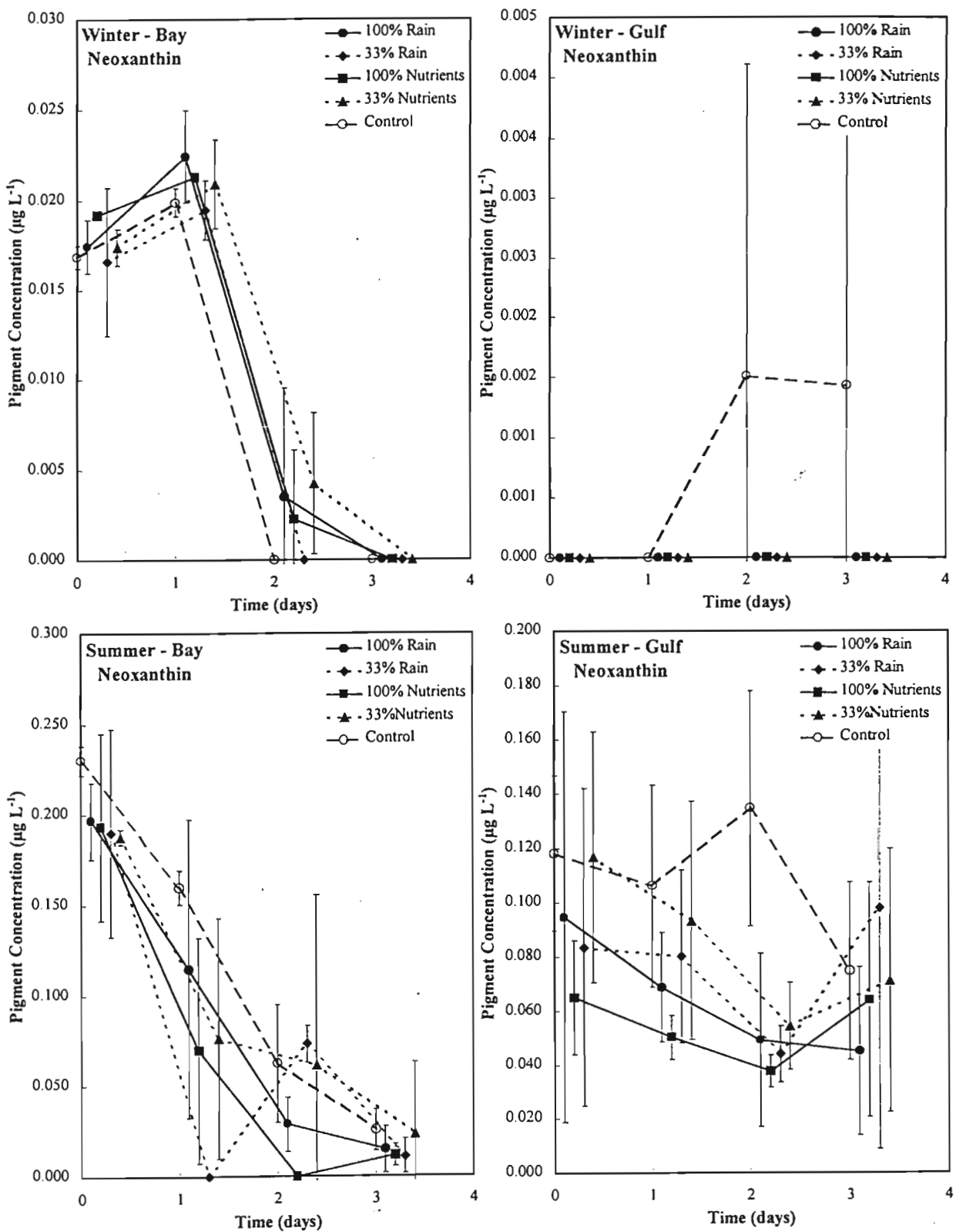


Figure 14. Neoxanthin concentrations (µg/l) for the winter (top) and summer (bottom) bioassays. Treatment legends are shown with each graph. Error bars are SEM (n=3). Note different vertical scales.

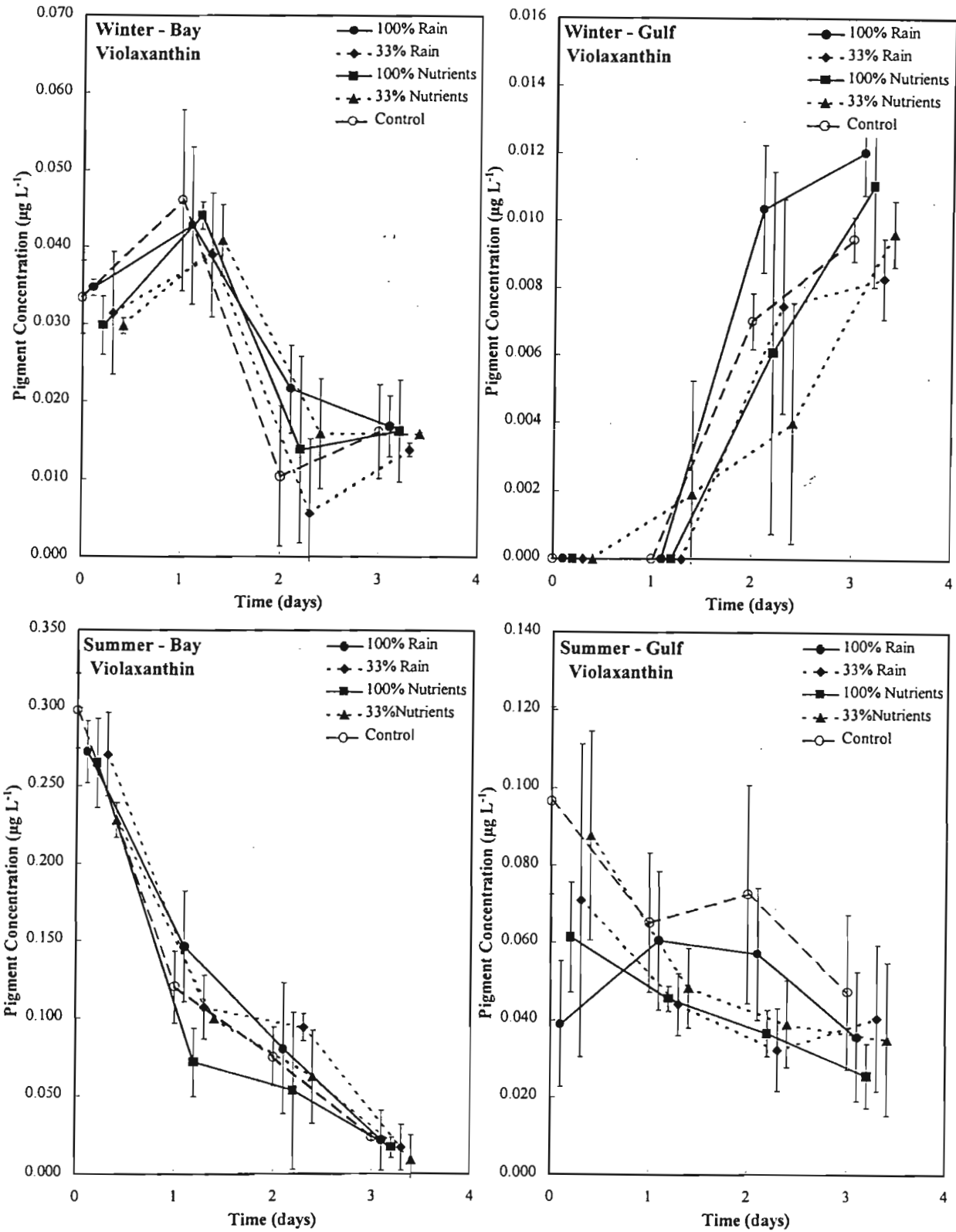


Figure 15. Violaxanthin concentrations ($\mu\text{g/l}$) for the winter (top) and summer (bottom) bioassays. Treatment legends are shown with each graph. Error bars are SEM ($n=3$). Note different vertical scales.

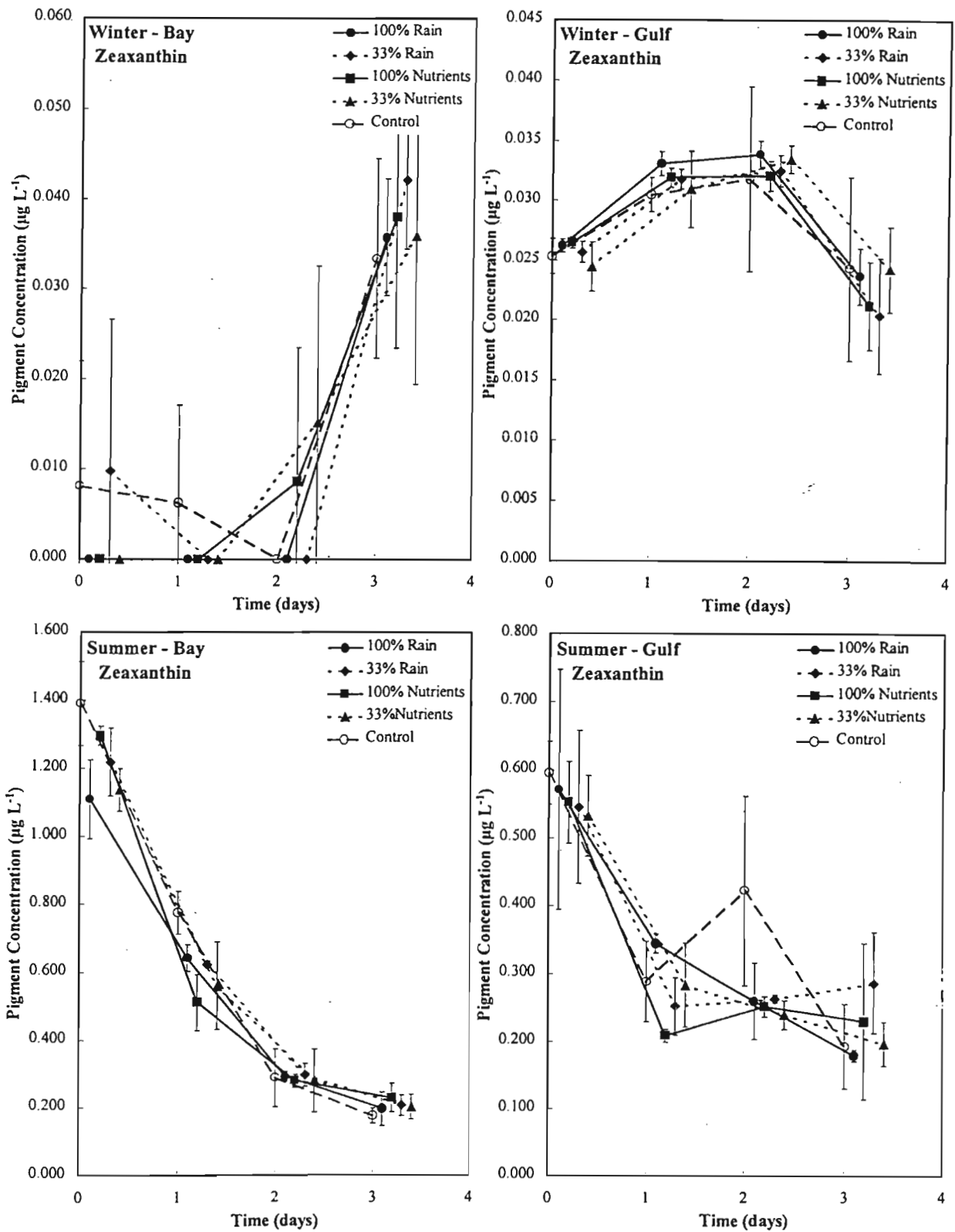


Figure 16. Zeaxanthin concentrations ($\mu\text{g/l}$) for the winter (top) and summer (bottom) bioassays. Treatment legends are shown with each graph. Error bars are SEM ($n=3$). When error bars are not visible, they are smaller than symbol. Note different vertical scales.

Table 6. Shifts in percent of Diatoms in each treatment versus the control during the specified time periods. A '+' indicates the change was an increase relative to the control and a '-' indicates the change was negative relative to the control. Significance: NS = not significant; * = $p < 0.05$. The layout of treatments in each major block is shown separately in the box at the bottom of the table.

Diatom	Bay - Winter		Bay - Summer		Gulf - Winter		Gulf - Summer	
	Day 0 – Day 1	+	+	NS	NS	NS	NS	NS
	*	**						
	NS	NS	NS	NS	NS	NS	NS	NS
Day 1 – Day 2	NS	NS	NS	NS	+	NS	NS	NS
					*			
	NS	NS	NS	NS	NS	NS	NS	NS
Day 2 – Day 3	NS	NS	NS	NS	NS	NS	NS	NS
	NS	NS	NS	NS	-	NS	NS	NS
					*			
Day 0 – Day 2	NS	NS	NS	NS	+	NS	+	NS
					*		**	
	NS	NS	NS	NS	NS	NS	NS	NS
Day 0 – Day 3	NS	-	NS	NS	NS	NS	NS	NS
		*						
	NS	-	NS	NS	NS	NS	NS	NS
		*						

100% Rain	100% Nutrient
33% Rain	33% Nutrient

Table 7. Shifts in percent of Cryptophytes in each treatment versus the control during the specified time periods. A '+' indicates the change was an increase relative to the control and a '-' indicates the change was negative relative to the control. Significance: NS = not significant; * = $p < 0.05$. The layout of treatments in each major block is shown separately in the box at the bottom of the table.

Crypto	Bay - Winter		Bay - Summer		Gulf - Winter		Gulf - Summer	
	Day 0 – Day 1	- *	NS	NS	NS	NS	NS	NS
	NS	NS	NS	NS	NS	NS	NS	NS
Day 1 – Day 2	NS	NS	NS	NS	NS	NS	NS	NS
	NS	NS	NS	NS	NS	NS	NS	NS
Day 2 – Day 3	NS	NS	NS	NS	NS	NS	NS	NS
	NS	- *	NS	NS	NS	NS	NS	NS
Day 0 – Day 2	- *	NS	NS	NS	NS	NS	NS	NS
	NS	NS	NS	NS	NS	NS	NS	NS
Day 0 – Day 3	NS	NS	NS	NS	NS	NS	NS	NS
	NS	NS	NS	- *	NS	NS	NS	NS

100% Rain	100% Nutrient
33% Rain	33% Nutrient

Table 8. Shifts in percent of Chlorophytes in each treatment versus the control during the specified time periods. A '+' indicates the change was an increase relative to the control and a '-' indicates the change was negative relative to the control. Significance: NS = not significant; * = $p < 0.05$. The layout of treatments in each major block is shown separately in the box at the bottom of the table.

Chloro	Bay - Winter		Bay - Summer		Gulf - Winter		Gulf - Summer	
Day 0 – Day 1	NS	NS	NS	NS	NS	NS	NS	NS
	NS	NS	NS	NS	NS	NS	NS	NS
Day 1 – Day 2	NS	NS	NS	NS	NS	NS	NS	NS
	NS	NS	NS	NS	NS	NS	NS	NS
Day 2 – Day 3	NS	NS	NS	NS	NS	NS	NS	NS
	NS	NS	NS	NS	NS	NS	NS	NS
Day 0 – Day 2	- *	NS	NS	NS	NS	NS	NS	NS
	NS	NS	NS	NS	NS	NS	NS	NS
Day 0 – Day 3	NS	NS	NS	NS	NS	NS	NS	NS
	NS	NS	NS	NS	NS	NS	NS	- *

100% Rain	100% Nutrient
33% Rain	33% Nutrient

Table 9. Shifts in percent of Cyanophytes in each treatment versus the control during the specified time periods. A '+' indicates the change was an increase relative to the control and a '-' indicates the change was negative relative to the control. Significance: NS = not significant; * = $p < 0.05$. The layout of treatments in each major block is shown separately in the box at the bottom of the table.

Cyano	Bay - Winter		Bay - Summer		Gulf - Winter		Gulf - Summer	
	Day 0 – Day 1	NS	NS	NS	NS	NS	NS	NS
	NS	NS	NS	NS	NS	NS	NS	NS
Day 1 – Day 2	NS	NS	NS	NS	NS	NS	NS	+ *
	NS	NS	NS	NS	NS	NS	NS	NS
Day 2 – Day 3	NS	NS	NS	NS	NS	NS	NS	NS
	NS	NS	NS	NS	NS	NS	NS	NS
Day 0 – Day 2	NS	NS	NS	NS	NS	NS	NS	NS
	NS	NS	NS	NS	NS	NS	NS	NS
Day 0 – Day 3	NS	NS	NS	NS	NS	NS	NS	NS
	NS	NS	NS	NS	NS	NS	NS	NS

100% Rain	100% Nutrient
33% Rain	33% Nutrient

DISCUSSION

Our bioassays were designed to simulate seasonal (dry and wet) phytoplankton growth response to atmospherically-derived precipitation in amounts typically experienced in local waters. A comparison of rainfall collected for this study with median seasonal values obtained from the National Atmospheric Deposition Project (NADP) Verna Wellfield site in Sarasota County revealed similar concentrations of primary inorganic nutrient species (~80% of NADP median concentrations). Seasonal concentrations of principal nitrogenous nutrients present in both Bay and Gulf waters from the current study also agreed well with recent data obtained independently from other sources (Sarasota County Ambient Water Quality Monitoring Program for Roberts Bay; MML Red Tide Monitoring Program for the Gulf of Mexico). Additionally, our bioassay treatments were designed to test the effects of rainfall at naturally occurring dilutions (one and three percent). Therefore, it is reasonable to conclude that the conditions under which our bioassays were performed are representative of natural meteorological and hydrological conditions commonly found in local waters and that the response of resident phytoplankton to these treatments were not the result of unnatural or unusual conditions.

Effect of Nutrients on Phytoplankton Growth *in situ*

Although there were not large growth responses to the addition of rainfall in our seasonal bioassays in the Gulf and Bay, there were some significant responses observed under specific conditions. The fact that any significant increases in phytoplankton populations resulting from rainfall additions were detected demonstrates that atmospherically derived nitrogen can be a significant nutrient supply for phytoplankton growth.

In the present study, the only stimulation of phytoplankton growth (measured as chlorophyll *a*) as a result of rainfall (and nutrient) enrichment occurred during the winter bioassays in the Gulf. Moreover, the growth response was greater in the two high nutrient treatments (100% rain and 100% nutrients). The fact that these two treatments resulted in similar growth responses indicates that the addition of inorganic nitrogen (and not some other component of the rainwater) was responsible for the stimulated growth. In both seasonal bioassays in the Bay, phytoplankton growth was not stimulated by nitrogen additions. In all Bay bioassays treatments, there was a rapid decline in chlorophyll *a* concentration within the first two days. The summer bioassays in the Gulf showed highly variable growth responses among the treatments, but in general, these bioassays exhibited declines in chlorophyll *a* concentrations in all treatments.

In performing similar bioassays using Neuse River estuarine water in North Carolina, Paerl *et al.* (1990) observed no net stimulation of chlorophyll *a* concentration when using rainfall with relatively low DIN concentrations. Only when rainwater was highly enriched in DIN did they see significant and prolonged stimulation of phytoplankton growth. Ambient nutrient concentrations of Neuse River waters was not determined prior to enrichment. In our Bay bioassays, ambient DIN concentration in the summer was already quite high (0.068 mg/l), which

is nearly twice the yearly median for these waters (Sarasota County monitoring program). The addition of 8% more DIN to these waters (the effect of a 6 cm rain event) did not stimulate phytoplankton growth in our bioassays. During the winter, the concentration of DIN in Roberts Bay was 0.024 mg/l, which was much lower than during the summer and below the average yearly mean. The DIN in the rainfall during winter was lower in DIN as well. However, even with the addition of 14% more DIN to Roberts Bay water (again, the effect of a 6 cm rain event), phytoplankton growth was not stimulated. These findings suggest that new nitrogen derived solely from atmospheric deposition may not be significant enough to stimulate primary production in our estuarine waters, but that additional sources of nitrogen (e.g., sediment resuspension or terrestrial runoff), which are also associated with storm events, may also be necessary to increase production.

Potential responses of phytoplankton in the more nutrient-poor waters of the nearshore Gulf of Mexico may be quite different from their estuarine counterparts. In this environment, atmospherically derived nitrogen can result in a substantial increase in available nutrients. In the present study, a 6 cm storm event led to increases in DIN concentration of 24 and 31% during summer and winter, respectively. These calculations were based on the assumption that the rain completely mixes within the top 2 meters of the water column. Bioassays conducted with Gulf water during winter conditions consisted of the lowest initial chlorophyll a concentrations (~ 0.1 µg/l) and lowest ambient nutrient concentrations (0.011 mg/l DIN). These results clearly show that the level of nitrogen derived from rainfall stimulated phytoplankton growth. This growth was primarily due to the diatom component, as detected by changes in chemotaxonomy, pigment increases and particle size distribution.

Other investigations on phytoplankton responses to nutrient enrichment have included additional nutrients, mainly phosphorus and trace metals, in addition to nitrogen. In Perdido Bay, Florida, phytoplankton responses to enrichment fell into two primary categories: (1) primary orthophosphate-P or nitrate-N stimulation (i.e., some growth with the primary nutrient but enhanced growth with the addition of both nutrients), and (2) apparent nitrate-N and orthophosphate-P co-stimulation (i.e., both nutrients were required to elicit a growth response (Flemer *et al.* 1998). Orthophosphate stimulated phytoplankton growth primarily during the cool season and nitrate-N during the warm season. In North Carolina, nitrate-N consistently stimulated growth, while occasionally the combination of nitrate-N and orthophosphate-P further stimulated growth (Paerl *et al.* 1990). Neither orthophosphate nor trace metals alone stimulated growth above controls (Paerl *et al.* 1990). Nitrogen was determined to be the primary growth stimulating nutrient (Paerl *et al.* 1990). Because phytoplankton productivity in coastal Sarasota waters is considered to be nitrogen limited (N:P mg:mg ratios generally less than 7; see Table 1), we did not consider orthophosphate to be a limiting nutrient in our bioassays.

Explanations for Observed Phytoplankton Growth Response During *in situ* Bioassays

Nutrient Depletion: When nutrients are suddenly added to depleted cells, the response is often a rapid uptake of the depleted nutrient. For example, when nitrogen was added to N-

limited diatom cultures, the nitrogen was taken up rapidly, and carbon assimilation was depressed (Falkowski and Stone 1975). Phytoplankton, if sufficiently N depleted, have the capacity to take up ammonium as much as 30 times faster than the steady-state growth rate when the cells are exposed briefly (5 min) to saturating ammonium concentrations (i.e. $> 1 \mu\text{M}$) (Falkowski and Stone 1975).

Paerl *et al.* (1990) observed deviations in chlorophyll *a* response compared to $^{14}\text{CO}_2$ assimilation in bioassays (similar in design to ours) from North Carolina. They explained that one possible explanation for the observed differences may be due to exhaustion of the added nitrogen. Phytoplankton may be adapted to a patchy nutrient distribution, rapidly assimilating nutrients before the temporary patches dissipate (McCarthy and Goldman, 1979; Turpin and Harrison, 1979; Lehman and Scavia, 1982). This enhanced uptake would result in rapid stimulation of algal growth and could quickly reduce the available DIN. Without further additions of nitrogen to support accelerated growth, phytoplankton could undergo death and decay, with a resultant rapid degradation of chlorophyll *a*. The ^{14}C content of the particulate fraction (not determined in the present study) would, in all likelihood, not have revealed a parallel decrease at this stage because much of the ^{14}C remained incorporated in organic carbon compounds, either in live cells or detritus. In this scenario, the highest N addition (100% Rain and 100% Nutrient treatments) could supply enough nitrogen such that the DIN would not be completely exhausted following the initial surge in enhanced uptake and would therefore support continued growth. Flemer *et al.* (1998), in a study of seasonal nutrient limitation of phytoplankton in Perdido Bay, FL, utilized a modified static renewal approach in their microcosm setup, whereby the daily removal of 10% of the volume was replaced with ambient source water, thus replenishing some of the nutrients to their microcosms. In the present study, DIN was depleted at the end of the first day in both Bay and Gulf bioassays during the summer. This could help explain the decline in chlorophyll *a* during these bioassays. However, during the winter bioassays, where DIN was not totally exhausted, only the Gulf bioassays exhibited positive growth. In addition, since all nutrient treatments (including the control) for the Gulf bioassays showed increased phytoplankton growth, other factors besides nutrient depletion were probably involved.

Zooplankton Grazing. A second explanation for the observed decrease in chlorophyll *a* relates to zooplankton grazing. In our bioassays, the source water was not prefiltered and thus, zooplankton were not excluded from the cubitainers. Under typical summer temperatures and favorable nutritional conditions, estuarine copepods can grow through more than one developmental stage per day (Heinle, 1996), while tintinnid protozoans can also grow very rapidly, with generation times from one to two days (Heinbolkel, 1988). Grazing by *Acartia* spp. can remove up to 30% of the standing stock of phytoplankton in estuarine waters (Deason, 1980), indicating that at times grazing pressure can be very significant. Feeding by resident Sarasota Bay zooplankton might have caused similar reductions in the algal biomass, especially during the summer, within the relatively small volumes of the cubitainers. Sorensson *et al.* (1989) found evidence of elevated flagellate grazing and enhanced ammonium regeneration after two days during mesocosm enrichment studies in the Baltic Sea. Flemer *et al.* (1998) filtered

water through nested plankton nets during water collection in order to remove larger zooplankton; Paerl *et al.* (1990) did not.

Photoacclimation. Phytoplankton adjust to changing light exposure over short time periods (seconds to days) in a variety of ways to optimize their existence under those conditions. This process of adjustment is referred to as photoacclimation. Typically, phytoplankton populations are lumped into ‘sun’ or ‘shade’ acclimated groupings, but they are usually found somewhere between these two acclimation endpoints. A shade acclimated phytoplankter has an elevated cellular concentration of chlorophyll *a* and associated photosynthetic accessory pigments to enhance its ability to capture photons in the low-light environment it occupies. The ‘shade’ acclimated phytoplankter will also exhibit depressed levels of photoprotective pigments since there is no light stress. A ‘sun’ acclimated phytoplankter will exhibit the opposite pigment complement, photosynthetic pigments will be depressed and photoprotective pigments will be elevated to mitigate light stress.

Light attenuation in bays and estuaries is very rapid relative to clear oceanic water. The dissolved color, detritus and phytoplankton in bays combine to strongly attenuate light over very short vertical distances. Therefore, the phytoplankton at the Bay site were closer to the ‘shade’ acclimated state with high cellular concentrations of chlorophyll *a* and other photosynthetic pigments. When the aliquots of water were distributed to the cubitainers and incubated at the surface of the water column, the ‘shade’ acclimated phytoplankton in those cubitainers were forced to remain in a high-light environment and had to photoacclimate to a ‘sun’ acclimated state. The rapid reduction of chlorophyll *a* concentration (Figure 9) seen in the Bay samples over the first two days can best be explained by photoacclimation. There was a concomitant reduction in the photosynthetic pigments lutein and alloxanthin (see Figures 11 and 12). Conversely, there was an increase in the concentration of the photoprotective pigments diadinoxanthin and diatoxanthin (Figures not presented). Because there was an increase in the photoprotective pigments, it seems unlikely that the decrease in chlorophyll *a* was due to nutrient reductions or grazing, but was rather due to this photoacclimation process.

Additional Causes: Finally, differential responses to various DIN enrichments may have indicated (1) compositional differences in phytoplankton species between bioassay dates, (2) contrasting DIN uptake and assimilation kinetics within similar phytoplankton assemblages on different dates, or (3) varying physical regimes (irradiance, turbulence, temperature or combinations thereof) controlling DIN uptake and assimilation dynamics (Paerl *et al.* 1990).

Conclusion

During the summer bioassays all added nutrients were essentially depleted by the end of the first day. There was also a rapid decline in chlorophyll *a*, which was a measure of the growth response of the algal assemblages. This short-term phytoplankton response was likely due to photoacclimation during the bioassays. These findings suggest that new nitrogen derived solely from atmospheric deposition may not be significant enough to stimulate primary production in

Sarasota estuarine waters, but that additional sources of nitrogen (e.g., sediment resuspension and terrestrial runoff), which are also associated with storm events, may also be necessary to increase production.

During the winter bioassays the positive growth response to rain and nutrient treatments in the Gulf was directly related to added nutrient concentration. Additionally, the growth response was similar between rainfall and nutrient treatments, strongly suggesting that the response was due primarily to nitrogen, and not some other component of the rainwater. The positive growth response was primarily due to the diatom fraction of the phytoplankton.

Although there was no consistent growth response to the addition of rainfall for all seasonal bioassays in the Gulf and Bay, there was a significant positive response under specific conditions. The fact that any significant increase in phytoplankton population growth resulting from rainfall additions was detected demonstrates that atmospherically derived nitrogen can be a significant nutrient supply for phytoplankton growth.

LITERATURE CITED

- Alderson, M. 1992. State of the Bay. Chapter 2 In: (P. Roat, C. Ciccolella, H. Smith and D. Tomasko, eds.) Framework for Action. Sarasota Bay National Estuary Program.
- Carpenter, E.J. and D.G. Capone. 1983. Nitrogen in the marine environment. Academic Press, New York.
- Deason, E.E. 1980. *Acartia hudsonica* grazing on *Skeletonema costatum*. Mar. Biol. 60: 101-113.
- Dixon, L.K., S. Murray, J.S. Perry, P.J. Minotti, M.S. Henry and R.H. Pierce. 1996. Assessment of bulk atmospheric deposition to the Tampa Bay watershed. Technical Publication #08-96 to the Tampa Bay National Estuary Program, prepared by Mote Marine Laboratory.
- Duce, R.A. 1986. The impact of atmospheric nitrogen, phosphorus, and iron species on marine biological productivity. In: The Role of Air-Sea Exchange in Geochemical Cycling. Ed. P. Buat-Menard.
- Falkowski, P.G. and D.P. Stone. 1975. Nitrate uptake in marine phytoplankton: energy sources and the interaction with carbon fixation. Mar. Bio. 32: 77-84.
- Fisher, D., J. Ceraso, T Mathew, and M. Oppenheimer. 1988. Polluted coastal waters: The role of acid rain. Environmental Defense Fund. New York, NY.
- Flemer, D.A., R.J. Livingston and S.E. McGlynn. 1998. Seasonal growth stimulation of sub-temperate estuarine phytoplankton to nitrogen and phosphorus: an outdoor microcosm experiment. Estuaries 21: 145-159.
- Frithsen, J.B., S.P. Schreiner, D.E. Strelbel, R.M. Laljani, D.T. Logan and H.W. Zarbock. 1995. Chemical contaminants in the Tampa Bay estuary: A summary of distributions and inputs. Technical Publication #01-95 of the Tampa Bay National Estuary Program, prepared by Versar, Inc.
- GESAMP. 1989. The atmospheric input of trace species to the world ocean. World Meteorological Organization No 38.
- Heinbokel, J.F. 1988. Reproductive rates and periodicities of oceanic tintinnine ciliates. Mar. Ecol. Prog. Ser. 47: 239-248.
- Heinle, D.R. 1996. Production of a calanoid copepod, *Acartia tonsa*, in the Patuxent River Estuary. Ches. Sci. 7: 59-74.
- Hicks, B., R. Artz, and R. Valigura. 1992. Atmospheric Nutrient Input to Coastal Areas (ANICA). NOAA Air Resources Laboratory. Silver Spring, MD.
- Lehman, J.T. and D. Scavia. 1982. Microscale patchiness of nutrients in plankton communities. Science: 216: 729-730.
- Lowrey, S. 1992. Physical and Chemical Properties- Bay Waters and Sediment Quality. Chapter 4 In: (P. Roat, C. Ciccolella, H. Smith and D. Tomasko, eds.) Framework For Action. Sarasota Bay National Estuary Program.
- Mackey, M. D., D.J. Mackey, H.W. Higgins and Wright S. W. 1996. CHEMTAX-a program for estimating class abundances from chemical markers: application to HPLC measurements of phytoplankton. Mar. Ecol. Prog. Ser. 144:265-283.
- Mann, K.H. 1982. Ecology of coastal waters. University of California Press, Berkeley.
- McCarthy, J.J. and J.C. Goldman. 1979. Nitrogenous nutrition of marine phytoplankton in

- nutrient-depleted waters. *Science*: 203: 670-672.
- Nixon, W.W. 1981. Nitrogen in estuarine and coastal marine ecosystems. In: E.J. Carpenter and D.G. Capone (eds.) Nitrogen in the marine environment. Academic Press, New York.
- Paerl, H.W. 1983. Factors regulating nuisance blue-green algal bloom potentials in the lower Neuse River, N.C. UNC Water Resources Research Institute, NC State University, Raleigh. Report No. 188.
- Paerl, H.W. 1985. Enhancement of marine primary production by nitrogen-enriched acid rain. *Nature* 316:747-749.
- Paerl, H.W. 1987. Dynamics of blue-green algal (*Microcystis aeruginosa*) blooms in the lower Neuse River, North Carolina: causative factors and potential controls. UNC Water Resources Research Institute. NC State University, Raleigh. Report No. 229.
- Paerl, H.W., J. Rudek and M.A. Mallin. 1990. Stimulation of phytoplankton production in coastal waters by natural rainfall inputs: nutritional and trophic implications. *Mar. Biol* 107: 247-254.
- Pedulla, J.C. 1988. A method for the measurement of total organic nitrogen in precipitation. Master Thesis. University of Virginia. Charlottesville, VA.
- Pinckney, J.L., D.F. Millie, K.E. Howe, H.S. Paerl and J.P. Hurley. 1996. Flow scintillation counting of ¹⁴C-labeled microalgal photosynthetic pigments. *J. Plankton Res.* 18: 1867-1880.
- Ryther, J.H. and W.M. Dunstan. 1971. Nitrogen, phosphorus, and eutrophication in the coastal marine environment. *Science* 171: 1008-1013.
- Sorensson, F., K. Pettersson, J. Selmer and E. Suhlster. 1989. Flows of nitrogen in a mesocosm experiment in the Baltic Sea. *Mar. Ecol. Prog. Ser.* 58: 77-88.
- Tester, P.A., M.R. Geesey, C. Guo, H.W. Paerl and D.F. Millie. 1995. Evaluating phytoplankton dynamics in the Newport River Estuary (North Carolina, USA) by HPLC-derived pigment profiles. *Mar. Ecol. Prog. Ser.* 124: 237-245.
- Turpin, D.H. and P.J. Harrison. 1979. Limiting nutrient patchiness and its role in phytoplankton ecology. *J. Exp. Mar. Bio. Ecol.* 39: 151-166.
- Tyler, M. 1988. Contribution of atmospheric nitrate deposition to nitrate loading in the Chesapeake Bay. Versar, Inc. Columbia, MD.
- Wright, S., S. Jeffrey, R. Mantoura, C. Llewellyn, T. Bjornland, D. Repeta and N. Welschmeyer. 1991. An improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. *Mar. Ecol. Prog. Ser.* 77: 183-196.
- Zarbock, H.W., A. Janicki, D. Wade, D. Heimbuch and H. Wilson. 1994. Estimates of total nitrogen, total phosphorus, and total suspended solids loadings to Tampa Bay, Florida. Technical Publication #04-96 of the Tampa Bay National Estuary Program, prepared by Coastal Environmental, Inc.