CONSEQUENCES OF NUTRIENT LOADING IN THE SUWANNEE RIVER AND ESTUARY, FLORIDA, USA

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2003

This document is dedicated to my parents.

ACKNOWLEDGMENTS

I extend my thanks and appreciation to my major professor, Dr. Edward Phlips, for his substantial contribution to my education. His patience and guidance made this work possible. In him I found enthusiasm, insight, resourcefulness, and a friend. I would also like to thank Dr. Shirley Baker, Dr. Charles Cichra, Dr. Thomas Crisman, and Dr. Thomas Frazer for their assistance and thought-provoking counsel while serving on my committee.

I gratefully acknowledge J.A. Hale, C. E. Jett, K. A. Donnelly, B.E. Sargent, T. Grosskopf, J. Frost, N. Love, S. Badylak, and M. Cichra for their invaluable assistance in the field and laboratory analysis. Special appreciation is directed toward the crew of the R/V Discovery for their helpful assistance during the cruises. I also wish to thank R. Mattson and D. Hornsby for their expertise on the Suwannee River and the Suwannnee River Water Management District for funding this project.

Finally, I want to express my gratitude to my family for their encouragement and my husband Michael, for his unconditional support, without him at my side nothing would have been possible.

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December 2003

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Major Department: Fisheries and Aquatic Sciences

Complex interactions among biological, physical, and chemical parameters within coastal ecosystems make it difficult to define the impacts of nutrient loading on phytoplankton biomass. Natural and anthropogenic surface runoff and groundwater discharge deliver elevated concentrations of nitrate and phosphorus to the Suwannee River, Florida, USA. These observations have precipitated interest in the potential impact of increased nutrient loads on algal biomass and composition in the Suwannee River and its estuary. To address the consequences of nutrient loading, the objectives of my study were the following:

- To define the relationships between changes in nutrient loading and phytoplankton biomass.
- To characterize the composition of the phytoplankton community within different hydrogeographical regions of the river and estuary.
- To determine the impact of micro-zooplankton grazers on phytoplankton standing crop in the estuary.

The results of my study indicated that phytoplankton biomass in the Suwannee River estuary was sensitive to changes in nutrient loads from the Suwannee River. For example, phytoplankton in the region seaward of the oyster reefs was most impacted by changes in nutrient loads due to the increased potential for nutrient limitation, specifically nitrogen. When the nutrient-rich freshwater plume was spatially restricted (i.e., low riverine discharge), phytoplankton standing crop remained low. During higher freshwater discharge, the nutrient-rich plume extended seaward and phytoplankton biomass increased. High phytoplankton biomass (>30 μg chl a L^{-1}) was also observed in the reef region after periods of southwesterly prevailing winds, which may have increased residence time.

The impact of nutrient loading on phytoplankton biomass was also viewed in the context of loss processes, such as mortality. Within the Suwannee River estuary (Site C2), the micro-zooplankton community was dominated by nauplii, hetero/mixotrophic dinoflagellates, and ciliates. The density of ciliates was comparable to densities found within highly eutrophic freshwater lakes and blackwater systems. Maximum phytoplankton growth rates ranged from 0.45 to 2.7 d^{-1} and grazing rate coefficients ranged from 0.12 to 1.4 d^{-1}, both of which were within the range reported for other estuarine/nearshore regions around the world. Micro-zooplankton grazing rates impacted phytoplankton standing crop as grazing loss rates of up to 75% per day were observed under laboratory conditions.

CHAPTER 1
INTRODUCTION

Like many estuaries, the ecology of the Suwannee River estuary is closely tied to water quality of the Suwannee River. Natural and anthropogenic surface runoff and groundwater inputs contribute high concentrations of nitrate and phosphorus to the Suwannee River before it discharges into the Gulf of Mexico. Nitrate levels within the Suwannee River have steadily increased over the last 50 years (Ham and Hatzell 1996, Katz et al. 1999), and concentrations near the mouth and in the upper reaches of the estuary can exceed 1 mg N L^{-1} (Bledsoe and Phlips 2000).

These observations have precipitated interest about the potential consequences of increased nutrient loading on the primary producer community of the river and estuarine waters. The Suwannee River estuary and surrounding coastal waters are productive nursery areas for marine invertebrates and fish in the Gulf of Mexico (Leadon 1979). The stability of this ecosystem hinges on maintaining a balance between the nutrients that contribute to primary productivity and avoiding excessive nutrient enrichment, which can lead to changes in phytoplankton bloom dynamics (e.g., frequency, duration, magnitude, and species composition).

A preliminary study of the factors that control the spatial and temporal composition and abundance of planktonic primary producers in the Suwannee River estuary was conducted in 1996-1997 and suggested that phytoplankton standing crops in the Suwannee River and its estuary were dependent on both light and nutrient availability (Bledsoe and Phlips 2000). Regional differences in light and nutrient availability

throughout the estuary produce favorable conditions for the accumulation of phytoplankton biomass and concern for harmful algal blooms. However, understanding the distribution of resources that support phytoplankton growth does not provide a complete picture of processes controlling the dynamics of phytoplankton populations in estuaries. The realization of increased biomass, represented by light and nutrient availability, must be viewed in the context of key loss functions, such as transport, dilution, and mortality. For example, high rates of grazer-induced mortality have been observed in estuarine and coastal environments (Dagg 1995) but not much is known about the contribution of grazing to the decline of phytoplankton blooms in river-dominated continental shelves (Ortner and Dagg 1995).

The goal of my dissertation research was to determine the relative role of key gain and loss functions in the control of phytoplankton standing crop in the Suwannee River and estuary. In this study, I completed an investigation of several issues related to the impact of nutrient enrichment on algal community structure and abundance in the Suwannee River estuary. The main objectives of this study were the following:

- To describe the spatial and temporal distribution of phytoplankton in the Suwannee River and its estuary in the context of changing nutrient loads, light availability, and hydrodynamic flushing.
- To characterize the composition of the phytoplankton community within different hydrogeographical regions of the river and estuary.
- To determine the impact of micro-zooplankton grazers on phytoplankton standing crop in the estuary.

The objectives were aimed at providing critical information about the influence of the Suwannee River on the overall ecology of the coastal ecosystem.

CHAPTER 2
RELATIONSHIPS AMONG PHYTOPLANKTON BIOMASS, NUTRIENT LOADING, AND HYDRODYNAMICS IN THE SUWANNEE RIVER ESTUARY, FLORIDA, USA

Introduction

The impact of increased nutrient loading on the density, biomass, and spatial distribution of phytoplankton in coastal ecosystems is an issue of global concern (Boynton et al. 1982, Paerl 1988, Rosenberg et al. 1990, Knoppers 1994, Abreu et al. 1995, Lapointe and Matzie 1995, Taylor et al. 1995, Richardson and Jørgensen 1996, Conley et al. 2000). Anthropogenically induced increases in nitrogen loading are of particular importance due to the predominance of nitrogen limitation in the marine environment (Hecky and Kilham 1988, Smayda 1990, Nixon 1995, Borum and Sand-Jensen 1996, Cloern 2001). Although increased nutrient delivery to coastal waters can enhance primary production, excessive nutrient enrichment can lead to undesirable changes in ecosystem structure and function. High levels of nutrient loading have been implicated in the formation of algal blooms in many regions throughout the world, and as a consequence, led to increases in the frequency and distribution of hypoxic events (Jørgensen 1980, Rosenberg et al. 1992, Paerl et al. 1998); toxin production (Smayda 1990, Hallegraeff 1993, Burkholder et al. 1995, Anderson et al. 2002); restriction of light availability for benthic production (Flindt et al. 1997, Borum 1996, Valiela et al. 1997); and changes in food web interrelationships (Shumway 1990, Turner and Tester 1997).

Research dealing with these issues has focused on defining the relationship between changes in nutrient loading and algal biomass, often using a comparative

approach between estuaries (e.g., Boynton et al. 1982, Monbet 1992). This has often been a difficult task, due to the complexity of ecological relationships in estuarine ecosystems (Cloern 2001). Basin morphology, residence time, light, grazing, temperature, and nutrient availability may individually, or in combination, constrain or magnify the response of phytoplankton to nutrient enrichment making the impact of nutrient loading change on the phytoplankton standing crop difficult to ascertain, especially in the absence of long-term databases (Boynton et al. 1982).

The goal of my study was to determine the relationship between spatial and temporal patterns of nutrient loading and the concentration and distribution of phytoplankton standing crops in a relatively open estuary located along the broad continental shelf of the eastern Gulf of Mexico. The major source of nutrients to this estuary is the Suwannee River, a blackwater river emanating from a sparsely populated watershed. A variety of agricultural activities (row crops, dairies, and poultry farms), along with atmospheric deposition have been implicated in the increase of nitrate concentrations in groundwater and surface water of the watershed (Ham and Hatzell 1996, Katz et al. 1999). The middle reach of the Suwannee River is characterized by porous karst topography (Katz et al. 1999), which allows for the relatively unrestricted movement of nitrate from the surface to the groundwater.

The permeable nature of karst topography has led to the contamination of groundwater sources in many parts of the world, including Australia (Harvey 1983), Denmark (Iverson et al. 1998), Israel (Burg and Heaton 1998), and southern France (Vaute et al. 1997). In the Suwannee River watershed, nitrate concentrations in the groundwater discharging from numerous springs along the Suwannee River have

increased substantially over the past 50 years, in some cases from less than 0.1 to more than 5 mg L^{-1} (Hornsby and Ceryak 1999). High concentrations of phosphorus are also found in the Suwannee River due to run-off from phosphate mining areas, discharge of sewage treatment plants, leaching and run-off of agricultural fertilizer, and weathering of natural phosphatic minerals (FDER 1985).

High nutrient concentrations, such as those observed in the Suwannee River, might be expected to support high phytoplankton standing crop. However, high flow rates, short residence times, and light limitation induced by colored dissolved organic matter (CDOM) restrict the development of phytoplankton biomass within the Suwannee River (Bledsoe and Phlips 2000). As a consequence, significant proportions of the bioavailable nutrients within the river are not assimilated by primary producers and are discharged into the Gulf of Mexico. It is logical to hypothesize that the greatest potential impact of nutrient loading on phytoplankton biomass should be expressed in the estuarine waters immediately adjacent to the Suwannee River (Bledsoe and Phlips 2000). To examine this hypothesis, the relationships among inter-annual variations in riverine discharge, changes in nutrient loading to the estuary, nutrient concentrations, nutrient-limiting status of phytoplankton production, and phytoplankton biomass in the estuary were investigated. These relationships were viewed within the context of changing water residence times within the estuary, which are a function of hydrological processes such as river discharge and wind-driven circulation.

Material and Methods

Study site description. The Suwannee River is a blackwater river that originates in the Okefenokee Swamp in southeastern Georgia and winds southward 394 km to the Gulf of Mexico. The Suwannee River, with its tributaries the Alapaha, Withlacoochee,

and Santa Fe rivers, drains approximately 28,500 km^2 of southern Georgia and north-central Florida. Freshwater flow is augmented by substantial groundwater contributions from numerous springs along the river. The Suwannee River has the second highest mean annual discharge of any river in Florida, i.e., 301 $m^3 s^{-1}$ (Palmer 1984).

Located at approximately latitude 29°17.50′ N and longitude 83°10.00′ W, the Suwannee River coastal area includes a delta region near the river mouth, which is characterized by a network of oyster reefs (Wolfe and Wolfe 1985). The region within the oyster reefs is approximately 70 km^2, with a volume of 88 million m^3 (mean high tide). This region is thought to be well-mixed due to its shallow nature (<1.5 m), although it can become vertically stratified depending on river discharge, tide stage, and wind velocity (Orlando et al. 1993).

Sampling regime. A network of 18 fixed sampling sites within the river and adjacent coastal waters was sampled monthly from April 1998 to April 2001 (Figure 2-1). The locations of the sampling sites were designed to incorporate ecologically distinct regions of the Suwannee River and adjacent estuary based on several key physical, chemical, and biological features (Bledsoe and Phlips 2000). Hierarchical cluster analysis was used to define these regions based on salinity, colored dissolved organic matter (CDOM), nutrient concentration, and chlorophyll a. Distance measurements were based on chi-squared coefficients. The site groups were evaluated with a nearest neighbor analysis. Any potential site groups, that were statistically similar, were grouped together.

Eight sites were collected from April 1998 to March 1999 on a monthly basis (Sites 1, 2, A1, A2, B1, B2, C2, and C3). An additional ten sites were added from April 1999 to April 2001 (3, 4, A3, A4, B3, C1, C4, D1, D2, and D3) (Figure 2-1). This basic sampling matrix provided an understanding of the overall seasonal patterns of variation in phytoplankton abundance and structure within different regions of the river and estuary.

Field measurements. Basic water-column characteristics were measured on site. Temperature, oxygen concentration, conductivity, and salinity were measured at 0.5-m depth intervals using either a Hydrolab Surveyor or YSI electronic meter. This information was used to describe the geographical, vertical extent, and variability of water masses, including locations of vertical discontinuity layers, presence of low oxygen zones, distribution of freshwater/saltwater mixing areas, and hydraulic flushing rates. Riverine stream flow data were obtained from the U.S. Geological Survey (2002) and meteorological data from the Cedar Key C-MAN station operated by NOAA (2002a).

The coastal waters adjacent to the Suwannee River receives freshwater from four outflows. Approximately 30% of the total discharge of the Suwannee River flows from East Pass. Of the remaining discharge, approximately 30% flows from Alligator Pass, 25% from Wadley Pass, and 15% from North Pass. The time taken for the total volume of freshwater inside the oyster reefs to be equaled by the freshwater input was estimated using the fraction of freshwater method (Dyer 1973) shown in Equation 2-1.

$$V_f = V_b * (1- S_b / S_o) \quad (2\text{-}1)$$

where V_f is the volume of freshwater, V_b is the volume of the reef region at mean high tide calculated from digitized NOAA navigational charts, S_b mean salinity of the reef

region, and S_o is the salinity of ocean water. The volume of the reef region was calculated at mean high tide because an effort was made to sample during high tide. The flushing time or the time taken for the total volume of freshwater in the reef region to be equaled by the freshwater input was calculated from Equation 2-2.

$$T = V_f / FW\ (86{,}400) \qquad (2\text{-}2)$$

where T is the flushing time in days (1 day = 86,400 seconds) and FW is the monthly mean riverine discharge (m^3s^{-1}) (USGS 2002). Flushing rate estimates were calculated for dates when the Suwannee Sound was vertically well-mixed as determined from physical measurements in the water column.

Water within the coastal area is moved also by the wind. Dominant wind characteristics, obtained from the Cedar Key C-MAN station operated by NOAA (2002a), were determined from averaging wind speed and direction for up to five days before the field-sampling event. I used five-day wind speed and direction means to determine if sustained winds could impact water movement and residence time in the Suwannee estuary thereby producing favorable conditions for phytoplankton biomass accumulation.

Water analyses. Water column samples (0.1 to 3-m depth) were collected at each site using an integrating sampling tube (7.3-cm i.d.). The tube was lowered to 0.1m from the bottom, a stopper was inserted into the top of the tube, the sampler raised, and the water collected into a bucket. One sample was used to rinse the bucket and tube, then disposed. At least 5 samples were collected into the bucket and mixed. Whole water samples were subdivided on site into aliquots for chlorophyll a (1 L), CDOM (125 ml), and water-chemistry analyses (125 ml). Chlorophyll a was used as the proxy for

phytoplankton abundance and estimated from samples (500 ml, 0.3-µm Gelman A/E glass-fiber filters) filtered on site, protected from sunlight and placed on ice. Samples were frozen immediately upon returning to the laboratory. Filters were placed in test tubes with 8.0 ml of 95% ethanol and heated in a water bath for 5 min at 78°C (Sartory and Grobbelaar 1984). After passive extraction (24 h), the filters were removed and samples were centrifuged to exclude particulate debris. Chlorophyll a concentrations were determined with a Hitachi U2000 dual beam spectrophotometer. The concentrations were corrected for phaeophytin using the acidification method (APHA 1989).

Inorganic nutrients ($NO_2^- + NO_3^-$, PO_4^+, and NH_4^+) from aliquots filtered on site (125 ml, 0.3-µm Gelman A/E glass fiber filters) were stored on ice, then refrigerated upon returning to the lab and analyzed within 24 hours of sampling. Concentrations were determined colorimetrically with a Technicon AutoAnalyzer (Armstrong et al. 1967, Strickland and Parsons 1972, APHA 1989). Silica (SiO_2) was determined from whole water samples using manual colorimetric methods (APHA 1989). Acidified sample blanks (APHA 1989) eliminated interference from colored dissolved organic matter (CDOM). Total nitrogen and total phosphorus were determined from whole water samples using the persulfate digestion method (APHA 1989) and analyzed with a Technicon AutoAnalyzer.

Colored dissolved organic matter (CDOM) was determined from filtered water (0.3-µm Gelman A/E glass fiber filters). Samples were analyzed using platinum cobalt standards from protocols described in Standard Methods (APHA 1989) on a Hitachi U2000 dual-beam spectrophotometer at 400 nm.

Light extinction coefficient - Light attenuation (K_t, m^{-1}) or Lambert-Beer's Law, (Equation 2-3), described the decrease in light penetration with depth.

$$K_t = \ln (I_o / I_z) / z \qquad (2\text{-}3)$$

where incident irradiance, I_o , and light intensity, I_z, at depth z (m), were determined with Li-cor Instruments Inc. (Lincoln, NE, USA) submersible quantum light probes (cosine corrected) which simultaneously recorded surface and downwelling light (μmole photons•$m^{-2}s^{-1}$ of photosynthetically active radiation, PAR) with a data logger. Surface reflection, based upon the angle and declination of the sun, was subtracted from the surface values prior to calculation.

Partial extinction coefficients caused by components of light attenuation (Kirk 1980, Verduin 1982) were estimated using conversion factors from the literature. Light attenuation by chlorophyll a containing phytoplankton, K_c, was estimated by multiplying chl-a concentrations by 0.030 m^2 mg^{-1} (Wolfsy 1983) and light attenuation attributed to seawater, K_w, was set at 0.038 m^{-1} (Lorenzen 1972). Light attenuation due to CDOM, K_{ac}, was estimated by multiplying platinum color units by 0.015 pt^{-1} m^{-1} (McPherson and Miller 1987). Light attenuation due to tripton, i.e., non-algal suspended solids, K_s, was calculated as $K_s = K_t - (Kc + K_{ac} + K_w)$ (Kirk 1983).

The mean light available in the mixed layer, I_m was estimated after Riley (1957) shown in Equation 2-4.

$$I_m = (I_0 / K_t Z_m) * (1\text{-}e^{-K_t Z_m}) \qquad (2\text{-}4)$$

where Z_m is the depth of the mixed layer (m), and I_o is the mean daily surface PAR irradiance as estimated from the literature (Oswald and Gotaas 1957). The depth of maximum change in salinity and/or temperature was used to estimate Z_m. The level of I_m,

at the onset of light limitation, has an estimated range of 0.9 to 4 mol•photons m^{-2} d^{-1} (Geddes 1984, Phlips et al. 1994).

Bioassay experiments. Nutrient limitation/growth bioassay experiments were performed on a monthly basis for eight sites within the study area (open circles identify the sites in Figure 2-1) during Year 1 (April 1998 to March 1999) and continued during Years 2 and 3 at sampling sites (Site 1, C2, and C3) (April 1999 to April 2001) (Figure 2-1). These sites were chosen based on location within the three regional designations. The experimental design was based on methods described by Aldridge et al. (1995). Assays were conducted under controlled laboratory conditions in 500-mL Erlenmeyer flasks with 400 mL of whole water. Treatments (in triplicate) included control (no additions), N addition (400 µg N L^{-1}), P addition (40 µg P L^{-1}), Si additions (400 µg Si L^{-1}), N + P addition (400 µg N L^{-1} + 40 µg P L^{-1}), and N+P+Si (400 µg N L^{-1} + 40 µg P L^{-1} + 400 µg Si L^{-1}).

Incubations were conducted in temperature-controlled water baths with bottom illumination. Incubation temperatures were maintained at ambient temperatures recorded on individual sampling dates. Light intensity was fixed at 120 µE m^{-2} s^{-1}. The photoperiod was 12/12 dark/light h, respectively, from October through March and 10/14 dark/light h from April through September. Algal biomass was estimated daily by net *in vivo* fluorescence (IVF) of chlorophyll a using a Turner Designs Model 10 fluorometer with a 1-cm path length. Ethanol extracted chlorophyll a concentrations were determined both at Time 0, and at the point when fluorescence values did not increase exponentially.

A nutrient was considered limiting when no growth occurred in the control and adding that nutrient, or combination of nutrients stimulated algal growth. When algal

growth in the control treatment, it was concluded that surplus bioavailable nutrients were present at the time of sampling. A growth response within the first 24 h established the primary limiting nutrient for all bioassay except the riverine bioassays, which tended to peak in growth after 96 h. Growth responses after 24 h were used to confirm the primary-limiting nutrient and establishing secondary-limiting conditions. The classification for the growth response was determined using Boolean logic (Aldridge et al. 1995). Significant differences among treatment groups were determined using ANOVA analysis. Post-hoc Tukey Tests were used to perform pair-wise comparisons of the means for specific treatments ($P < 0.05$) (SAS 2002).

Results

Cluster analysis based on physical, chemical, and biological parameters grouped the sites into three major regions (Figure 2-2):

- The freshwater reach of the river (Site 1).
- The reef region located just outside the river and characterized by well-defined oyster bars (Sites 2, A1, B1, C1, C2, and D1).
- The nearshore region seaward of the oyster bars, and extending 16 km offshore (Sites 3, 4, A2, A3, A4, B2, B3, C3, C4, D2, and D3).

Physical parameters. Long-term (1932-1999) mean-monthly flow rates in the Suwannee River indicate peak flow rates in spring (Figure 2-3A) (USGS 2002). Lowest flow commonly occurs during summer. Annual riverine flow rates for April 1998 to March 1999, April 1999 to March 2000, and April 2000 to April 2001 were 277, 102, and 108 $m^3 s^{-1}$, respectively (USGS 2002). A substantial decrease in riverine discharge occurred during the last 2 years of the study due to reduced rainfall within the watershed (NOAA 2002b) (Figure 2-3B).

Flushing rates in the reef region of the Suwannee River estuary decrease exponentially with increasing discharge from the Suwannee River (Figure 2-4A). During the highest flow rates reported during my study (811 $m^3 s^{-1}$, April 1998), flushing time was less than 1 day, while under the lowest flow conditions (i.e., less than 100 $m^3 s^{-1}$), flushing rates ranged from 4 to 6.5 days. The overall mean flushing time in the reef region for the 3-year study was 3.4 ± 1.4 days. Flow rates were higher in the first-year (April 1998 to March 1999) than in the second (April 1999 to March 2000) and third year (April 2000 to April 2001) (Figure 2-4B) and consequently the first year flushing times averaged 2.2 days, which was significantly lower than in the second (3.8 days) and third years (3.9 days) of the project ($P < 0.05$).

Salinities in the Suwannee River were less than 0.6 ‰ during all sampling periods. Mean salinities within the reef region were significantly lower than outside the reef region (Figure 2-5). During the 1998-1999 sampling period, mean salinity in the reef region was 19.4‰ (n = 48, SD = 1.1‰), while in the nearshore region, seaward of the oyster bars and extending to 16 km outward from the shore, it was 23.4‰ (n = 36, SD = 1.4‰). During the drought years of 1999 through 2001, the mean salinity in the reef region was 24.5 ‰ (n = 144, SD = 0.7 ‰) and 32.1‰ in the nearshore region (n = 264, SD = 0.2‰).

Nutrient concentrations and loading rates. Mean monthly nutrient loads to the reef region from the Suwannee River were estimated by multiplying the mean monthly river discharge by monthly river nutrient concentrations. Nitrate + nitrite contributed up to 80% of the total nitrogen in the Suwannee River (Figure 2-6A). Nitrogen loading increased with increasing riverine discharge, i.e., during the high/medium flow year

(April 1998 to March 1999) the mean monthly input of nitrate + nitrite was 219 ± 62 g N s^{-1} (n = 13) (Figure 2-7A). The mean annual total nitrogen rate for 1998-99 was 320 ± 150 g N s^{-1} (Table 2-1). The following 2 years (April 1999 to April 2001) saw a three-fold decrease in riverine flow and a corresponding decrease in both the nitrate + nitrite loading rate (87 ± 20 g N s^{-1}, n = 24) (Figure 2-7A). Mean annual total nitrogen loading rates for 1999-00 and 2000-01 were 110 ± 24 g N s^{-1} and 130 ± 58 g N s^{-1}, respectively (Table 2-1).

Suwannee River phosphorus loads followed a pattern similar to nitrogen loads. Soluble reactive phosphorus (SRP) comprised up to 90% of the total phosphorus in the river (Figure 2-6B), and loading rates were generally reflective of discharge rates. The annual loading rate of SRP into the coastal waters adjacent to Suwannee River from April 1998 to March 1999 was 21 ± 11 g P s^{-1} (n = 12) (Figure 2-7B). Mean total phosphorus load for April 1998 to March 1999 was 35 ± 28 g P s^{-1} (Table 2-2). Loading rates decreased substantially during the following years (April 1999 to April 2001) to an two-year mean of 9 ± 5 g P s^{-1} (n = 24) (Figure 2-7B). Mean annual total phosphorus loading rates for 1999-00 and 2000-01 were 11 ± 4 g P s^{-1} and 14 ± 10 g P s^{-1}, respectively (Table 2-1).

Nutrient limitation bioassays. Phytoplankton growth in the Suwannee River was not limited by nutrients (Figure 2-8, Table 2-2), as there was significant ($P < 0.01$) growth within the control treatment during each monthly experiment. Nitrogen was the first nutrient to become exhausted in 50% of the riverine bioassays after several days of unlimited growth (up to 8 days). In the remaining experiments, no nutrient limitation was observed during the course of the incubations.

Four bioassay stations were selected in the reef region during the first year of the study (Sites 2, A1, B1, and C2). There was significant ($P < 0.01$) growth within the first 24 h for all treatments (including controls) in 75% of the 48 experiments, indicating surplus nutrients. However, in each of these bioassays, nitrogen limitation was evident within 48 h. In the remaining 25% of the experiments, there was a significant ($P < 0.05$) growth response within the first 24 h with the addition of nitrogen, indicating nitrogen limitation. During the second and third year, a centrally located reef station (Site C2) contained surplus nutrients in 70% of the 23 months sampled, though nitrogen was the first nutrient to become exhausted during these periods. As in the first year, nitrogen was limiting in the remaining experiments within the first 24 h of incubation (Figure 2-8, Table 2-2).

Three stations were selected for the bioassays in the nearshore region seaward of the oyster reefs from April 1998 to March 1999. In 44% of the 36 nearshore bioassays, there were surplus nutrients available to sustain significant growth ($P < 0.05$). Nitrogen became limiting after 24 to 48 h in these experiments. The remaining bioassays were limited by nitrogen within the first 24 h. During the second and third years (April 1999 to April 2001) of my study, Site C3 was used to estimate nutrient limitation in the nearshore region. Within the first 24 h of the bioassays, nutrients were in surplus in only 25% of the experiments. Nitrogen was frequently the first nutrient to become limiting in these experiments. Nitrogen was also the primary-limiting nutrient in 38% of the bioassays. During December 2000 and April 2001, there was significant ($P < 0.05$) algal growth in the phosphorus addition treatments and no growth in the control and nitrogen

addition treatments. The nearshore station was co-limited by nitrogen and phosphorus in the remaining months (Figure 2-8, Table 2-2).

Phytoplankton standing crop. Chlorophyll a concentrations ranged from 0.49 to 12.6 μg L^{-1} in the Suwannee River. In the reef region, chlorophyll a concentrations ranged from 0.34 to 80.0 μg L^{-1}, while in the nearshore region, concentrations ranged from 0.34 to 31.3 μg L^{-1}. Mean chlorophyll a concentrations were significantly higher in the reef region than in either the river or nearshore regions of the estuary ($P < 0.05$) (Figure 2-9A).

There was a two-fold difference in mean annual chlorophyll a concentrations in the reef region of the estuary over the course of the study period. There was a significant difference in the mean annual chlorophyll a concentration in the nearshore region, which decreased by three-fold from the high/medium flow year (1998-1999) to the low flow years (1999-2000 and 2000-2001) ($P < 0.05$) (Figure 2-9B, Table 2-2).

Chlorophyll a concentrations greater than 30 μg L^{-1} in the reef region were observed after periods of southwest wind (averaged over 5 days) (Figure 2-10, Table 2-3). The exception to this pattern occurred in October 7,1999 following tropical storm Harvey in late September. The dissipation of elevated phytoplankton biomass was observed with a shift in wind direction from the southwest to an easterly direction (Figure 2-10).

Light availability. Vertical light extinction coefficients, K_t, and the relationships between light extinction and the three major components of light absorption; tripton (non-algal suspended solids), CDOM, and chlorophyll a (an estimate of living phytoplankton in the water column) showed regional differences (Figure 2-11). Mean annual K_t values

were generally highest in the river, followed by the reef and nearshore regions. In the river, K_t was correlated with tripton, and strongly correlated with CDOM. K_t was not correlated with chlorophyll a in the river during my study. In the reef region, K_t was correlated with tripton, CDOM, and chlorophyll a (Table 2-4). Similarly, in the nearshore region, K_t was most strongly correlated with tripton, followed by CDOM and chlorophyll a (Table 2-4).

Mean light availability in the mixed-layer (I_m) was lowest in the Suwannee River (Table 2-5). The threshold level of mean light availability in the mixed layer for the onset of light limitation has been estimated at between 2 and 4 mol photons m^{-2} d^{-1} (Geddes 1984, Phlips et al. 1995). Values for I_m in the Suwannee River fell within or below this threshold range in the fall and winter of 1998 (Table 2-5), when river flows were greatest (Figure 2-3). The reef and nearshore regions, within the estuary, remained above this threshold for all years sampled.

Discussion

The central goal of our study was to determine whether the abundance of phytoplankton in the estuary is linked to spatial and temporal patterns of nutrient loading and availability. The results support the hypothesis that algal abundance is sensitive to changes in nutrient concentration and loading rates (Table 2-1). However, the effects of nutrient loading should be viewed within the context of other factors that regulate losses and gains to the algal community. For example, river outflow levels directly affect nutrient loading but also impact water turnover rates, changing the residence time of water and associated phytoplankton biomass. Similarly, the principal causes of changes in flow, namely regional rainfall patterns, also result in changes in river CDOM which alters light availability for primary production. Therefore, light availability and water

turnover rates should be considered alongside nutrient concentrations in the broader discussion of nutrient affects on algal communities.

Effects of nutrient availability. Nutrient limitation bioassays demonstrated that nutrient concentrations within the river near the mouth are sufficient to sustain more than a week of phytoplankton growth in a controlled laboratory environment. These results indicate that the sources of nutrient input to the river, such as groundwater and surface run-off, exceed the demands of the primary producer community within the river. This phenomenon is based on two key factors:

- Low residence time of water in the river, which limits the time available for nutrient consumption by phytoplankton (Lewis 1988, Allen 1996).
- Limitation of light available for phytoplankton and benthic algal growth due to high CDOM levels in the river, as observed in other blackwater rivers in Florida (Phlips et al. 2000) and around the world (Lewis 1988, Meyer 1990).

Excess nutrients are discharged and become available for primary production in the estuary. The amount of nutrients transported to the coastal waters adjacent to the Suwannee River is substantial. Nitrogen loading from the Suwannee River is similar to discharges from the Apalachicola River in northwest Florida (Mortazavi et al. 2000), even though the Apalachicola River has a flow rate and drainage basin more than twice that of the Suwannee River. Nitrate + nitrite, ammonium, and soluble reactive phosphate loads are similar to those observed within the Neuse River estuary in North Carolina (Paerl et al. 1998) and within the ranges of bioavailable nutrients observed within Danish estuaries (Conley et al. 2000). Accelerated eutrophication of the Neuse River estuary and many Danish estuaries has been attributed to nutrient run-off and atmospheric deposition of agricultural, urban, and industrial origin. These systems have also experienced increased water quality degradation including the proliferation of nuisance

algal blooms (e.g., dinoflagellates and cyanobacteria) and increased hypoxia and anoxia associated fish kills (Iverson et al. 1998, Paerl et al. 1998, Conley et al. 2000). Although nutrient loading rates to the Suwannee River estuary are comparable to that of some of the aforementioned estuaries, the more dramatic negative impacts of eutrophication have not yet been widely observed, possibly due to some of the unique properties of the Suwannee region, including high water turn-over rates.

To explore the relationship between changes in nutrient loading and phytoplankton standing crop, an empirical approach similar to that used by Boynton et al. (1982) in the Chesapeake Bay was employed. The *El Niño* period during 1997-1998 increased rainfall in the watershed of the Suwannee River. During the spring of 1998, river water discharge into the estuary was 75% higher than the historical mean (USGS 2002). The low rainfall encountered during the post *El Niño* period subjected the Suwannee River watershed to prolonged drought conditions resulting in flow rates up to 80% less than historical averages (USGS 2002) (Figure 2-3B). The low level of surface water input from the watershed and the accompanying reduction in water discharge to the estuary resulted in reduced loading rates of nitrogen and phosphorus into the estuary. Phosphorus concentrations in the river and loading rates to the estuary decreased during the drought years. This was likely due to decreased surface run-off in the phosphatic areas of the watershed. The relatively high concentration of phosphorus in September 2000, followed heavy rains and consequential surface run-off of Hurricane Gordan, which tracked up the Suwannee River watershed the week prior to sampling.

By comparison, changes in riverine nitrogen concentrations were not as large during drought years. The primary source of nitrogen in the Suwannee River during base

riverine flow is groundwater spring inflows (Pittman et al. 1997). Although the groundwater levels were also reduced during the drought, the overall nitrate concentration in the river was not significantly lower than the medium/high flow year 1998-99 (Figure 2-6). However, the total nitrogen loading rate into the estuary was significantly lowered by the reduction of river flow. Thus groundwater inputs of nitrogen to the river are an issue of particular concern for two primary reasons:

- The porous karst topography of the region has facilitated in increased nitrate concentrations in the groundwater inflow to the river stemming from increases in anthropogenic activities (Katz et al. 1999).
- The Suwannee River estuary appears to be sensitive to increased nitrogen loading due to the predominance of nitrogen-limiting conditions in the estuary.

Nitrogen and phosphorus loading estimates had significant positive relationships on chlorophyll a concentrations (Table 2-1). Flow-adjusted estimates for nitrogen and phosphorus loading from the Suwannee River showed a three-fold decrease in loading of both nitrogen and phosphorus from the first year (April 1998 to March 1999) to the second and third years (April 1999 to April 2001) of the study. The decrease in mean annual nutrient loading rates was matched by a three-fold decrease in mean annual chlorophyll a values in the nearshore region seaward of the oyster reefs (Table 2-1). Correlation analyses of specific loading rates and chlorophyll concentrations for the study period (April 1998 to March 2001) showed a strong positive relationship between loading and phytoplankton biomass for the nearshore region of the estuary (Pearson $r = 0.58$ for nitrogen loading, $P < 0.001$ and Pearson $r = 0.47$ for phosphorus loading, $P < 0.001$) (Figure 2-12A). The nutrient-biomass correlation was concordant with bioassay experiments, which showed that nutrients (both in many cases) were limiting in 75% of the experimental bioassays during the drought period (1999-2001). During periods of

increased rainfall (1998-1999), the nutrient-rich riverine plume extended beyond the oyster reefs of the estuary, and nutrient limitation decreased to only 56% of the bioassays. Similar results have been observed in other rivers after high flow events enrich the coastal waters with nutrients, e.g., the Neuse River in North Carolina, USA (Rudek et al. 1991) and the River Po in Italy (Revelante and Gilmartin 1976).

The correlations were not as significant in the reef region, where only a two-fold change occurred between drought and wet years and correlations of nitrogen with biomass (Pearson $r = 0.12$, $P < 0.001$) and phosphorus (Pearson $r = 0.11$, $p = 0.06$) were weak (Figure 2-12B), reflecting the fact that this region seems to be less frequently nutrient-limited and more subject to light limitation (Bledsoe and Phlips 2000). The results of the experimental bioassays support this conclusion, with only 25% of the reef bioassays showing nutrient limitation during 1998-1999 and 30% of the reef bioassays during the following two years.

These observations suggest that changes in flow rates and nutrient concentrations in the Suwannee River can impact phytoplankton standing crop in the coastal region. These results are analogous to those observed for the coastal waters around Funen, Denmark (Rask 1999). Low phytoplankton biomass in Funen was observed in years with low precipitation and consequently low nutrient run-off to the coastal areas.

Effects of water turnover rates. The time frame of our study provided an opportunity to consider the role of changes in meteorological conditions, such as *El Niño,* on features of the river other than nutrient loads, including CDOM, flow rate, depth, and water residence times. In the Suwannee River estuary, hydrodynamic properties such as tidal amplitude, riverine outflow, and current patterns can influence phytoplankton

abundance as in many estuaries (Monbet 1992, Lucas et al. 1999, Cloern 2001, Phlips et al. 2002). The region of the Suwannee River estuary most directly impacted by freshwater flow from the Suwannee River is semi-enclosed by oyster reefs. These oyster reefs restrict movement and exchange of water, as suggested by low salinities measured during this study, and can provide the time needed for accumulation of phytoplankton biomass. Even outside the reef region, the broad extent of the continental shelf within the Big Bend of Florida (i.e., often extending >100 km offshore), as well as local coastal topography, may contribute to a longer residence time. Therefore, regional variations in water residence periods are a major consideration in defining potential phytoplankton standing crop.

During the course of the study, chlorophyll a concentrations within and outside of the Suwannee reef region exceeded 20 $\mu g\ L^{-1}$ on numerous occasions and reached as high as 80 $\mu g\ L^{-1}$. In contrast, annual mean estimates of phytoplankton biomass entering the estuary from the river and offshore were low during the high flow year (1998-1999), (1.1 $\pm$ 1.4 and 7.5 $\pm$ 4.5 $\mu g\ L^{-1}$, respectively). Therefore, it could be hypothesized that high phytoplankton standing crops in the reef region are a product of nutrient availability and favorable hydrodynamic conditions. Based on a simple freshwater fraction model, the flushing rates in the Suwannee estuary have been estimated to range from 2 to 3 days (Solis and Powell 1999), similar to the overall results of the initial estimates in my study (Figure 2-4A and 2-4B). Short flushing times would seem to preclude the buildup of the high phytoplankton standing crops observed in my study. However, a possible solution to this apparent dilemma could be inclusion of wind and wave elements to the modeling of residence time.

Ward (1980) described wind events as a dominant force in water circulation in many estuaries. Wind-generated estuarine circulation is a driving factor in phytoplankton distribution in estuaries such as the Laguna Madre, Texas (Smith 1988) and Narragansett Bay, Rhode Island (Pilson 1985). Based on shallow depth (<2m), sustained winds could have a considerable impact on water movement and residence time in the Suwannee estuary. I hypothesized that prolonged wind events blowing onshore could increase retention times within the reef and nearshore regions, enhancing the potential for high phytoplankton standing crop. High phytoplankton biomass (>40 μg chl a L^{-1}) in the Suwannee River estuary was observed after periods of sustained (5-8 days) southwest winds (Table 2-3, Figure 2-10). During onshore wind conditions, water within the reef can be forced or entrained within the oyster reefs, extending the residence time to 6 to 8 days (Figure 2-4). Hypothetically, a phytoplankton population with doubling times of 2 days could increase in biomass from 2 μg chl a L^{-1} to over 30 μg chl a L^{-1} within 8 days. Therefore, relatively short-lived wind events, which increase the retention time by several days, could also have significant impacts on phytoplankton abundance in the Suwannee River estuary.

Effects of light availability. Light availability is important in controlling algal biomass in rivers and estuaries (Hitchcock and Smayda 1977, Cole and Cloern 1984, Thom and Albright 1990, Phlips et al. 2000). Prior studies on the Suwannee River and its estuary suggest that light limitation contributes to low phytoplankton standing crops in both the river and certain areas within the river plume (Bledsoe and Phlips 2000).

A large component of light attenuation in the river and parts of the estuary is colored dissolved organic matter (CDOM). Color increases during medium to high flow

events in the Suwannee River due to loading of tannins to the river, particularly in the upper reaches of the watershed and reduces light availability in both the river and parts of the estuary. The drought conditions of 1999-2001 diminished surface runoff and flow from tannin-rich source waters such as the Okefenokee Swamp. The reduction of these inputs decreased the CDOM in the river from its characteristic 150+ Pt-Co Units to less than 10 Pt-Co Units (Hornsby and Raulson 2000). The substantially lower CDOM levels resulted in a general increase in mean light availability in the mixed layer relative to previous years. With the lowered potential for light limitation, phytoplankton standing crops increased in the river from an annual mean of 1.1 ± 1.4 μg chl a L^{-1} in 1998-1999 to 2.78 ±2.53 μg chl a L^{-1} in 1999-2001.

Reduced riverine CDOM also caused changes in the major light-absorbing components within the plume region. I predicted an overall increase in light availability with the corresponding decrease in CDOM, yet light attenuation (K_t) did not change significantly within the reef region between high and low flow years. One explanation for this observation is related to an increase in the total particulate fraction in the water column during the low flow years of the study. It could be suggested that medium to high discharge events produce freshwater induced stratification, which may reduce the role of wind resuspension and dampen the suspended particulate component of light attenuation in the upper water column. When discharge is low, the water column is less stratified and more polymictic, enhancing sediment suspension. This phenomenon has been observed in other riverine plumes such as the Frazer River plume in the Georgia Straits, British Columbia (Beamish et al. 1994), the Connecticut River plume in Long Island Sound, Conneticut (Bowman and Iverson 1978), and in the Southern Bight of the

Northern Sea (Wild-Allen et al. 2002). Additionally, short-term stratification may increase phytoplankton biomass development similar to that observed within the Roskilde Fjord, Denmark (Møhlenberg 1995). In the Roskilde Fjord, stratification allows phytoplankton biomass to increase in the upper water column by excluding benthic suspension feeders. Phytoplankton abundance in the Suwannee River estuary may increase in the upper water column during periods of stratification, but decline during mixing due to mixing of low phytoplankton biomass water from the lower water column and exposure of the entire water column to suspension-feeding bivalves (e.g., American oysters, Crassostrea virginica and hard clams, Mercenaria mercenaria).

Table 2-1. Mean annual values for phytoplankton abundance (chlorophyll a) in the 'reef' and 'nearshore' regions of the Suwannee River estuary, Florida, USA and discharge-weighted nutrient loading rates (g s^{-1} of TN and TP) from the Suwannee River. Standard errors are in parentheses.

	Reef chl a µg L^{-1}	Nearshore chl a µg L^{-1}	TN Load g N s^{-1}	TP Load g P s^{-1}	Discharge
4/98-3/99	18 (2.1)	9.8 (0.84)	320 (150)	35 (28)	Medium-high
4/99-3/00	13 (1.5)	3.3 (0.27)	110 (24)	11 (4.0)	Low
4/00-4/01	11 (1.1)	3.1 (0.27)	130 (58)	14 (10)	Low

Table 2-2. Percent of bioassays with nutrient limitation observed within the first 24 h from April 1998 to April 2001 in the Suwannee River and its estuary, Florida, USA.

Limiting Nutrient	Suwannee River	Reef Region	Nearshore Region
Unlimited	100	70	25
Nitrogen	0	30	38
Phosphorus	0	0	8
Silica	0	0	0
Co-limitation N and P	0	0	30

Table 2-3. Wind dynamics and phytoplankton abundance in the Suwannee River estuary, Florida, USA.

Date	Site	Chlorophyll μg chl a L^{-1}	Heading (degrees)	Wind velocity ($m\ s^{-1}$)	Prevailing Wind Direction
5/18/98	2	36	211	2.4	SSW
7/20/98	C2	46	210	3.3	SSW
9/23/98	A1	57	174	2.4	S
5/26/99	C1	80	221	2.1	SW
6/16/99	C1	31	213	0.4	SW
7/29/99	A1	36	260	3.2	W
10/7/99	C1	49	72	4.7	NE
8/16/99	A1	33	249	1.6	SW
8/19/99	A1	24	171	1.5	S
8/24/99	A1	16	175	2.6	S
8/26/99	A1	14	172	1.8	S
4/15/00	C1	5	50	1.5	NE
4/16/00	C1	6	51	1.2	NE
4/19/00	C1	7	314	1.6	NW
4/20/00	C1	15	283	2.2	NW
4/26/00	C1	29	261	3.3	SW
4/28/00	C1	30	251	3.4	SW
7/19/00	A1	22	249	4.6	SW
7/20/00	A1	56	246	4.4	SW
7/26/00	A1	16	197	2.2	SW
7/29/00	A1	10	154	1.5	SE

Table 2-4. Light extinction coefficient (K_t), and Pearson correlation coefficients for three major components of light absorption: tripton, CDOM, and phytoplankton abundance (as chlorophyll a) in the Suwannee River and its estuary, Florida, USA.

Year	Region	K_t	Tripton	CDOM	Chl a
1998-99	Suwannee River	1.8	0.72 **	0.92 ***	-0.51
	Reef	1.5	0.66 ***	0.58 ***	0.52 ***
	Nearshore	1.4	0.72 ***	0.71 ***	0.39 *
1999-00	Suwannee River	1.3	0.29	0.66 *	0.11
	Reef	1.5	0.78 ***	0.25 *	0.70 ***
	Nearshore	0.5	0.92 ***	0.29 **	0.49 ***
2000-01	Suwannee River	2.0	-0.29	0.90 ***	-0.41
	Reef	1.7	0.68 ***	0.28 *	0.54 ***
	Nearshore	0.9	0.88 ***	0.23 *	0.11
Overall	Suwannee River	1.8	0.34	0.87 ***	-0.30
	Reef	1.5	0.69 ***	0.57 ***	0.40 ***
	Nearshore	0.9	0.81 ***	0.52 ***	0.45 ***

Note: Asterisks denote the level of significance; those marked with a single asterisk indicate significance at the 5% level, a double asterisk denotes significance at the 1% level, and a triple asterisk denotes significance at the 0.1% level. Empty cells indicate that the significance of the difference was above the 5% level.

Table 2-5. Seasonal mean light availability in the mixed-layer, I_m, (mol photons m^{-2} d^{-1}) in the three regions of the Suwannee River and its estuary, Florida, USA. Standard deviations are shown in parentheses. An asterisk (*) indicates values within the range of potential light limitation (0.9 to 4 mol photons m^{-2} d^{-1}).

	Suwannee River	Reef Region	Nearshore Region
Spring 98	5.3	15.0	12.0
	(3.4)	(9.7)	(14.0)
Summer 98	15.0	25.0	21.0
	(3.2)	(9.9)	(15.7)
Fall 98	*2.6	19.0	12.0
	(1.2)	(13.3)	(4.1)
Winter 98	*3.5	24.0	31.0
	(0.60)	(20.9)	(26.0)
Spring 99	12.0	30.0	27.0
	(8.03)	(10.6)	(14.0)
Summer 99	14.0	23.0	23.0
	(3.2)	(3.5)	(0.57)
Fall 99	7.4	19.0	14.0
	(1.3)	(5.09)	(0.42)
Winter 99	5.6	14.0	11.0
	(1.1)	(4.03)	(3.5)
Spring 00	9.6	16.0	17.0
	(2.1)	(0.35)	(3.1)
Summer 00	14.0	24.0	18.3
	(3.01)	(9.8)	(0.99)
Fall 00	6.1	21.0	21.0
	(1.3)	(0.57)	(11.0)
Winter 00	8.3	30.0	21.0
	(1.7)	(2.5)	12.0)
Spring 01	7.0	23.0	17.0
	(2.7)	(7.6)	(3.4)

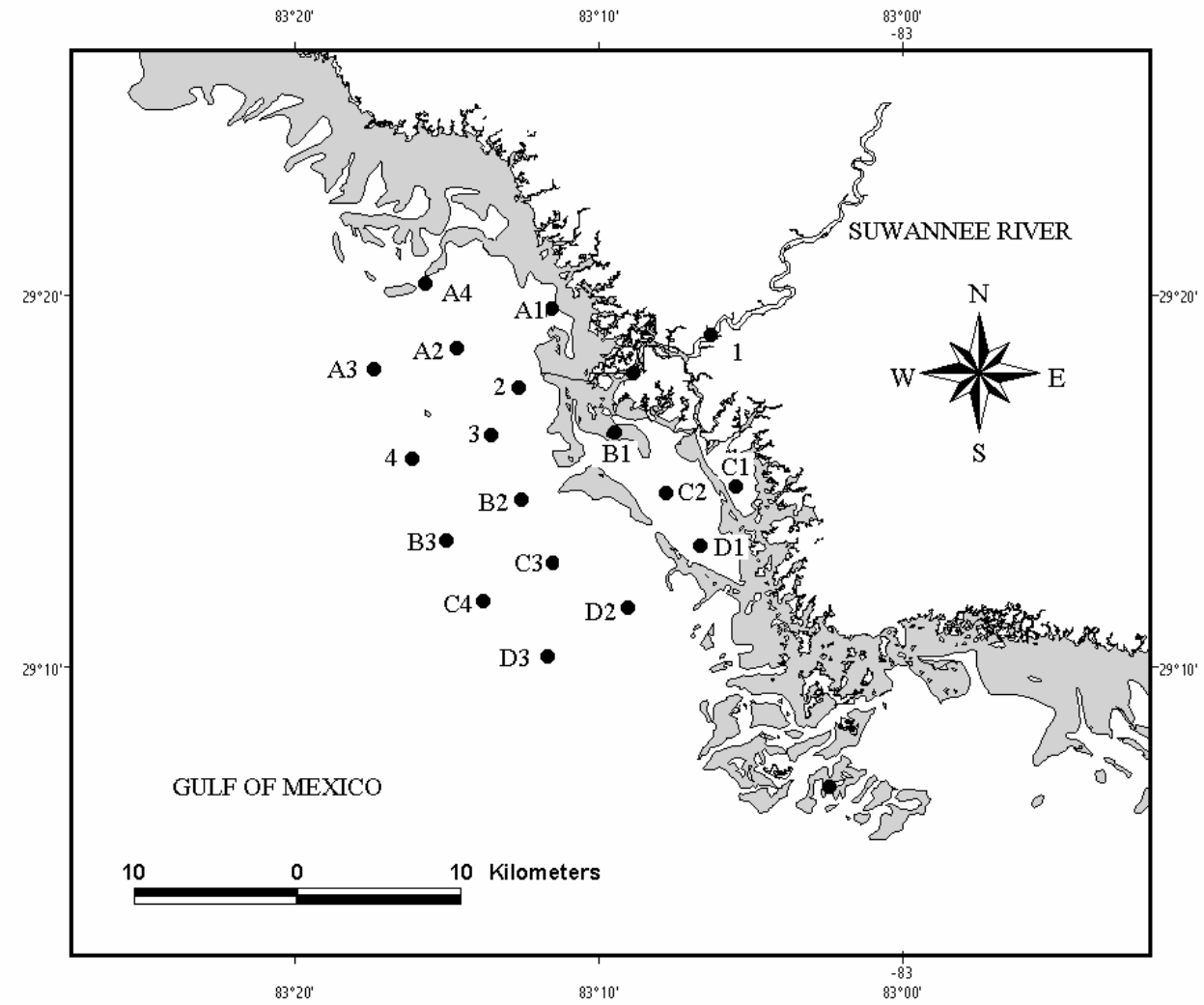

Figure 2-1. Sites sampled monthly from April 1998 to April 2001 in the Suwannee River and its estuary, Florida, USA. Oyster reefs and mud flats (<1 meter MLW) are indicated in light gray.

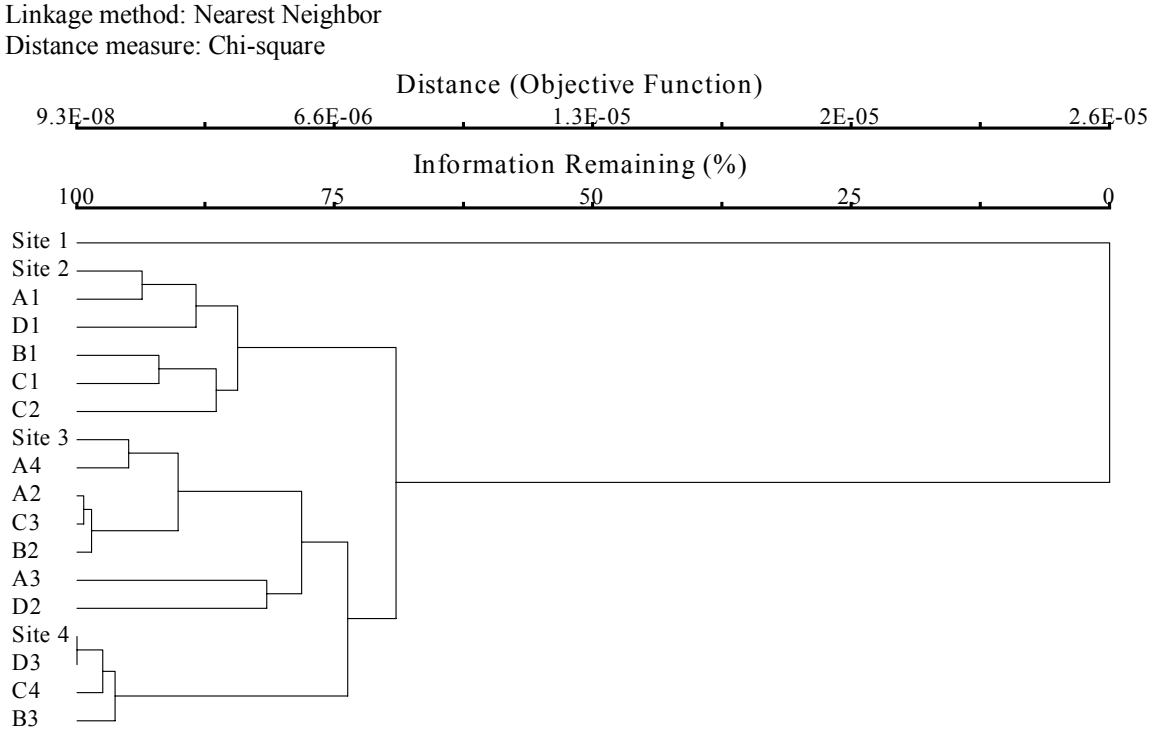

Figure 2-2. Distance dendrogram for classification of the major geographic regions within the Suwannee River and its estuary, Florida, USA based on physical, chemical, and biological characteristics (i.e., salinity, CDOM, nutrients, and chlorophyll a) from April 1998 to April 2001. Site 1 represents the 'river' region; Sites 2, A1, D1, B1, C1, and C2 the 'reef' region; and Sites 3, A4, A2, C3, B2, A3, D2, 4, D3, C4, and B3 the 'nearshore' region.

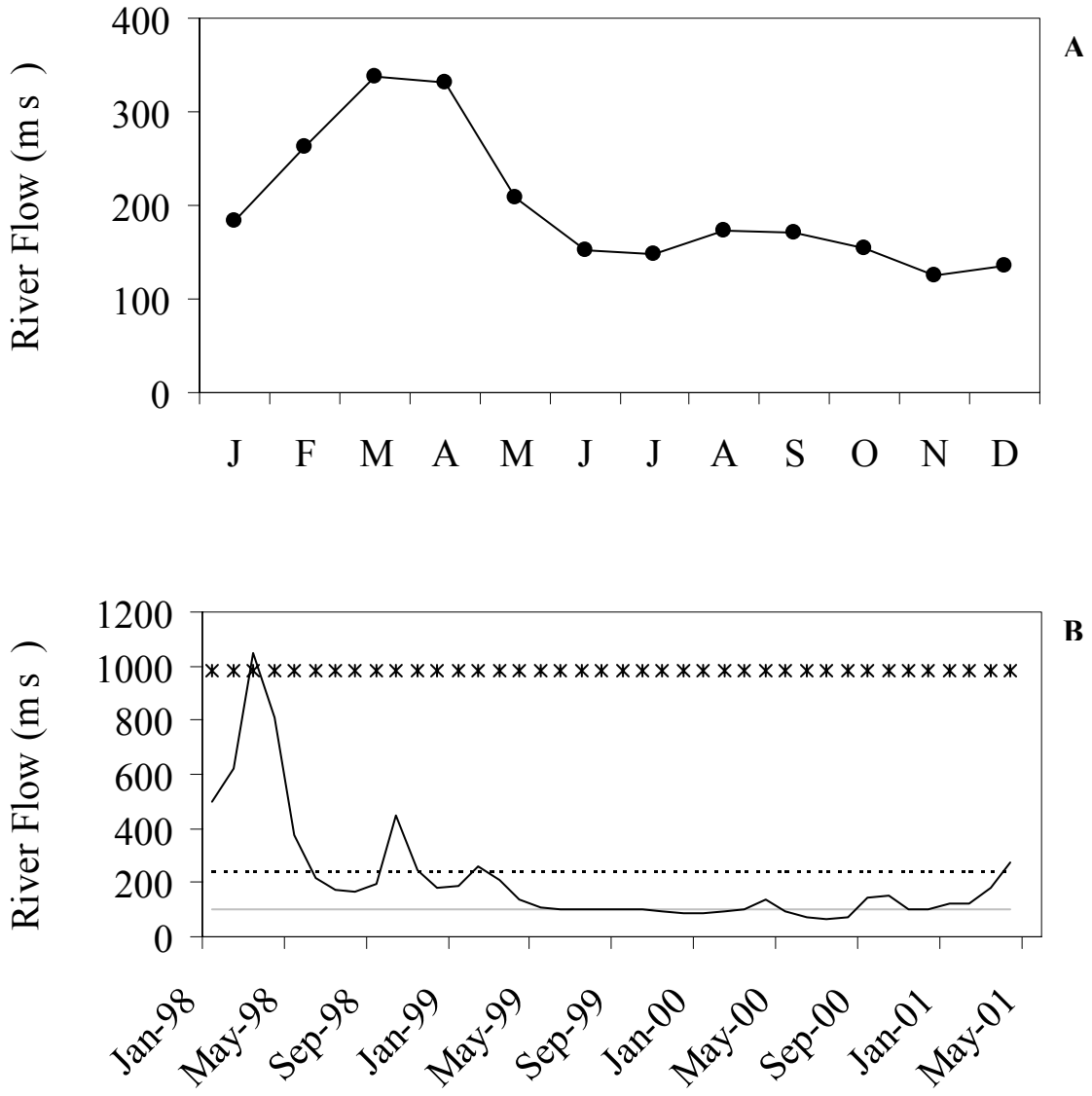

Figure 2-3. Flow-rates in the Suwannee River, Florida, USA at the Wilcox gauging station. A) Long-term mean monthly flow-rates from 1932 to 1999. Drawn from data reported in USGS (2002). B) Mean monthly flow-rates for the Suwannee River from 1998 to 2001. The dash line (---) indicates median flow rate for the Suwannee River. The gray line represents the flow-rate exceeded 99% of all dates from 1960 to 2001 (minimum flow) and the asterisk (***) line represents the flow-rate exceeded only 1% of all dates from 1960 to 2001 (maximum flow). Drawn from data reported (USGS 2002).

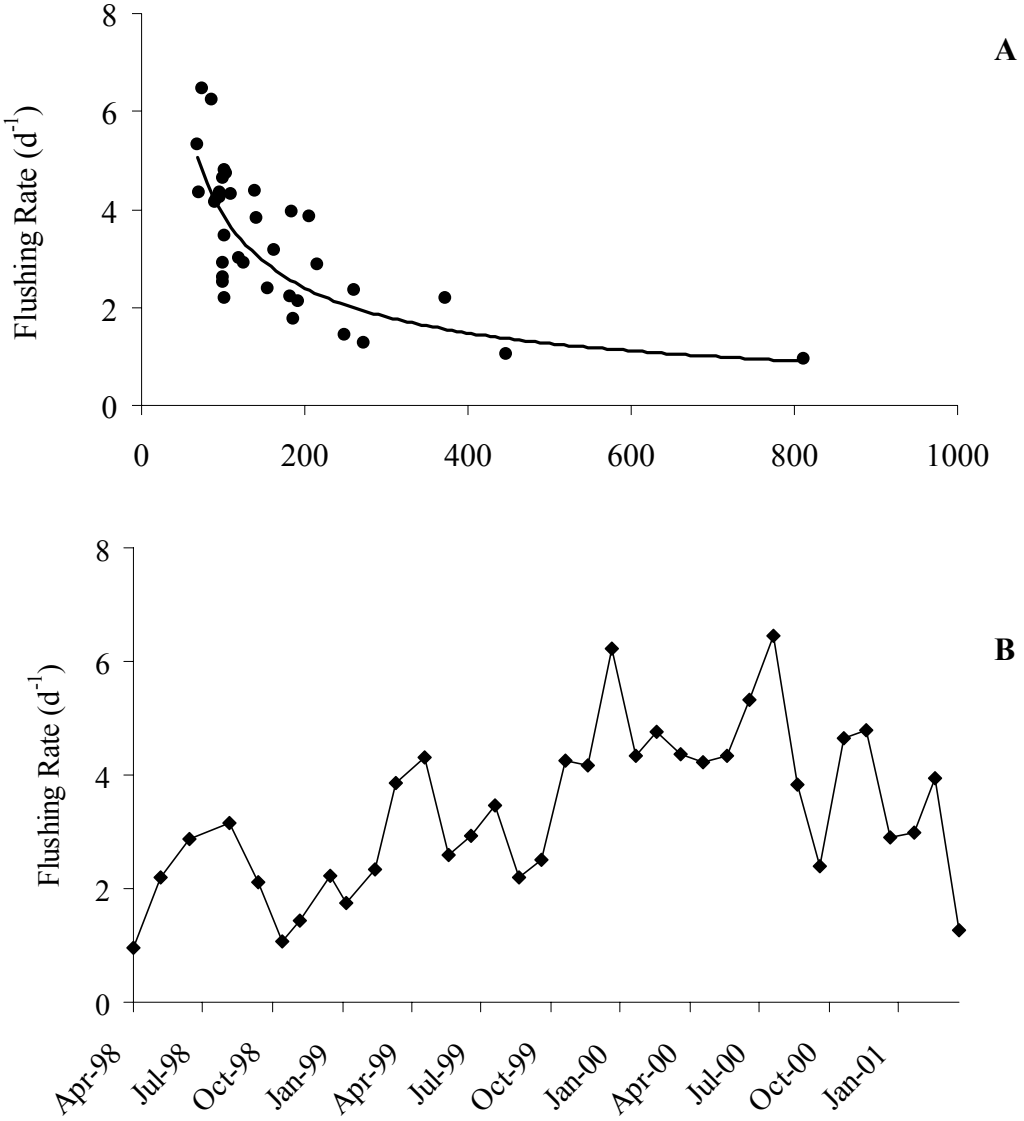

Figure 2-4. Flushing rates in the reef region of the Suwannee River estuary, Florida, USA. A) Calculated flushing rate (d^{-1}) of the reef region of the Suwannee River estuary, Florida, USA based on riverine flow from April 1998 to April 2001. The points are the flushing rates derived by the fraction of freshwater method (Dyer 1973). The line is the least squares fit of the estimated flushing rates based on riverine flow ($y = 97.496x^{-0.6992}$, $R^2 = 0.66$). B) Flushing rate (d^{-1}) in the reef region from 1998-2001 (mean 3.4 ± 1.4 days, n = 35).

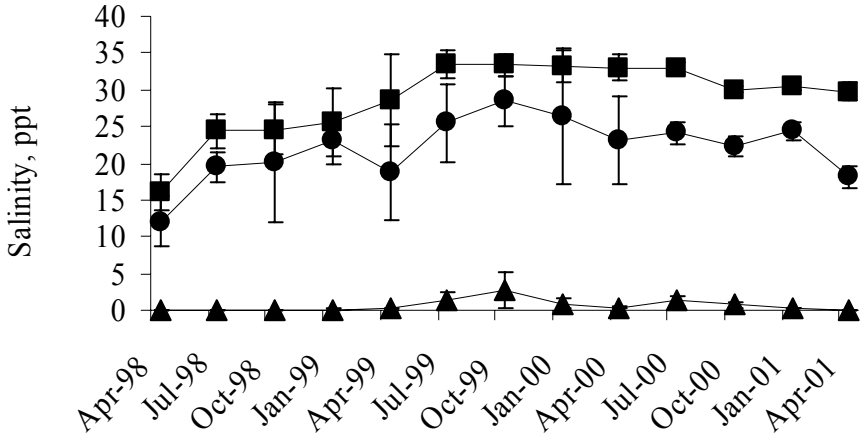

Figure 2-5. Seasonal salinities (ppt) for the three regions within the Suwannee River and its estuary, Florida, USA: Suwannee River (▲), Reef Region (●) and Nearshore Region (■). Error bars represent one standard deviation.

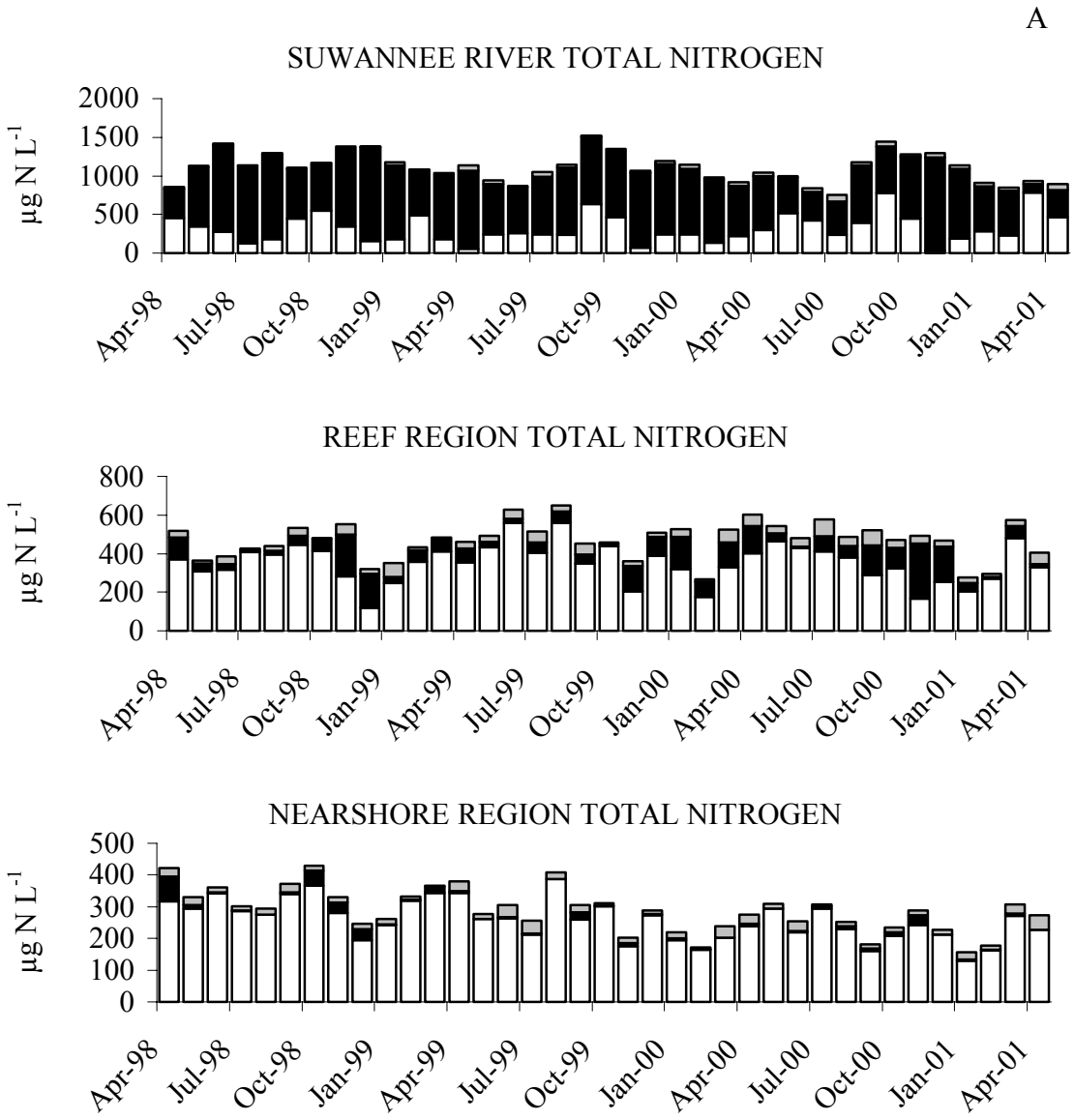

Figure 2-6. Nutrient concentrations in the three regions of the Suwannee River and its estuary, Florida, USA April 1998 to April 2001. A) Contribution of nitrate + nitrite-N (black) and ammonium-N (gray) to total nitrogen concentrations (µg N L^{-1}). B) Contribution of soluble reactive phosphorus (black) to total phosphorus concentrations (µg P L^{-1}).

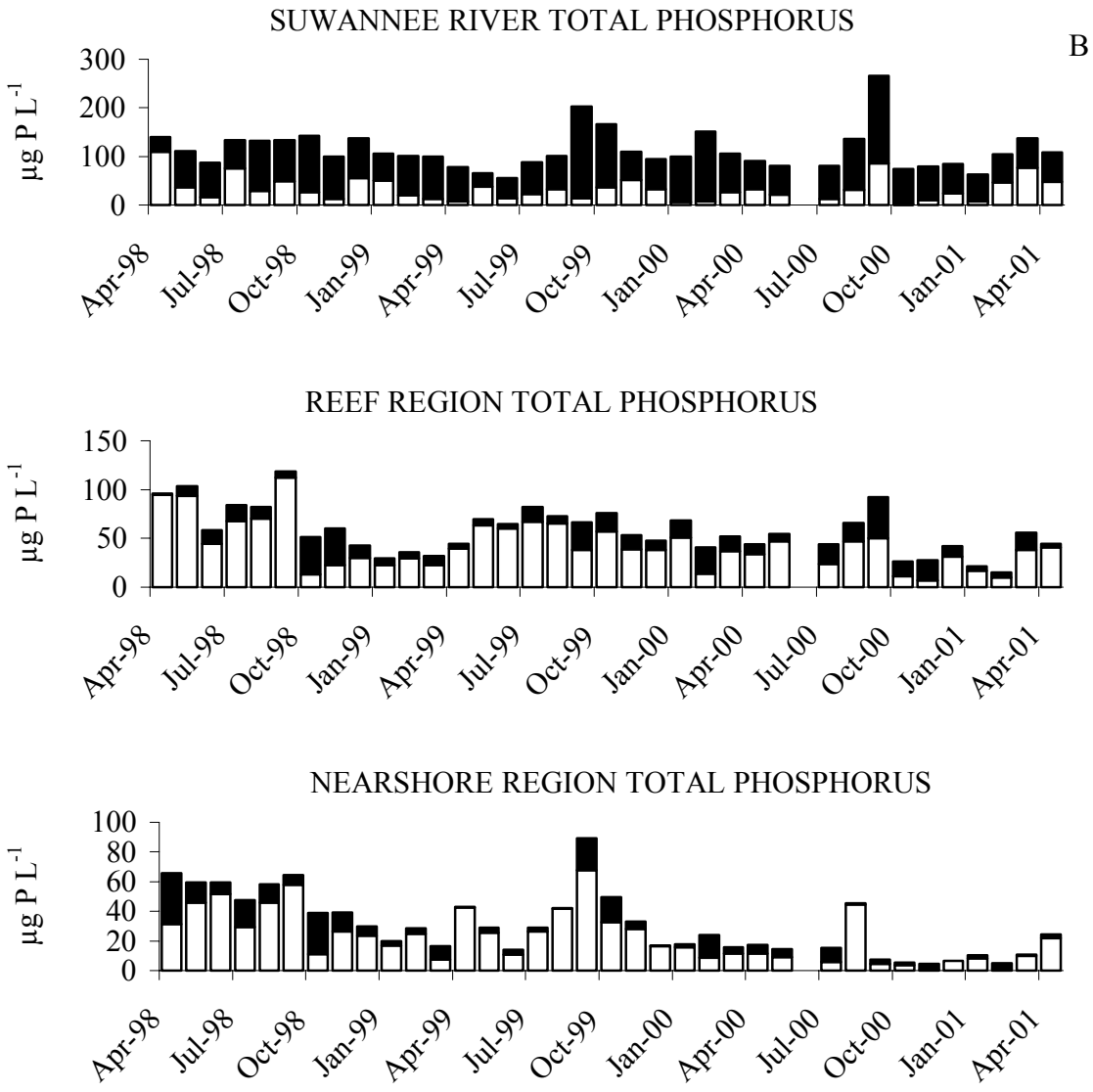

Figure 2-6. Continued

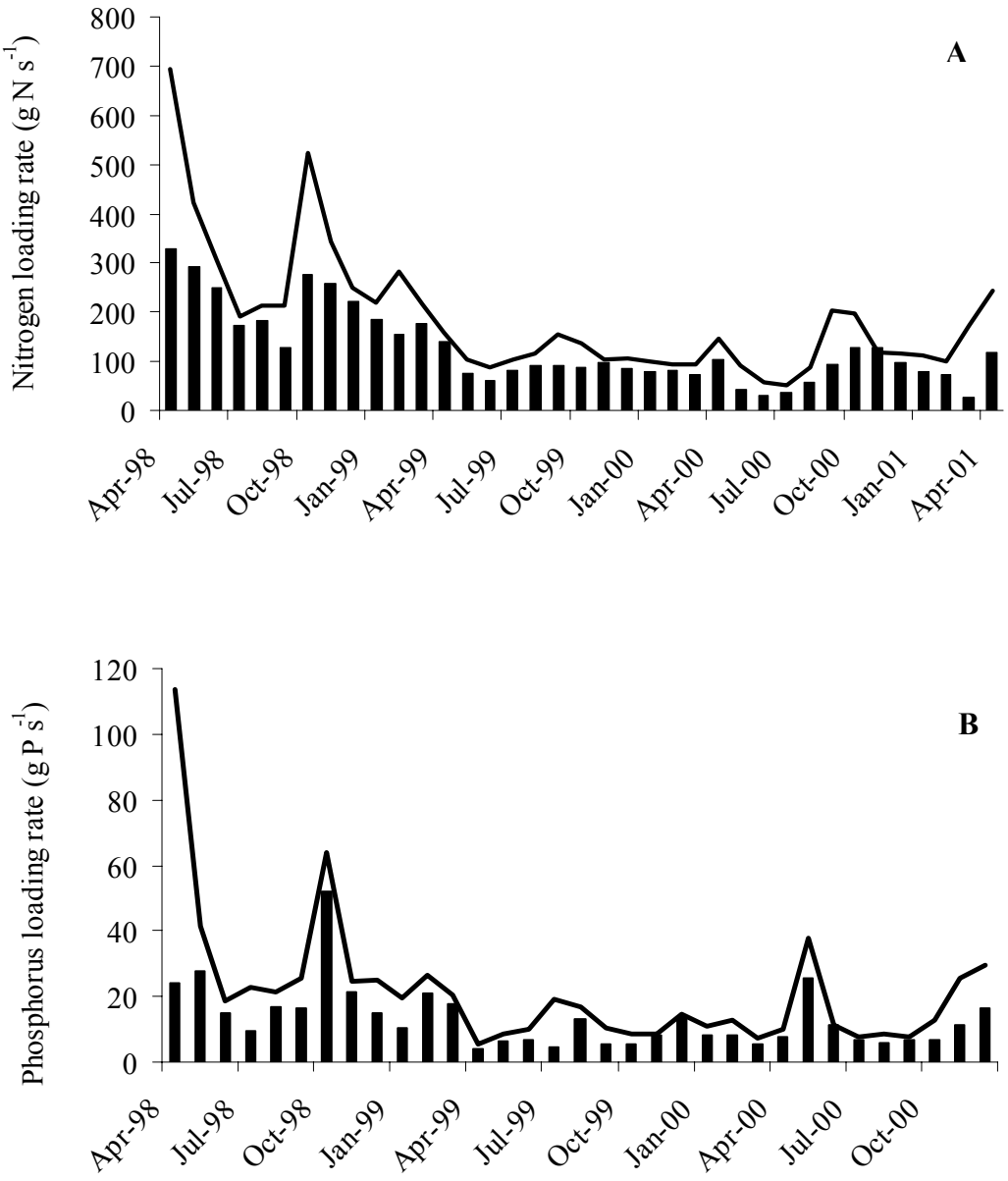

Figure 2-7. Nutrient loading rates of the Suwannee River, Florida, USA. A) Loading rates of total nitrogen (line) and nitrate + nitrite (bars) from the Suwannee River, Florida (g N s^{-1}). B) Loading rates of total phosphorus (line) and soluble reactive phosphorus (bars) from the Suwannee River, Florida (g P s^{-1}).

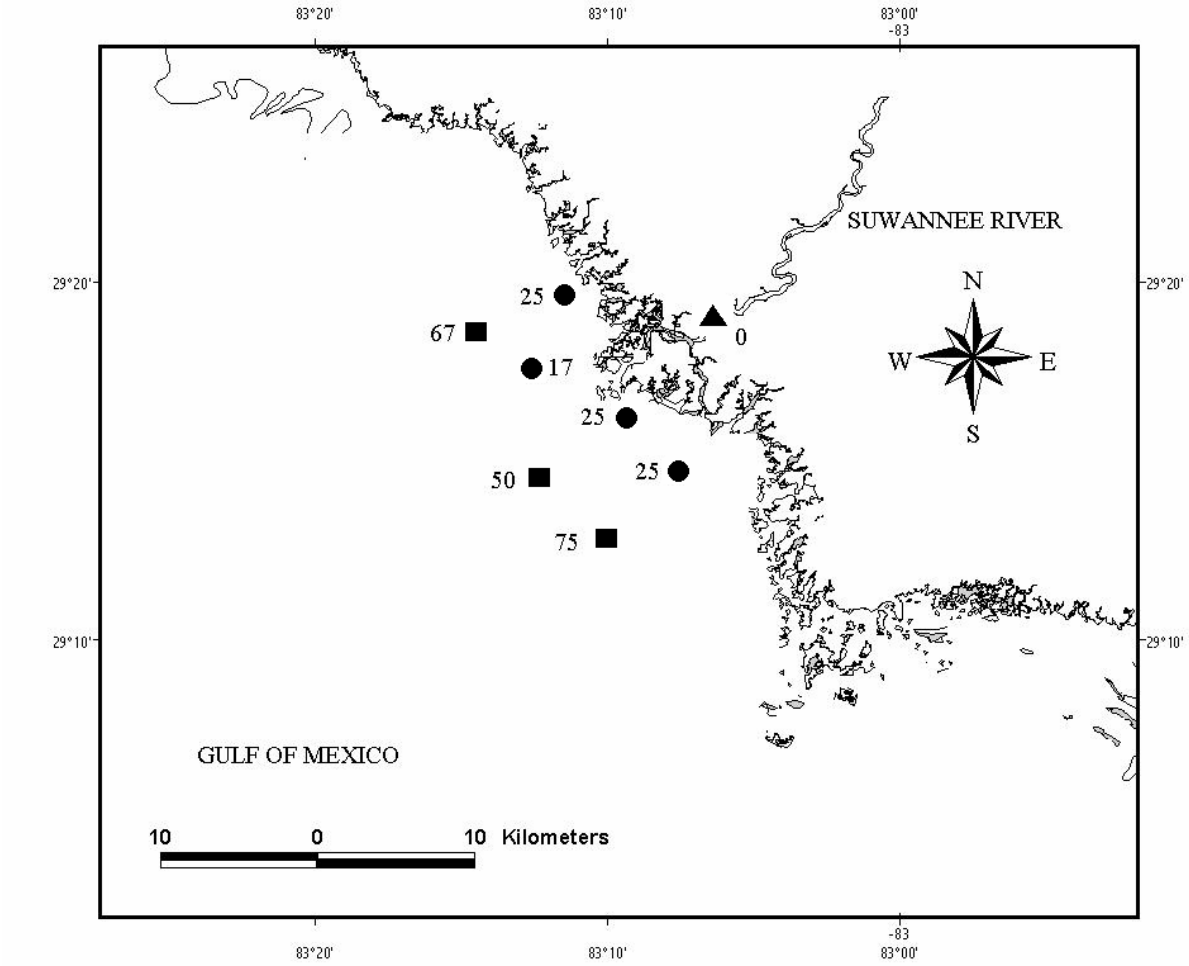

Figure 2-8. Percentage (%) of samples exhibiting nutrient limitation within the three main regions of the Suwannee River and its estuary, Florida, USA from April 1998 to April 2001: Suwannee River (▲) (n=36), Reef Region (●) (n=71), and Nearshore Region (■) (n=59).

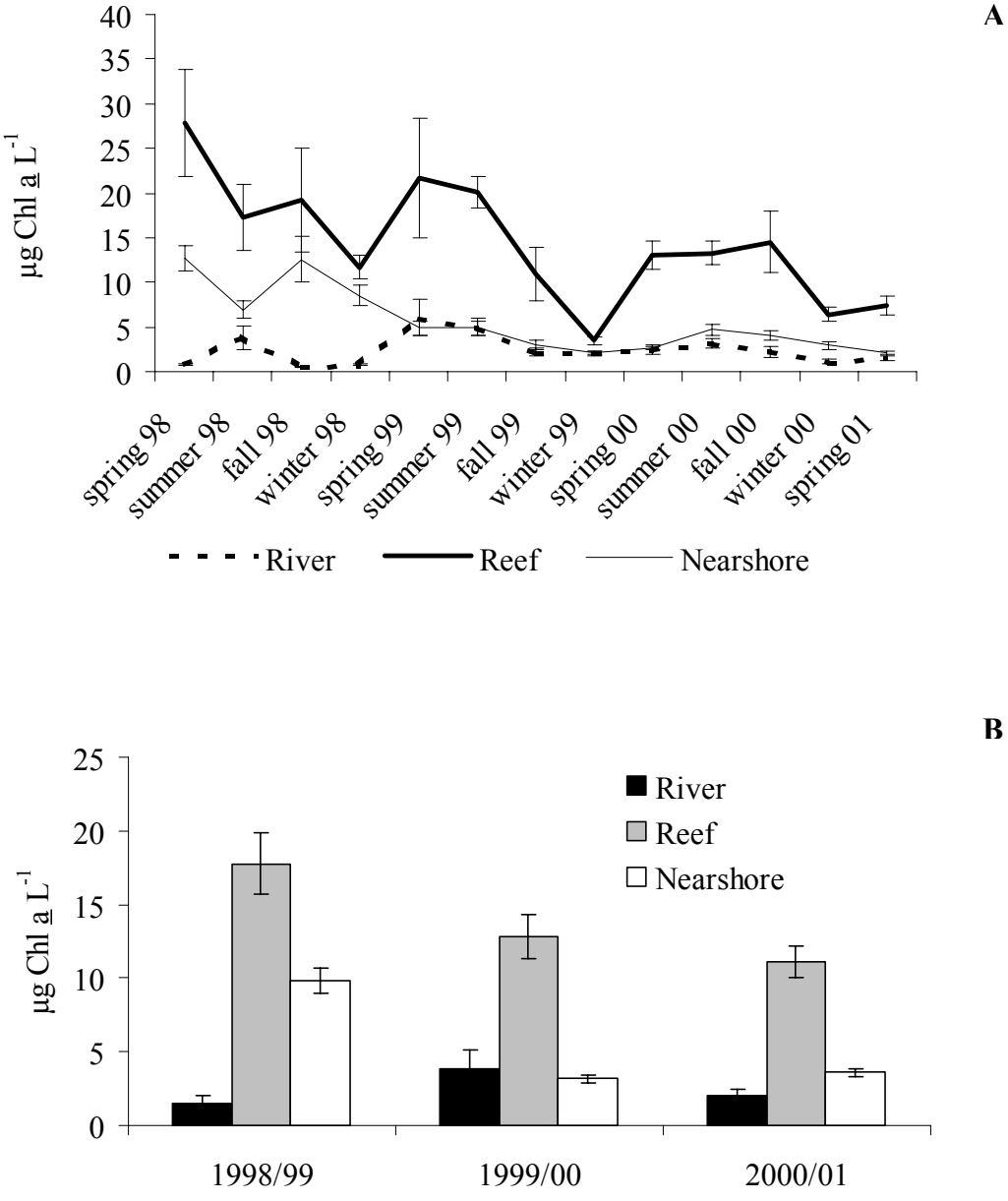

Figure 2-9. Chlorophyll a concentrations in the three regions of the Suwannee River and its estuary, Florida, USA. A) Seasonal chlorophyll a concentrations (µg L^{-1}). B) Mean annual chlorophyll a concentrations (µg L^{-1}). Errors bar equal one standard deviation.

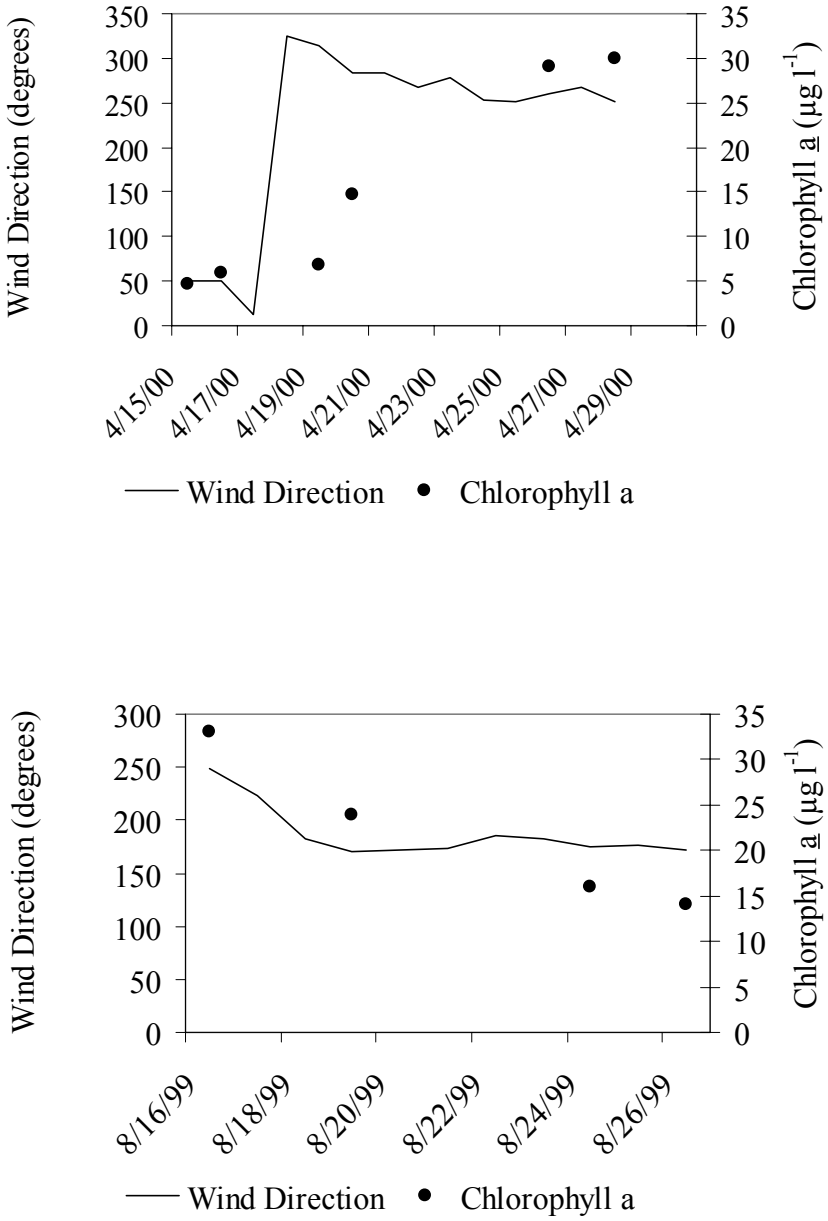

Figure 2-10. Selected sampling dates illustrating changes in chlorophyll a concentration ($\mu g\ L^{-1}$) with wind direction in the Suwannee River estuary, Florida, USA. Five-day mean wind direction was calculated from NOAA (2002) observations from Cedar Key, Florida.

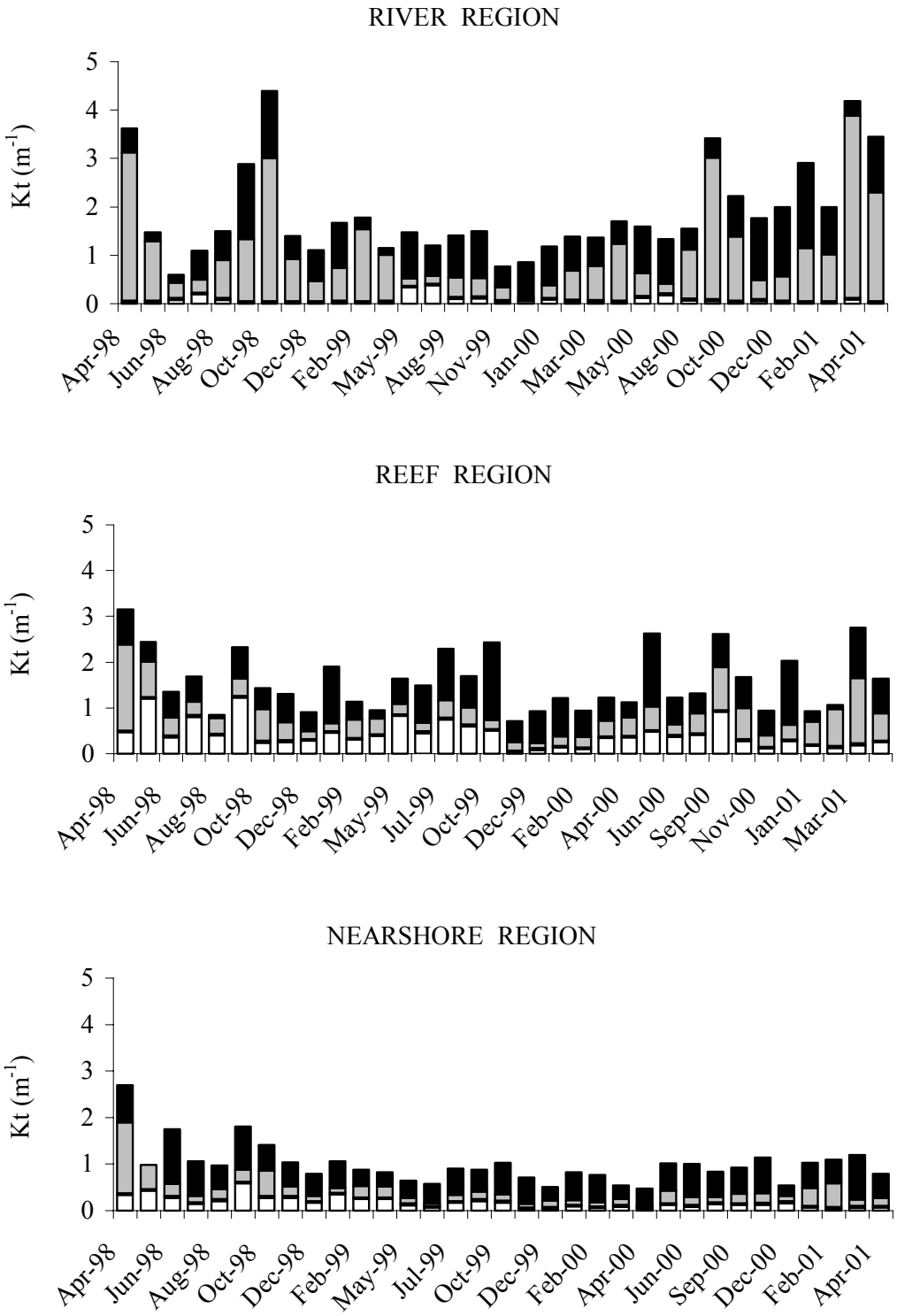

Figure 2-11. Light attenuation K_t (m^{-1}) in the three regions of the Suwannee River and its estuary, Florida, USA April 1998 to April 2001. The major components of light extinction due to phytoplankton (white), CDOM (gray) and tripton (black).

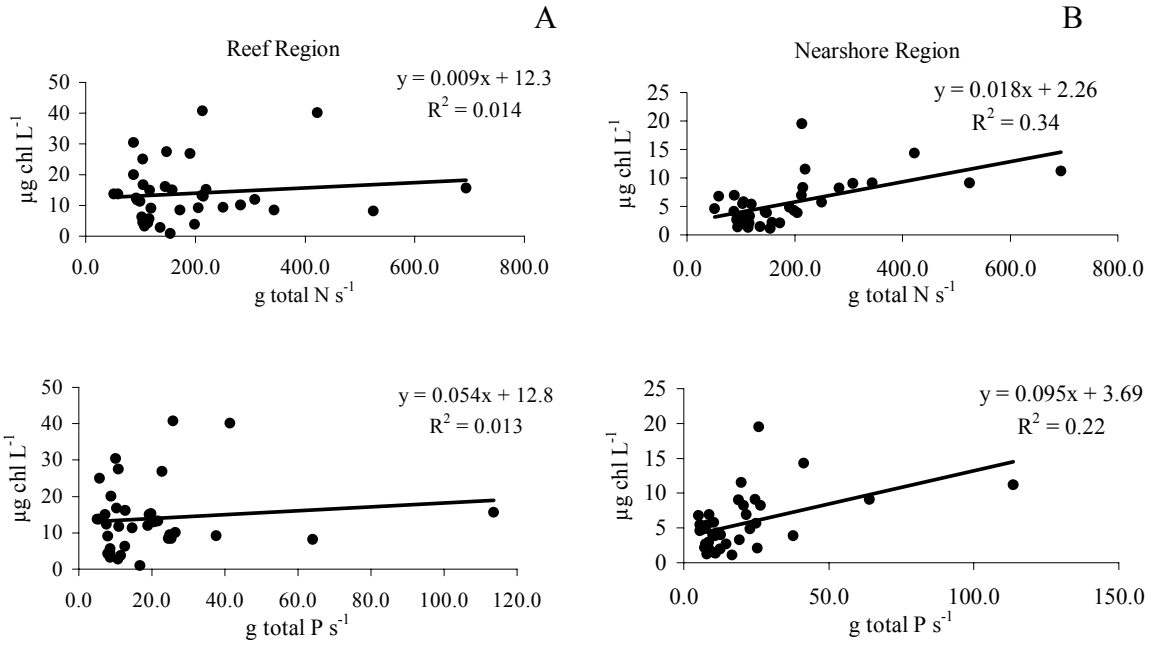

Figure 2-12. Mean monthly chlorophyll a concentrations by region (µg L^{-1}) and nutrient loading from the Suwannee River, Florida, USA April 1998 to April 2001. A) Mean reef region chlorophyll a µg L^{-1} versus total nitrogen loading (g N s^{-1}) and total phosphorus loading (g P s^{-1}). B) Mean nearshore region chlorophyll a concentrations (µg L^{-1}) versus total nitrogen loading (g N s^{-1}) and total phosphorus loading (g P s^{-1}). Lines indicate the best least squares regression fit to the data.

CHAPTER 3
PHYTOPLANKTON DISTRIBUTION AND COMPOSITION IN THE ESTUARINE WATERS ADJACENT TO THE SUWANNEE RIVER DURING CONDITIONS OF REDUCED FRESHWATER DISCHARGE

Introduction

The Suwannee River estuary and surrounding Big Bend region is one of the largest and most productive nursery areas for marine invertebrates and fish in the Gulf of Mexico (SRWMD 1979). Historical records indicate that the Big Bend region has supported a variety of important fisheries since the 19th century (Fishburne 1982). The basis for much of this productivity has been inputs of nutrient-rich water from major rivers such as the Suwannee, Apalachicola, and the Mississippi. Excess nutrient loading has been linked to changes in the abundance and composition of algal biomass; an increase in the frequency of harmful algal blooms (Smayda 1990, Hallegraeff 1993, Margalef 1998); loss of benthic communities (Valiela et al. 1997); an increase in the duration and frequency of oxygen depleted zones (Rosenberg et al. 1992, Paerl et al. 1998); and changes in food-web structure (Shumway 1990, Turner and Tester 1997).

Researchers and water resource managers in the Big Bend region have observed signs of nutrient enrichment in the Suwannee River as nitrate concentrations increased. For example, a large proportion of the overall flow of the Suwannee River originates as groundwater either by seepage or springs (Florida Department of Environmental Resources 1985). During the past 50 years, nitrate-N concentrations in groundwater discharge from several springs that flow into the river have increased from less than 0.1 mg L^{-1} (Katz 1992) to more than 5 mg L^{-1} (Pittman et al. 1997). Although, the drainage

basin is considered sparsely populated (Grubbs 1998); a variety of anthropogenic activities (e.g., fertilizer, animal wastes), along with atmospheric deposition, have been implicated in this increase (Katz et al. 1999, Katz et al. 2001).

Increased nitrogen loading rates within the Suwannee River have raised concerns about the impacts of nutrient enrichment on the phytoplankton community within the estuary. The potential response of the algal community to increased nutrients is dependent on a number of both abiotic (i.e., flushing rates, salinity, temperature, light availability, wind resuspension) and biotic factors (i.e., growth, competition, grazing). Few studies have extensively examined the spatial and temporal distribution and composition of phytoplankton within the Suwannee River estuary (Wolfe and Wolfe 1985, Bledsoe and Phlips 2000). The objectives of this study were to describe the phytoplankton communities within hydrological distinct regions of the estuary and to determine the effect of flushing rates, salinity, temperature, light availability, and nutrient loading on the spatial and temporal distribution.

Material and Methods

Study site description. The Suwannee River is a blackwater river, which originates in the Okeefenokee Swamp in southeastern Georgia before winding southward 394 km to the Gulf of Mexico (Bass and Cox 1988). Along with its tributaries the Alapaha, Withlacoochee, and Santa Fe Rivers, drains 28,500 km^2 of southern Georgia and north-central Florida (Wolfe and Wolfe 1985). Freshwater surface flow is augmented by substantial groundwater contributions of numerous springs along its course (Florida Department of Environmental Resources 1985) and has the second highest mean annual discharge of any river in Florida (301 $m^3 s^{-1}$) (Palmer 1984).

Located at approximately latitude 29° 17.50′ N and longitude 83° 10.00′ W, the Suwannee River coastal area includes a delta region near the river mouth, which is characterized by a network of oyster reefs. The river estuarine area is thought to be well-mixed due to its shallow nature, although it can become vertically stratified depending on tide, wind, and river discharge (Orlando et al. 1993).

Sampling regime. A network of fixed sampling sites within the Suwannee River and adjacent coastal waters was sampled monthly from May 1999 to April 2001. Thirty sites (Figure 3-1) were sampled on a monthly basis. The locations of the sampling sites were designed to incorporate regions of the Suwannee River and estuary previously characterized as ecologically distinct based on several key physical, chemical, and biological features (Bledsoe and Phlips 2000). For the sake of consistency and logistical considerations, an effort was made to sample at or near high tide. Seven of the thirty samples collected each month were analyzed for phytoplankton composition. These sites were based on unique geographical conditions. The Suwannee River region encompassed the lower reaches of the river (Site 1). Sampling sites A1, C1, and C2 represented an area landward of the oyster reefs, and sites A3 and C3 represented the region just seaward of the oyster reefs. Site 10 represented the site farthest from the influence of the Suwannee River.

Field measurements. A number of basic water column characteristics were measured on site. Temperature, oxygen concentration, conductivity, and salinity were measured at regular depth intervals using Hydrolab Surveyor units and YSI electronic meter. Light attenuation was determined by measuring quantum flux at 0.5-m intervals

with an array of Li-cor PAR probes; 2π surface, 2π underwater upwelling, 2π underwater downwelling, and 4π underwater scalar (Phlips et al. 1995) (Chapter 2).

Phytoplankton analyses. Water column samples (0.1 to 3 m depth) were collected at each site using an integrating sampling tube (7.3 cm i.d.). The tube was lowered to 0.1m from the bottom, a stopper was inserted into the top of the tube, the sampler raised, and the water collected into a bucket. One sample was used to rinse the bucket and tube, then disposed. At least 5 samples were collected into the bucket and mixed. Whole water samples were subdivided on site into aliquots for picoplankton (immediately stored on ice) and Lugol's preserved phytoplankton samples.

Fluorescence microscopy of unpreserved samples was used to enumerate picoplanktonic cyanobacteria and green algae (Fahnenstiel and Carrick 1991). Subsamples of unpreserved samples were filtered onto 0.2-μm pore nucleopore filters immediately upon returning to the laboratory, mounted between a microscope slide and cover slip with immersion oil, and frozen. All samples were counted within 72 h using a Nikon research microscope equipped with autofluorescence (green light 530-560-nm excitation and >580-nm excitation). Numerical abundance of picophytoplankton cells was determined by counting a minimum of five ocular-micrometer grids at 1000x. The number of grids counted was determined by cell density. Counts were completed upon reaching a minimum of 100 cells.

Phytoplankton composition was characterized microscopically using the Utermohl settling method (Utermohl 1958). Lugol's preserved samples were settled in 19-mm diameter cylindrical glass chambers. Phytoplankton cells were identified and counted at 400x and 100x with a Nikon phase-contrast inverted microscope. At 400x, a minimum of

100 cells of a single taxa and at least 30 grids were counted. At 100x, a total bottom count was completed for taxa greater than 30 μm at the longest dimension.

Counts for individual taxa were converted to cell volumes using the closest geometric shape method (Smayda 1978). Mean volume was calculated for each species from specific phytoplankton dimensions measured for at least 30 randomly selected cells. The total biovolume per sample was the sum of the estimated cell volumes for each species.

For comparative purposes within our study, species "blooms" were defined as a single species total biovolume exceeding a threshold level established using 1% exceedence criteria, i.e., the biovolume level that was exceeded in fewer than 1% of the samples for each region (Badylak and Phlips 2003).

Statistical analysis. Hierarchical cluster analysis was used to define biologically distinct areas within the estuary based on the composition of phytoplankton (McCune and Mellford 1999). Distance measurements were based on chi-squared coefficients. The site groups were evaluated with a nearest neighbor analysis. Any potential site groups, that were statistically similar, were grouped together.

The correlation analysis was used to analyze the statistical relationships between phytoplankton composition and abundance and environmental variables. All taxonomic data were transformed ($\log_{10}$ [x + 1]). The relationship between species composition and environmental variables was analyzed using canonical correspondence analysis ($P < 0.05$). Significant differences among regions were determined with Student t-test analysis ($P < 0.05$) (SAS 2000).

Results

The major algal groups observed within the Suwannee River and estuary during the study period included: cyanobacteria (11 taxa), diatoms (85 taxa), dinoflagellates (52 taxa), green algae (16 taxa), euglenoids (3 taxa), cryptomonads (2 taxa), chrysophyta (2 taxa), and prasinophytes (2 taxa) (Table 3-1).

Cluster analysis, based on phytoplankton composition, produced four major site groups (Figure 3-2). The major divisions occurred between the tidal freshwater site in the Suwannee River (Site 1) and the estuarine and marine sites (Sites 10, A3, C3, A1, C1, and C2). The latter sites were different enough to form three separate groups. The 'reef' group included sites A1, C1, and C2, located landward of the oyster reefs. The marine sites, seaward of the oyster reefs, were differentiated into two groups, the 'nearshore' region represented by sites A3 and C3, and the 'offshore' region indicated by site 10.

Phytoplankton Composition

Suwannee River region. The Suwannee River is located within subtropical to temperate climate. Water temperature varied seasonally from a mean low of 17.5ºC in the winter months (December to February) to a mean high temperature of 28.6 ºC in the summer months (June to August) (Table 3-2). Although the riverine sampling site was tidally influenced, salinities remained below 0.2 ppt. Due to drought conditions from May 1999 to April 2001, riverine discharge was reduced to less than 50% of the historical mean (244,000 L s^{-1}) with a mean flow rate of 114,000 L s^{-1} (Figure 3-3). Seasonal mean chlorophyll concentrations ranged from 0.44 to 12.6 µg chl a L^{-1} and maximum concentrations were observed from May to September (Figure 3-4).

Bloom conditions within the river corresponded to approximate total biovolumes of 160,000 μm^3 ml^{-1} or more. On seven sampling dates, from May to August, bloom levels

were observed (i.e., up to 1.2 million μm^3 ml^{-1}) (Figure 3-5). Spherical cyanobacteria (1-µm diameter, Cyanobacterium A) containing phycocyanin comprised as much as 99% of the biomass during these blooms (CY = cyanobacteria, Figure 3-5). In May 1999 and June 2000, densities reached 2.2 million cells per milliliter. Increased biomass was also attributed to the diatom, Melosira distans, which reached bloom concentrations in June 1999 (i.e., 230,000 μm^3 ml^{-1}) (DT = diatoms, Figure 3-5). Centric and pennate diatoms, the small (<5-µm) spherical green alga Nannochloris c.f., and phytoflagellates (i.e., Spermatozoopsis exultans, Pyramimonas spp., and cryptomonads) increased in abundance on a biovolume basis following the summer cyanobacteria blooms (CH = green algae, FL = phytoflagellates, Figure 3-5). Detached ephiphytic diatoms (Terpinsoe americana, Licomorpha sp., and Melosira spp.) were major contributors to phytoplankton biomass in the river throughout the sampling period.

Reef region. Sampling sites A1, C1, and C2 were identified by cluster analysis as being significantly different from the other estuarine sites, based on phytoplankton composition (Figure 3-2). These sites fell within the region of the estuary semi-enclosed by oyster reefs. Water temperature ranged from a mean low 15.4ºC in winter months to mean high temperature of 29.9ºC during the summer months (Table 3-2). Salinities within this region varied with riverine flow and tidal stage, with overall seasonal means ranging from 18.5 ppt in the spring (March to May) to 23.1 ppt in the summer months (Table 3-2). Mean monthly chlorophyll concentrations for the region ranged from 0.85 to 37.0 µg chl a L^{-1} with peak individual concentrations observed in May 1999 (Site C1, 79.9 µg chl a L^{-1}) and September 2000 (Site A1, 55.3 µg chl a L^{-1}) (Figure 3-4).

Bloom conditions within the reef region corresponded to total individual biovolume exceeding 1 million μm^3 ml^{-1}. Bloom-forming phytoplankton included Cyanobacterium A, as in the river, and Cyanobacterium B, which falls within the same size range and is phycoerythrin-rich rather than phycocyanin-rich. Cyanobacteria frequently exceeded 1 million μm^3 ml^{-1} during summer (Figure 3-5). However, diatoms were more commonly the dominant algal group on a biovolume basis. Bloom-forming species included Skeletonema menzellii, Skeletonema costatum, Rhizosolenia imbricata, Thalassionema nitzschioides, Thalassiosira sp., and centric diatoms (5 to 15-µm diameter). In October 1999, the biovolume of R. imbricata increased to >6 million μm^3 ml^{-1} at two of the three reef sites. Nannochloris c.f. was also a biovolumetrically important contribution to the phytoplankton biomass in late spring and summer. Picophytoplankton concentrations decreased in the fall, and diatoms (Rhizosolenia spp., Thalassionema nitzschioides, and Thalassiosira sp.) dominated the phytoplankton biomass. Algal biomass decreased at all sites within the oyster reefs during winter. Small dinoflagellates, such as Katodinium rotundatum and Katodinium glaucum and other phytoflagellates (e.g., Eutreptia lanowii and Eutreptia globulifera) increased during the winter, reaching near-bloom proportions at all sites within the reef region (DF = dinoflagellates, Figure 3-5).

Nearshore region. Sites A3 and C3 had similar phytoplankton composition based on the cluster analysis (Figure 3-2). These sites were located seaward of the oyster reefs in a region extending 16 km from shore. Seasonal mean water temperature ranged from 14.9ºC in the winter to 29.7ºC in the summer (Table 3-2). Seasonal mean salinities within this region varied from 31.2 ppt in the fall (September to November) to 32.9 ppt in the summer (Table 3-2). Monthly chlorophyll concentrations for the nearshore region

were significantly lower ($P < 0.001$) than those observed for the reef region, ranging from 0.74 to 6.5 µg chl a L^{-1} with peak concentrations observed during summer and fall (Figure 3-4).

Bloom conditions within the nearshore region corresponded to total individual biovolumes of approximately 400,000 μm^3 ml^{-1}. Taxa exceeding this bloom level were primarily diatoms, although Cyanobacterium B, was observed to form blooms during summer (Figure 3-5). Bloom-forming diatoms included Proboscia alata, Guinardia striata, Skeletonema costatum, Hemiaulus hauckii, Pseudosolenia calcar-avis, and Leptocylindrus danicus.

The general seasonal succession pattern of the dominant phytoplankton taxa was as follows; Cyanobacterium B and Nannochloris sp. in the spring and early summer, followed by the diatoms Proboscia alata, Hemiaulus hauckii, Pseudo-nitzschia sp., and Pseudosolenia calcar-avis in summer. Cerataulina pelagica and Guinardia striata dominated phytoplankton biomass in fall. Like the reef region, phytoplankton standing crop in the nearshore region decreased in winter with S. costatum, G. striata, R. setigera, and L. danicus being dominant.

Offshore region. The station farthest from shore (Site 10, approximately 31.5 km from the coast), which was significantly different from all other sites (Figure 3-2), was used to characterize the phytoplankton community of the offshore region. Seasonal mean water temperatures ranged from 14.8ºC in winter to 29.3ºC in summer. Seasonal mean salinities ranged from 34.9 ppt in the fall to 36.0 ppt in spring (Table 3-2). Chlorophyll concentrations ranged from 0.14 to 1.1 µg chl a L^{-1} (Figure 3-4).

Bloom conditions in the offshore region corresponded to phytoplankton biovolumes exceeding 400,000 μm^3 ml^{-1} for a single sampling event. Diatoms were the only group to exceed this threshold and included Proboscia alata, Guinardia striata, Cerataulina pelagica, Hemiaulus hauckii, and Leptocylindrus danicus. The picoplanktonic green algae that were major components of the phytoplankton community in the reef and nearshore regions were largely absent offshore. Seasonally, Cyanobacterium B and L. danicus dominated the spring season. By summer, Rhizosolenia imbricata, H. hauckii, P. alata, and G. striata, as well as L. danicus represented a high percentage of total biovolume. P. alata and C. pelagica dominated in winter.

Relationships Between Species Composition and Environmental Variables

Canonical correspondence analysis suggested that salinity, light, and temperature play dominant roles in the distribution of the major bloom forming species within the estuary combining all regions (Figure 3-6A). Within each region, individual species are associated with each environmental variable. For example, in the reef region, Katodinium spp. and Eutreptia spp. were commonly observed during winter and occur in the quadrant opposite to the temperature vector. The temperature vector is found within the same quadrant as Nannochloris c.f., Cyanobacterium A and B, which are frequently found during the warmer months (Figure 3-6B).

In the nearshore region, nutrients, temperature, and salinity were associated with the highest abundance of phytoplankton. Light was not limiting in this region of the estuary (Chapter 2), and the light vector was shown as less important (e.g., shortest vector). The nearshore region is frequently nutrient limited (Bledsoe and Phlips 2000) and as expected, canonical analysis showed that TN and TP were associated with certain

phytoplankton species. For example, Cyanobacterium B, Thalassiosira sp. and Cerataulina pelagica were correlated with high nutrient availability (Figure 3-6C).

The offshore region was characterized by stable salinities, high light availability, and low nutrient availability. Skeletonema costatum, Eutreptia spp., and Cerataulina pelagica were found at low water temperatures and therefore opposite the temperature vector. Several species were correlated with higher nutrient concentrations (e.g., Cyanobacterium B and Thalassiosira sp.), while others were correlated with nutrient limiting conditions (e.g., Rhizosolenia imbricata) (Figure 3-6D). Species within the center of the ordination diagram may be indifferent with regard to the gradients used for this analysis. Many of these species were observed under the entire range of environmental conditions throughout the year.

Discussion

A variety of ecological processes regulate phytoplankton community-structure in natural systems. Selective grazing, toxin production and allelopathy, competition for nutrients and light, and differences in temperature and salinity preferences are among the factors that influence the structure of phytoplankton communities in estuaries associated with large rivers like the Suwannee. To complicate matters further, meteorological events such as *El Niño*/*La Niña* can significantly alter the degree and relative importance of these factors in time and space.

One of the main factors impacting primary productivity in estuaries is the quality and quantity of freshwater discharge. Each hydrogeographically distinct region of the estuary and coastal region has a unique set of forces acting on the phytoplankton community. It is therefore useful to view the phytoplankton community in this context. For the purpose of this discussion, four distinct regions were identified: river, reef,

nearshore, and offshore regions. Nutrient availability within the river is high, however low light availability and short water residence periods limit phytoplankton standing crop (Bledsoe and Phlips 2000). During the post-*El Niño* drought of 1999, river discharge rates were 50% of the historical mean. Although both nitrogen and phosphorus concentrations in the river remained elevated, the spatial distribution of the nutrient-rich water plume was significantly reduced. In addition, the tannin-rich color of the Suwannee River was absent, reducing attenuation of light due to color within the river and estuary (Chapter 2). Therefore, the results of our study represent the phytoplankton dynamics in the Suwannee River and estuary during a period of relatively low riverine discharge.

River region. The Suwannee River has many of the physical and chemical attributes of other blackwater rivers of the southeastern USA (Meyer 1990). It is located in close proximity to Florida's other major blackwater river, the St. Johns River, located on the east coast of the Florida peninsula. Both have drainage basins with numerous groundwater inputs along their reach, and have high bioavailable nutrient concentrations. The composition of phytoplankton, however, is significantly different. The St. Johns River is dominated by cyanobacteria such as Cylindrospermopsis, Oscillatoria, Lyngbya, and Microcystis (Cichra and Phlips, unpublished data). By comparison, displaced epiphytic diatoms and phytoflagellates (e.g., Spermatozoopsis, Chlamydomonas, and cryptophytes) primarily dominate the phytoplankton composition in the Suwannee River, with high densities (>1 million cells ml^{-1}) of picoplanktonic cyanobacteria during the warmer months. These phytoplankton groups are more similar to those found in the Orinoco River, Venezuela, where periods of peak biomass contained large numbers of

phytoflagellates, chain-forming diatoms, and coccoid or filamentous blue-green algae (Lewis 1988).

The biomass of phytoplankton within these rivers is also significantly different. The phytoplankton biomass within the St. Johns River can reach levels exceeding of 100µg chl a L^{-1} while the phytoplankton biomass of the Suwannee River rarely exceeds 1 µg chl a L^{-1} under normal flow conditions (Phlips et al. 2000, Phlips et al. 2003). These differences are primarily due to high flow rates and low residence times within the Suwannee River (301 m^3 s^{-1}, less than 10 days during mean flow). In contrast, the St. Johns River has flow rates less than 1 m^3 s^{-1} (25 year average 1970 to 2000, USGS 2003) and water residence times that can exceed two months (Phlips et al. 2000). The short residence times in the Suwannee River limits the accumulation of phytoplankton standing crop. During periods of reduced flow, like those observed during our study, phytoplankton standing crop can increase to >10 µg chl a L^{-1} (Table 3-2). This increase can be attributed to increased residence time within the river and increased light availability due to the reduction of dissolved organic matter (Chapter 2). Lewis (1988) observed similar trends in the Orinoco River, Venezuela, where significant algal biomass accumulated during the interval of low flow due to higher transparency, lower average water-column depth, and longer residence times. Short residence time may reduce the presence of many common riverine phytoplankton species found in Florida and elsewhere.

Reef region. If the majority of bioavailable nutrients are not utilized within the river, then increased nutrient loads may be expressed within the estuary as increased phytoplankton biomass with potential negative consequences (e.g., anoxia, loss of

benthic habitat, harmful species, alteration of food chains). These impacts are likely to be most profound near the outflow of the river, especially in the reef region.

There are three main factors that distinguish the reef region from the other three regions in the study. First, the reef region is semi-enclosed by oyster reefs, which restrict the movement of water, and result in increased residence times. In general, riverine estuaries, where flushing is constrained by barrier islands support the highest phytoplankton production (Paerl 1988, Monbet 1992). However, well-flushed sounds or unconstrained rivers maintain much lower phytoplankton biomass. For example, the estuaries in North Carolina have significantly different flushing rates. The Cape Fear system is relatively fast flushing, as it is open to the sea, rather than enclosed by a protected sound (e.g., Pamlico Sound), and it has significantly lower phytoplankton biomass than nearby enclosed estuaries (Mallin et al. 2000). The typical flushing rates for the Suwannee River estuary are relatively short by comparison to some other estuaries along the Gulf of Mexico (3 days versus up to several months) (Solis and Powell 1999), yet large standing crops of phytoplankton were observed in the reef region of the estuary. It has recently been suggested that this apparent contradiction is associated with periods of elevated residence time caused by the intensity and orientation of prevailing winds (Chapter 2). Since doubling times for phytoplankton can be on the order of days, even small increases, due to onshore wind events, can have a major impact on the accumulation of phytoplankton biomass.

Another distinguishing feature of the reef region is that it receives direct nutrient-rich input from the Suwannee River (Bledsoe and Phlips 2000, Chapter 2). High nutrient inputs persist throughout the year in the reef region, which reduces nutrient limitation of

phytoplankton growth. As a consequence, annual peak biomasses correlated best with temperature and light rather than nutrient concentration (Table 3-2, Figure 3-5). Furthermore, the results of canonical correspondence analysis suggest that nutrients (i.e., nitrogen, phosphorous, and silica) were not the principal driving factors for the phytoplankton species composition of the reef region. These results suggest that nutrient availability within the reef region remained sufficient to support high phytoplankton standing crop, and that physical variables (e.g., light, salinity, and temperature) within this region determine species composition.

The importance of light availability in the reef region is supported by another unique feature of the region, namely, high dissolved organic matter discharging into the coastal region from the river, which is a major contributors to low light availability in the river and at times the reef region (Bledsoe and Phlips 2000). The reef region is also shallow (<2m), which increases the potential for sediment resuspension events via wind and tidal forcing. Light was not limiting to phytoplankton growth in the reef region in 1999-2001 likely due to the relatively low dissolved organic matter concentrations in the Suwannee River. In fact, mean light availability in the mixed layer, I_m, was significantly higher within the reef region than other coastal regions during the sampling period (Table 3-2) because of the shallow mixing depths (<2m) by comparison to the offshore site (>10m).

Based on the above considerations, it is not surprising that peak phytoplankton biomass in the reef region coincided with high water temperatures and light levels in summer and early fall. Conversely, biomass minima occurred in winter as water temperatures and day-length decreased. A distinct assembly of phytoplankton developed

during winter, dominated by the dinoflagellates Katodinium rotundum and K. glaucum, the euglenoid Eutreptia lanowii and E. globulifera, and cryptophytes. This assembly of phytoplankton is a common feature of North Carolina estuaries (Mallin 1994). Pinckney et al. (1998) suggested that these types of species assemblages are not consumed by microzooplankton due to reduction of grazers and grazing rates during periods of low water temperature in the Neuse Estuary. Although winter also correlates with reduced microzooplankton grazing in the Suwannee River estuary (Chapter 4), the implication that the microzooplankton community affects phytoplankton species composition should be viewed with caution. For example, although K. rotundum was found primarily during winter in the Suwannee River, it has also been observed in spring and fall in the Neuse Estuary (Mallin 1994). Due to latitudinal differences, winter temperatures for the Suwannee River estuary may coincide with spring and fall temperatures for North Carolina estuaries, suggesting a narrow temperature preference range for this assemblage of phytoplankton.

During summer and fall, individual phytoplankton species that reached bloom concentrations (i.e., biovolume >1 million μm^3 ml^{-1}) included the centric diatoms Skeletonema spp., Thalassiosira spp. Thalassionema nitzschioides, and Rhizosolenia imbricata. Each of these taxa have been found in other southeastern estuaries including Apalachee Bay, Florida (Curl 1959) and North Carolina estuaries (Mallin 1994). Two unidentified coccoid cyanobacteria (Cyanobacterium A and B) were also observed at bloom concentrations. The phycocyanin-rich cells appear to be flushed into the estuary via the river during the warmer months, while the phycoerythrin-rich cells maintain a presence throughout the year, peaking in abundance during the spring and summer.

Positive correlations between cyanobacteria picoplankton and temperature have also been reported in other marine systems including the Mediterranean (Caroppo 2000) and the Great Barrier Reef (Ayukai 1992). Although never reaching bloom conditions, several dinoflagellates persisted in the estuary throughout the year including Akashiwo sanguinea, Prorocentrum micans, Prorocentrum minimum, Gyrodinium estuariale, and Ceratium hircus.

The reef region is considered euryhaline, in that salinities ranged from 7.1 to 34.5 ppt. Therefore, it is not surprising that many of the major phytoplankton species found at the reef sites have been previously characterized as euryhaline including Sketetonema costatum, Akashiwo sanguineum, Prorocentrum micans, Prorocentrum minimum, and Scripsiella trochoidea (Braarud 1951, Kain and Fogg 1960, Qasim 1972, Brand 1984). Neritic species, such as Rhizosolenia imbricata, Hemiaulus hauckii, Guinardia striata, and Psuedosolenia calcar-avis, were also observed in the reef region. Although typically found in marine regions, R. imbricata, was found in bloom concentrations within the reef region during this study. The appearance of this species coincided with reduction of freshwater discharge from the Suwannee River (Chapter 2), which may have permitted neritic species to move farther into the estuary. This phenomenon is well documented for other estuaries including San Francisco Bay (Cloern et al. 1983) and Chesapeake Bay (Marshall and Alden 1993). For example, neritic species found in the clear low nutrient waters of the continental shelf enter and proliferate in the Chesapeake Bay during periods of low rainfall (Marshall and Alden 1993).

Marine region. The nearshore and offshore environments of the Suwannee River estuary have considerably higher salinities (25 to 37 ppt) than the reef region. These

regions also have relatively low nutrient concentrations, particularly during periods of low riverine discharge (Table 3-2). Under high flow conditions, the riverine plume can extend beyond the offshore site (Bledsoe unpub. data) and thereby increase nutrient availability beyond the reef region. Drought conditions prevailed during this sampling period, and nutrient limitation of phytoplankton growth was prevalent within the nearshore and offshore region. Nitrogen was the primary limiting nutrient in the nearshore region, while co-limitation of nitrogen and phosphorus was common offshore (Chapter 2).

Phytoplankton composition within the nearshore region exhibited seasonal trends similar to those in the reef region, with peak phytoplankton biomass in summer and fall. Offshore, overall phytoplankton biomass was low and lacked the larger bloom events characteristic of the reef and nearshore regions. Typical marine diatom genera for these regions included Rhizosolenia imbricata, Probisca alata, Hemiaulus hauckii, Psuedonitzchia spp., and Pseudosolenia calcar-avis, which are all considered warm-water neritic assemblages (Mallin et al. 2000). Similar diatom assemblages have been identified in the Mediterranean and Gulf of California in summer both during periods of stratification and subsequent nutrient depletion in the upper-layer of the water column (Kemp et al. 2000) and under oligotrophic conditions in the Mediterranean (Ignatiades 1969), North Pacific (Brzezinski et al. 1998) and along the northwest African coast (Lange et al. 1998).

Diatom assemblages in regions depleted of nutrients generally have a large cell size and have distinct characteristics designed to exploit such conditions (Kemp et al. 2000). Under conditions of nitrogen limitation, diatoms may use symbiosis with nitrogen-fixing

cyanobacteria as a mechanism for survival (Villareal 1991). Diatoms are also capable of growing deep in the water column under conditions of low light to utilize nutrients released at the water-sediment interface (Goldman 1993). Kilham and Kilham (1980) have also noted that large diatoms persist, if not flourish, under nutrient-poor oceanic conditions because of their ability to "store" nutrients. Through vertical migration by regulating buoyancy, diatoms may take up nutrients trapped in a stratified water column or at the water-sediment interface and utilize them for growth under more favorable light conditions in the euphotic zone (Moore and Villareal 1996, Villareal and Carpenter 1994).

Canonical correspondence analysis results suggest a correlation between Cyanobacterium A and high nutrient availability. Generally, Cyanobacterium A blooms in the Suwannee River estuary were restricted to regions of low salinity and high nutrient availability such as in the river and reef region. Therefore, the relationship suggested by the canonical correspondence analysis may be a consequence of high nutrient, low salinity water moving into the nearshore region (Bledsoe and Phlips 2000). The extent and direction of the freshwater plume is determined by riverine discharge, wind direction, tidal mixing, and long-shore currents. Strong offshore winds can deflect the plume seaward out from the reef region and into the nearshore region, increasing the abundance of Cyanobacterium A. In contrast, the phycoerythrin-containing coccoid cyanobacteria (Cyanobacterium B), which are frequently observed in open waters (Waterbury et al. 1986), can appear in the reef region during periods of low riverine flow and prevailing onshore winds.

Toxic phytoplankton. Beyond the relationship between nutrient loading and algal blooms, another issue that has come to the forefront in dealing with coastal eutrophication is the presence of toxic algal species (Smayda 1990, Hallegraeff 1993, Margalef 1997). A number of potentially toxic phytoplankton species were observed below bloom proportions in the Suwannee River estuary. Based on the listing of harmful algae by the Intergovernmental Oceanographic Commission of UNESCO and Florida Marine Research Institute, potentially toxic algae present in some of the samples, included Alexandrium monilatum, Dinophysis acuminata, Dinophysis caudata, Karenia brevis, Prorocentrum mexicanum, and Prorocentrum minimum (IOC 2002, FMRI 2002).

Although inorganic nutrient loads from the Suwannee River are high and episodes of high phytoplankton biomass do occur, bloom events involving toxic algal species are not common. Temperature and salinity restrictions may reduce the impact of common Florida harmful algal species to proliferate within the reef region of the estuary. For example, Bledsoe and Phlips (2000) observed high densities of Karenia brevis (formerly Gymnodinium breve) in the waters offshore of the Suwannee River estuary in 1996. Long-shore currents, transporting water masses from southwest Florida where K. brevis is a common bloom forming species, presumably introduced the dinoflagellate into the region. Blooms originating off the southwest coast of Florida have also been carried into the warm waters of the Gulf Stream around Florida and up the east coast into North Carolina waters (Tester et al. 1991). Since that single event, K. brevis has only been observed within the estuary on two occasions, and only at densities <100 cells L^{-1}. In comparison, background concentrations of 1,000 cells L^{-1} of K. brevis are common along the southwest coast of Florida, south of the Suwannee estuary and along the Florida

Panhandle north of the estuary (Tester and Steidinger 1997). In addition to unfavorable currents, absence of high densities of K. brevis within the reef region suggests the presence of low salinity inhibition resulting from freshwater outflow from the Suwannee River. Kim and Martine (1974) found that salinities <23 ppt and >43 ppt resulted in death within 24 h of inoculation for K. brevis. During periods of normal to high Suwannee River flow; salinities are lower than the potential threshold for K. brevis, ranging from 15.9 to 28.6 ppt in the reef region (Table 3-2).

Another dinoflagellate, Pyrodinium bahamense, is frequently observed in many regions of Florida, but has not been observed within the Suwannee River estuary. Recently, this dinoflagellate was found in bloom concentrations in the Indian River Lagoon during summer (Badylak and Phlips 2003) and reported to be toxic (Landsberg et al. 2002). P. bahmense has also been found throughout the year in Florida Bay (Badylak and Phlips, unpublished data). The distribution and seasonality of these observations lead to the hypothesis that P. bahamense is restricted to warmer waters, and the water temperatures of the Suwannee River estuary are below this threshold.

Table 3-1. Species observed in the Suwannee River and its estuary, Florida, USA (May 1999 to April 2001). The letter 'r' indicates 'rare' species, occurring in less than 3 samples. The letter 'c' indicates 'common' species, occurring in greater than 3 samples. The numerals indicate the number of samples in which the species appeared at biovolumes (μm^3 ml^{-1}) exceeding the 1% threshold criteria for a bloom in each region.

Phytoplankton Species	Region			
	River	Reef	Nearshore	Offshore
Cyanobacteria – Cyanophyceae				
Anabaena sp	r	c	c	c
Cyanobacterium A -phycocyanin	c^6	c^2	c	c
Cyanobacterium B - phycoerythrin	c	c^2	c^3	c
Gomphosphaeria	r	c	c	c
Lyngbya sp	r	r	r	
Merismopedia sp	r	r	c	
Microcystis aeragenosa (Kuetzing)	r		r	
Microcystis incerta (Lemmermann)	r	c	c	c
Oscillatoria sp (Vaucher ex Gomont)	c	r	r	
Spirolina sp	r			
Synechococcus elongates (Nageli)	r	c	c	
Green algae - Chlorophyceae				
Ankistrodesmus convolutes (Corda)	c			
Ankistrodesmus falcatus (Corda) Ralfs	c			
Chlamydomonas sp	c	c	c	c
Cosmarium sp	r			
Crucigenia crucifera (Wolle) Collins	r			
Dictyosphaerium sp	c			
Eudorina sp	c			
Nannochloris c.f.	c	c	c^2	c
Oltmannsiellopsis viridis (Chihara and Inouye)		r	r	
Pandorina sp	r			
Pediastrum duplex (Meyen)	c			
Scenedesmus histrix (Lagerheim)	c			
Scenedesmus quadricata (Turp.) de Brébisson	c			
Spermatozoopsis exultans (Korsch)	c	r	r	r

Table 3-1. Continued

Phytoplankton Species	Region			
	River	Reef	Nearshore	Offshore
Prasinophyceae				
Pyramimonas sp	c	c[1]	c	c
Tetraselmis sp		r	r	
Euglena - Euglenophyceae				
Euglena sp	r	c		
Eutreptia globulifera (van Goor)	r	c[1]	c	c
Eutreptia lanowii (Steur)	r	c[1]	c	c
Dinoflagellates - Dinophyceae				
Akashiwo sanguinea (Hirasaka) Hansen & Moestrup		c	c	c
Alexandrium cf. monilatum (Howell) Taylor		r		
Balechena coerulia (Dogiel) Taylor			r	r
Ceratium declinatum (Karsten) Jörgensen				r
Ceratium fusus (Ehrenberg) Dujardin		r	c	c
Ceratium hircus (Schröder)		c	c	c
Ceratium lineatum (Ehrenberg) Cleve				r
Ceratium macroceros (Ehrenberg) Vanhöffen			r	r
Ceratium symmetricum (Pavillard)				r
Ceratium trichocerus (Ehrenberg) Kofoid		r	c	r
Ceratium tripos (O.F. Müller) Nitzsch		r	c	c
Dinophysis acuminate (Claparède & Lachmann)				r
Dinophysis cauldata (Saville-Kent)		c	c	r
Dinophysis diegens (Kofoid)			r	
Diplosalis sp			c	
Goniodoma polyedricun (Pouchet) Jorgensen		r	r	
Gonyaulax scrippsae (Kofoid)		c	r	
Gonyaulax spinifera (Claparede & Lachmann) Diesing		c	c	c
Gyrodinium c.f. uncatenum (Hulburt)		r	r	r
Gyrodinium estuariale (Hulburt)		c[1]	c	c
Gyrodinium spirale (Berg) Kofoid & Swezy		r	c	r
Heterocapsa niei (Loeblich) Morrill & Loeblich III		c	c	c
Karenia brevis (Davis) Steidinger			r	r
Katodinium glaucum (Lebour) Loeblich III		c	r	
Katodinium rotundroum (Lohmann) Loeblich III		c	c	r

Table 3-1. Continued

Phytoplankton Species	Region			
	River	Reef	Nearshore	Offshore
Oxytoxum scolopax (Stein)		r		
Oxytoxum sp				r
Peridinium sp.	r			
Polykrikos koifoidii (Chatton)		r		
Polykrikos schwartzii (Bütschli)			r	
Prorocentrum emargenallum (Fukuyo)		r		
Prorocentrum gracile (Schütt)		r	r	r
Prorocentrum mexicanum (Tafall)		r	c	r
Prorocentrum micans (Ehrenberg)		c	c	c
Prorocentrum minimum (Pavillard) Schiller		c	c	c
Prorocentrum minimum var. triangulum (Martin)		c	r	
Prorocentrum scutellum (Schröder)		r	r	r
Protoperidinium claudicans (Paulsen) Balech			c	
Protoperidinium depressum (Bailey) Balech		r	r	r
Protoperidinium divergens (Ehrenberg)		c	c	c
Protoperidinium grande (Kofoid) Balech				r
Protoperidinium leonis (Pavillard) Balech		r	c	
Protoperidinium oblongum (Parke & Dodge)			c	
Protoperidinium pellucidum (Bergh)			c	r
Protoperidinium pentagonium (Gran) Balech		r	c	r
Pyrocystis noctiluca (Murray ex Haeckel)		c	c	r
Pyrophacus horologicals (Stein)		c	c	c
Scrippsiela trochoidea (Stein) Loeblich III		r	r	r
Spirolax koifoidii (Graham)			r	
Torodinium terado (Pouchet) Kofoid & Swezy		r	c	r
Unidentified Gymnodinium		r	r	c
Chromophyta - Cryptophyceae				
Unidentified Cryptophyta	c	c	c	c
Hillea sp	c	c	c	c
Chromophyta - Chrysophyceae				
Dinobryon sp	c			
Dictyocha fibula (Ehrenberg)			r	

Table 3-1. Continued

Phytoplankton Species	Region			
	River	Reef	Nearshore	Offshore
Diatoms - Bacillariophyceae				
Asterionellopis glacialis (Castracane) Round	r	c	c	r
Aulacosira granulate (Ehrenberg) Simonsen	c			
Bacillaria paxillifera (O.F. Müeller) Hendry	c	c	c	c
Bacteriastrum delicatum (Cleve)			r	
Bacteriastrum hyalinum (Lauder)		r	c	r
Bellerochea horologicalis (von Stosch)		r	r	
Bellerochea malleus (Brightwell) Van Heruck		c	c	r
Biddulphia puchella (Smith) Boyer		c	c	
Biddulphia rhomubus (Ehrenberg) Smith		c	r	
Campylosira cymbelliformis (A. Schimdt)Grunow			r	r
Cerataulina pelagica (Cleve) Hendey			c[1]	c[1]
Chaetoceros affinis (Lauder)			r	r
Chaetoceros convolutes (Castracane)			r	
Chaetoceros danicus (Cleve)		r	c	r
Chaetoceros decipiens (Cleve)		r	c	r
Chaetoceros didymus (Ehrenberg)			c	
Chaetoceros lorenzianus (Grunroe)		r		
Chaetoceros peruvianus (Brightwell)		r	r	r
Chaetoceros simplex (Ostenfeld)		r	c	
Chaetoceros sp - 10 µm	r	r	c	r
Chaetoceros sp - 20 µm		r	r	
Chaetoceros sp - 5 µm		r	c	r
Chaetoceros didymus (Ehrenberg)			c	
Chaetoceros lorenzianus (Grunroe)		r		
Chaetoceros tenuissimus (Meunier)		r	r	
Corethron criophilum (Castracane)			r	
Coscinodiscus granii (Gough)		c		
Coscinodiscus sp		c	c	c
Dactosolenia fragilissimus (Bergon) Hosle		r	c	c
Diploneis sp		r		
Ephemera planamembranacea (Hendey) Paddock	r	c	c	c
Eucampia zodiacus (Ehrenberg)			r	

Table 3-1. Continued

Phytoplankton Species	Region			
	River	Reef	Nearshore	Offshore
Eunotia sp	r			
Fragilaria sp	r	r	r	
Guinardia flaccida (Castracane) H. Peragallo		c	c	c
Guinardia striata (Stolterfoth) Hasle comb nov.		c	c[1]	c
Gyrosigma fascicola (Ehrenberg) Cleve		c		
Haslea trompii (Cleve) Simonsen		r	c	r
Haslea wawrikae (Hustedt) Simonsen		r	c	c
Hemialus hauckii (Grunrow in Van Heurck)		r	c	c
Hemialus sinensis (Greville)		r	c[1]	c[1]
Leptocylindricus minimus (Gran)	r	c	c	r
Leptocylindrus danicus (Cleve)	r	c	c[1]	c[4]
Licomorphora sp	r	c	c	r
Lithodesmium undulatum (Ehrenberg)			c	
Melosira nummuloides (C.A. Agardh)		c		
Melosira sp	c	r		
Melosira distans (Agardh)	c[1]	r		
Navicula septentrionalis (Grunow) Gran			r	r
Navicula sp - 5µm transapical axis	r	c	r	
Navicula sp - 10µm transapical axis	r	c	c	
Navicula sp - 20µm transapical axis	c	r	r	
Neostreptotheca sp	r	c	c	r
Nitzchia sigma (Kütz) W. Smith	r	c	c	r
Nitzschia closterium (Ehrenberg) W. Smith		c		r
Nitzschia longissma (Brébisson) Ralfs in Pritchard	c	c	c	r
Nitzschia sp	c	c	c	c
Odontella aurita (Lyngbye) C.A. Agardh		c	c	
Odontella mobiliensis (Bailey) Grunow		r	r	
Odontella sinesis (Greville) Grunow		r	r	r
Paralia sulcata (Ehrenberg) Cleve	r	c	c	r
Pennate diatoms - <5µm transapical axis	c	c	c	c
Pennate diatoms - 5 to 10µm transapical axis	c	c	c	c
Pennate diatoms - 20µm transapical axis	c	c[1]	c	c
Pennate diatoms - 30µm transapical axis	c	c	c	c

Table 3-1. Continued

Phytoplankton Species	Region			
	River	Reef	Nearshore	Offshore
Pennularia sp	c			
Phaeodactylum triconutum (Bohlin)	r	c	c	r
Pleurosigma / Gyrosigma sp	c	c	c	r
Pleurosigma normaii (Ralfs in Pritchard)	c	c	c	r
Proboscia alata (Brightwell) Sundström	r	c	c[2]	c[1]
Pseudo-nitzschia sp		c	c[1]	r
Psuedosolenia calcar-avis (Schultze) Sundström		c	c[2]	c
Rhizosolenia imbricata (Brightwell)		c[2]	c	r
Rhizosolenia robusta (Norman in Pritchard)				r
Rhizosolenia setigera (Brightwell)		c	c	r
Skeletonema costatum (Greville) Cleve	r	c	c[2]	r
Synedra sp	c			
Terpinsoe Americana (Bailey) Ralfs	c			
Thalassionema nitzschioides (Grunow) Mereschkowsky	r	c	c	r
Thalassiosira sp		c[3]	c	r
Thalassiothrix gibberula (Hasle)			r	r
Triceratium alternans (Bailey)		r	r	r
Trigonium sp			r	r
Unidentified Centric diatom - >10 µm <50 µm	r	c[1]	c	r
Unidentified Centric diatom - 5 µm	c	c	c	c
Unidentified Pennate diatom	c	c		

Table 3-2. Regional seasonal mean physical and chemical parameters within the Suwannee River and its estuary, Florida, USA, (May 1999 to April 2001). Standard deviations are indicated in parenthesis.

Region		T ºC	Sal ppt	I_m	PO_4 µg P L^{-1}	NO_3+NO_2 µg N L^{-1}	NH_4 µg N L^{-1}	SiO_2 mg Si L^{-1}
Suwannee	Spring	23.2	0.1	4.4	57.5	484.3	46.6	6.7
		(3.9)	(0.1)	(1.7)	(16.8)	(233.2)	(23.3)	(1.0)
	Summer	28.6	0.2	7.6	70.7	579.7	44.4	5.7
		(1.2)	(0.0)	(2.3)	(26.1)	(195.1)	(26.1)	(1.9)
	Fall	21.8	0.2	4.3	92.7	891.3	33.0	6.5
		(4.4)	(0.0)	(1.5)	(51.7)	(197.7)	(24.8)	(2.0)
	Winter	17.5	0.2	4.0	78.5	773.0	41.5	6.5
		(2.7)	(0.0)	(0.8)	(34.5)	(153.3)	(7.9)	(1.4)
Reef	Spring	23.4	18.5	20.2	9.9	65.2	56.1	3.3
		(3.6)	(6.3)	(8.5)	(9.8)	(88.8)	(31.0)	(2.0)
	Summer	29.9	23.1	29.0	86.9	57.4	56.2	4.5
		(1.6)	(5.8)	(11.2)	(148.0)	(94.4)	(41.6)	(1.8)
	Fall	21.0	22.8	17.4	28.1	133.0	45.0	2.6
		(5.9)	(5.5)	(7.6)	(25.4)	(141.5)	(42.6)	(2.1)
	Winter	15.4	21.0	24.3	14.8	113.9	22.2	2.9
		(3.3)	(5.7)	(10.3)	(15.3)	(126.3)	(17.8)	(1.2)
Nearshore	Spring	22.6	31.5	17.9	5.1	2.3	21.9	0.8
		(4.1)	(2.3)	(4.3)	(3.9)	(3.5)	(18.0)	(0.6)
	Summer	29.7	32.9	19.2	15.7	1.9	30.6	0.7
		(1.0)	(3.0)	(8.7)	(29.4)	(1.2)	(17.8)	(0.3)
	Fall	21.6	31.2	17.6	7.5	16.4	8.7	0.7
		(4.8)	(2.5)	(9.3)	(6.9)	(22.0)	(6.0)	(0.6)
	Winter	14.9	31.8	22.7	3.5	1.5	8.2	0.5
		(2.8)	(2.8)	(14.1)	(5.2)	(1.8)	(8.7)	(0.4)
Offshore	Spring	22.5	36.0	13.8	3.8	1.8	29.1	0.1
		(2.5)	(1.5)	(5.7)	(1.5)	(2.9)	(16.6)	(0.0)
	Summer	29.3	35.8	11.1	6.7	9.3	17.5	0.1
		(1.7)	(0.6)	(10.0)	(11.6)	(15.2)	(15.0)	(0.1)
	Fall	23.5	34.9	8.2	7.9	4.8	18.0	0.3
		(3.0)	(1.0)	(2.7)	(6.3)	(4.1)	(14.9)	(0.1)
	Winter	14.8	35.4	8.3	5.5	2.1	14.5	0.2
		(1.7)	(0.2)	(3.0)	(9.2)	(2.6)	(12.3)	(0.2)

Note: T, water temperature; Sal, salinity; I_m, mean irradiance in the mixed layer (mol photons m^{-2} d^{-1}); Spring, March to May; Summer, June to August; Fall, September to November; Winter, December to February.

Table 3-3. Mean biovolume of major algal taxa for the four major regions of the Suwannee River and its estuary, Florida, USA (May 1999 to April 2001). The two year means are presented as million μm^3 ml^{-1}.

	Region			
	Suwannee	Reef	Nearshore	Offshore
Cyanobacteria	0.23	0.19	0.16	0.07
Cryptomonads	0.02	0.12	0.03	0.00
Diatoms	0.08	1.15	0.78	0.53
Dinoflagellates	0.00	0.09	0.10	0.05
Euglena	0.00	0.04	0.00	0.00
Microflagellates	0.03	0.14	0.04	0.00
Chlorophytes	0.04	0.15	0.08	0.01
Total	0.40	1.88	1.18	0.65

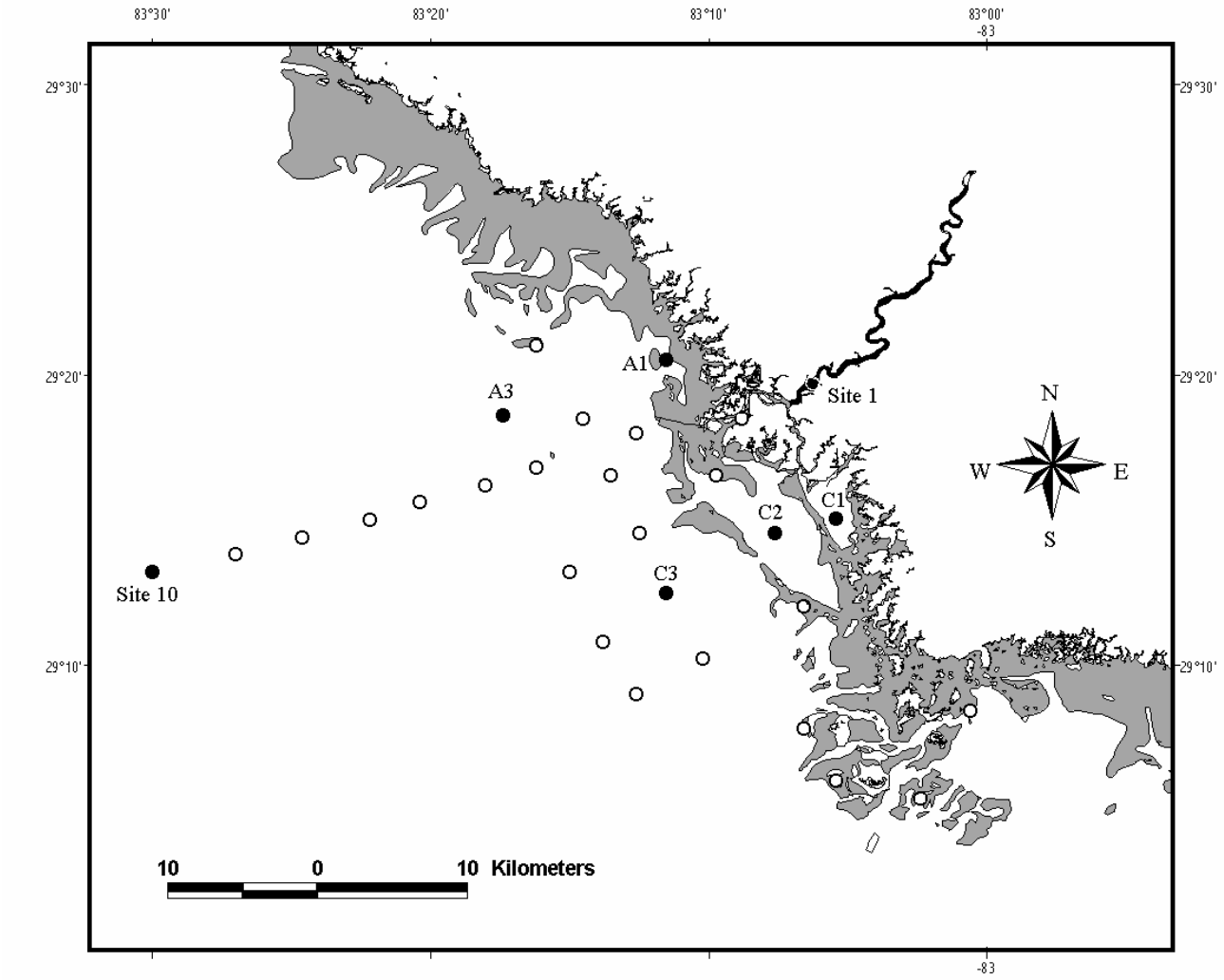

Figure 3-1. Sites sampled monthly from May 1999 to April 2001 in the Suwannee River and its estuary, Florida, USA (all circles). Black circles indicate sites sampled for phytoplankton composition. Oyster reefs and mud flats (<1 meter MLW) are indicated in light gray.

Linkage method: NEAREST NEIGHBOR
Distance measure: Chi-squared

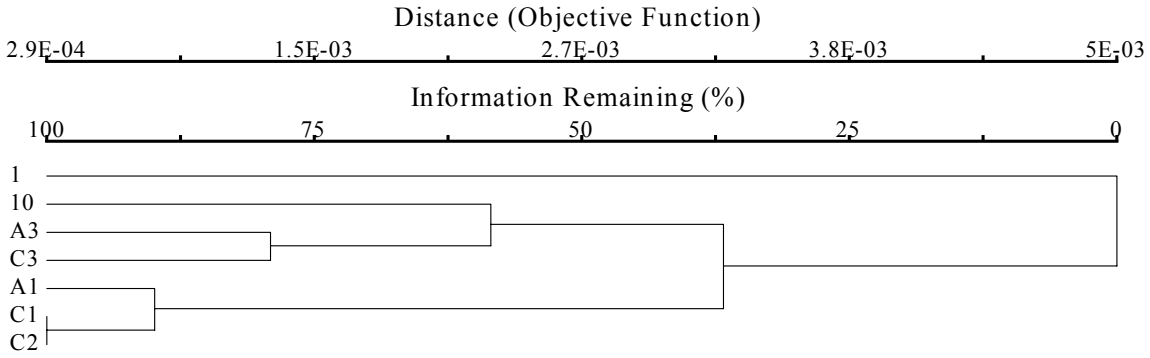

Figure 3-2. Distance dendrogram for classification of the major geographic regions within the Suwannee River and its estuary, Florida, USA based on phytoplankton composition from May 1999 to April 2001. Site 1 represents the river phytoplankton community; Site 10 the 'offshore' region; Sites A1, C1 and C2 the 'reef' region; and Sites A3 and C3 the 'nearshore' region.

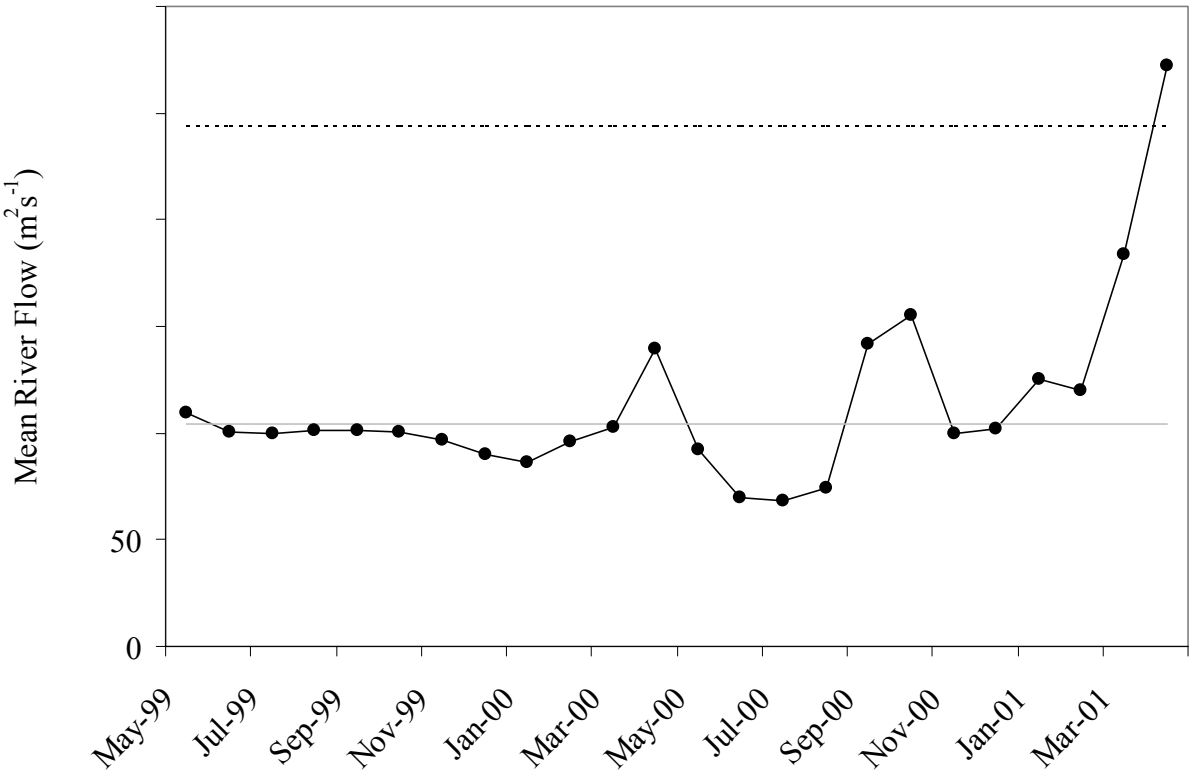

Figure 3-3. Mean monthly flow rates for the Suwannee River, Florida, USA at the Wilcox gaging station from 1999 to 2001. The slashed line indicates median flow rate for the Suwannee River. The gray line represents the flow rate exceeded 99% of all dates from 1960 to present (minimum flow). Redrawn from USGS (2002).

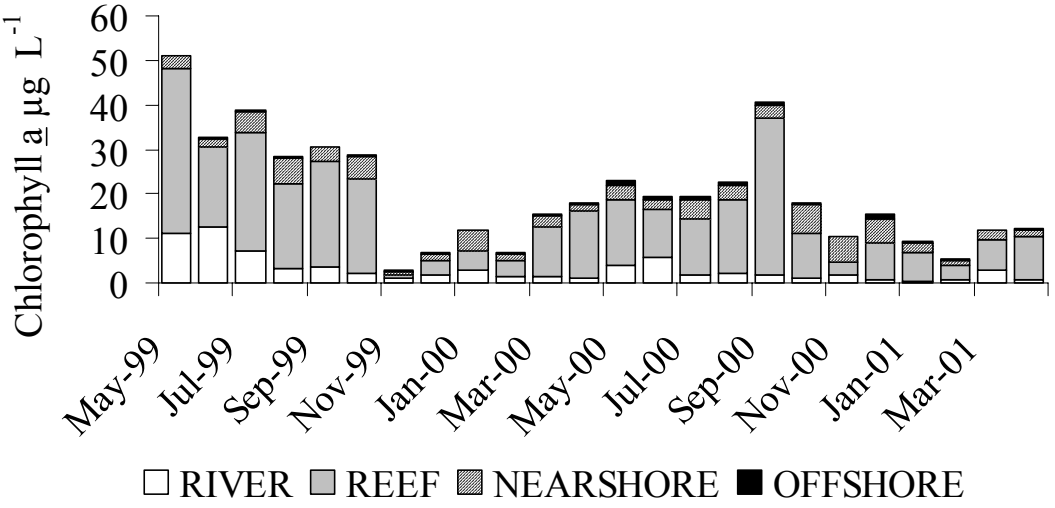

Figure 3-4. Mean monthly chlorophyll a (µg chl a L^{-1}) for each region of the Suwannee River and its estuary, Florida, USA May 1999 to April 2001.

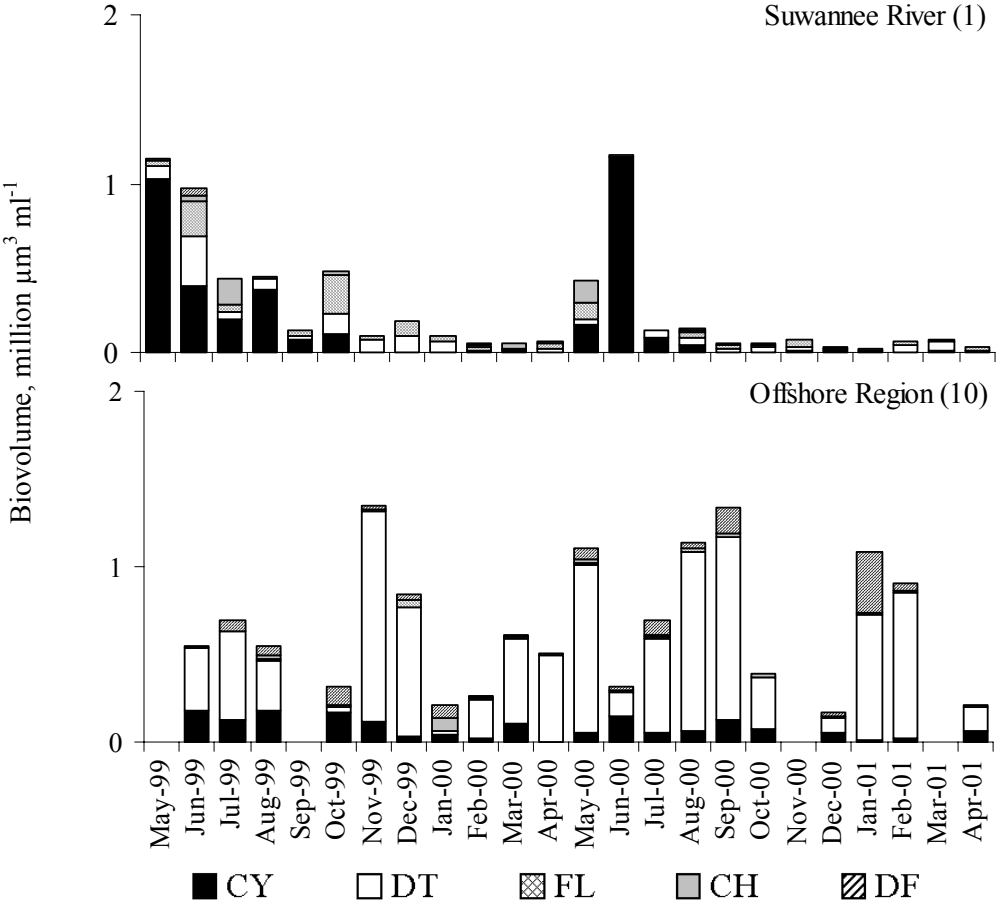

Figure 3-5. Biovolumes (million μm^3 ml^{-1}) of the major phytoplankton taxa observed at seven sampling sites in the Suwannee River and its estuary, Florida, USA (May 1999 to April 2001). Cyanobacteria (CY) are indicated in black, diatoms (DT) are indicated in white, phytoflagellates (FL) are indicated by cross-hatch, chlorophytes (CH) are indicated in gray, and dinoflagellates (DF) are indicated by stripes.

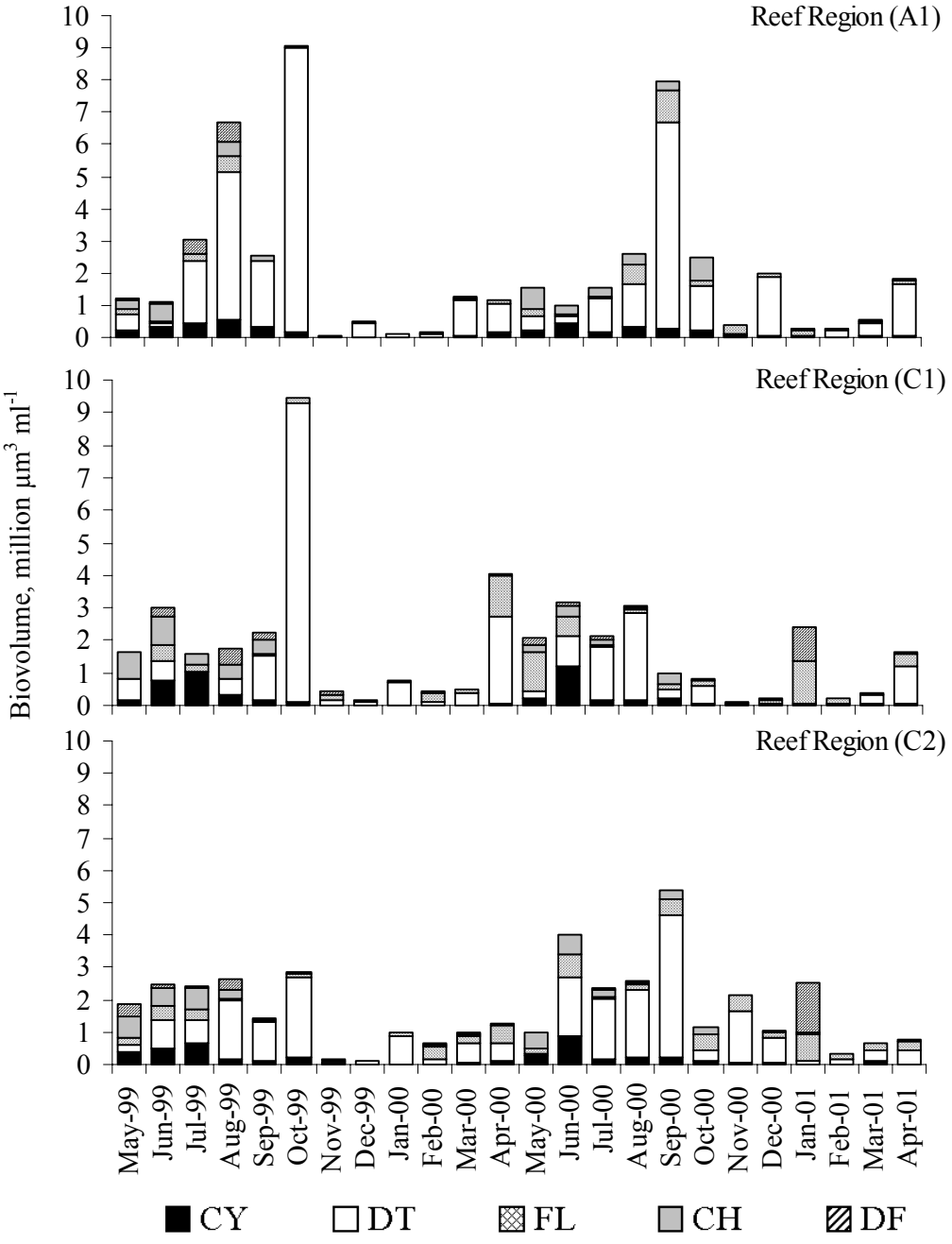

Figure 3-5. Continued

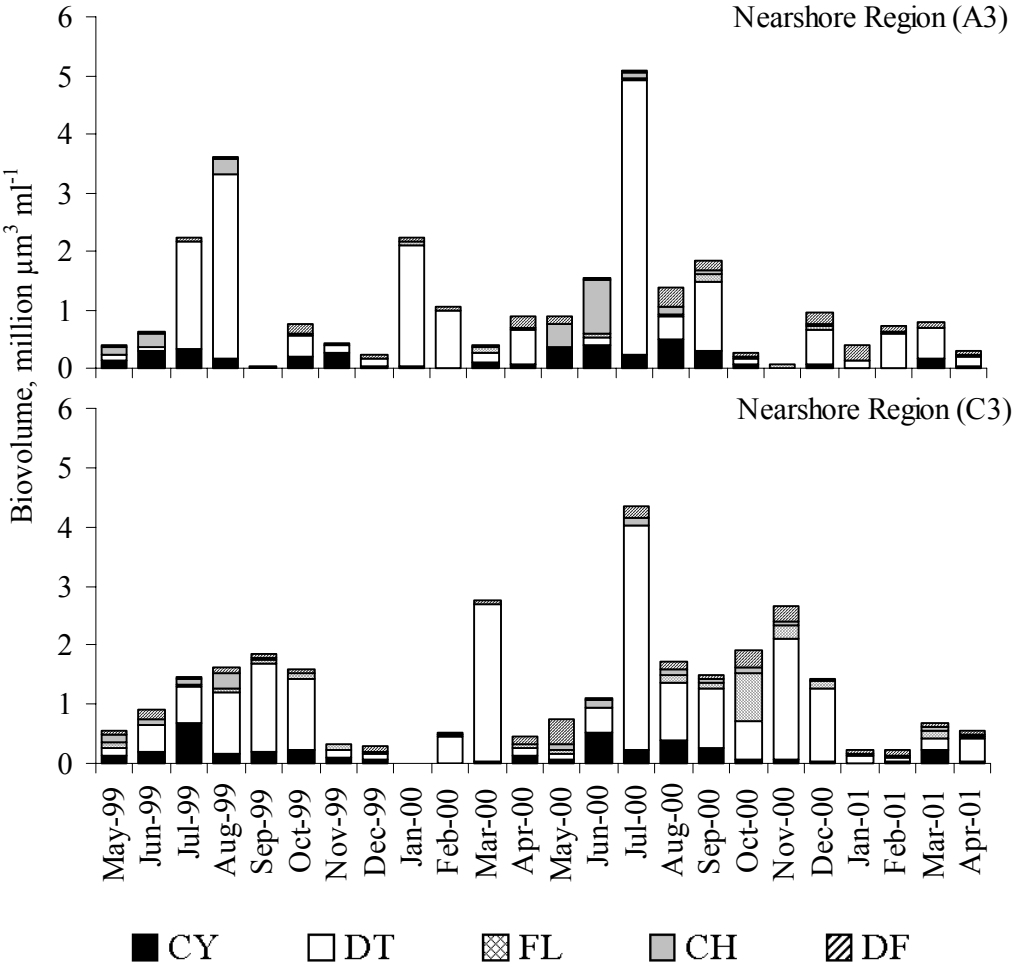

Figure 3-5. Continued

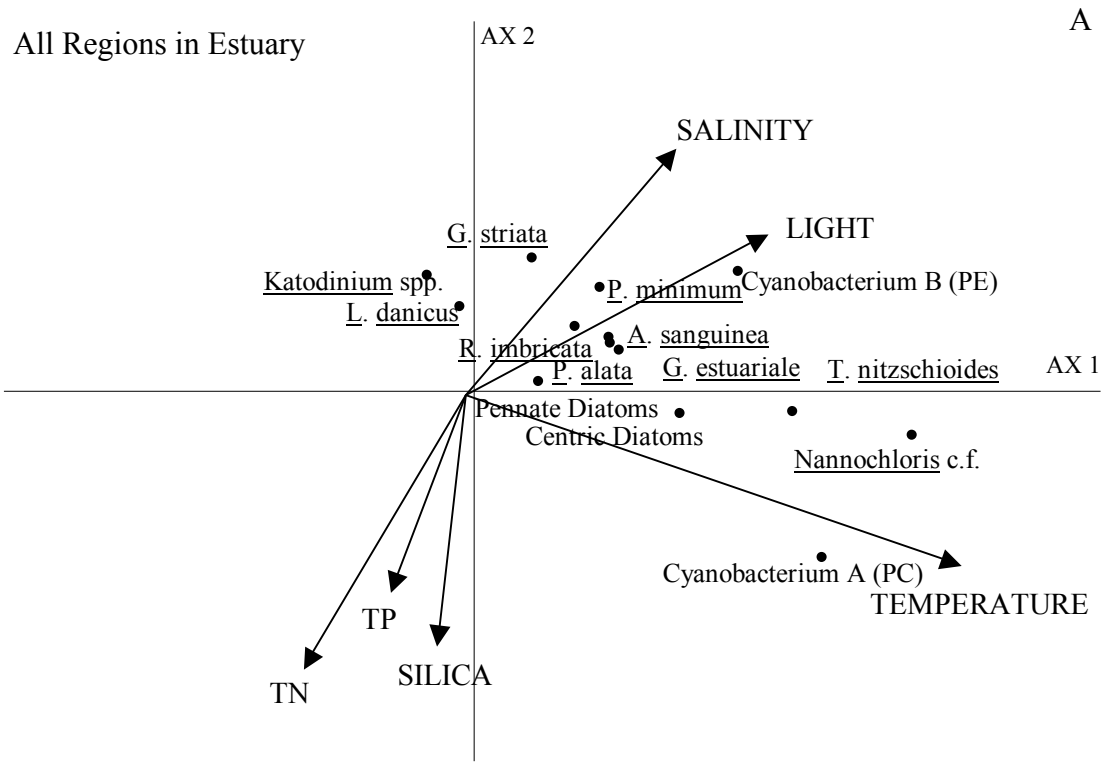

Figure 3-6. Canonical correspondence analysis of phytoplankton and environmental characteristics in the Suwannee River and its estuary, Florida, USA. A) Scores of overall environmental variables and species in the plane of the first two axes of the CCA ordination for all regions in the estuary, B) reef region, C) nearshore region, and D) offshore region.

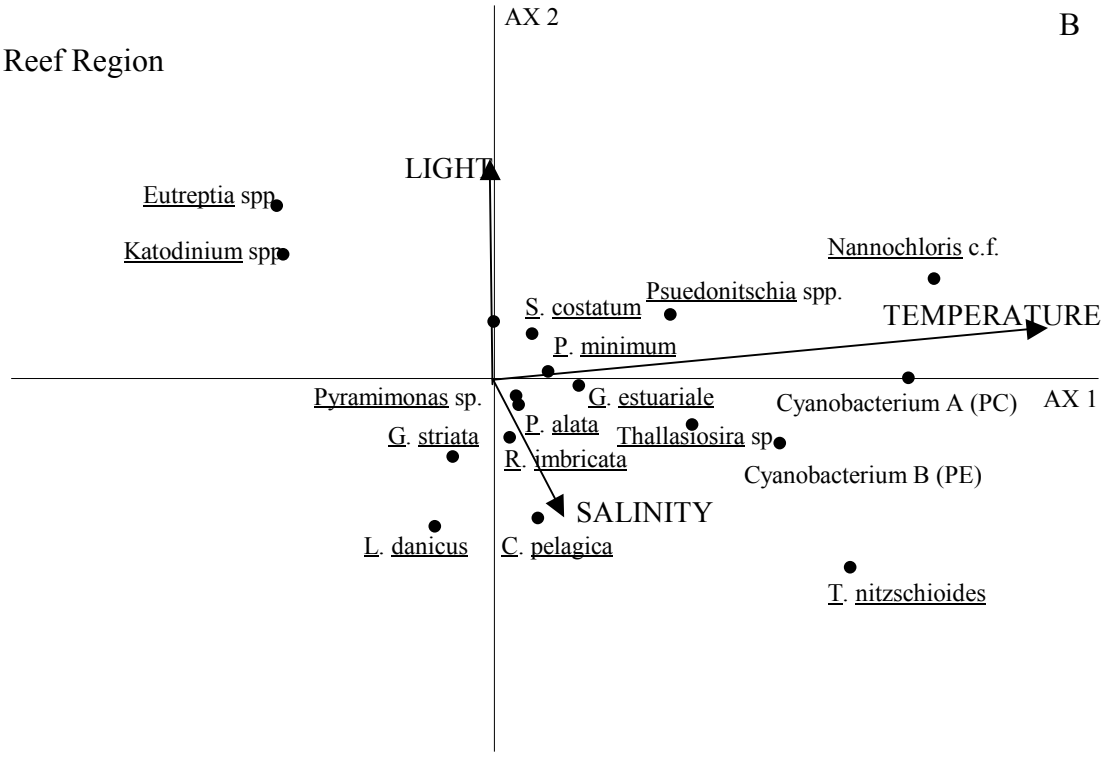

Figure 3-6. Continued

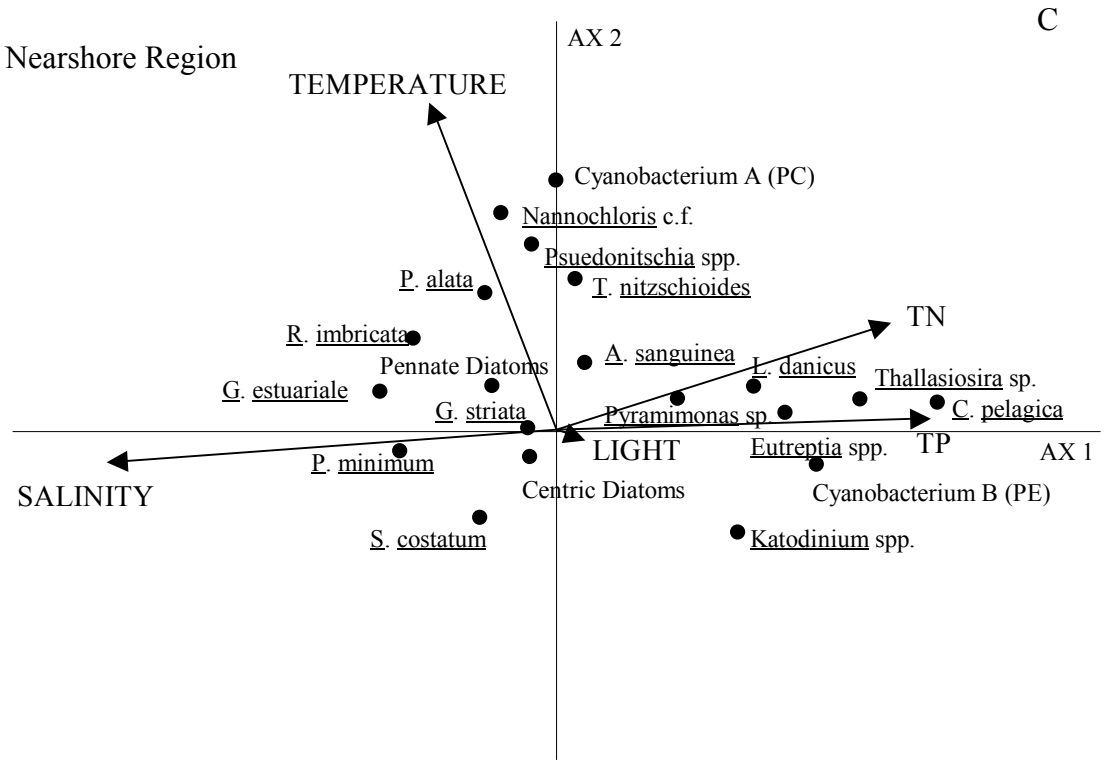

Figure 3-6. Continued

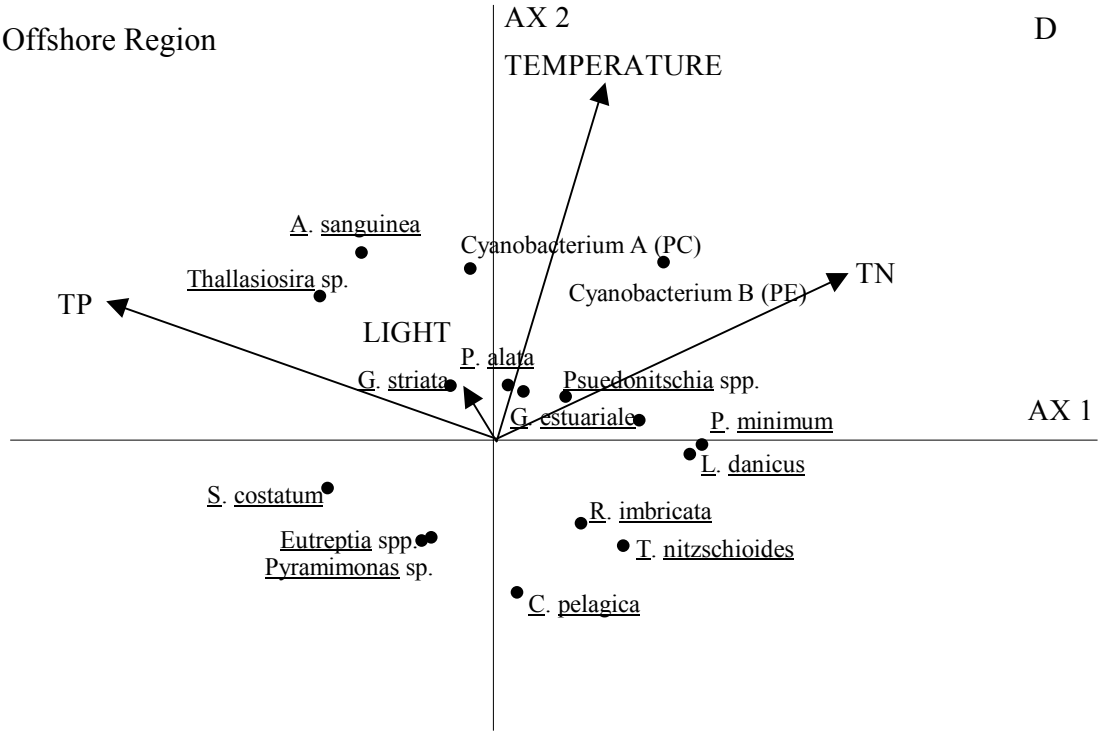

Figure 3-6. Continued

CHAPTER 4
MICROZOOPLANKTON GRAZING AND THE CONTROL OF PHYTOPLANKTON BIOMASS IN AN ESTUARY INFLUENCED BY BLACKWATER DISCHARGE, SUWANNEE RIVER ESTUARY, USA

Introduction

During the past century, coastal nutrient loading has significantly increased, resulting in elevated phytoplankton biomass in many regions of the world (Revelante and Gilmartin 1976, Nixon 1995, Valiela et al. 1997, Paerl et al. 1998). Increased input of nutrients has also been implicated in the increase of harmful algae blooms, hypoxia, diminished harvest of invertebrates, and changes in the dynamics of local food webs (Jørgensen 1980, Shumway 1990, Smayda 1990, Turner and Tester 1997, Anderson et al. 2002). Historically, much of the research on the eutrophication of marine waters has focused on the impact of nutrient availability on algal biomass and phytoplankton species composition (Ingrid et al. 1996). However, the potential impacts of increased nutrient loads must also be viewed in the context of loss processes such as transport, dilution, sinking, and mortality (Banse 2002). A change in the magnitude in any of these factors can enhance or diminish the potential of algal blooms resulting from nutrient enrichment.

Mortality due to zooplankton grazing is a critical factor in phytoplankton dynamics. In the open ocean, grazing plays a large role in the control of phytoplankton standing crop, as rates of phytoplankton growth and grazer-induced mortality can nearly balance each other (Banse 1992). High rates of grazer-induced mortality on phytoplankton have also been observed in many estuarine and coastal environments, including the New York Bight (Dagg and Turner 1982), the northern Gulf of Mexico

(Strom and Strom 1996), the plume of the Hudson River, USA (Malone and Chervin 1979), Hiroshima Bay, Japan (Kamiyama 1994), and the Celtic Sea (Burkill et al. 1987). As in freshwater lakes (Carpenter and Kitchell 1993), micro-zooplankton grazing may induce cascading effects on the trophic status of coastal systems impacted by nutrient loading, as observed in the Rhode River, Maryland (Gallegos 1989) and the northern Gulf of Mexico (Dagg 1995), where micro-zooplankton removed up to 100% of phytoplankton production per day. The potential for micro-zooplankton to control phytoplankton biomass is strengthened by the fact that some micro-zooplankton grazers have growth rates similar to phytoplankton (Montagnes 1996), which enables them to exploit pulses of phytoplankton growth.

The goal of this study was to determine the seasonal impacts of micro-zooplankton (>5 to 200 μm) grazing on phytoplankton biomass in the Suwannee River estuary. Prior studies in the Suwannee River estuary suggest that phytoplankton dynamics may be subject to "top-down" controls as well as chemical and physical forcing factors (Bledsoe and Phlips 2000). This suggestion is based on observations of high densities of micro-zooplankton within this region. In addition, estuarine waters adjacent to the river are subject to periodic inflows of highly colored freshwater, which may play a role in determining the composition of the micro-zooplankton community. Protozoans have been shown to play a dominant role in the grazing activities in many blackwater systems (Meyer 1990) and can facilitate the transfer of energy to higher organisms. In addition, protozoans can be an important food source for some larval fish species (James and Hall 1995) and bivalves (LeGall et al. 1997). The hypothesis that protozoans play a

significant role in the Suwannee River due to the blackwater nature of the Suwannee River, is explored in this paper.

Material and Methods

Study site description. Micro-zooplankton grazing experiments were conducted monthly from January 2001 to January 2002 using whole water samples collected from the Suwannee River estuary (Figure 4-1). Located at approximately latitude 29°17.50′ N and longitude 83°10.00′ W, the Suwannee River coastal area includes a delta region near the river mouth, which is characterized by a network of oyster reefs. This coastal region is generally well mixed due to its shallow depth (<1.5 m), although it can become vertically stratified depending on tide, wind, and river discharge (Orlando et al. 1993).

Experimental procedure. Micro-zooplankton grazing rates and phytoplankton growth rates were estimated under laboratory conditions using a dilution technique (Landry and Hassett 1982). Seawater (approximately 80 to 90 liters) was collected with a water column integrating tube (DeVries and Stein 1991), which captures water from the surface to 0.1 m above the sediment, and transported in acid-washed carboys to the laboratory. Half of the seawater was filtered through a 0.2-µm Millipore filter with a peristaltic pump and used as the dilution medium. The remaining seawater was combined with filtered seawater in dilutions of 100, 80, 60, 50, 40, and 30 % for a total volume of 2-liters. High densities of large gelatinous zooplankton (e.g., ctenophores) in the fall were excluded from all dilutions using a 1000-µm-mesh screen. Removal was necessary due to difficulty in replicating treatments with similar gelatinous zooplankton densities. Experiments were conducted in 3-liter polycarbonate incubation containers in duplicate. A second series of dilution experiments was performed monthly from August

2001 to January 2002 with samples collected at the same time and station. These samples, however, were pre-filtered with a 202-µm mesh Nitex screen to exclude meso-zooplankton before incubation.

Incubations were conducted in a temperature-controlled room set at field water temperatures recorded on each sampling date (Table 3-1). Full spectrum light intensity was fixed between 100 and 120 µE m^{-2} s^{-1}. Photoperiod was 12/12 dark/light h, respectively, from October through March and 10/14 dark/light h from April through September. As nutrient limitation has been observed in this region of the estuary (Bledsoe and Phlips 2000), excess nutrients (KNO_3, KPO_4, and $NaSiO_4$) were added to yield a final concentration of 400 ug N L^{-1}, 40 ug P L^{-1}, and 400 ug Si L^{-1} in each container to prevent nutrient limitation of phytoplankton growth. The dilution containers were incubated for 24 h under gentle stirring using stir bars (78 x 12mm) at 60 rpm.

Sample analysis. Water samples were subdivided on site into aliquots for chlorophyll a, phytoplankton and zooplankton composition, color and water chemistry analyses. Color was analyzed spectrophotometrically against platinum cobalt standards. Total nitrogen, nitrate + nitrite, ammonium, total phosphorus, soluble reactive phosphorus, and silica were analyzed using standard methods for water analysis (APHA 1995).

Chlorophyll a concentrations were used to estimate phytoplankton biomass in the initial and final samples. Subsamples (500 ml) were filtered onto 0.3-µm glass-fiber filters (Gelman A/E) then frozen for at least 24 h. Filters were thawed, placed in test tubes with 8.0 ml of 95% ethanol and heated in a water bath for 5 min at 78 °C. After passive extraction (24 h), the filters were removed, and samples were centrifuged to

exclude particulate debris. Chlorophyll a concentrations were determined with a Hitachi U2000 dual-beam spectrophotometer (Sartory and Grobbelaar 1984). The concentrations were corrected for phaeophytin using the acidification method (APHA 1989). Initial and final algal biomass was also estimated by net *in vivo* fluorescence of Chl-a (*IVF*) using a Turner Designs Model 10 fluorometer with a 1-cm path length.

Fluorescence microscopy was used to enumerate small-celled cyanobacteria and chlorophytes (Fahnenstiel and Carrick 1992). Subsamples of station water were filtered onto 0.2-µm pore Nucleopore filters and analyzed with a Nikon research microscope equipped with autofluorescence for phycobilins (530 to 560-nm excitation and >580-nm emission) within 72 h. Numerical abundance of cyanobacteria cells was determined by counting a minimum of five ocular micrometer grids at 1000x. The number of grids counted was determined by cell density. Counts were completed on reaching a minimum of 100 cells.

Phytoplankton and micro-zooplankton (<50-µm) composition were determined using the Utermohl method (1958). Lugol's preserved samples were settled in 19-mm diameter cylindrical chambers. Phytoplankton cells were identified and counted at 400x and 100x with a Nikon phase-contrast inverted microscope. At 400x, counts were completed upon reaching 100 cells of a single dominant taxa, but a minimum of 30 ocular micrometer grids was counted. Large-celled phytoplankton taxa (>30 µm) were enumerated by a complete count of the settling chamber at 100x. Larger zooplankton (>50 µm) were concentrated from 1000 ml to 100 ml using a 41-µm-mesh Nitex screen and enumerated by a complete count of the settling chamber at 100x. Counts were completed upon reaching 100 individuals of the dominant taxa.

Nutrient limitation bioassay experiments were performed each month to determine the potential factors limiting phytoplankton growth. The experimental design was based on methods described by Aldridge et al. (1995). Assays were conducted under laboratory conditions in 500-mL Erlenmeyer flasks with 400 mL of seawater. Treatments (in triplicate) included control (no additions), N addition (400 µg N L^{-1}), P addition (40 µg P L^{-1}), Si additions (400 µg Si L^{-1}), N + P addition (400 µg N L^{-1} + 40 µg P L^{-1}), and N+P+Si (400 µg N L^{-1} + 40 µg P l^{-1} + 400 µg Si L^{-1}).

Incubations were conducted in temperature-controlled water baths with bottom illumination under the same conditions as the dilution experiments. Algal biomass was estimated by net *in vivo* fluorescence of Chl-a (*IVF*) using a Turner Designs Model 10 fluorometer with a 1-cm path length at time for up to 7 days, or until fluorescence values peaked. Ethanol-extracted chlorophyll a concentrations were determined at time 0, and at the point where fluorescence values did not increase exponentially.

Under nutrient-limiting conditions, the addition of that nutrient, or combination of nutrients, resulted in significantly greater growth than the control ($P < 0.05$). When algal growth in the control treatment increased relative to the initial control, it was concluded that surplus bioavailable nutrients were present at the time of sampling. The classification for the growth response was determined using Boolean logic (Aldridge 1995). Relationships between different variables and treatments were determined using ANOVA analysis.

Micro-zooplankton grazing was determined from measurements of the apparent growth rate of phytoplankton using the exponential growth equation of Landry and Hassett (1982) shown in Equation 4-1.

$$P_t = P_0 \exp [(\underline{k} - \underline{g}) t] \quad (4\text{-}1)$$

where $\underline{k}$ is the exponential growth rate of phytoplankton (day^{-1}), $\underline{g}$ is the specific grazing rate of the micro-zooplankton (day^{-1}), t is the time in days, P_t and P_0 are the final and the initial chlorophyll a concentration, based on initial whole water chlorophyll a concentration and dilution factor. Values of $\underline{k}$ and $\underline{g}$ were determined from linear regression of the apparent phytoplankton growth rate against the fraction of undiluted seawater. Linearity of this relationship was subjected to a lack-of-fit test at the 0.05 level (Evans and Paranjape 1992). If the regression analysis of the apparent growth rates against the dilution factor was non-linear, phytoplankton growth rate ($\underline{k}$) was calculated from the three-point method of Gallegos (1989). Under these conditions, grazing rate ($\underline{g}$) was estimated as shown in Equation 4-2.

$$\underline{g} = -[P_t / P_0 - e^{(\underline{k}t)}] * [(\mu_z - \underline{k}) / (e^{(\mu_z t)} - e^{(\underline{k}t)})] \quad (4\text{-}2)$$

where μ_z is net micro-zooplankton growth rate (day^{-1}) in undiluted bottles, and $\underline{k}$ is calculated using the three-point method. This equation accounts for biases in estimates of $\underline{g}$ due to micro-zooplankton growth under conditions of grazing saturation.

Zooplankton and phytoplankton biomass was expressed as biovolumes (cubic microns ml^{-1}) based on geometric shape. Equivalent spherical diameters (ESD) were used to express predator and prey sizes and defined as the diameter of a sphere with equal volume (biovolume = $\pi/6ESD^3$).

Results

Environmental conditions. The sampling site in the Suwannee River estuary ranged from 1.4 to 2.8 m (mean = 2.2 ±0.4 m) in depth. Water temperature during the experiments ranged from 8.9 to 30.5 ºC (mean = 21.5 ± 7.8 ºC) and salinity from 14.6 to

30.6 ppt (mean = 24.1 ± 4.9 ppt) (Table 4-1). The relatively high salinity range is indicative of variation in the flow rates of the Suwannee River. The lowest salinities were observed during periods of relatively high freshwater discharge.

Due to proximately of the Suwannee River outflow, the highest concentrations of bioavailable nutrients were observed during periods of high riverine flow. Nitrate + nitrate concentrations ranged from 0 to 181 μg N L^{-1}, ammonium ranged from 9 to 211 μg N^{-1} L^{-1}, and soluble reactive phosphorus from 1 to 46 μg P L^{-1}. Nutrients were limiting on several occasions corresponding to lower riverine flow and lower nutrient inputs (Table 4-2). On all dates of nutrient limitation, nitrogen was the primary limiting nutrient.

Phytoplankton community. Phytoplankton biomass estimated by chlorophyll a varied between 2.92 and 21.1 μg chl L^{-1}. Peak phytoplankton biomass was observed during September and October 2001. Rates of nutrient-enhanced phytoplankton growth (k) ranged between 0.41 to 2.74 day^{-1} (Table 4-3). Phytoplankton growth rates in the experimental containers were generally lower in January, February, and March (mean = 1.06 day^{-1}) when water temperatures were lowest (~13 ºC). Higher phytoplankton growth rates were observed during warmer periods (mean = 2.23 day^{-1}, water temperature ~ 27 ºC).

Microscopic analysis of the samples indicated that diatoms accounted for the highest percentage (>70%) of the phytoplankton biomass (biovolume in μm^3 ml^{-1}) in the Suwannee River estuary during the dilution experiments (Figure 4-2A). Common taxa included Chaetoceros spp., Thallassionema nitzschioides, Rhizosolenia spp., Leptocylindrus danicus, and centric diatoms 5 to 10 μm in diameter. Picoplanktonic

cyanobacteria 1 to 2 µm and chlorophytes 2 to 4 µm (c.f. Nannochloropsis sp.) accounted for up to 15% and 55% of total phytoplankton biomass, respectively, during summer and fall. These taxa also dominated overall phytoplankton abundance (cells ml^{-1}) throughout the year and accounted for up to 78% of the variability in chlorophyll a (Figure 4-2B and Figure 4-3.). Autotrophic dinoflagellates were generally low (<10% of the biomass). Other phytoflagellates such as cryptophytes, Eutreptia spp. and Pyramonis sp. accounted for up to 30% of the total phytoplankton biomass in spring. Overall, the composition of the phytoplankton community was dominated by taxa <5 µm equivalent spherical diameter (ESD), which accounted for 91 to 99% of total phytoplankton abundance. Picoplanktonic cyanobacterium 1 to 2 µm and chlorophytes 2 to 4 µm comprised the majority of phytoplankton within this size class (Figure 4-4).

Growth rates of individual taxon varied during the experiment. Many genera increased over the 24-h incubation period; however, pico-phytoplankton including cyanobacteria and chlorophyta exhibited distinct seasonal differences in growth rates. For example, during the majority of the year, pico-phytoplankton had some of the highest growth rates (e.g., >1 doubling day^{-1}). Similar or even higher growth rates were expected during the warmer months; however, negative growth rates were observed during these periods indicating a loss potentially due to grazing or other mortality (Figure 4-5).

Micro-zooplankton populations. Aloricate ciliates and tintinnids dominated the micro-zooplankton community throughout the year. The most common taxa included oligotrichs of the genera Strombidium, Strobilidium, and Halteria. These ciliates accounted for up to 52% of total biomass (Figure 4-6A), with densities ranging from 32 to 56 individuals ml^{-1} throughout the year (Figure 4-6B). Tintinnid genera were also

numerous throughout the year and included Eutintinnus, Salpingella, Amphorella, Favella, and Tintinnopsis. Tintinnid ciliates accounted for up to 50% of the total micro-zooplankton biomass. Heterotrophic dinoflagellates were generally low in abundance, (i.e., <20% of the total biomass); however, high densities of Katodinium rotundum and K. glaucum were observed during January 2001 and 2002 (up to 44% of the total biomass, 700 individuals ml^{-1}). The most frequently occurring dinoflagellates included Gymnodinum, Gyrodinium, Torodinium, Protoperidinium, and Prorocentrum. Copepod nauplii and copepodites were also observed in the micro-zooplankton community, accounting for up to 67% of the total biomass during the warmer months. The highest densities were observed during June 2001 (860 individuals L^{-1}). Overall, the composition of the micro-zooplankton community was dominated by taxa <25 μm equivalent spherical diameter (ESD), which accounted for 78 to 99% of total micro-zooplankton abundance. Ciliates (including tintinnids) and heterotrophic dinoflagellates comprised the majority of micro-zooplankton within this size class (Figure 4-7B).

Meso-zooplankton populations. Meroplanktonic larvae (e.g., echinoderm, bivalve, polychaete, and gastropod) were observed within water samples used for the dilution experiments, and were numerous during summer (Figure 4-8). Gelatinous zooplankton were prevalent during the spring and fall and included larvaceans, medusa, chaetognaths, and salps. All taxa were observed in both the whole and pre-filtered (202-μm mesh) samples during the initial and final counts. Ctenophores (e.g., Mnemiopsis sp.) occurred in high densities during the fall and were removed from all samples collected from August to November 2001.

The abundance of adult copepods in the dilution experiments varied throughout the year. The three major groups of copepods were represented. Calanoids were dominated by Acartia spp., cyclopoids were dominated by Oithona spp., and harpactacoids were dominated by Microsetella spp. in the experiments. The highest densities were observed during June 2001 due to a large number of Oithona spp. (Figure 4-9).

It was not possible to remove all meso-zooplankton prior to the grazing experiments using 202-μm mesh screening, due to the varying size and orientation of the individuals. The mesh screening procedure also removed a significant proportion of phytoplankton biomass (10% of the chlorophyll a), primarily large cells and chain-forming diatoms. Therefore, all experiments were conducted on whole water with the consequence of increased micro-zooplankton mortality due to predators.

Growth and grazing rates. The results of micro-zooplankton grazing experiments are summarized in Table 4-3 and Figure 4-10. The relationship between the apparent growth rate of phytoplankton and dilution factor appeared non-linear in several of the dilution experiments. However, none of the data sets could be rejected by the lack-of-fit test for linearity ($P < 0.05$) (Evans and Paranjape 1992). The potential for saturation of micro-zooplankton grazing is consistent with the high initial phytoplankton standing crops observed during the warmer months. Although all experiments fit the linear model, phytoplankton growth rate (k) and grazing rate (g) were also calculated using the three-point method of Gallegos (1989) even though no incubations were carried out at the recommended high dilutions. The instantaneous grazing rate (g) for this method required an estimate of the net rate of increase of micro-zooplankton during incubation, which was highest during summer and fall (mean community growth rates ~ 1.7 day^{-1}, max = 1.95

day^{-1}) (Table 4-3). The three-point estimates of g were within 20% of the estimates based on linear regression in ten of the experiments. In the remaining experiments, three-point estimates of g were within 40% of the values derived from linear regression.

Micro-zooplankton grazing rate coefficients ranged from 0.11 to 1.41 day^{-1} based on linear models. The highest grazing rates occurred during the spring and early summer experiments of April, May and June (mean = 1.36 day^{-1}) and were equivalent to 50-60% of nutrient enhanced phytoplankton growth (Table 4-3) corresponding to grazing impacts of 73 to 76 % of phytoplankton standing crop per day. Following Landry and Hasset (1982), and assuming (1) steady state and (2) growth rates are similar to those at ambient nutrient levels, the grazing rates estimated for April, May, and June correspond with 17, 19, and 15% of the daily phytoplankton production, respectively.

The net growth rates of the micro-zooplankton community ranged from 0.27 to 1.75 day $^{-1}$ peaking during June through August (Table 4-3) and were positively correlated with phytoplankton growth (Figure 4-11). Growth rates of the individual micro-zooplankton taxa ranged from –0.6 to 2.5 day^{-1}. Ciliates (aloricate and loricate) showed exceptionally high growth rates (>1 day^{-1}), while heterotrophic dinoflagellates showed negative rate of increase for most of the experiments (Table 4-4). In addition, growth rates of ciliates showed a significant relationship to both initial phytoplankton biomass (Table 4-5) and initial pico-phytoplankton densities (Pearson $r^2 = 0.58$, $P < 0.001$) (Figure 4-12).

Discussion

Composition of the micro-zooplankton community. The composition of the micro-zooplankton community was dominated by nauplii, hetero/mixotrophic dinoflagellates, and ciliates. From the standpoint of grazing impact, it is useful to

examine these major micro-zooplankton components in terms of feeding guilds. Although nauplii were not as abundant as ciliates and dinoflagellates, they did comprise a large percentage of overall micro-zooplankton biomass in the warmer months. The major phytoplankton taxa preyed upon by nauplii were examined using the optimal predator-to-prey size ratio method proposed by Hansen et al. (1994). Copepod nauplii have an optimal predator-to-prey equivalent spherical diameter (ESD : ESD, see Hansen et al.1994) near 18 ± 4:1. Based on the average copepod nauplii ESD in the experiments, the optimal ESD for phytoplankton ranged from 4 to 12 μm and included copepod nauplii, small dinoflagellates (e.g., Katodinium, Gyrodinium, Scrippsiella), other phytoflagellates (e.g., cryptophytes, Pyramonis), diatoms (e.g., Cyclotella, Thalassiosira, Thallassionema, Skeletonema), and small ciliates (e.g., less than 10 μm ESD). Ciliates have predator-to-prey ratios similar to those of copepod nauplii, 2.5:1 to 30:1. In addition to the phytoplankton taxa available to copepod nauplii, ciliates are able to utilize smaller phytoplankton prey including picoplanktonic cyanobacteria and chlorophytes, as well as some bacteria (Callieri et al. 2002).

Dinoflagellates differ considerably as the predator-to-prey range extends from 0.4:1 to 7:1. Thus, the prey available to dinoflagellates includes the same taxa as naupllii and ciliates, but can also include prey larger than the actual predator. The size range of the heterotrophic dinoflagellates observed within the experiments, 10 to 45 ESD, implies prey sizes within the range of <1-μm to >100-μm ESD. That size range includes a number of larger diatoms, e.g., Rhizosolenia spp., Coscinodiscus spp., as well as longer chain-forming species, e.g., Skeletonema costatum, Leptocylindrus danicus. Strom and Strom (1996) observed ingestion of chain-forming diatoms by the dinoflagellate,

Gyrodinium. Of the three dominant micro-zooplankton taxa, the prey sizes are capable of consuming small bacteria-sized prey to some of the largest diatom chains.

Food-web structure is dependent on the actual abundance of predators with preference for certain prey size ranges (Hansen et al. 1994). Micro-zooplankton <25-µm ESD, including ciliates and heterotrophic dinoflagellates, accounted for up to 99% of total abundance at one site within the estuarine waters adjacent to the Suwannee River (Figure 4-6). The prey size class, under the greatest grazing pressure, should therefore include the smaller phytoplankton (e.g., pico-cyanobacteria and pico-chlorophytes). Some ciliates and heterotrophic flagellates prefer to consume pico-cyanobacteria rather than bacteria (Pernthaler et al. 1996, Callieri et al. 2002). This is of particular importance to the Suwannee River estuary, where the phytoplankton community is sometimes dominated by pico-cyanobacteria and 2 to 4-µm spherical green algae, particularly during warmer months (Chapter 3). Based on the idea that ciliates and heterotrophic dinoflagellates are considerable consumers of pico- and nano-sized fractions of the plankton, high grazing mortality on this size fraction of the phytoplankton standing crop should occur during periods of high predator abundance. This relationship appears evident as pico-cyanobacteria densities declined during the dilution experiments from May to September 2001 (Figure 4-3).

A further basis for suggesting the importance of micro-zooplankton grazing lies in the fact that ciliates and dinoflagellates have growth rates similar to those of phytoplankton (Montagnes 1996). Rapid grazer response to prey availability potentially enables micro-zooplankton to exploit small and ephemeral patches of phytoplankton. In this study, grazing rate coefficients (g) increased significantly with net rates of

phytoplankton growth (k) (Pearson $r^2 = 0.84$, $P < 0.001$) (Figure 4-11). Further, ciliate growth rates were significantly correlated with initial densities of pico-phytoplankton (Pearson $r^2 = 0.58$, $P < 0.001$) suggesting a response by the ciliates to increasing prey availability (Figure 4-12). In addition, standing crops of zooplankton have also been positively correlated with chlorophyll concentrations in the tropical Pacific (Beers and Stewart 1971) and in experimental manipulations with nutrient enrichments (Fulton 1984).

The classic food chain model in which phytoplankton are consumed by large zooplankton, which are then preyed on by larval and small fish, has been modified to incorporate the role of ciliates in sequestering bacteria and pico-phytoplankton, therefore adding loops to the more classic food chain (Pomeroy 1974, Azam et al. 1983). The "microbial loop" has been debated as an energy sink, as part of the energy entering the loop is lost via respiration, thereby lowering the overall efficiency of energy transfer to higher trophic levels. However, the microbial foodweb may also act as a link to larger consumers. For example, ciliates feeding on pico-phytoplankton are utilizing a food source that is difficult for the classic grazers (i.e., copepods) to consume (Nival and Nival 1976, Porter et al. 1979). Since small ciliates have higher metabolic efficiencies than larger zooplankton on a body-size basis (Findlay 1978), the "microbial loop" has also been considered an energy link to higher trophic levels. This has important consequences as several species of copepods can feed and proliferate on ciliates (Gifford and Dagg 1988, Pierce and Turner 1992, Sherr et al. 1986, Stoecker and Capuzzo 1990).

The importance of ciliates as prey extends to the benthic invertebrate community in the Suwannee River estuary. Adult bivalves do not efficiently retain pico-phytoplankton

(<2μm) as an energy source (Pouvreau et al. 1999). However, LeGall et al (1997) reported significant retention and ingestion of ciliates by oysters, supporting the role of protozoa as a realistic trophic link between pico-phytoplankton and higher organisms.

Importance of ciliates within the Suwannee River estuary. Ciliates are an important component of the micro-zooplankton in the Suwannee River estuary, as in many marine waters (Pierce and Turner 1992). However, total ciliate abundance in the water column from 0-3m (9,400 – 72,800 L^{-1}) is significantly higher than that reported for several well-studied marine ecosystems; Narragansett Bay, USA (5 to 2,800 L^{-1}; Verity 1986), the Mediterranean (200 to 20,000 L^{-1}; Sherr et al. 1989), Oslofjord, Norway (2,000 to 10,500 L^{-1}; Paasche and Kristiansen 1982), and the California Current (2,400 to 18,000 L^{-1}; Beers and Stewart 1971). The annual mean abundance of ciliates within the Suwannee River estuary (53,000 L^{-1}) is comparable to densities observed in meso- to eutrophic environments such as Dalnee Lake, USSR (18,000 to 42,000 L^{-1}; Sorokin and Paveljeva 1972), Damariscotta estuary, USA (20 to 46,000 l^{-1}; Sanders 1987), eutrophic Florida lakes (annual mean 55,000 L^{-1}; Beaver and Crisman 1982), and Tokyo Bay, Japan (10,000 to 100,000 L^{-1}; Kume 1979).

Beaver and Crisman (1982) suggested that protozoans played an increased role in freshwater systems with increasing nutrient concentrations. Similarly, Park and Marshall (2000) observed an increase in the percent contribution of micro-zooplankton to the total zooplankton community with increasing trophic status in the lower Chesapeake Bay and Elizabeth River, USA. However, it is not clear whether high nutrient, organic carbon, or riverine ciliate inputs to the estuarine waters adjacent to the Suwannee River are responsible for the high concentrations of ciliates.

It is possible that high ciliate biomass in the estuary is a consequence of transport from the Suwannee River. However, Suwannee River ciliate populations are relatively low (i.e., <100 L^{-1}, Bledsoe unpublished data), which does not support this hypothesis. The low abundance of ciliates within the river can be attributed to relatively high discharge rates of the river (mean discharge of 301 m^3s^{-1}), which reduce prey availability and residence period. Alternatively, high ciliate abundance in other blackwater systems has been linked to high dissolved organic carbon (e.g., Florida lakes; Beaver and Crisman 1982 and blackwater rivers; Findlay et al. 1986). Large inputs of humic substances are a likely source of carbon for bacteria. Carlough and Meyer (1989) reported that ciliate concentrations were significantly correlated with dissolved organic carbon and river discharge, and ranged from 600 to 150,000 ciliates L^{-1} in the blackwater Ogeechee River, Georgia. Colored dissolved organic matter in the Suwannee River is derived from the leaching of humic substances from the soils within the watershed. Carlsson et al. (1995) observed utilization of humic substances by marine bacteria and stimulation of the 'microbial loop' in the form of increased ciliate abundance. It is possible that the high concentrations of ciliates within the Suwannee estuary may be a secondary effect of increased microbial production due to humic inputs. However, the ciliate abundance in the Suwannee River estuary could not be correlated with color (as a proxy for humic substances) or riverine discharge in this study. Similar arguments could be made for the impact of high levels of nutrient loads from the Suwannee River on estuarine pico-phytoplankton production. However, this argument is also limited by the apparent lack of a positive relationship between pico-phytoplankton biomass and nutrient loading.

Control of phytoplankton production by micro-zooplankton grazing in the Suwannee River estuary. The observed ranges for maximum phytoplankton growth ($\underline{k}$) (0.45 to 2.7 d^{-1}) and grazing ($\underline{g}$) (0.12 to 1.4 d^{-1}) were within the ranges reported for other estuarine/nearshore regions globally (Table 4-6). Under laboratory conditions, micro-zooplankton grazing rates were high enough to impact phytoplankton standing crop significantly. Grazing loss rates of up to 75% per day were observed during the one-year study period; yet, phytoplankton growth rates were greater than micro-zooplankton grazing rates (i.e,. $\underline{k} > \underline{g}$) in all experiments.

Murrell et al. (2002) compiled a number of micro-zooplankton grazing studies across a wide range of habitats. Micro-zooplankton grazing rates exceeded phytoplankton growth rates in only a small fraction of studies (e.g., Tomales Bay; Murrel and Hollibaugh 1998, Celtic Sea / Carmarthen Bay; Burkill et al. 1987, and in the Sargasso Sea; Lessard and Murrell 1998). This is an indication that the loss of phytoplankton standing crop may be attributed to other factors such as export, sinking, dilution, senescence, and other grazing activities beyond the micro-zooplankton community. For example, cell lysis accounted for 75% of phytoplankton death at the end of the spring bloom in the North Sea (Brussaard et al. 1995) and up to 50% in the Mediterranean Sea (Agustí et al. 1998).

The fact that phytoplankton growth rates exceeded grazing rates in the laboratory under nutrient enhanced conditions indicates that there was always a potential for an increase in phytoplankton biomass. However, such optimal conditions may not exist *in situ*. There are several reasons why the impact of grazing may be more or less significant in the natural environment. For example, phytoplankton standing crops within the

estuary are often subjected to nutrient limitation (Table 4-2). In addition, ambient light availability in the Suwannee River estuary can also be limiting during periods of high river discharge and/or sediment resuspension (Bledsoe and Phlips 2000). Under these conditions, phytoplankton growth can be inhibited, and the stress of nutrient and light limitation can increase the potential impact of grazing and cell mortality on phytoplankton dynamics (Berges and Galkowski 1998). In addition, benthic invertebrates (e.g., Crassostrea virginica and Mercenaria mercenaria) in the estuary are likely to contribute to the overall loss of phytoplankton standing crop in the water. Møhlenberg (1995) found reduced phytoplankton biomass in the lower water column directly above mussel beds in the Roskilde Fjord, Denmark and similar findings were reported for San Francisco Bay, USA (Cloern 1982).

Micro-zooplankton grazing may be less significant under conditions of vertical stratification in the water column. During periods of stratification, phytoplankton biomass may increase in the upper water column in response to enhanced light and nutrient availability with reduced mixing depth, reducing the impact of grazing loss on phytoplankton biomass. Predation by meso-zooplankton on micro-zooplankton may further reduce the impact of grazing on phytoplankton. Meso-zooplankton are a significant source of micro-zooplankton mortality (e.g., calanoid copepods; Dagg and Turner 1982, appendicularians; Alldredge 1984, and salps; Perissinotto and Pakhomov 1998). Many of these organisms were frequently observed in the estuary and may have contributed to a loss in micro-zooplankton. For example, a common cyclopoid copepod, Oithona, was frequently observed in the estuary. It has been suggested that this copepod preferentially preys upon ciliates (Nielsen and Sabatini 1996). In addition, a noticeable

decrease in micro-zooplankton was observed during the fall 2001, following a period of high densities of meroplanktonic larvae (e.g., cnidarian planula 485 L^{-1}) and gelatinous zooplankton (e.g., ctenophores).

From the results of this study it appears micro-zooplankton grazing is likely to have a significant impact the phytoplankton biomass within the Suwannee River estuary. The magnitude of this impact, however, is dependent on a range of physical, chemical and biological conditions. Therefore, the experimental grazing estimates obtained from this study represent the minimum impact potential of grazing activities on phytoplankton biomass.

Table 4-1. Environmental conditions for the dilution experiments and initial chlorophyll concentration at Site C2 in the Suwannee River estuary, Florida, USA (µg Chl a L^{-1}).

	Total Depth (m)	Temperature (ºC)	Salinity (ppt)	Chlorophyll a (µg Chl a L^{-1})
1/24/01	1.5	11.2	22.3	4.65
2/26/01	2.1	18.9	25.5	4.74
3/26/01	2.0	17.3	20.2	7.52
4/23/01	2.8	23.4	25.1	7.24
5/21/01	2.5	26.3	28.9	9.36
6/18/01	2.4	29.3	26.5	10.70
7/23/01	2.3	29.4	15.7	9.43
8/20/01	2.1	30.5	14.6	12.30
9/24/01	1.4	28.9	25.2	21.10
10/23/01	1.8	23.8	27.0	16.20
11/27/01	2.6	22.3	30.6	7.17
1/28/02	2.5	8.9	23.0	2.92
1/9/02	2.4	9.0	28.8	2.92

Table 4-2. Initial nutrient concentrations measured for the dilution experiments at site C2 in the Suwannee River estuary, Florida, USA.

	Nitrate $\mu g\ N\ L^{-1}$	Nitrite $\mu g\ N\ L^{-1}$	Ammonium $\mu g\ N\ L^{-1}$	Phosphorus $\mu g\ P\ L^{-1}$	Silica $\mu g\ Si\ L^{-1}$	Limiting Nutrient
1/24/01	*65	-	25	5	2.4	U
2/26/01	*7	-	9	4	1.3	N
3/26/01	*52	-	46	7	1.1	U
4/23/01	*0	-	55	3	0.3	N
5/21/01	*2	-	9	1	0.9	N
6/18/01	*0	-	17	3	1.2	N
7/23/01	*181	-	211	46	5.7	U
8/20/01	163	37	180	43	6.1	U
9/24/01	0	2	30	5	1.5	N
10/23/01	16	4	49	12	0.9	N
11/27/01	36	4	49	12	0.9	U
1/9/02	61	2	30	12	2.3	U
1/28/02	25	2	22	11	2.0	U

Note: The (*) indicates the nitrogen measurement of µg Nitrate + Nitrite-N L^{-1}. The limiting nutrient is based on nutrient limitation bioassays, where U indicates that nutrients were not limiting during the bioassays, N indicates nitrogen was the primary limiting nutrient, P as phosphorus limited and Si as silica limitation.

Table 4-3. Dilution experiment estimates of phytoplankton growth coefficients (k) (day $^{-1}$) and grazing rate coefficients (g) (day $^{-1}$) at site C2 in the Suwannee River estuary, Florida, USA, obtained using linear regression of apparent growth rate with dilution factor.

Whole Water Dilutions

		linear regression					3-point
	Initial Chl a	k	g	n	k	g	mz
1/24/01	4.65	0.41	0.31	8	0.80	0.56	0.47
2/26/01	4.74	0.45	0.12	12	0.46	0.11	1.48
3/26/01	7.52	0.81	0.27	12	0.67	0.15	1.43
4/23/01	7.24	2.34	1.34	12	2.66	1.52	1.17
5/21/01	9.36	2.74	1.33	12	2.99	1.67	1.22
6/18/01	10.7	2.37	1.41	12	2.76	1.47	1.41
7/23/01	9.43	2.49	1.02	12	3.34	1.66	1.75
8/20/01	12.3	2.67	1.22	6	2.48	0.97	1.55
*9/24/01	21.1	0.89	0.37	12	1.80	0.81	1.51
10/23/01	16.2	1.24	0.32	6	1.41	0.53	0.59
11/27/01	7.17	-	-	-	-	-	-
1/9/02	2.92	0.85	0.25	12	1.37	0.79	0.30
1/28/02	2.92	1.57	1.03	12	1.49	1.03	0.27

Pre-Filtered Water Dilutions

		linear regression		
	Initial Chl a	k	g	n
1/24/01	-	-	-	-
2/26/01	-	-	-	-
3/26/01	-	-	-	-
4/23/01	-	-	-	-
5/21/01	-	-	-	-
6/18/01	-	-	-	-
7/23/01	-	-	-	-
8/20/01	12.3	2.67	1.22	6
*9/24/01	20.1	1.74	1.45	12
10/23/01	14.8	1.42	0.68	6
11/27/01	7.17	1.51	0.90	8
1/9/02	2.92	0.93	0.97	12
1/28/02	2.92	1.74	0.56	12

Note: Although none of the experiments failed the lack-of-fit test ($P < 0.05$), (*) indicates non-linear feeding kinetics was observed, and growth and grazing estimates were obtained using the 3-point method (Gallegos 1989); m_z: micro-zooplankton net growth rates incorporated into grazing estimates. All experiments conducted using seawater pre-filtered with 202-µm mesh were not rejected by the lack of fit test for linear regression.

Table 4-4. Individual net growth rates (day $^{-1}$) for loricate (tintinnids), aloricate (other ciliates), nauplii, and heterotrophic dinoflagellates (Hdino) at site C2 in the Suwannee River estuary, Florida, USA.

	Loricate	Aloricate	Nauplii	Hdino
1/24/01	0.98	0.21	0.43	-0.15
2/20/01	1.32	1.71	0.35	-0.29
3/26/01	1.90	1.13	0.05	-
4/23/01	0.69	1.06	0.32	-0.01
5/21/01	1.64	0.69	0.00	0.68
6/18/01	2.47	1.07	-0.25	0.69
7/23/01	1.51	1.87	-0.23	1.37
8/20/01	2.50	0.77	0.55	-
9/24/01	0.79	1.60	0.03	-
10/23/01	0.69	0.55	0.18	-0.60
11/27/01	0.92	0.09	0.27	-
1/9/02	0.95	0.10	0.95	-
1/24/02	0.10	0.70	1.05	0.00

Note: A dash line indicates heterotrophic dinoflagellates were absent from the sample on this date.

Table 4-5. Regression analysis of chlorophyll a (independent variable) as an estimate of phytoplankton biomass against densities and growth rates of zooplankton.

Dependent variables	r	slope	intercept
Abundance			
Aloricate ciliates	*0.44	0.22	1.43
Loricate ciliates	-0.15	-0.21	1.29
Rotifers	*0.40	0.02	-0.01
Copepod nauplii	*0.46	0.14	-0.05
Total Mesozooplankton	*0.32	0.11	1.65
Total Microzooplantkon	*0.34	0.14	1.60
Growth Rates			
Tintinnid growth	*0.31	0.13	0.23
Ciliate growth	*0.63	0.26	0.03

Note: Values listed are the Pearson correlation coefficients (r) for the major zooplankton groups, with an asterisk (*) indicating significance at $\alpha = 0.05$. All data were transformed prior to analysis, $\log_{10} (x + 1)$.

Table 4-6. Literature value for average phytoplankton growth (k), micro-zooplankton grazing (g), and their respective ranges. Standard errors in parentheses. Selected data redrawn from Murrell et al. (2002).

Environment	k	range	g	range	Reference
Suwannee River Estuary	1.58 (0.83)	0.27 - 1.75	0.78 (0.44)	0.11 - 1.41	***
Chesapeake Bay, Upper	1.37 (0.69)	0.00 - 2.15	0.24 (0.46)	-0.22 - 1.60	McManus and Ederinton-Cantrell 1992
Halifax Harbor, Nova Scotia	0.77 (0.24)	0.24 - 1.68	0.34 (0.12)	0.02 - 0.72	Gifford 1988
Mobile Bay, Lower,	1.27 (0.19)	0.25 - 2.87	0.97 (0.18)	-0.03 - 2.44	Lehrter et al. 1999
Mundaka Estuary, Lower	2.35 (0.22)	1.21 - 3.41	0.94 (0.19)	0.18 - 2.22	Ruiz et al. 1998
Pensacola Bay	1.01 (0.05)	0.33 - 1.66	0.53 (0.07)	0.08 - 1.25	Murrell et al. 2002
Rhode River	1.23 (0.42)	0.48 - 2.30	1.05 (0.30)	0.41 - 1.60	Gallegos 1989
San Francisco Bay, Northern	0.15 (0.04)	-0.31 - 0.44	0.06 (0.03)	0.00 - 0.37	Murrell and Hollibaugh 1998
San Francisco Bay, Southern	0.74 (0.14)	0.22 - 1.23	0.41 (0.14)	0.00 - 1.19	Murrell and Hollibaugh 1998
Kaneohe Bay, Hawaii	1.59 (0.01)	1.58 - 1.60	0.41 (0.06)	0.35 - 0.47	Landry et al. 1984
Hiroshima Bay, Japan	0.90 (0.09)	0.00 - 1.88	0.25 (0.05)	0.00 - 1.23	Kamiyama 1994

Note: The results of this study are indicated by ***.

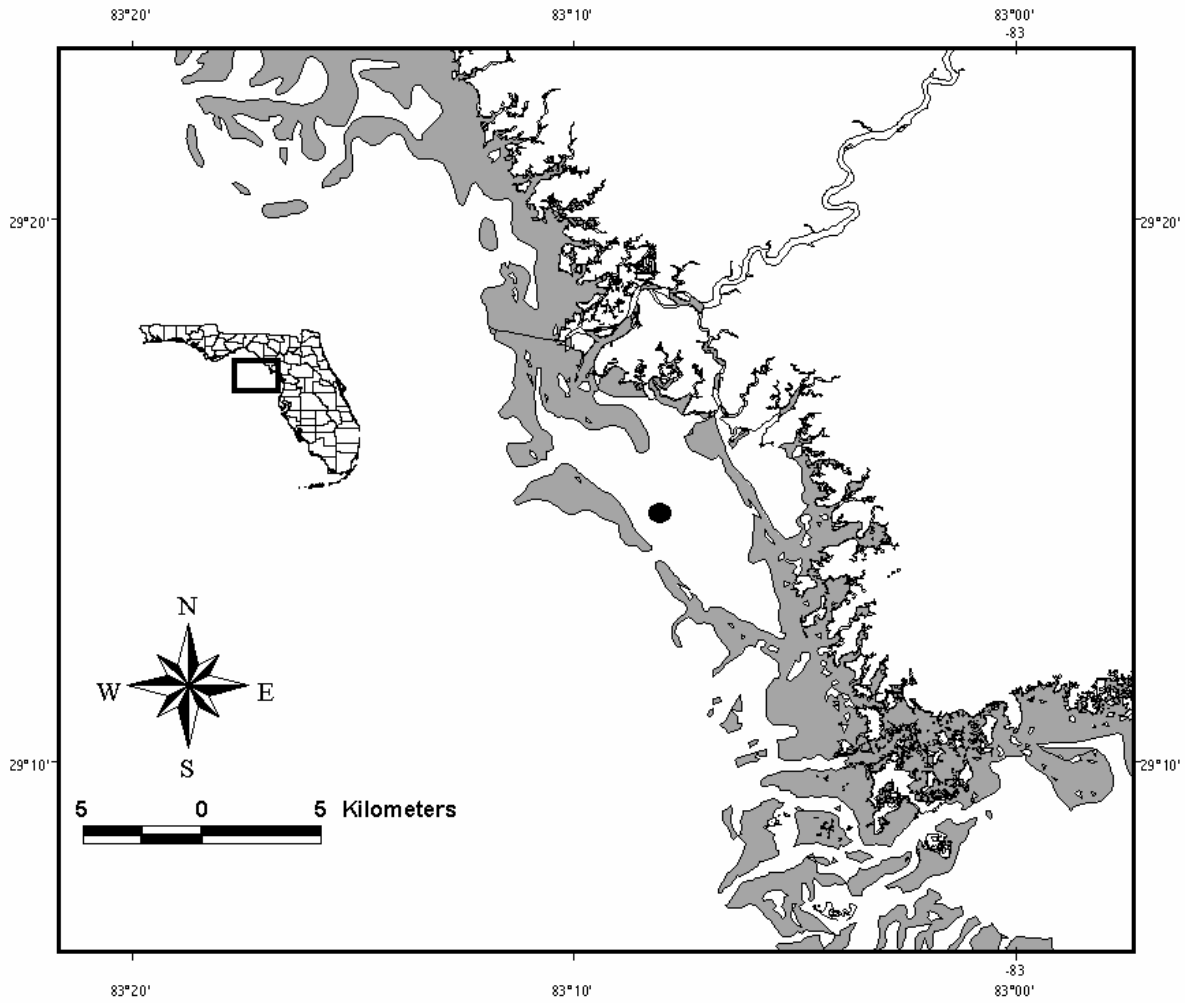

Figure 4-1. Map of the Suwannee River and its estuary, Florida, USA showing station (Site C2) location for micro-zooplankton grazing experiments (●) conducted from January 2001 to January 2002. Oyster reefs and mud flats (<1 meter MLW) are indicated in light gray.

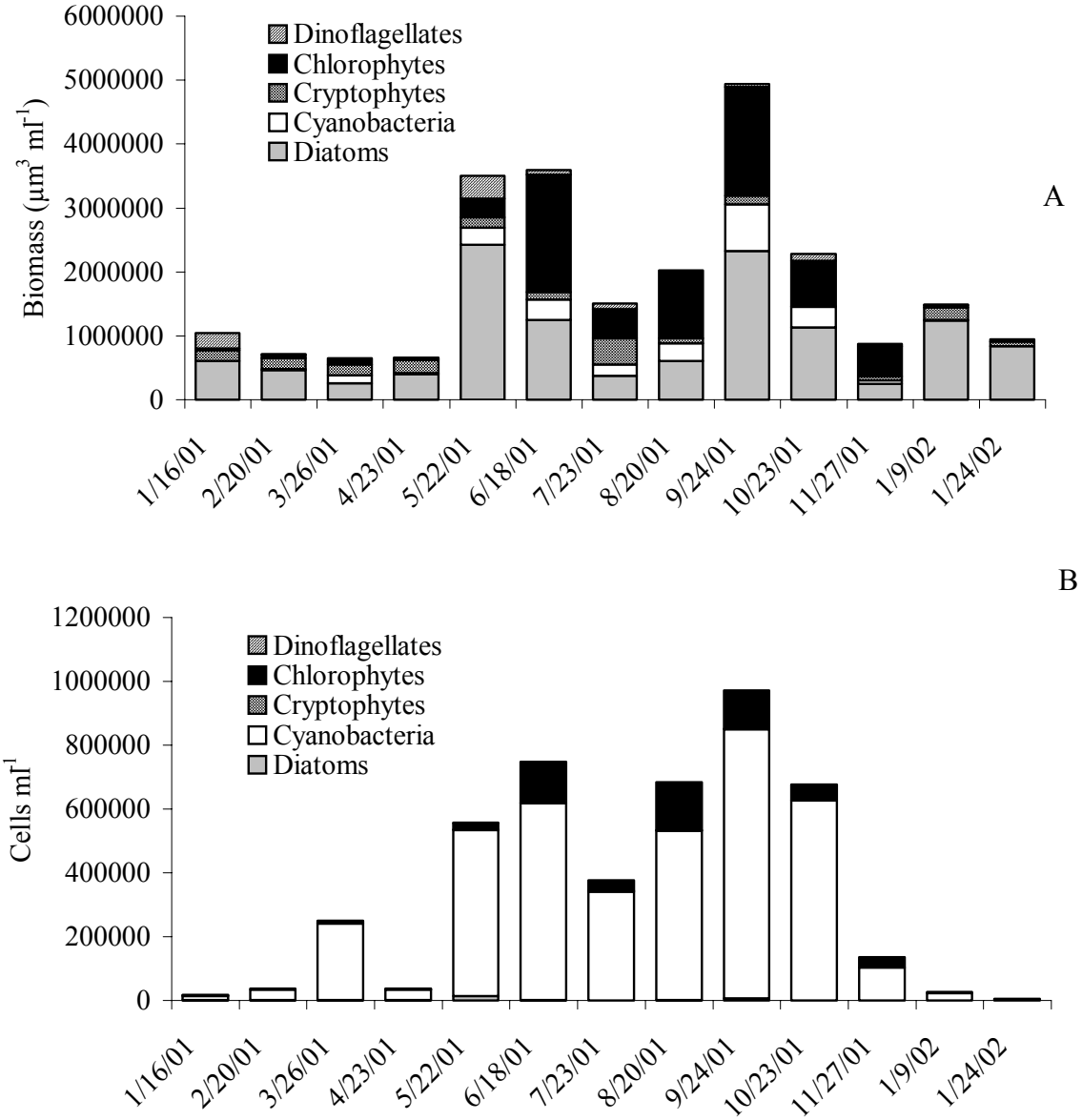

Figure 4-2. Phytoplankton concentration by taxa in the Suwannee River estuary (Site C2) from January 2001 to January 2002. A) Initial phytoplankton biomass (μm^3 ml^{-1}) B) Initial phytoplankton density (cells ml^{-1}).

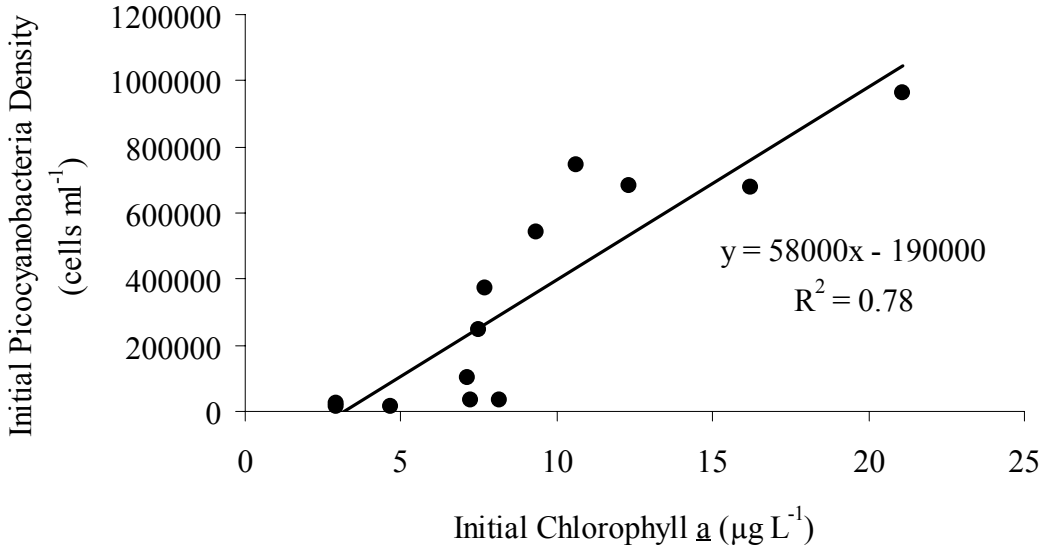

Figure 4-3. Relationship between initial pico-phytoplankton density (cells ml^{-1}) and initial chlorophyll concentrations (chl a µg L^{-1}) in the Suwannee River estuary, Florida, USA from January 2001 to January 2002.

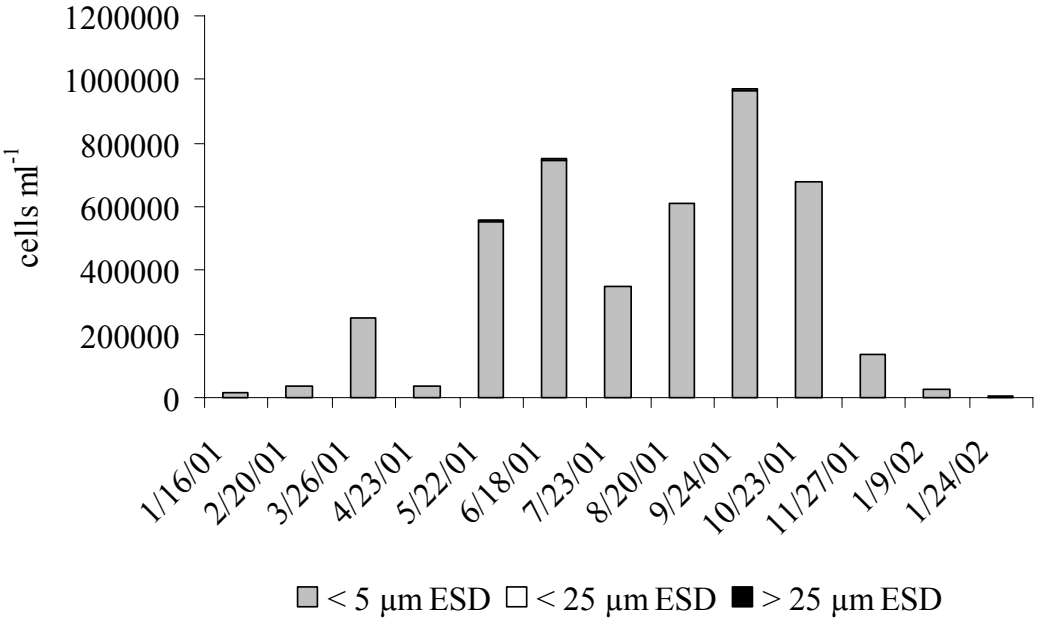

Figure 4-4. Initial phytoplankton densities (cells ml^{-1}) in the Suwannee River estuary, Florida, USA (Site C2) from January 2001 to January 2002 based on equivalent spherical diameters (µm ESD) [ESD = (biovolume/0.523) 0.33] (Hansen et al.1994).

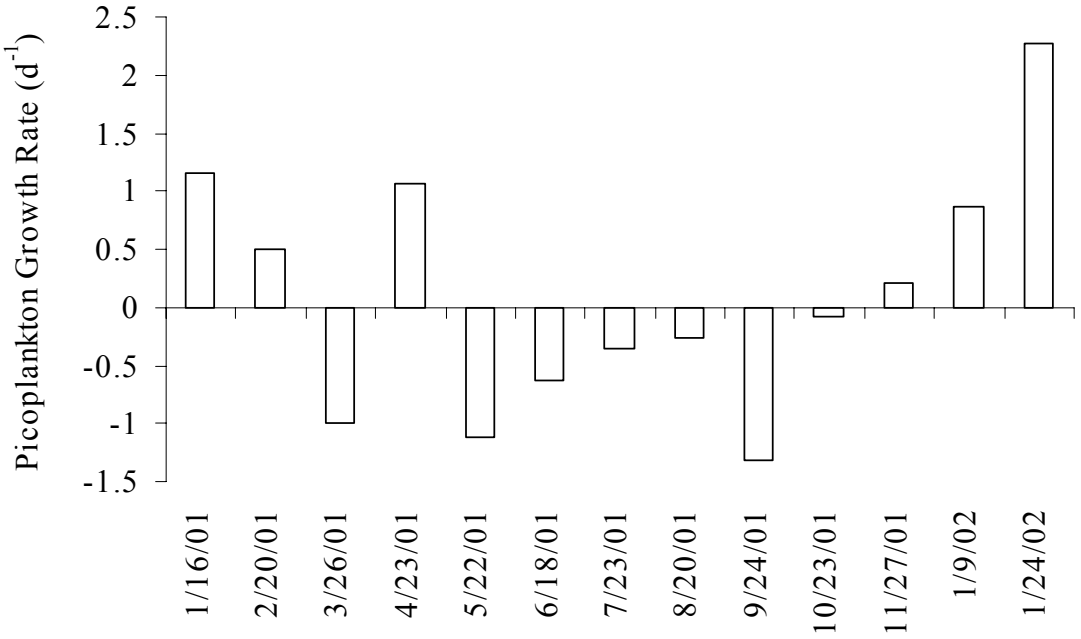

Figure 4-5. Pico-phytoplankton growth rate (day^{-1}) in the Suwannee River estuary, Florida, USA (site C2) observed in the dilution experiments for all sampling dates .

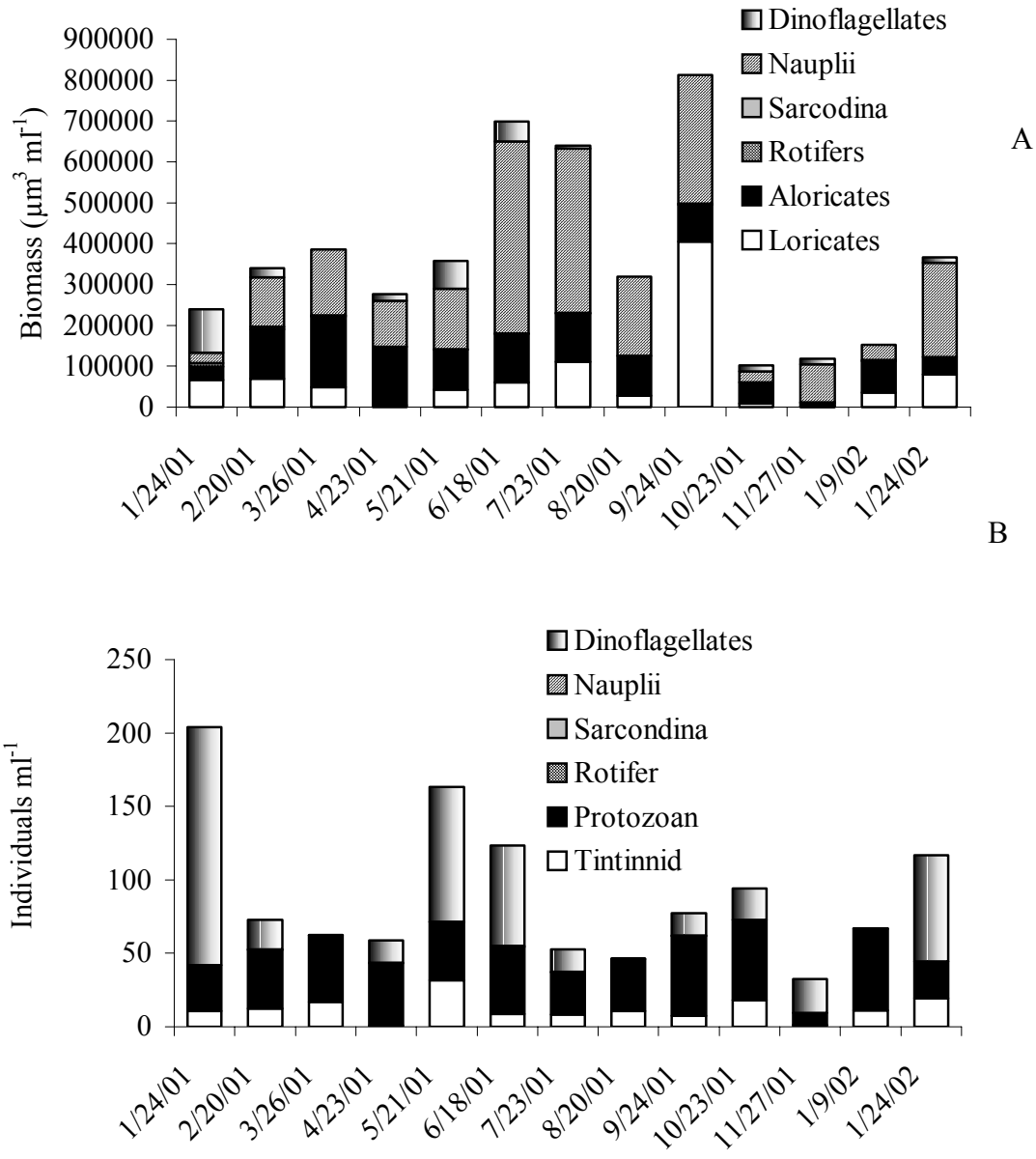

Figure 4-6. Micro-zooplankton concentrations by taxonomic group in the Suwannee River estuary, Florida (Site C2) from January 2001 to January 2002. A) Initial micro-zooplankton biomass (µm3 ml-1). B) Initial micro-zooplankton density (indivduals ml-1).

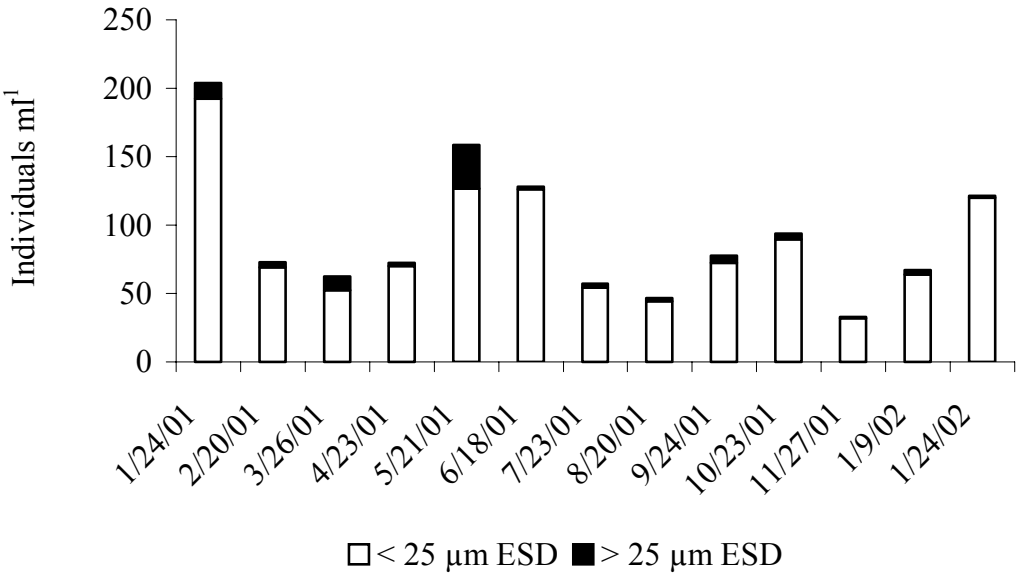

Figure 4-7. Initial micro-zooplankton densities (individual ml^{-1}) based on equivalent spherical diameter (ESD) in the Suwannee River estuary, Florida (Site C2) from January 2001 to January 2002.

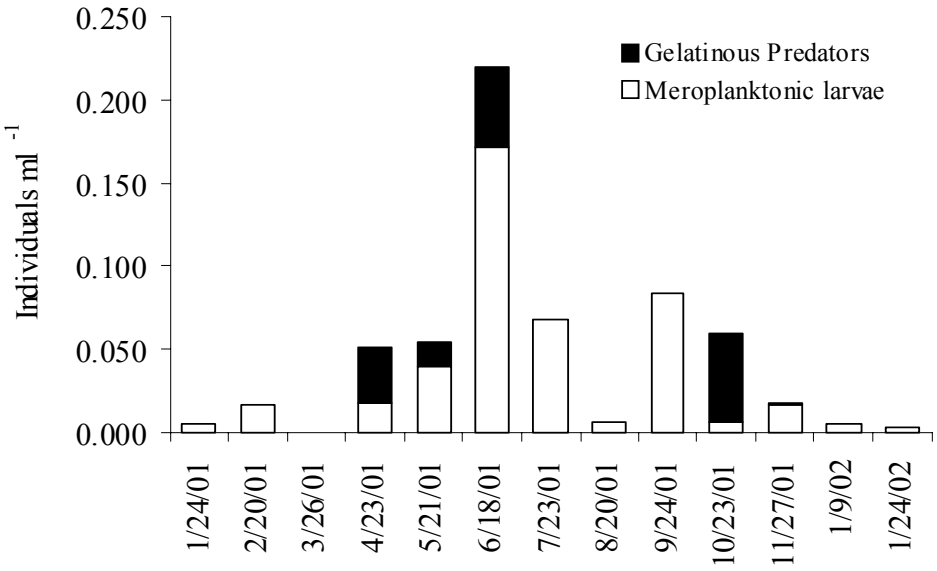

Figure 4-8. Initial concentrations (indivuduals ml^{-1}) of meroplanktonic larvae and gelatinous zooplankton in the Suwannee River estuary, Florida, USA (site C2).

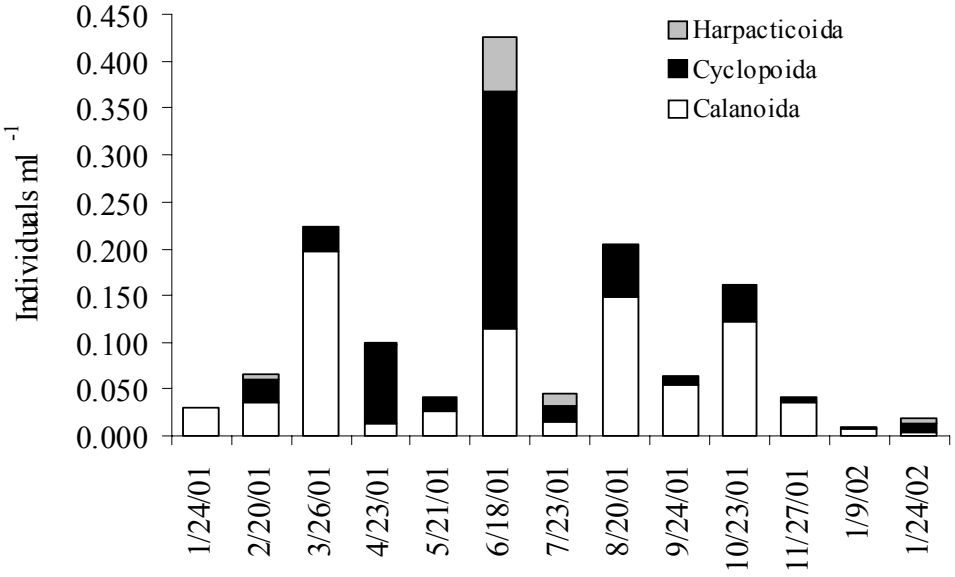

Figure 4-9. Initial densities of adult copepods (individuals ml^{-1}) in the Suwannee River estuary, Florida, USA (site C2).

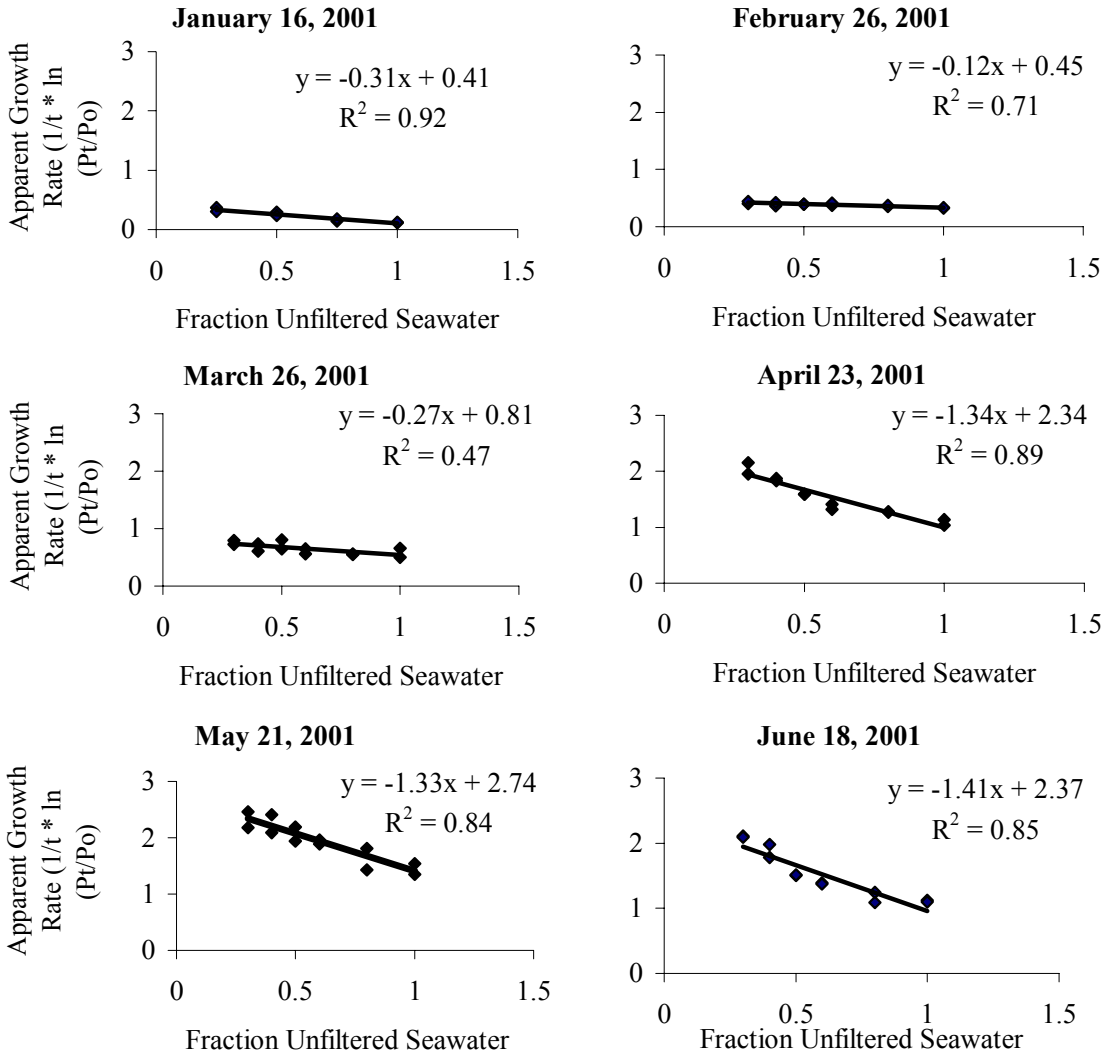

Figure 4-10. Dilution plots of phytoplankton apparent growth (y-axis) versus fraction of unfiltered seawater (x-axis). The line is the least squares regression of the data. Open circles indicate plots using pre-filtered seawater.

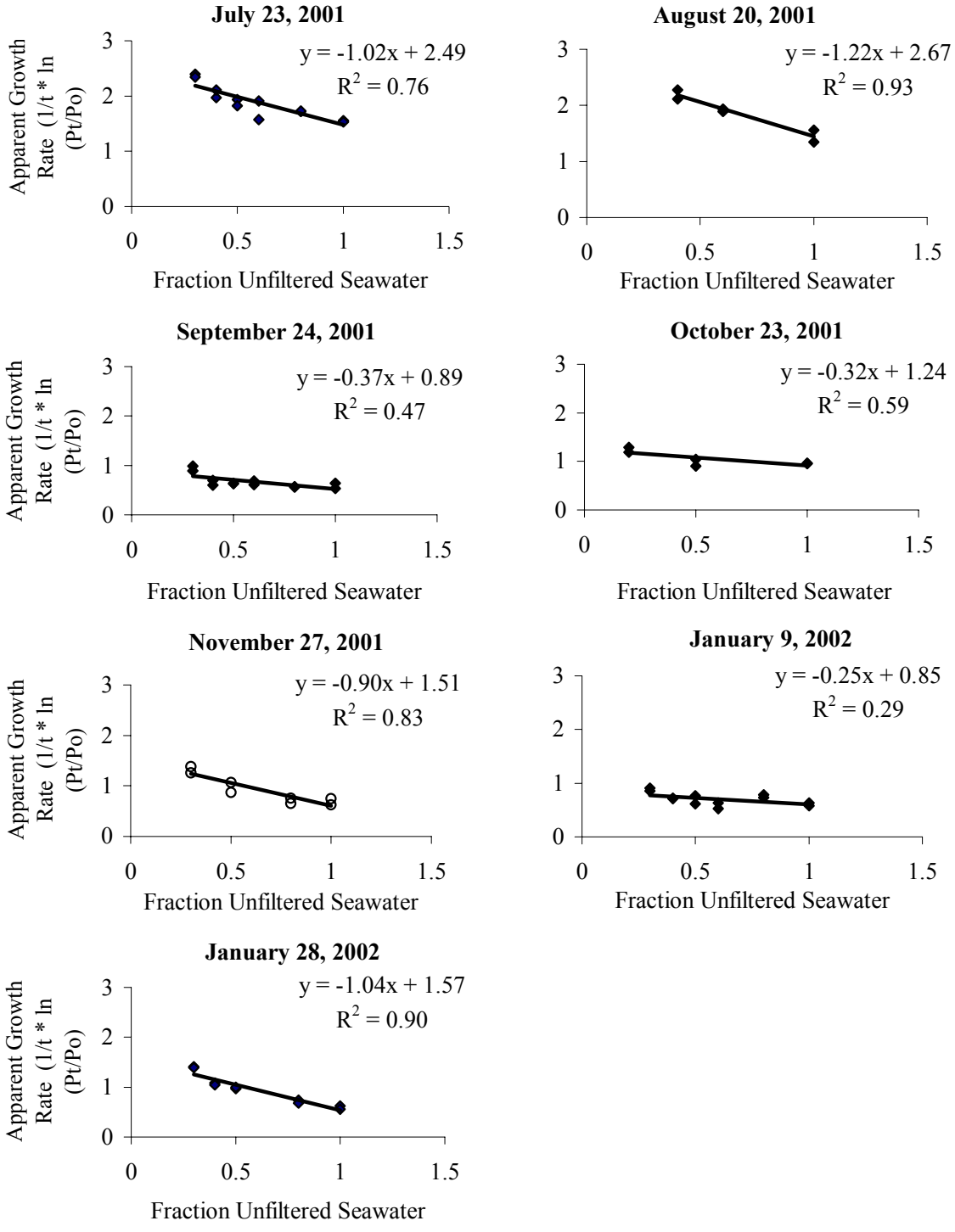

Figure 4-10. Continued

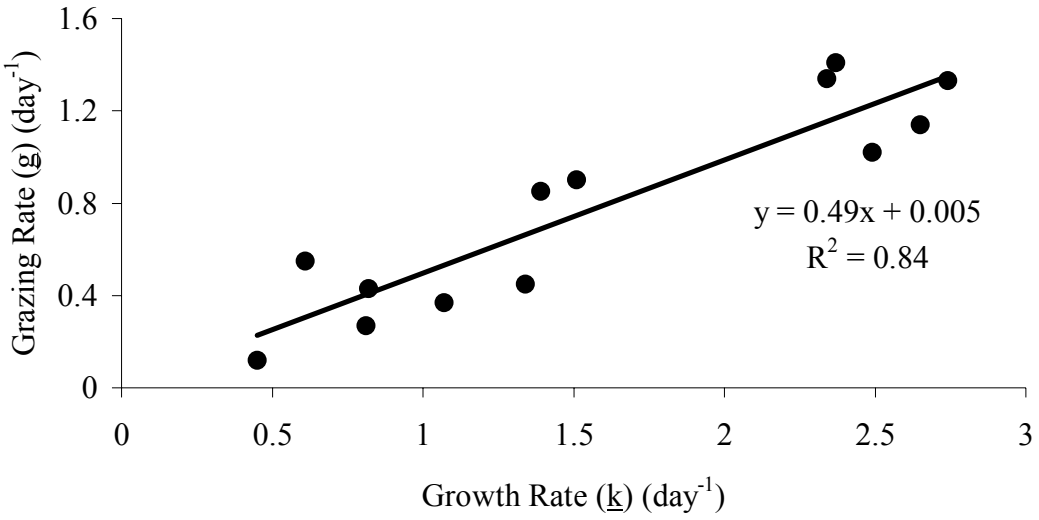

Figure 4-11. Correlation of phytoplankton growth rates (k, day^{-1}) against micro-zooplankton grazing rates (g, day^{-1}) at site C2 in the Suwannee River estuary, Florida, USA, from January 2001 to January 2002.

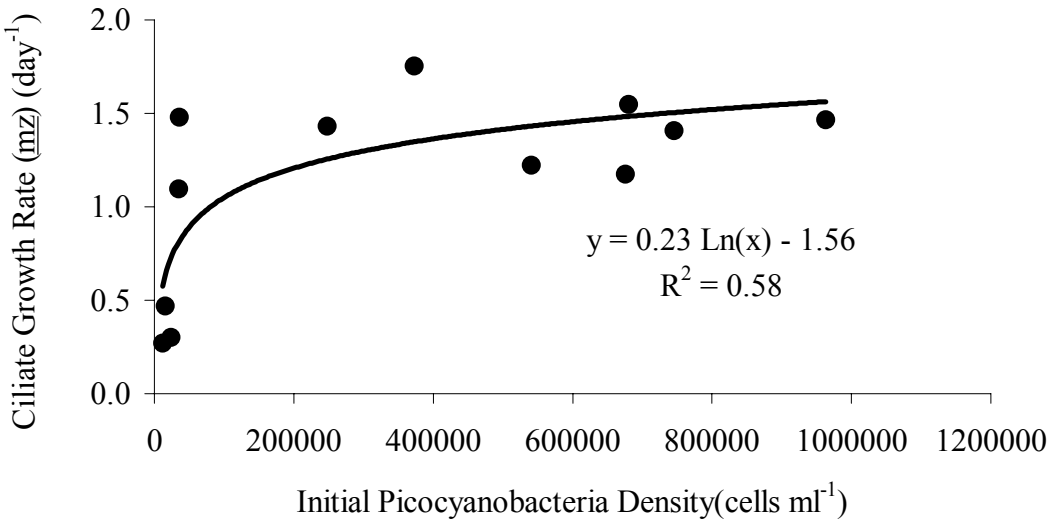

Figure 4-12. Relationship between initial picocyanobacteria density (cell ml^{-1}) and ciliate growth rate (mz, day^{-1}) at site C2 in the Suwannee River estuary, Florida, USA, from January 2001 to January 2002.

CHAPTER 5
SUMMARY

Shifts in phytoplankton biomass and composition are often the first signs of changing water conditions. However, in spite of the potential as biological indicators, it can be difficult to determine the effects of increased nutrient loads on phytoplankton abundance and composition in estuaries (Cloern 2002). It is often the development of problematic algal blooms, which stimulate ecological investigations of phytoplankton. The outflow of nutrient-rich riverine water into coastal ecosystems, in addition to increased phytoplankton biomass, has been linked to an increase in harmful algal species, disruption of trophic pathways, increased frequency of anoxia/hypoxia, and loss of benthic habitat.

The objective of the research presented within my dissertation was to determine the relative role of key gain and loss functions in the control of phytoplankton standing crop in the Suwannee River and its estuary. The objectives were aimed at providing critical information about the influence of the Suwannee River on the overall ecology of the coastal ecosystem, which is currently a highly productive estuary.

The results of my study indicated that phytoplankton dynamics in the Suwannee River estuary are sensitive to changes in nutrient loads from the Suwannee River. Patterns of annual mean algal biomass were correlated with changes in nutrient loading from the Suwannee River. Due to the increased potential for nutrient limitation, specifically nitrogen, the region seaward of the oyster reefs was most impacted by shifts in nutrient loading. For example, when the nutrient-rich freshwater plume was spatially

restricted (i.e., low riverine discharge) phytoplankton standing crop outside of the plume was limited by nutrients and the biomass remained low. During higher freshwater discharge the nutrient-rich plume extended seaward and phytoplankton biomass increased because of increased nutrient availability.

Although there is potential for negative responses in both the Suwannee River and its estuary due to high nutrient availability and nutrient loading, undesirable conditions in ecosystem structure and function were not observed in this study. It appears that several factors may have contributed to the absence of nuisance and/or harmful algal blooms and the ecological consequences of such blooms in the Suwannee River and estuary. These factors included fast flushing rates, potential light limitation, and grazing mortality.

One of the key factors in the distribution and composition of phytoplankton standing crop in the Suwannee River and the adjacent coastal waters seemed to be the physical characteristics of freshwater discharge. The average discharge from the Suwannee River is relatively high in comparison to other riverine systems within the southeastern states, i.e., 301 m^3s^{-1} (Palmer 1984). Although bioavailable nutrients remained high, fast flushing rates precluded the accumulation of phytoplankton standing crop within the Suwannee River from April 1998 to March 1999. In addition, light conditions were unfavorable for phytoplankton growth due to high concentrations of colored dissolved organic matter (CDOM). The exception to this was during drought conditions (May 1999 to April 2001), where residence times increased and CDOM concentrations diminished resulting in increased phytoplankton biomass.

The impact of riverine discharge was also observed in the coastal waters adjacent to the Suwannee River, which is semi-enclosed by oyster reefs. The reefs acted as physical

barriers to the exchange of freshwater and seawater, which resulted in slower flushing rates. Flushing rates within this region lengthen exponentially with decreased riverine discharge. However, even during the lowest riverine discharge (April 1999 to April 2001), the period of time for the complete exchange of freshwater within this region was only 4 to 6.5 days. These rates are significantly faster than other estuaries within the Gulf of Mexico (Solis and Powell 1999). Like the river, fast flushing rates within the estuary may restrict the accumulation of phytoplankton biomass and reduce the potential for negative environmental consequences including anoxia/hypoxia, benthic habitat loss, and changes in foodwebs.

The results of my study suggest that the development of problematic algal blooms in the Suwannee River and estuary would be more likely to occur during periods of reduced freshwater discharge. During periods of low riverine discharge, residence times increase and phytoplankton may have sufficient time to utilize nutrients and accumulate biomass. This has important implications for managers in their effort to set meaningful and reasonable targets for nutrient loading, as well as, minimum flows and levels within the Suwannee River. Prior studies of the impacts of minimum flows and levels have addressed changes in benthic and terrestrial habitat (Leadon 1979, Darst et al. 2002), but have not addressed the consequences of reduced flow on the spatial and temporal distribution and composition of phytoplankton standing crop.

In addition, empirical data suggested that periods of exceptionally high phytoplankton standing crops (>30 μg chl a L^{-1}) were associated with periods of southwesterly prevailing winds that increased residence time of water within this region. It is likely that wind-forcing increased retention times within the reef and nearshore

regions, enhancing the potential for high phytoplankton standing crops to develop. Therefore relatively short-lived wind events, which increased the retention time by several days, had significant impacts on phytoplankton abundance in the Suwannee estuary.

Historically, research on the eutrophication of marine waters has focused on the impact of nutrient availability on algal biomass (Ingrid et al. 1996). However, the impact of nutrient loading on phytoplankton biomass must also be viewed in the context of loss processes, such as mortality. Mortality due to zooplankton grazing is a critical factor in phytoplankton dynamics. In the open ocean, grazing plays a large role in the control of phytoplankton standing crop as rates of phytoplankton growth and grazer-induced mortality are nearly balanced (Banse 1992).

Within the Suwannee River estuary (Site C2) the micro-zooplankton community was dominated by nauplii, hetero/mixotrophic dinoflagellates, and ciliates. The density of ciliates was comparable to densities found within highly eutrophic freshwater lakes and blackwater systems. The results also suggested that the ciliates may be utilizing a prey source, picoplanktonic algae, which are unavailable to larger zooplankton and benthic invertebrates.

The observed range for maximum phytoplankton growth ($\underline{k}$) (0.45 to 2.7 d^{-1}) and grazing ($\underline{g}$) (0.12 to 1.4 d^{-1}) is within the range reported for other estuarine/nearshore regions around the world (Murrell et al. 2002). Under laboratory conditions, micro-zooplankton grazing rates were high enough to impact phytoplankton standing crop as grazing loss rates of up to 75% per day were observed. However, phytoplankton growth rates were greater than micro-zooplankton grazing rates (i.e., $\underline{k} > \underline{g}$) in all experiments.

In situ impacts of micro-zooplankton grazing on phytoplankton could be higher. For example, nutrients and light availability may be limiting for phytoplankton growth in certain areas of the estuary. Under these conditions it might be expected that micro-zooplankton could have a greater impact on the control of phytoplankton biomass. Furthermore, many micro-zooplankton are diurnal and migrate from the sediments to the water column at night. The sampling design and collection methods used were not appropriate to account for this phenomenon. Micro-zooplankton grazing may significantly impact the phytoplankton biomass within the Suwannee River estuary. The magnitude of this impact, however, is dependent on a range of physical, chemical, and biological conditions. Therefore, the experimental grazing estimates obtained from this study represent the minimum impact potential of grazing activities on the mortality of phytoplankton.

The general picture of phytoplankton dynamics in the Suwannee River and its estuary emerging from this study is one of a nutrient-rich environment, where phytoplankton standing crops can reach high levels, but can also be held below their potential by several key loss functions, including hydraulic flushing and grazing. The specific potential for algal blooms is probably dictated by the later factors, as well as, spatial and temporal patterns of external and internal loading of bioavailable nutrients. Nitrogen appears to be the most widely limiting nutrient in the estuary; therefore future increases in nitrogen loading are an issue of concern for the ecology of the system.

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BIOGRAPHICAL SKETCH

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