Florida Bay microalgae blooms: Physiological characteristics and competitive strategies of bloom forming cyanobacteria and diatoms of Florida Bay

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Florida Bay Microalgae Blooms:
Physiological Characteristics and Competitive Strategies
of Bloom Forming Cyanobacteria and Diatoms
of Florida Bay

by

Ralph William Richardson

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
College of Marine Science
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Dedication

This dissertation is dedicated to my wife and parents without whose continuous support and encouragement I would not have been able to complete my formal studies in Marine Science.
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FLORIDA BAY MICROALGAE BLOOMS:
PHYSIOLOGICAL CHARACTERISTICS AND COMPETITIVE STRATEGIES
OF BLOOM FORMING CYANOBACTERIA AND DIATOMS
OF FLORIDA BAY

Ralph William Richardson

ABSTRACT

Areally expansive, persistent and recurring blooms frequently dominated by cyanobacteria have
developed primarily in the north-central region of Florida Bay since approximately 1991. This part of the
bay has a history of the following: periodic hypersalinity, high sediment-derived turbidity, P limitation, N
limitation, light limitation and long water residence time. Clonal isolates of selected dominant bloom
species of cyanobacteria (*Synechococcus* cf. *elongatus* and *Synechocystis* sp.) and diatoms (*Chaetoceros* cf.
salsugineus and *Thalassiosira* cf. *oceanica*) from Florida Bay were examined in an effort to explain their
relative dominance of the phytoplankton community.

The following physiological characteristics and nutrient strategies of the study species were
examined: (1) salinity-growth response; (2) light-growth response; (3) phosphorus-dependent growth
kinetics; (4) ERC-theory phosphorus competitiveness; (5) cellular quotas and luxury storage capabilities of
N and P; (6) optimal N:P ratios; (7) P and N-limited competitiveness under various salinities, N:P ratios,
forms of N and P, and rates of nutrient delivery; (8) aerobic nitrogen fixation; (9) production of
allelochemic compounds, and (10) response to resuspended sediment.

This study identified salinity and nutrient limitation as the factors having the greatest potential to
regulate the development of cyanobacteria and diatom bloom dominance in Florida Bay. The results
strongly suggest that the frequent dominance of *Synechococcus* cf. *elongatus*, and *Synechocystis* sp. in the
recurring phytoplankton blooms of the north-central region of Florida Bay can be attributed to their
superior P-competitiveness and to a lesser degree to their greater salinity tolerance limits.
CHAPTER 1
GENERAL INTRODUCTION

Introduction

Florida Bay is a semi-tropical, shallow water lagoon located at the southern tip of Florida, between the Florida Keys and the Everglades (Fig. 1-1). This approximately one thousand square mile area has been historically characterized by a clear water column supporting a low phytoplankton biomass and large regions of lush seagrass meadows fringed by mangrove communities (Zieman et al. 1989). While the majority of the bay is dominated by seagrasses (Fourquarean et al. 1993) hard-bottom areas occur that contain coral, sponges, macroalgae and a thin layer of carbonate sediment (Butler et al. 1995). It has an average depth of less than three meters and is underlain by carbonate bedrock and sedimentary deposits. The bay's sediment is dominated by carbonate that is largely biogenic in origin and most developed in the western part of the bay (Halley et al. 1995). The bay is physically subdivided into a mosaic of sub-basins separated by a network of shallow submerged mudbanks (<0.5m) and mangrove islands, and interconnected by a series of narrow channels (Fourquarean et al. 1992).

The climate is subtropical with a mean annual water temperature of 26 °C (Boyer et. al 1999). Florida Bay waters are influenced by exchange with Gulf of Mexico waters to the west, Atlantic waters to the south, and freshwater delivery along the northern boundary. Circulation patterns in the bay indicate that the net transport of marine water through Florida Bay begins as Gulf of Mexico water enters the bay along the northwestern boundary, flows south and east and exits the bay along the southwest boundary as well as along the southern boundary through passages between the Florida Keys. At the same time, freshwater enters along the northern boundary and promotes water flow both south and west eventually exiting along the southern boundary into the Atlantic Ocean. Accompanying the marine coastal Gulf of Mexico shelf water entering the bay along the western boundary is freshwater runoff from the Shark, Broad, and Lostman’s Rivers draining from the Ten Thousand Islands area (Johns et al. 2001, Lee et al. 2002). Freshwater also enters the bay along the northern boundary, as runoff from the Everglades primarily through Taylor Slough and the South Dade Conveyance System network of canals (Brand 2002). Tidal exchange is greatest nearest the Gulf of Mexico and Atlantic Ocean boundaries and decreases towards the interior of the bay (Lee et al. 2002), and although greatly diminished, it still remains an important mechanism of bay water exchange in the northern and western regions of the bay (Smith and Pitts 2002). The flow of water into the bay and between basins is restricted by the mudbanks and
mangrove islands resulting in long and variable water residence times within the different basins of the bay. The high salinities found in the central and northeast regions of the bay are indicative of the long residence time and poor exchange of water occurring between these regions and adjacent regions within the bay (Johns et al. 2001, Lee et al. 2002). The prevailing winds are from the east/southeast but shift to northeasterly winds during the winter-spring period (Phlips et al. 1999, Lee et al. 2002). The regional rainfall pattern consists of a wet season during the summer-fall period and a dry season during the winter-spring period, but the alternating wet and dry seasons may be interrupted by abnormally extended periods of drought (e.g. La Nina-enhanced dry season), extended periods of rainfall (e.g. El Nino-enhanced wet season) and/or large rainfall events (e.g. tropical storms) (Lee et al. 2002,). The high rates of evaporation, coupled with extended periods of drought and long water residence times have periodically created large areas of hypersaline water particularly within the central region of the bay (Fig. 1-2).

The spatial patterns of water column concentrations of total phosphorus and total nitrogen within the bay are approximately the inverse of each other (Fig. 1-3). Phosphorus is lowest in the east and increases along a transect from east to northwest across the bay, while nitrogen is generally lowest in the western region of the bay and increases eastward into the bay being highest along the northeastern boundary of the bay. The spatial gradients of phosphorus and nitrogen have been hypothesized to reflect their west Florida shelf and freshwater runoff source waters, respectively (Fourquarean et al. 1993, Brand 2002). The concentration of phosphorus in Florida Bay, particularly in the eastern region, has been historically low, as phosphate is efficiently stripped from the freshwater before it enters the bay as it flows over carbonate substrate on its way to the bay. At the same time, phosphate levels in the bay are maintained at continually low levels by the high affinity of the bays carbonate sediments for phosphate. The east to west gradient of increasing phosphorus across the bay has also been recorded in the longer time-averaged records of the sediment (Chambers et al. 1999, Yarbro and Carlson 1998) as well as the tissues of the dominant seagrass *Thalassia testudinum* (Fourquarean et al. 1992). Average water column N:P ratios increase from the western to the central region of the bay (Figs. 1-4 and 1-5).

The results of numerous studies have led to a spatial division of the bay into three broad geographic and ecological zones; the eastern, central, and western zones, defined by water quality parameters (Boyer et al. 1999) and the results of microalgal nutrient bioassays (Tomas et al. 1999). Microalgal distribution patterns suggest a subdivision of the central region into a north-central and a south-central region (Steidinger and Phlips 1996) (Fig. 1-6). Although the bay can be discussed in terms of these three major zones, each zone is further subdivided by mudbanks into a network of sub-basins. The mudbanks restrict water exchange between the basins effectively allowing some sub-basins to develop substantial differences in water quality, water residence time, phytoplankton biomass and composition which, creates a mosaic of physical, chemical, and biological characteristics across Florida Bay.

Significant ecological calamities and anomalies including massive seagrass die-offs, sponge mortalities, large and persistent phytoplankton blooms often dominated by cyanobacteria, increased turbidity and overall decline in water quality beginning in 1987 led to a heightened concern for the health
of the bay and stimulated the search for the cause of these ecological disturbances. It was initially hypothesized that the disturbances were largely the result of the drastic changes to the natural flow of water entering and exiting the bay as well as the anthropogenic nutrient enrichment of bay waters (Lapointe and Matzie 1996). The natural overland sheet flow of freshwater to the bay originating largely from the Lake Okeechobee drainage basin has been greatly reduced as water has been diverted away from the Everglades and replaced to a great extent with the regulated delivery of agriculturally nutrient enriched freshwater via canals. The construction of overland transportation routes along the Florida Keys has also led to the constriction of water passages between the Keys that have significantly reduced the natural exchange of Florida Bay with Atlantic Ocean waters.

Although it is accepted that the man induced alterations in water flow and nutrient enrichment of bay waters have affected the ecology of the bay, the magnitude of their influence as well as their role in the recent ecological perturbations is not known. The diversion of freshwater, considered to be a contributing factor in the recent extensive hypersaline conditions, and the anthropogenic nutrient enrichment of freshwater entering the bay were both suspected to be directly or indirectly the principal factors triggering the cascade of recent ecological disturbances in Florida Bay. The search for the cause of these recently observed perturbations led also to the consideration that the causes might be natural in origin and not the result of man-induced alterations to the environment.

The historical salinity record and the recent geologic record indicate that the bay has experienced cyclic extensive hypersaline events during the past century, prior to the major man-induced changes in water flow (Brewster-Wingard and Ishman 1999, Swart et al. 1999, Robblee et al. 2001). This suggests that the man-induced changes in water flow, although exacerbating the recent hypersaline conditions brought about by drought conditions, may not be the primary force behind the cascade of disturbances, although they are suspected to be a contributing factor.

Early hypotheses implicated the phytoplankton blooms as a primary factor influencing the mass sponge mortality event (Butler et al. 1995), seagrass declines resulting from light-stress induced mortalities, as well as increased turbidity resulting from the resuspension of exposed sediments following massive seagrass mortality (Fig. 1-7) (Durako et al. 1996). Early hypotheses incorporated eutrophication (Lapointe and Matzie 1996) and hypersalinity (Phlips and Badylak 1996) as potential factors contributing to the development of the large and persistent cyanobacteria dominated algal blooms, particularly in the north-central region of the bay.

The phytoplankton community of Florida Bay is both taxonomically diverse and species rich, estimated collectively at more than 250 species. Phytoplankton blooms for the most part consist of a mix of centric and pennate diatoms, cyanobacteria, dinoflagellates, and flagellates. Temporally and spatially distinct blooms reoccur annually in the bay and are typically dominated by species of cyanobacteria and/or diatoms belonging to the following taxonomic groups; *Synechococcus*, *Synechocystis*, *Chaetoceros*, *Cyclotella* and *Rhizosoleniaceae*. Three species that have been found to occur commonly throughout the bay and regularly as dominant components of algal blooms in the north-central region are; *Synechococcus*
cf. *elongatus*, *Synechocystis* sp. (spherical picoplankter), and *Chaetoceros* cf. *salsugineus* (Steidinger et al. 2001). Other small centric diatoms including *Thalassiosira* spp. are also found in the bay but do not commonly form large blooms or dominate the phytoplankton community (Steidinger et al. 1995).

Although the phytoplankton community and small short-lived blooms have likely always been a natural component of the Florida Bay ecosystem, it was the recurrence of large and persistent cyanobacterial blooms often dominated by species of *Synechococcus* beginning in the spring of 1991 (Fourquarean et al. 1992), that suggested a distinct change in the ecology of the bay.

There are seasonal and spatial patterns to the phytoplankton blooms in Florida Bay (Steidinger and Phlips 1996) (Fig. 1-8), and there may be a successional pattern as well. Large recurring seasonal blooms of cyanobacteria and/or diatoms have been documented since approximately 1991 in both the western and central zones. In contrast, the eastern zone has been infrequently influenced by microalgae blooms (Steidinger and Phlips 1996). These blooms are for the most part both spatially and temporally distinct, and characteristically dominated by different taxa. By and large, the dominant phytoplankton group in the western zone blooms is diatoms, although cyanobacteria have at times dominated samples, particularly where the western and central zones converge. The western zone blooms occur predictably in fall and winter and they are most often dominated by open water shelf species (e.g. Rhizosoleniaceae spp.), while smaller blooms in the summer are commonly composed of *Chaetoceros* spp. (Steidinger et al. 1995). The central zone blooms are composed of both diatoms (e.g.'s *Cyclotella* spp., *Chaetoceros* spp.) and cyanobacteria (e.g. *Synechococcus* spp. and *Synechocystis* sp.) and are commonly dominated numerically and by biovolume by the cyanobacteria species (Steidinger et al. 1998; Phlips et al. 1999). Central zone blooms tend to originate in the north-central region in the summer, spread primarily southward into the south-central region as the bloom grows during the fall, and ultimately dissipate during the winter-spring period. During the winter-spring decline, the bloom normally shrinks back in size to a small bloom located in the north-central zone from which it appears to have originated (Fig. 1-8) (Phlips et al. 1999, Steidinger et al. 1998).

The perceived declining health of the bay seen as the newly observed occurrence of large and persistent cyanobacteria dominated algal blooms, sponge mortalities, seagrass die-offs, increased turbidity, hypersaline waters and overall decline in water quality, prompted public concern and a strong interest in the mechanisms responsible for the formation of these blooms. The Florida Bay Scientific Program Management Committee (PMC) cited the following question as one of the five questions central to understanding the problems affecting Florida Bay; “What regulates the onset, persistence and fate of planktonic algal blooms in Florida Bay?” (Armentano et al. 1997).

Phytoplankton blooms are a function of both the environmental conditions and the resource requirements of the organism. In order to bloom, a phytoplankton species must be able to optimize resource capture, efficiently utilize resources, and minimize losses (Oliver and Ganf 2000). Bloom dominance by a single species or group of species (numerically and/or by biovolume), suggests that the dominant species are able to maximize their net growth to a greater degree than any of the other
phytoplankton species, under the prevailing ambient environmental conditions, by virtue of their physiological and ecological attributes.

An understanding of bloom dominance by cyanobacteria and/or diatom species in Florida Bay should consider the environmental factors and species physiological and ecological characteristics that may convey a competitive advantage to the dominant species. To examine the factors that may convey a competitive advantage to the dominant species, the relationships between selected environmental factors and ecophysiological attributes of the following cyanobacteria and diatom taxa of Florida Bay were examined: the often numerically dominant bloom species *Synechococcus* cf. *elongatus*, *Synechocystis* sp., and *Chaetoceros* cf. *salsugineus* and the less common and non-dominant *Thalassiosira* cf. *oceanica*. Experiments were performed to determine each species light and salinity growth relationships, nutrient kinetics, nutrient competitiveness, nutrient cell quotas, optimal nutrient ratios and luxury nutrient consumption. In addition, several hypothesized ecophysiological adaptations that were suspected to play an influential role in species dominance were examined. The results of these experiments are presented and discussed in the following chapters.
Figure 1-1. Florida Bay, in South Florida, USA.

Figure 1-2. Salinity distribution in Florida Bay illustrating the typical occurrence of hypersaline conditions in the central region of the bay. Data represent averages from eight cruises conducted between June 1989 and July 1990. (Redrawn from Fourqurean et al. 1992).
Figure 1-3. Average concentrations of (A) dissolved inorganic N, (B) total P measured in monthly sampling of Florida Bay from 1991 to 1998. Data from SFWMD and plotted using nearest neighbor surface interpolation. (Redrawn from Brand 2002).
Figure 1-4. Average ratio of total nitrogen to total phosphorus in the water column at each station across all sampling dates in Florida Bay (June 1989-August 1990). (Redrawn from Fourquean et al. 1993).

Figure 1-5. Average molar ratios of dissolved inorganic N to total P measured in monthly sampling of Florida Bay from 1991 to 1998. Data from SFWMD and plotted using nearest neighbor surface interpolation. (Redrawn from Brand 2002).
Figure 1-6. The ecological regions that have been used to characterize Florida Bay. (Redrawn from Philipps et al. 1995).

Figure 1-7. Location of the major seagrass die-off in 1987. (Redrawn from Robblee et al. 1991).
Figure 1-8. Seasonal mean Synechococcus biovolumes in Florida Bay in (A) Summer (B) Fall (C) Winter (D) Spring. Unshaded regions not included. (Redrawn from Phlips et al., 1999).

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CHAPTER 2
EFFECTS OF SALINITY AND IRRADIANCE ON THE GROWTH OF
SYNECHOCoccus CF. ELONGATUS, SYNECHOCystis SP.,
THALASSIOSira CF. OCEANICA, AND CHAETOCEROS CF. SALSUGINEUS

Abstract

Laboratory experiments determined the salinity- and light-growth relationships of the following taxa from Florida Bay: the cyanobacteria *Synechococcus* cf. *elongatus* and *Synechocystis* sp., and the diatoms *Chaetoceros* cf. *salsugineus* and *Thalassiosira* cf. *oceanica*. The species responses closely followed taxonomic lines. Cyanobacteria species had broader salinity tolerance ranges and growth rates that were at least 60% \( U_m \) from 5 °/oo to 60 °/oo. In contrast, the diatoms had narrower salinity tolerances with *T. cf. oceanica* unable to grow at salinities of 5, 10 or 55 °/oo, while *C. cf. salsugineus* grew at 5 °/oo but barely grew at 60 °/oo. Although all four species had relatively broad optimum salinity ranges for growth, indicative of their euryhaline nature, the cyanobacteria species salinity optima for growth (10-20 °/oo), was lower than *T. cf. oceanica*’s (25-40 °/oo) and *C. cf. salsugineus*’s (15-30 °/oo). The maximal growth rates in divisions da\(^{-1}\) for each species at its salinity optimum were 1.06 for *S. cf. elongatus*, 0.97 for *Synechocystis* sp., 2.11 for *C. cf. salsugineus* and 2.46 for *T. cf. oceanica*. Between 15 and 50 °/oo the diatom species maximal growth rates exceeded those of the cyanobacteria species, while at 5 and 60 °/oo the maximal growth rates of both cyanobacteria exceeded those of the diatoms.

The species growth-irradiance (U-E) curves also followed taxonomic lines in that both cyanobacteria species responded alike, as did both diatom species. Values of \( \alpha_g \) in m\(^2\) E\(^{-1}\) indicated that growth per unit PAR of both diatoms (0.33, 0.35) was greater than the cyanobacteria (0.23, 0.23). No interspecific differences in \( E_c \) were detectable indicating that all species have similarly low minimum light requirements. \( E_c \)’s indicated lower light saturation intensities for the cyanobacteria (70-80 µEm\(^{-2}\)sec\(^{-1}\)) than the diatoms (121-133 µEm\(^{-2}\)sec\(^{-1}\)). No photoinhibitory response was observed in any of the species growth rates up to an irradiance of 350 µEm\(^{-2}\)sec\(^{-1}\).

The results suggest that physiological differences between the cyanobacteria and the diatoms in their response to salinity and light may be influential in determining their relative dominance in Florida Bay algal blooms. At more moderate salinities (10 °/oo – 50 °/oo) the much greater \( U_m \)’s and greater \( \alpha_g \)’s of the diatom species (*C. cf. salsugineus* and *T. cf. oceanica*) place them at a competitive advantage over the cyanobacteria species (*S. cf. elongatus* and *Synechocystis* sp.), thereby enhancing the potential for the
development of diatom dominated blooms under both light limiting and light saturating conditions. The cyanobacteria's much greater tolerance of hypersaline conditions may play a significant role in the development and persistence of the cyanobacteria dominated blooms in the north-central region during periods of hypersalinity.

**Introduction**

Phytoplankton blooms dominated by a single or several species occurs when the dominant species maximize their net growth to a greater degree than any of the other co-occurring phytoplankton species. The ecological success of the more abundant and dominant phytoplankton bloom species of Florida Bay may be viewed as a reflection of the overall good fit of their physiological and ecological characteristics to the prevailing environmental conditions of the bay. The occurrence of cyanobacteria dominated blooms and diatom dominated blooms in Florida Bay implies not only that the environmental conditions are within their physiological limits of adaptation, but that under the prevailing environmental conditions the bloom forming cyanobacteria and diatom species are the superior competitors. Although nuisance cyanobacteria dominated blooms have traditionally been more of a problem in freshwater environments and have therefore been studied extensively, large and persistent cyanobacteria blooms occur in marine environments as well (Paerl 2000).

In attempting to explain the occurrence of cyanobacterial dominated blooms, efforts have been made to identify ecophysiological characteristics that would give the cyanobacteria a competitive advantage over other competing eukaryotic phytoplankton. Explanations for the abundance and frequent dominance of cyanobacteria in phytoplankton communities include; N$_2$-fixing ability, high nutrient affinities, enhanced uptake and storage capabilities, ability to assimilate diverse organic compounds, low grazing losses, resistance to viral infection, and an ability to flourish under physical and chemical environmental conditions that would be sub-optimal for other algae such as high pH, low light, low dissolved oxygen, high sulfides, and extremes in salinity (Oliver and Ganf 2000, Paerl 2000, Stockner 1988, Lavrentyev et al. 1998).

Among the many environmental variables influencing phytoplankton growth in Florida Bay, the two considered to be influential in bloom dominance and examined in detail in this first chapter are salinity and light. Initially it was suspected that populations of picoplanktonic *Synechococcus* and *Synechocystis* may be at a competitive advantage in regions of Florida Bay that experience low light and high salinities. Salinity was chosen because cyanobacteria are known to tolerate and even flourish under adverse environmental conditions including extremes in salinity (Stockner 1988; Sorokin et al. 1996; Phlips and Badylak 1996) and large blooms often dominated by the cyanobacteria species of *Synechococcus* and *Synechocystis* regularly reoccur in the north-central region (Steidinger and Phlips 1996) which commonly experiences hypersaline conditions (Robblee et al. 2001). Although hypersaline conditions are not unique to the north-central region of the bay, it is in this central region of the bay that hypersaline conditions...
usually appear first and are most persistent (Robblee et al. 2001). The frequent co-occurrence of large and persistent cyanobacteria dominated blooms and hypersaline conditions in the north-central region of the bay led to an early hypothesis that hypersaline conditions may contribute to the development of large and persistent cyanobacteria dominated algal blooms in that region of the bay.

Light was chosen because some marine species of *Synechococcus* have been described as being particularly well adapted to low irradiances (Stockner 1988) including saturation of photosynthesis and growth at very low irradiances (Glover et al. 1987, Morris and Glover 1981) and Florida Bay is characterized by large temporal and spatial differences in water column clarity and irradiance levels (Boyer et al. 1999, Phlips et al. 1995). Although the potential for phytoplankton light limitation would be considered unlikely in a bay as shallow as Florida Bay (0-4 m), mean daily light levels in the mixed layer ($I_m$) approach the threshold level for the onset of phytoplankton light limitation (Phlips et al. 1995). These light limiting conditions are most common in the north-western and north-central regions and it is in the north-central region in particular, that large and persistent cyanobacterial blooms develop.

The recurring growth and development of blooms typically dominated by cyanobacteria in primarily the north-central region of Florida Bay, an area which has a history of hypersaline conditions, high turbidity and periodically may be light limited, led to the formation of the following hypotheses concerning the light- and salinity-based physiological attributes of the cyanobacteria bloom species (*S. cf. elongatus* and *Synechocystis* sp.) that may contribute to their dominance over the diatom bloom species (*C. cf. salsugineus* and *T. cf. oceanica*). The hypotheses are:

- **H$_0$:** The cyanobacteria species have broader salinity tolerance ranges and higher optimal salinity ranges for growth than the diatoms species.
- **H$_1$:** The maximal growth rates (nutrient and light saturated) of the diatoms are greater than those of the cyanobacteria at low to moderate salinities (15-35 $\%_o$), while the maximum growth rates of the cyanobacteria are greater than those of the diatoms under hypersaline conditions (40-60 $\%_o$).
- **H$_2$:** The cyanobacteria are more efficient in light utilization for growth (higher $\alpha_g$) than the diatoms.

The objective of this chapter was to characterize the physiological responses of four dominant bloom species from Florida Bay to gradients of salinity and light. The species responses and the prevailing spatial and temporal patterns of salinity and light in Florida Bay were then used to explain in part the spatial and temporal patterns of cyanobacteria and diatom bloom dominance in the bay.
Methods

The following clonal isolates of three numerically dominant and one non-dominant microalgal species from Florida Bay were established in 1995 using the pipet technique (Guillard 1973): *Synechococcus* cf. *elongatus* (Naegeli), *Synechocystis* sp., *Chaetoceros* cf. *salsugineus* (Takano), and *Thalassiosira* cf. *oceanica* Hasle (synonymous with *Cyclotella nana* Hustedt Guillard clone 13-1). They were maintained in glass fiber filtered (GF/F) seawater collected in the Gulf of Mexico approximately 40 miles offshore, diluted to 25 % with distilled water, autoclaved and enriched to "f/2" (Guillard and Ryther 1962) nutrient levels.

Basal growth media used in the experiments was artificial seawater prepared from deionized water and reagent grade chemicals (Parsons et al. 1984) or seawater collected in the Gulf of Mexico approximately 40 miles offshore. A combination of autoclaving and sterile filtration were used to sterilize experimental culture vessels, seawater and nutrient stock solutions. Desired salinities were obtained by the addition of the appropriate amount of de-ionized water (Brand 1984).

A series of laboratory experiments examining the effect of salinity and light on the growth rates of the clonal isolates were conducted in a growth chamber, at a constant temperature of 25 °C, with a light-dark cycle of 12L :12D, and light provided by cool white fluorescent bulbs. Aseptic techniques were used throughout.

**Salinity growth response**

Each species salinity growth response curve was determined individually under acclimated static salinity conditions. The experimental irradiance of 150 µEm⁻²sec⁻¹ was chosen to avoid both light limitation and light inhibition. Prior to the initiation of the experiment, populations of each species were established at each of the experimental salinities from stock cultures maintained at 25 °C by gradually increasing or decreasing the salinity in increments of 3 %. Populations were then allowed to fully acclimate to the experimental salinities of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 % for three weeks prior to the initiation of growth rate measurements. Experimental cultures of each species at each salinity were initiated by inoculating a dilute suspension of acclimated cells into replicate test tubes (25 X 125-mm Pyrex) and flasks (250 ml Pyrex) containing 30 ml and 50 ml of "f/2" growth media, respectively. Twice daily, tubes and flasks were inverted and swirled, respectively.

Batch culture technique was used in the determination of species exponential growth rates. Salinity was determined by use of a hand-held refractometer. Irradiance, as quantum scalar irradiance, was measured inside the flasks using a biospherical QLS-100 PAR irradiance meter with a 4π sensor, and as photon flux densities using a LiCor PAR meter with a cosine corrected 2π sensor when the source of the light was strictly unidirectional. The growth rate at each salinity was monitored daily by the measurement of *in vivo* fluorescence (Brand et al. 1981) using a Turner Designs 10 AU fluorometer. Calculation of the
average specific growth rates (u) and average divisions per day or doublings per day (k) were made using the following equations:

\[ u = \frac{1}{t} \cdot \ln \left( \frac{C_f}{C_i} \right) \]  

\[ k = \left[ \frac{1}{t} \cdot \ln \left( \frac{C_f}{C_i} \right) \right] \cdot \left( \frac{1}{\ln 2} \right) \]

where \( u \) = specific growth rate \( da^{-1} \); \( k \) = growth rate as divisions \( da^{-1} \) or doublings \( da^{-1} \); \( t \) = time between readings expressed in days; \( C_i \) is the initial fluorescence reading; and, \( C_f \) is the final fluorescence reading.

Simultaneous growth rate determinations made on each species using cell counts verified that the in vivo fluorescence methodology produced comparable growth rates for these species. The actual exponential growth rates of each replicate were determined by a linear least squares regression of time vs. the natural log of the in vivo fluorescence value. The growth rates of all of the replicates were then used to calculate mean growth rates.

**Irradiance growth response**

Populations were acclimated to a range of experimental light conditions ranging from 11 to 350 \( \mu E m^{-2} sec^{-1} \) for at least 2 weeks prior to the measurement of growth rates. Different light intensities were achieved by the use of neutral density screen cloth. Steady-state growth rates were maintained using continuous batch culture technique (Brand et al. 1981). All other experimental growth conditions and methodologies were the same as those used for the salinity growth rate experiments. Each species irradiance growth response curve was determined for the ambient experimental salinity of 25 \( 0/0 \). The irradiance growth curve (U-E curve) similar to its photosynthesis-irradiance (P-E curve) counterpart was determined by fitting the growth vs. irradiance data to the hyperbolic tangent function, as it has been used to successfully describe the growth irradiance relationship in phytoplankton (Yoder et al. 1979). The function

\[ U = U_m \cdot \text{Tanh} \left( \alpha_g \cdot E \cdot U_m^{-1} \right) \]

is defined by the following function parameters: \( U \), the growth rate \( da^{-1} \); \( U_m \), the maximum growth rate \( da^{-1} \); \( \alpha_g \), the light utilization growth efficiency or slope of the initial linear portion of the curve \( \frac{\text{growth} \ da^{-1}}{\text{Em}^2 \ da^{-1}} \); and, \( E_k \), the irradiance approximating the onset of light-saturated growth, defined as \( U_{max} \cdot \alpha_g^{-1} \frac{\text{growth} \ da^{-1}}{m^2 E^{-1}} \). The function parameters \( U_m \) and \( \alpha_g \) for each species were derived from the species U-E curves. The parameter \( \alpha_g \) in \( \text{Em}^2 \ da^{-1} \) was calculated using the average specific growth rate \( u \) \( da^{-1} / \mu E^{-1} m^2 sec^{-1} \) and the 12 hour experimental photoperiod. Linear, least squares regression of growth rate vs. irradiance in the light-limited regions of the growth curves \( \left( E < 40 \ \mu E m^2 sec^{-1} \right) \) was used to estimate the compensation growth-irradiance levels \( (E_c) \) (i.e. E at \( u=0 \)) and the initial slope of the u-E curves \( (\alpha_g) \). \( E_c \) estimates were calculated using the recommendation of Geider et al. (1985) in which measurements are made during balanced growth at very low specific growth rates (i.e. at \( <20\% \) of the light saturated growth rate).
Results

Salinity

The reproduction rates and standard errors at the various experimental salinities are presented for each species in Table 2-1 and Figure 2-1. The species responses closely followed taxonomic lines in that both diatom species responded similarly, as did both cyanobacteria species. The cyanobacteria species had the broadest salinity tolerance range and maintained growth rates that were no less than ~60% $U_m$ over the entire experimental salinity range of 5 $/\text{oo}$ to 60 $/\text{oo}$ (Table 2-2). In contrast, the diatoms had narrower salinity tolerance ranges. The salinity growth curves suggest that the absolute lower salinity tolerance limit is well below 5 $/\text{oo}$ for both cyanobacteria species, near 5 $/\text{oo}$ for C. cf. *salsugineus* and between 10 $/\text{oo}$ and 15 $/\text{oo}$ for *T*. cf. *oceanica*. Both cyanobacteria species had salinity optima for growth (10-20 $/\text{oo}$), that were lower than the optima of *T*. cf. *oceanica* (25-40 $/\text{oo}$) and *C*. cf. *salsugineus* (15-30 $/\text{oo}$). The relatively broad salinity optimum displayed by all four species is indicative of their euryhaline nature, while the flatter salinity growth curve of the cyanobacteria species shows the smaller influence that salinity has on their growth rates (Fig. 2-1). The maximal growth rate of each species at its salinity optimum in divisions da$^{-1}$ was 1.06 for *S*. cf. *elongatus*, and 0.97 for *Synechocystis* sp. both at 15 $/\text{oo}$ and 2.11 for *C*. cf. *salsugineus* at 25 $/\text{oo}$ and 2.46 for *T*. cf. *oceanica* at 40 $/\text{oo}$ (Table 2-1). Between 15 $/\text{oo}$ and 50 $/\text{oo}$ the diatom species maximal growth rates exceeded those of the cyanobacteria species, while at 5 $/\text{oo}$ and 60 $/\text{oo}$ the growth rates of the cyanobacteria exceeded those of the diatoms.

The results may be summarized as follows: the cyanobacteria species have broader tolerance ranges and higher optimal salinity ranges for growth than the diatoms species; the diatom species $U_m$'s are greater than those of the cyanobacteria species at low to moderate salinities (15-35 $/\text{oo}$); and under hypersaline conditions (45-60 $/\text{oo}$) the $U_m$'s of the cyanobacteria approach and/or exceed those of the diatoms.

Light

The hyperbolic tangent function effectively described the growth vs. irradiance (U-E) responses of both cyanobacteria and diatom species (Fig. 2-2). The species responses once again followed taxonomic lines in that both cyanobacteria species responded alike, as did both diatoms. Values of $\alpha$, $E_c$, and $E_k$ for *S*. cf. *elongatus* are depicted in Figure 2-3 and listed for all species in Table 2-2. The $\alpha$ values in m$^2$ E$^{-1}$ of the cyanobacteria species (0.23 for *S*. cf. *elongatus*, 0.23 for *Synechocystis* sp.) were lower than those of the diatom species, (0.35 for *C*. cf. *salsugineus*, and 0.33 for *T*. cf. *oceanica*). No differences were detected in $E_c$'s between the species (Table 2-2). The $E_k$'s indicated that the cyanobacteria (70-80 $\mu$Em$^{-2}$sec$^{-1}$) saturate at a much lower light intensity than the diatom species (121-133 $\mu$Em$^{-2}$sec$^{-1}$) (Table 2-2). No photoinhibitory response was observed in any of the species growth rates up to an irradiance of 350 $\mu$Em$^{-2}$sec$^{-1}$ (Fig. 2-2).
The results may be summarized as follows: the diatoms are more efficient in light capture and utilization (higher $\alpha_g$’s) than the cyanobacteria, the cyanobacteria species growth rates saturate at lower irradiances (lower $E_k$’s) than the diatom species; and the cyanobacteria and diatoms have similarly low minimum light levels for net growth ($E_c$’s).
Table 2-1. Mean growth rates in divisions per day and standard errors (SE) for each species at each experimental salinity.

<table>
<thead>
<tr>
<th>Salinity (‰)</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
<th>40</th>
<th>45</th>
<th>50</th>
<th>55</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S. cf. elongatus</strong></td>
<td>0.82 (0.11)</td>
<td>1.03 (0.01)</td>
<td>1.06 (0.03)</td>
<td>0.97 (0.06)</td>
<td>0.89 (0.04)</td>
<td>0.77 (0.04)</td>
<td>0.67 (0.02)</td>
<td>0.68 (0.02)</td>
<td>0.70 (0.01)</td>
<td>0.64 (0.01)</td>
<td>0.56 (0.02)</td>
<td>0.60 (0.01)</td>
</tr>
<tr>
<td><strong>Synechocystis sp.</strong></td>
<td>0.86 (0.02)</td>
<td>0.90 (0.01)</td>
<td>0.97 (0.02)</td>
<td>0.95 (0.02)</td>
<td>0.93 (0.02)</td>
<td>0.89 (0.01)</td>
<td>0.86 (0.01)</td>
<td>0.81 (0.01)</td>
<td>0.77 (0.01)</td>
<td>0.73 (0.01)</td>
<td>0.67 (0.02)</td>
<td>0.58 (0.01)</td>
</tr>
<tr>
<td><strong>T. cf. oceanica</strong></td>
<td>0.00 (0.08)</td>
<td>0.00 (0.04)</td>
<td>1.85 (0.10)</td>
<td>2.35 (0.11)</td>
<td>2.45 (0.12)</td>
<td>2.46 (0.07)</td>
<td>2.45 (0.07)</td>
<td>2.48 (0.12)</td>
<td>2.16 (0.07)</td>
<td>1.00 (0.07)</td>
<td>0.00 (0.07)</td>
<td>0.00 (0.07)</td>
</tr>
<tr>
<td><strong>C. cf. salsugineus</strong></td>
<td>0.53 (0.04)</td>
<td>1.81 (0.03)</td>
<td>2.05 (0.05)</td>
<td>2.09 (0.03)</td>
<td>2.11 (0.03)</td>
<td>2.03 (0.03)</td>
<td>1.91 (0.01)</td>
<td>1.70 (0.02)</td>
<td>1.61 (0.02)</td>
<td>1.40 (0.02)</td>
<td>0.95 (0.01)</td>
<td>0.08 (0.04)</td>
</tr>
</tbody>
</table>
Figure 2-1. Salinity growth curves plotted as mean growth and standard error at each salinity. *S. cf. elongatus* = ▲, *Synechocystis* sp. = ●, *C. cf. salsugineus* = ♦, and *C. cf. oceanica* = ■.
Figure 2-2. Effect of irradiance on cell division rate. All curves are non-linear least squares regression fit to equation 1.
Figure 2-3. U-E Curve of *Synechococcus* cf. *elongatus*. Triangles are single observations. Curve fit by non-linear least squares to the hyperbolic tangent function (equation 1).
Table 2-2. Light growth-curve parameters for each species and standard errors (S.E.). Results of linear regressions are based on measurements of growth at irradiances under 36 μEm²sec⁻¹, while results of the non-linear least squares fit to the hyperbolic tangent function (equation 1) are based on all measured growth rate values.

<table>
<thead>
<tr>
<th>Species</th>
<th>Linear Regression</th>
<th>Nonlinear Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\alpha_g$ (m²E⁻¹)</td>
<td>$I_c$ (μEm²sec⁻¹)</td>
</tr>
<tr>
<td>S. cf. elongatus</td>
<td>0.23 (0.02)</td>
<td>5.01 (5.17)</td>
</tr>
<tr>
<td>Synechocystis sp.</td>
<td>0.23 (0.02)</td>
<td>2.75 (5.97)</td>
</tr>
<tr>
<td>T. cf. oceanica</td>
<td>0.33 (0.02)</td>
<td>6.5 (6.70)</td>
</tr>
<tr>
<td>C. cf. salsugineus</td>
<td>0.35 (0.08)</td>
<td>8.13 (11.33)</td>
</tr>
</tbody>
</table>
Table 2-3. $E_k$, $E_c$ and $\alpha_g$ of the study species and selected freshwater and marine cyanobacteria and diatoms.

<table>
<thead>
<tr>
<th></th>
<th>$E_k$ ($\mu$Em$^{-2}$sec$^{-1}$)</th>
<th>$E_c$ ($\mu$Em$^{-2}$sec$^{-1}$)</th>
<th>$\alpha_g$ ($m^2Em^{-1}$)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cyanobacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Synechococcus</em> sp.</td>
<td>45</td>
<td>-</td>
<td>0.14a</td>
<td>Morris and Glover (1981)</td>
</tr>
<tr>
<td><em>Synechococcus</em> sp.</td>
<td>55</td>
<td>-</td>
<td>0.28a</td>
<td>Morris and Glover (1981)</td>
</tr>
<tr>
<td><em>Synechococcus</em> sp.</td>
<td>-</td>
<td>-</td>
<td>0.13</td>
<td>Kana and Glibert (1987a)</td>
</tr>
<tr>
<td><em>Synechococcus</em> cf. elongatus</td>
<td>75</td>
<td>5.01</td>
<td>0.23</td>
<td><em>this study</em></td>
</tr>
<tr>
<td><em>Synechocystis</em> sp.</td>
<td>80</td>
<td>2.75</td>
<td>0.23</td>
<td><em>this study</em></td>
</tr>
<tr>
<td><em>Synechococcus</em> linearis</td>
<td>150</td>
<td>-</td>
<td>0.41a</td>
<td>Healy (1985)</td>
</tr>
<tr>
<td><em>Oscillatoria agardhii</em></td>
<td>-</td>
<td>0.7</td>
<td></td>
<td>Lee and Rhee (1999)</td>
</tr>
<tr>
<td><em>Anabaena flos-aquae</em></td>
<td>-</td>
<td>3.4</td>
<td>0.08</td>
<td>Lee and Rhee (1999)</td>
</tr>
<tr>
<td><strong>Diatoms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Thalassiosira</em> cf. oceanica</td>
<td>133</td>
<td>6.5</td>
<td>0.33</td>
<td><em>this study</em></td>
</tr>
<tr>
<td><em>Chaetoceros</em> cf. salsugineus</td>
<td>121</td>
<td>8.13</td>
<td>0.35</td>
<td><em>this study</em></td>
</tr>
<tr>
<td><em>Thalassiosira</em> oceanica</td>
<td>-</td>
<td>17</td>
<td>-</td>
<td>Sakshaug et al. (1987)</td>
</tr>
<tr>
<td><em>Thalassiosira</em> pseudonana</td>
<td>-</td>
<td>5.1</td>
<td>-</td>
<td>Hobson and Guest (1983)</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>0.74b</td>
<td>-</td>
<td>Thompson (1999)</td>
</tr>
<tr>
<td><em>Thalassiosira</em> fluviatilis</td>
<td>-</td>
<td>1.7b</td>
<td>-</td>
<td>Thompson (1999)</td>
</tr>
<tr>
<td><em>Thalassiosira</em> weisflogii</td>
<td>-</td>
<td>1.7</td>
<td>-</td>
<td>Bannister (1979)</td>
</tr>
<tr>
<td><em>Leptoclintris danicus</em></td>
<td>-</td>
<td>23</td>
<td>0.12</td>
<td>Falkowski et al. (1985)</td>
</tr>
<tr>
<td><em>Phaeodactylum tricornutum</em></td>
<td>-</td>
<td>1</td>
<td>0.14 - 0.9</td>
<td>Verity (1982)</td>
</tr>
<tr>
<td><em>Ditylum brightwelli</em></td>
<td>-</td>
<td>0.5</td>
<td>0.29</td>
<td>Geider et al. (1985)</td>
</tr>
<tr>
<td><em>Nitzschia turgidula</em></td>
<td>-</td>
<td>-</td>
<td>0.22</td>
<td>Paasche (1968)</td>
</tr>
<tr>
<td><em>Nitzschia turgidula</em></td>
<td>-</td>
<td>-</td>
<td>0.4</td>
<td>Paasche (1968)</td>
</tr>
<tr>
<td><em>Skeletonema costatum</em></td>
<td>-</td>
<td>0.3</td>
<td>-</td>
<td>Falkowski and Owens (1980)</td>
</tr>
<tr>
<td><em>Skeletonema costatum</em></td>
<td>-</td>
<td>1.1</td>
<td>-</td>
<td>Langdon (1987)</td>
</tr>
<tr>
<td><em>Skeletonema costatum</em></td>
<td>-</td>
<td>4.4</td>
<td>-</td>
<td>Yoder (1979)</td>
</tr>
</tbody>
</table>

a Values calculated using authors data.

b $E_c$’s determined under different photoperiods.
Discussion

**Salinity**

The typical phytoplankton salinity vs. growth response is a curve with a narrow or broad peak that decreases at both the higher and lower salinities. The characteristics of the curve are species specific, but the general trend is for estuarine species to have broad curves and oceanic species to have narrower curves. Overall, the salinity growth response curves of the four study species (Fig. 2-1) agree with the general curves of estuarine and coastal phytoplankton species. Both estuarine and coastal species have relatively broad salinity optima for growth, with most estuarine isolates reproducing well between 5 \(^{0}/\text{o}_{00}\) and 45 \(^{0}/\text{o}_{00}\), and most coastal isolates growing well between 15 and 45 \(^{0}/\text{o}_{00}\), but unable to grow at 5 \(^{0}/\text{o}_{00}\) (Brand 1984).

Both cyanobacteria species and *C. cf. salsugineus* fit the estuarine pattern with growth at 5 \(^{0}/\text{o}_{00}\), while *T. cf. oceanica* resembles that of a coastal species. Despite some differences in methodology, there was good agreement between the salinity growth response of the Florida Bay clones in my study and the salinity growth characteristics reported in the literature. For example, *Synechococcus elongatus* from Tampa Bay was found to be a euryhaline species growing well between 5 and 35 \(^{0}/\text{o}_{00}\) with a broad salinity optimum of 15-25 \(^{0}/\text{o}_{00}\) (Erickson and Farrow 1965). A natural population of *Synechococcus* sp. from Florida Bay was found to have a broad salinity tolerance with cell densities of at least 50% maximum between 10 and 55 \(^{0}/\text{o}_{00}\) (Phlips and Badyk 1996).

Although there was good agreement between literature values and the value in my study, discrepancies are not uncommon as large differences in salinity tolerance ranges and optimal salinity ranges for growth have been documented between clones of the same species and are likely to be the result of genetic differences (Brand 1984).

In order to evaluate the role of salinity as a major regulatory factor in the large persistent and recurring cyanobacteria (*S. cf. elongatus*, *Synechococcus spp.* and *Synechocystis* sp.) dominated blooms in Florida Bay, the following should be considered: the bay-wide salinity patterns, the salinity tolerance ranges and optimal salinity ranges for growth of the dominant cyanobacteria and diatom bloom species, and the spatial and temporal patterns of the cyanobacteria dominated and diatom dominated blooms in the bay.

Salinity patterns since 1955 indicate that Florida Bay as a whole has not acted like an estuary, but more like a large marine lagoon. Estuarine conditions in the bay are mainly limited to the northeast margin of the bay nearest the major sources of freshwater (Robblee et al. 2001). Distinct salinity patterns are found in each of Florida Bay’s three ecological zones. The salinity in the western zone is the most stable, being strongly moderated by the coastal waters of the Gulf of Mexico, while the eastern and central zone salinities fluctuate widely in response to evaporation, direct freshwater runoff from the Everglades, and restricted circulation (Boyer et al. 1999). In general salinity variation in the bay is greatest in the northeast.
and decreases westward. The central zone is distinguished by periodic dramatic hypersaline events. Hypersaline conditions usually appear first and are most persistent in the central zone where salinities have exceeded 40% for 60% of the months since 1955 (Robblee et al. 2001).

The ranges of salinities commonly found in Florida Bay are suitable for the growth of both estuarine and coastal phytoplankton species. For the 45 year record, the mean monthly salinities of the western, central and eastern zones were respectively approximately 36%, 42%, and 33%; the range of monthly average salinities were approximately 28-40%, 21-57%, and 13-51%; and the median monthly salinities ranged from approximately 27-50%, 17-61%, and 14-53%.

A recent review of the salinity patterns during which the bay experienced large and persistent algal blooms of concern, reveals that the monthly median salinities during a ten year period (1989-99) ranged from approximately 27-50% in the western zone, 17-61% in the central zone, and 14-53% in the eastern zone (Boyer and Jones 2000). Since the lower salinity range values in all three zones were above 14% and the lower salinity tolerance limits of the four study species are below 14%, on average the lower salinity tolerance limits of the species used in this study were not exceeded. Similarly, the upper salinity range values of 50% and 53% for the western and eastern zones, respectively, did not exceed the salinity tolerances of any of the study species. Only in the central zone with a monthly median upper salinity range value of 61% were the diatom species salinity tolerance ranges exceeded (Fig. 2-1). These monthly median salinity ranges indicate that on average only in the central region may salinity periodically favor bloom dominance by the cyanobacteria species (S. cf. elongatus and Synechocystis sp.) by exceeding the tolerance limits of the diatom species (C. cf. salsugineus and T. cf. oceanica).

A comparison of each species optimal salinity for growth with the bay’s salinity patterns from 1989-99 indicate that median monthly salinity values of approximately 35%, 34% and 28% for the western, central, and eastern zones respectively (Boyer and Jones 2000), overlap or approximate the optimal ranges for T. cf. oceanica (25-40%) and C. cf. salsugineus (15-30%), and are higher than the optimal growth ranges for both cyanobacteria species (10-20%). Although the median monthly values for all three zones are above the optimal salinity range of the cyanobacteria, the growth rates of the cyanobacteria clones at these higher salinities still remains near their maximal growth rate (Table 2-1, Fig. 2-1).

The salinity growth curves of the species suggest that in order for reduced salinity to selectively enhance the growth of the cyanobacteria species over the growth of T. cf. oceanica and C. cf. salsugineus, the salinity must be below 10% or 5% respectively. Salinity conditions in the bay below 5% or 10% would most likely be found nearest the freshwater sources, along the northern boundary near the mangrove-lined creeks draining Taylor Slough and the SDCS canal system during periods of freshwater runoff (Robblee et al. 2001). Blooms dominated by cyanobacteria have not been documented as either originating or persisting in these potentially low salinity regions of the bay (Phlips et al. 1999), and long-term salinity patterns indicate that these very low salinities are not commonly found in the bloom-forming central and western regions of the bay (Boyer and Jones 2000). Although the bay experiences large variations in
salinity, estuarine conditions across Florida Bay are rare and usually associated with periods of above average rainfall or episodic high rainfall events (Robblee et al. 2001). As a result, the potential advantage that the cyanobacteria would have over the diatom species under very low salinities does not appear to be a factor in the development of the large cyanobacteria dominated blooms in the north-central region and in the transition zone between the north-central and north-western regions of the bay.

The salinity growth curves also indicate that hypersaline conditions above 50 \%/00 and between 55 and 60 \%/00 will selectively enhance the growth of both cyanobacteria populations over those of T. cf. oceanica and C. cf. salsugineus, respectively. Between 50 \%/00 and 60 \%/00 the diatom species growth rates decrease and approach or fall below those of the normally slower growing cyanobacteria species.

The potential role of hypersalinity in determining bloom dominance is supported by the greater tolerance of the cyanobacteria species than the diatom species of very high salinities, and the common occurrence of cyanobacteria dominated blooms in the north-central zone of the bay, which has a history hypersaline events. Although blooms dominated by cyanobacteria have been documented on multiple occasions in all three zones, the largest and most persistent cyanobacteria dominated blooms have been consistently found in the north-central zone (Steidinger and Phlips 1996, Steidinger et al. 1995). If hypersalinity plays a major role in the development of cyanobacteria dominated blooms in the north-central region, then the eastern region, which also experiences hypersaline conditions, would also be expected to have cyanobacteria dominated blooms. Although the eastern region does not experience large blooms like the north-central region, the phytoplankton community is nonetheless at times still dominated by cyanobacteria (Steidinger et al. 1995). On the other hand, the occasional cyanobacteria dominated blooms that occur in the western zone which typically has diatom dominated blooms and is for the most part not hypersaline, must be attributed to factors other than hypersalinity such as wind and/or tidal driven advection of bloom water westward from the north-central zone.

An important consideration when evaluating the role of interspecific differences in salinity-growth response curves (determined in this study), as a factor contributing to the development and persistence of cyanobacterial dominated blooms in Florida Bay would be that the growth rates and physiological tolerance limits of the bloom species in this study were determined under saturating light and nutrient conditions. Salinity tolerance limits of species would be expected to narrow under the added stress imposed by nutrient and or light limiting conditions. But the more euryhaline response and superior limiting-nutrient growth characteristics of the cyanobacteria (Chapter II) suggest that their upper and lower salinity tolerance limits would be reduced proportionately less than those of the diatom species. This may be of significance during hypersaline non-saturating light and/or nutrient conditions, as the ambient salinity may not have to be greater than 50 \%/00 in order to begin to shift the competitive advantage from the diatom species to the cyanobacteria species, as was the case under nutrient and light saturating conditions.

In summary, the interspecific differences in salinity-growth curves indicate that salinity, by impacting the survival and growth of each species differently, may influence the composition of the phytoplankton community in Florida Bay. Under nutrient and light sufficient conditions, salinities <10 \%/00
and >50 °/oo begin to favor the development of cyanobacteria (S. cf. elongatus and Synechocystis sp.) dominated blooms, while salinities between 15 °/oo and 45 °/oo, which encompasses the majority of the salinities occurring in the bay, favor the development of diatom (C. cf. salsugineus and T. cf. oceanica) dominated blooms, since the growth rates of the diatom species are consistently much higher than that of the cyanobacteria throughout this salinity range. The obvious conclusion is that for most of Florida Bay, excluding the lower salinity fringe environment along the northern boundary where salinities may regularly be 15 °/oo or less, and the regularly hypersaline north-central zone where salinities often exceed 50 °/oo, the bay-wide salinities do not selectively favor the development of cyanobacteria (S. cf. elongatus and Synechocystis sp.) dominated blooms, but instead favor the development of diatom (C. cf. salsugineus and T. cf. oceanica) dominated blooms.

Light

In order to evaluate the role of light as a major regulatory factor in the large persistent and recurring cyanobacteria dominated blooms in Florida Bay, the following should be considered: the bay’s water column irradiance patterns, the light growth responses of the dominant cyanobacteria and diatom bloom species, and the temporal and spatial patterns of the cyanobacteria dominated and diatom dominated blooms in the bay.

The water column irradiance patterns in Florida Bay are characterized by large temporal and spatial differences (Boyer et al. 1999). These differences are largely due to the extinction of light by resuspended benthic material and planktonic algae. The shallow depth of Florida Bay (0-4 m) allows for easier mixing of the entire water column, which in turn can lead to the resuspension of unconsolidated benthic material. The amount of sediment and detritus subject to resuspension greatly increased following the seagrass die-offs that began in 1987 mainly in and around the north-central zone (Rankin Lake and Rabbitt Key Basin) and in the transitional area between the central and western zones (Johnson Key Basin) (Robblee et al. 1991). Periodic dense algal blooms that began occurring around 1991, along with the sediment-derived turbidity have altered both the water clarity and the average water column irradiance in the bay, especially in the regions in which major seagrass die-offs occurred.

The results of a seven-year study (1991-98) of Florida Bay indicated that turbidity has a strong seasonal signal in all three Florida Bay ecological zones, with a maximum during the dry winter-spring season resulting largely from the resuspension of fine sediments by strong northeasterly winds. There was also a seasonal signal in phytoplankton biomass with peaks in the central and western zones occurring in the fall and fall-winter periods, respectively. The high turbidity values found in the central and western zones were a product of both algal biomass and resuspended sediment (Boyer et al. 1999). When water column turbidity and phytoplankton biomass patterns in Florida Bay are used as indicators of water column irradiance levels they predict that reduced light environments are most likely to occur in all three zones during the winter, with the lowest light environments in the north-central and western zones.
Other studies have found similar seasonal and spatial patterns of light availability. Phlips et al. (1995) observed that the mean daily light level in the mixed layer, \( I_m \), displayed a seasonal signal being generally lowest during the windy winter-spring season and was in general lowest in the north-western zone, higher in the central zone, and highest in the eastern zone. Using 2-5 Em\(^2\)da\(^{-1}\) as an estimate of the threshold level for the onset of light limitation, the \( I_m \) values in Florida Bay indicated that certain areas in the western and central zones were at or near the threshold level for the onset of phytoplankton light limitation. Consequently, the potential for light limitation of phytoplankton is greatest in the western and north-central zone, while most of the south-central zone and eastern zone exhibit a low potential for light limitation.

In another more recent study of continuous PAR measurements from 1998 - 2000, Carlson et al. (2001) also found the same pattern of reduced water column irradiances in the fall-winter, but only on three occasions was the mean daily photon flux near the benthos less than or equal to 5 Em\(^2\)da\(^{-1}\) and this occurred at stations in the north-central and western regions.

The U-E growth curves of the Florida Bay species indicate that overall all four species have the genetic potential to acclimate and grow successfully at both high and low irradiance levels. This is not unexpected, as studies have found species of both cyanobacteria and diatoms that grow well at both high and low irradiances (Morris and Glover 1981, Kana and Glibert 1987a, Brand and Guillard 1981). The initial expectation of this study was that the dominant Florida Bay cyanobacteria bloom species would have the characteristics of a low light adapted species and owe their dominance in part to the reduced light conditions found particularly in the turbid north-central region. The cyanobacteria were also hypothesized to have low light requirements as it has been speculated that they have a benthic stage (Phlips et al. 1999). In addition, \textit{Synechococcus} species have generally been described as having relatively low light requirements for growth, owing to lower maintenance energy requirements (Mur 1983; Richardson et al. 1983). On the other hand, the cyanobacteria bloom species \( E_{c}'s \) could just as easily have had high values due to the very high light environment of the bay, as has been found to be the case with many surface-blooming cyanobacteria (Reynolds 1988).

Light is highly variable in most aquatic environments and as a result, phytoplankton have evolved numerous adaptations to help them optimize their use of this resource, that include physiological adaptations for growth at both low and high irradiances. Together, light availability and differences in species physiological adaptations to light have been found to influence the composition and structure of phytoplankton communities (Oliver and Ganf 2000). Physiological adaptations to low light environments include low saturation irradiance for growth (\( E_k \)), low minimum irradiance for growth (\( E_c \)), high light utilization growth efficiency (\( a_g \)), and low photoinhibition irradiance. In contrast, the physiological adaptations to high light environments would include a high \( E_k \), a high \( E_c \), a low \( a_g \) and high photoinhibition irradiance. The occurrence of both high and low light environments in Florida Bay and the interspecific differences in the \( E_k, E_c, a_g \) as well as the photoinhibitory response of the study species
through the relative competitive advantage each offers, suggests that light availability may play a role in determining phytoplankton species bloom dominance in Florida Bay.

One strategy that may be utilized by dominant bloom species in Florida Bay to compensate for low light intensities is low saturation irradiance for growth, which allows them to attain their maximal growth rate under relatively low irradiances. The similarity of the lower $E_k$ values among the two Florida Bay cyanobacteria isolates (75 and 80 $\mu \text{Em}^{-2} \text{sec}^{-1}$) compared with the higher $E_k$ values shared by the Florida Bay diatom isolates (121 and 133 $\mu \text{Em}^{-2} \text{sec}^{-1}$) suggests a physiological difference between the two dominant groups of bloom species in the bay. The lower $E_k$'s of the cyanobacteria species indicates that they are genetically adapted to grow at low irradiances, while the higher $E_k$'s of the diatoms indicate a genetic predisposition for optimal growth at higher irradiances. This is not unexpected as species of marine *Synechococcus* in general have been found to saturate photosynthesis and growth at relatively low irradiances (Table 2-3). The Florida Bay cyanobacteria species $E_k$'s for growth fall approximately midway between literature $E_k$'s for growth for species of marine *Synechococcus* and freshwater *Synechococcus* (Table 2-3). Keeping in mind that P-E and U-E relationships may be comparable when photosynthesis and growth are tightly coupled, the saturation growth irradiance of *S. cf. elongatus* used in my study is lower but still comparable to the photosynthetic saturation irradiance of 100 $\mu \text{Em}^{-2} \text{sec}^{-1}$ that was found by Phlips and Badylak (1996) for a natural phytoplankton field population from Florida Bay, dominated by *Synechococcus* spp. (~93% of total biovolume). The $E_k$'s for growth for the two diatom bloom species are also well within the 100-200 $\mu \text{Em}^{-2} \text{sec}^{-1}$ range commonly reported for planktonic diatoms (Furnas 1991).

Although the lower $E_k$'s of the cyanobacteria species in my study indicate they have the ability to physiologically adapt to low irradiances that occur periodically in the north-central and north-western regions of Florida Bay, the U-E responses (Fig. 2-2) clearly indicate that this ability does not translate into a competitive advantage over the diatom species under low light conditions.

A second strategy that may be utilized by Florida Bay phytoplankton to compensate for low light intensities is a low minimum light level for net growth ($E_c$). While a typical $E_c$ value for diatoms is ~6 $\mu \text{Em}^{-2} \text{sec}^{-1}$, other observations indicate minimum light levels for growth above 5 $\mu \text{Em}^{-2} \text{sec}^{-1}$ as characteristic of Cyanophyceae, Dinophyceae, and Bacillariophyceae, and 20 $\mu \text{Em}^{-2} \text{sec}^{-1}$ for Chlorophyceae (Richardson et al. 1983). More recent observations indicate that representatives of all major algal groups can be grown at light levels on the order of 1 $\mu \text{Em}^{-2} \text{sec}^{-1}$ (Geider et al. 1985). The $E_c$ values determined in my study ranging from 2.75 - 8.13 $\mu \text{Em}^{-2} \text{sec}^{-1}$ are comparable to the wide range of reported literature values of other similar coastal and estuarine phytoplankton (Table 2-3). The $E_c$ of 17 $\mu \text{Em}^{-2} \text{sec}^{-1}$ for *T. oceanica* (Sakshaug et al. 1987) is also reasonably comparable to the $E_c$ estimate of 6.5 $\mu \text{Em}^{-2} \text{sec}^{-1}$ obtained in this study for the *T. cf. oceanica* isolate from Florida Bay. But the wide range in $E_c$'s that diatoms and cyanobacteria share (Table 2-3) prevents any generalization regarding the competitive advantage of one group over the other at extremely low irradiances. Instead, comparisons can only be made on a species basis. Given the difficulty of measuring precisely such low growth and irradiance values, as well as the estimated error associated with each $E_c$ value, no detectable difference was found
between the $E_c$ values of the Florida Bay clones. Any competitive advantage that a low $E_c$ may give to a species would have to be realized in an extremely low light environment. But in a two-year study (1998 - 2000) in Florida Bay, weekly mean mid-day PAR values near the sediment surface in the periodically light-limited north-central and western regions were for the most part above 250 µEm$^{-2}$sec$^{-1}$ (Carlson et al. 2001), which greatly exceeds all of the study species $E_c$'s. The conclusion that can be reached is that all four Florida Bay isolates have similarly low minimum irradiance requirements and any interspecific differences in $E_c$'s that may not have been detected in this study would not be expected to influence their relative dominance in Florida Bay.

A third strategy that may be employed by Florida Bay phytoplankton to optimize a low light environment is to increase their efficiency of light capture and utilization. The similarly higher light utilization growth efficiencies ($\alpha_g$’s) of the diatom species compared to the lower $\alpha_g$’s shared by the two cyanobacteria species (Table 2-2) suggests a physiological difference between the two groups of dominant bloom species in Florida Bay. Although no literature values of the study species $\alpha_g$ were available for comparison, the $\alpha_g$ values found in this study are all well within the range reported for other similar coastal and estuarine cyanobacteria and diatoms (Table 2-3).

The lower $\alpha_g$’s of the cyanobacteria clones compared to the $\alpha_g$’s of the diatom clones may reflect a genetic adaptation by the cyanobacteria to the potentially very high light environment of Florida Bay, in which there is a decreased reliance on irradiance-growth efficiency as a competitive strategy. The apparent decreased reliance on irradiance-growth efficiency seen in the cyanobacteria study species is also shared by many surface-blooming cyanobacteria, which have poor light-utilization efficiencies (Reynolds 1988).

The higher $\alpha_g$’s of the two diatoms in my study compared to the lower $\alpha_g$ shared by the two cyanobacteria species indicate that per unit increase in PAR, under light limiting conditions, the diatom clones grow slightly faster than the cyanobacteria clones. Alternatively, under non-limiting light conditions, when the diatoms higher $\alpha_g$’s would not give them any advantage over the cyanobacteria, the diatoms still remain at a competitive advantage due to their much higher light saturated $U_m$’s (Table 2-2). The competitive advantage that the two diatom study species have over both cyanobacteria study species under both limiting and non-limiting light conditions due to their higher $\alpha_g$’s and $U_m$’s, respectively, may help explain patterns of bloom species dominance in low and high light environments within the bay. The northwestern region typically experiences large blooms dominated not by cyanobacteria, but by species of diatoms (Phlips et al. 1999). The north-western region also frequently experiences high sediment-derived turbidity as a result of massive seagrass die-offs (Robblee et al. 1991) and increased winter mixing of the water column. The resulting $I_m$ values may be at times light limiting (Phlips et al. 1995). The higher $\alpha_g$’s of the diatoms may then contribute to the development of these diatom-dominated blooms as opposed to cyanobacteria dominated blooms in winter particularly in the north-western region. Although other factors are likely to play more significant roles in determining diatom bloom dominance in the northwestern region in winter, the potential influence of light limitation on the bloom species competitive interactions in this region during the winter cannot be discounted.
In contrast, the north-central region which experiences light limiting conditions second to the north-western region in winter is typically dominated by cyanobacteria (Phlips et al. 1995). But mixed blooms of cyanobacteria and diatoms as well as diatom-dominated blooms occur in the north-central region during winter as well (Steidinger et al. 1995, Phlips et al. 1999) and light limitation may therefore play a role in the development of increased diatom dominance in the north-central region during the winter. But the cyanobacteria dominated blooms must be promoted by some factor(s) other than the availability of light as the lower $\alpha_g$'s of the cyanobacteria bloom species place them at a competitive disadvantage with respect to the diatoms under light limiting conditions.

The common occurrence of diatom dominated blooms throughout much of Florida Bay may be attributed in part to their much higher light saturated growth rates ($U_m$'s), particularly during the summer when solar irradiances are high and sediment derived turbidity is low. For example, the occurrence of small diatom blooms in the western zone in summer usually dominated by species of *Chaetoceros* spp. (Steidinger et al. 1995) rather than cyanobacteria species may be due in part to the selective action of light availability on bloom species dominance, as light is not expected to be a significantly limiting resource during this period of low turbidity and high water column irradiance. The U-E relationship (Fig. 2-2) shows how the diatoms outcompete the cyanobacteria under both limiting and saturating irradiances. Diatom species growth rates exceed those of the cyanobacteria species by $\approx$0.6 da$^{-1}$ at 75 $\mu$Em$^{-2}$sec$^{-1}$ (the saturating growth rate for the cyanobacteria) and $\approx$1.5 da$^{-1}$ at 175 $\mu$Em$^{-2}$sec$^{-1}$ (the saturating growth rate for the diatoms) giving them a marked competitive advantage. Although they have higher $\alpha_g$'s and $U_m$'s, the higher $U_m$'s would be expected to play a proportionately greater role since on average bay-wide water column irradiances considerably exceed the diatom $U_m$ saturating irradiance of 175 $\mu$Em$^{-2}$sec$^{-1}$. The magnitude of the competitive advantage that the diatoms have over the cyanobacteria is proportionately reduced with decreasing irradiance (Fig. 2-2) and it is only at low irradiances of $\approx$50 $\mu$Em$^{-2}$sec$^{-1}$ and below when the difference in growth rate is $\approx$0.2 da$^{-1}$ or less that the cyanobacteria may be considered to remain somewhat competitive with the diatoms. Consequently, the success of the diatoms and their relative dominance in Florida Bay may be attributed in part to their higher $U_m$'s and the high ambient light levels of the bay which for the most part allow them to attain their light saturated $U_m$'s, except on occasion in the northwestern and north-central regions during winter.

While light limited environments may play a role in structuring phytoplankton communities through interspecific differences in the response parameters ($E_k$'s, $E_c$'s and $\alpha_g$'s), light saturated environments also influence community structure through interspecific differences in $U_m$ and photoinhibition. Photoinhibition results in a decrease in growth or photosynthesis at increasing irradiances when populations acclimated to lower irradiances are suddenly exposed to much higher irradiances or when populations are physiologically unable to maintain maximal growth rates at higher irradiances. Phytoplankton have developed diverse strategies to compensate for high light intensities such as decreased size and number of photosynthetic units, increased amounts of photoprotective pigments, and regulation of their vertical position in the water column through buoyancy regulation (Oliver and Ganf 2000).
prevailing view has been that marine *Synechococcus* species are adapted to grow at low light intensities and grow best deep in the euphotic zone. This view was based on field and laboratory observations of *Synechococcus* spp., in which growth and photosynthesis were inhibited at moderate to high irradiances (Platt et al. 1983; Barlow and Alberte 1985). But more recent field studies indicate that populations of *Synechococcus* spp. exhibit good growth in surface mixed layers (Landry et al. 1984), and natural populations grown at surface irradiance generally grow faster (Campbell and Carpenter 1986). The inhibition found at higher irradiances in the earlier laboratory studies of cyanobacteria has been attributed primarily to a lack of adequate pre-conditioning at higher irradiances prior to measurements of photosynthesis or growth (Kana and Glibert 1987b).

The absence of a photoinhibitory response in all four species in my study after the species were fully acclimated to the experimental irradiances indicates that they all have the genetic potential to maintain maximal growth rates at irradiances exceeding their saturating irradiance, at least as high as 350 µEm⁻² sec⁻¹ (Fig 2-2). An example of a *Synechococcus* species that is very well adapted to a shallow-water high light environment similar to that found in Florida Bay, is the *Synechococcus* strain BG0011, which was isolated from a lagoon in the Florida Keys and found to photosynthesize over a wide range of light intensity, with no sign of photoinhibition up to 2100 µEm⁻² sec⁻¹ (Phlips et al. 1989). Other studies have also found no indication of photoinhibition at irradiances up to 2000 µEm⁻² sec⁻¹ for species of marine *Synechococcus* (Kana and Glibert 1987b). Consequently, it has been suggested that the presumed preference for low light by *Synechococcus* spp. should be re-evaluated, and that *Synechococcus* should be considered euryphotic, since it can grow well over a broad range of irradiances (Kana & Glibert, 1987a). In contrast, measurements of photosynthetic oxygen evolution by a natural phytoplankton field population from Florida Bay, dominated by *Synechococcus* sp. (~93% of total biovolume), indicated saturation at approximately 100 µEm⁻² sec⁻¹ and decreasing gross photosynthesis above 300 µEm⁻² sec⁻¹ (Phlips and Badylak 1996). The P-E response in this study indicates that the natural population was adapted to a mean water column light regime that was less than 300 µEm⁻² sec⁻¹ and is not an indication of the genetic potential of this population to adapt to irradiances in excess of 300 µEm⁻² sec⁻¹.

The results reported by Kana and Glibert (1987a) and Phlips and Badylak (1996) are significant because PAR levels may reach 2000 µEm⁻² sec⁻¹ near the sediment surface in shallow clear Florida Bay waters (Carlson et al. 2001). Consequently, species that have the potential to not only adapt to high irradiances but to acclimate rapidly, will have a competitive advantage.

The relevance of irradiances in excess of the saturation irradiance for the cyanobacteria study species and the diatom study species may also be important in terms of dominance because of the potential differential effect of photoinhibition on growth rate and even survival of these major bloom species. The potentially very high light environment found in the surface layer in Florida Bay would be expected to negatively impact the cyanobacteria populations more than the diatom populations, because the lower *Eₚₛ*’s of the cyanobacteria species than the diatom species found in my study suggest that these cyanobacteria species are better suited to lower irradiances than the diatoms as well as the observation that in general the
level for photoinhibition is generally lower for cyanobacteria than diatoms (Richardson et al. 1983). Although exposure to very high irradiance has been known to cause cell death in surface blooming populations of cyanobacteria (Abeliovich and Shilo 1972), the Florida Bay cyanobacteria bloom species used in this study do not accumulate at the surface like other surface blooming cyanobacteria, and as such, the influence of high irradiances on cyanobacteria cell mortality in Florida Bay may be expected to be minimal. Overall the potential for a large photoinhibitory response by natural populations of cyanobacteria in Florida Bay to the high ambient irradiances is not supported by the typical development and growth of large cyanobacteria dominated blooms during periods of high solar input and irradiance, as occurs during the summer and early fall, when the cyanobacteria dominated blooms are typically experiencing significant growth.

Although inhibition of growth and photosynthesis in *Synechococcus* spp. at moderate to high irradiances has been observed under experimental conditions when low light adapted cells were subjected to large and sudden increases in irradiance, the effect of photoinhibition on natural populations is difficult to determine, as a phytoplankter’s light exposure is normally constantly changing as they are circulated in the water column. The degree of water column turbulence and cell buoyancy will together largely determine the duration of exposure to high light levels. The reduced rate of photosynthesis resulting from photoinhibition may also be quickly reversed as the cells are circulated to more optimal lower light levels. Although the cyanobacteria and diatom isolates are likely to have the genetic potential to overcome the potential photoinhibitory effects of high surface irradiances when suitably conditioned, the rate at which they are able to adjust and effectively utilize fluctuating irradiances may be particularly influential in determining phytoplankton bloom composition in the bay, as the light field in Florida Bay can change quite rapidly in intensity and spectral composition as a result of mixing and sediment resuspension, as wind events can very rapidly influence sediment derived turbidity levels.

Cyanobacteria, in general, have been observed not to adapt to a changing light environment as rapidly as some other phytoplankton species (Tomas 1980). The success of *Synechococcus* may be influenced by the stability of the light field, as the time required for photoadaptation by species of *Synechococcus* seems to be longer than their generation time (Kana and Glibert 1987b). There is some evidence that suggests eukaryotes are able to respond to changes in irradiance more quickly than cyanobacteria. This may allow them to outcompete the cyanobacteria in a turbulent environment where cells are rapidly circulated through a strong vertical light gradient (Flameling and Kromkamp 1997, Ibelings 1992).

Although the study species growth responses to light and salinity may play significant roles in determining their distributions and relative abundances in Florida Bay, additional factors such as nutrients may also selectively influence species bloom dominance in the bay and these will be discussed in the following chapters.
Conclusions

1) Interspecific differences in the growth response of S. cf. elongatus, Synechocystis sp., C. cf. salsugineus and T. cf. oceanica to gradients of light and salinity followed taxonomic lines with the cyanobacteria species having broader salinity tolerances and lower optimum salinities for growth than the diatoms.

2) Interspecific differences in responses to salinity and light when considered in light of the prevailing spatial and temporal patterns of salinity, light and bloom species dominance in Florida Bay, suggests that salinity and light may be influential in determining diatom and cyanobacteria bloom dominance in the bay. Under moderate salinities (15-35 0/00), the diatom species much larger U_m’s places them at a competitive advantage over the cyanobacteria species, while the advantage begins to shift to the cyanobacteria below 10 0/00 and above 50 0/00. Although the cyanobacteria species lower E_k’s indicate that they are better suited to low irradiances, their lower α_g’s and lower U_m’s place them at a competitive disadvantage to the diatom species in both light limiting (< 75µEm^-2sec^-1) and light saturating (≥150 µEm^-2sec^-1) environments, respectively.

3) The development of diatom (C. cf. salsugineus and T. cf. oceanica) dominated blooms would be favored over cyanobacteria (S. cf. elongatus and Synechocystis sp.) dominated blooms under both light limiting and light saturating moderate salinity (10 0/00 - 50 0/00) conditions, which characterize the majority of Florida Bay. In contrast, the development of cyanobacteria (S. cf. elongatus and Synechocystis sp.) dominated blooms over diatom dominated blooms (C. cf. salsugineus and T. cf. oceanica) would begin to be favored above salinities of 50 0/00, as well as below salinities of 10 0/00. The development and persistence of cyanobacteria dominated blooms predominantly in the north-central region which has a history of recurring hypersaline conditions, may be in part due to the cyanobacteria isolates much greater tolerance of hypersaline conditions.

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CHAPTER 3
PHOSPHORUS-DEPENDENT ALGAL GROWTH KINETICS AND
ERC-THEORY INDICES OF COMPETITIVE ABILITIES OF
SYNECHOCOCCUS CF. ELONGATUS, SYNECHOCYSTIS SP.,
THALASSIOSIRA CF. OCEANICA, CHAETOCEROS CF.
SALSUGINEUS AND THE OUTCOMES OF N- AND P-LIMITED COMPETITION
EXPERIMENTS UNDER VARIOUS AMBIENT SALINITIES

Abstract

Phosphate-dependent growth kinetic parameters ($U_m$ and $K_{up}$) were determined for Synechococcus cf. elongatus, Synechocystis sp., Chaetoceros cf. salsugineus and Thalassiosira cf. oceanica as a function of the external PO$_4^{3-}$ concentration at 15, 25 and 50 °/oo. While $U_m$ varied between species and with salinity, $K_{up}$ values, when detectable, did not differ between species at each experimental salinity nor were they influenced by salinity. $K_{up}$ values for $S$. cf. elongatus and Synechocystis sp. often could not be determined, being below the limit of analytical detection (0.03 µM PO$_4^{3-}$). While species did not differ in their equilibrium phosphate competitiveness ($R_p^*$ values), $R_p^*$ was influenced by salinity. Qualitative trends suggested interspecific differences in $K_{up}$ and $R_p^*$ values, at 15, 25 and 50 °/oo. The trends in $K_{up}$ and $R_p^*$ from smallest to largest was: $S$. cf. elongatus < Synechocystis sp. # $C$. cf. salsugineus < $T$. cf. oceanica.

The pattern predicts that under steady state P-limiting conditions $S$. cf. elongatus is the superior PO$_4^{3-}$-affinity competitor, $T$. cf. oceanica is the most inferior competitor, and Synechocystis sp. and $C$. cf. salsugineus are intermediate competitors.

The competitive outcomes of P-limited competition experiments at 15, 25 and 50 °/oo in which $S$. cf. elongatus dominated by biovolume, followed by decreasing biovolumes of both Synechocystis sp. and $C$. cf. salsugineus and the early competitive exclusion of $T$. cf. oceanica, was in agreement with the competitive outcomes predicted by the qualitative trend in $R_p^*$ values.

The competitive outcomes of additional P-limited competition experiments found that the P-competitive ranking from strongest to weakest competitor remained largely the same ($S$. cf. elongatus > Synechocystis sp. > $C$. cf. salsugineus > $T$. cf. oceanica), whether the P was delivered semicontinuously (daily pulse); pulsed (6 day intervals); accompanied by a periodic cyclic salinity fluctuation (~1 °/oo da$^{-1}$); or delivered as PO$_4^{3-}$ or glycerophosphate. But the rate at which Synechocystis sp. was competitively excluded was greatly accelerated when P was pulsed as glycerophosphate. P-limited competition
experiments of paired diatom species conducted only at 25 °/oo found a clonal isolate of *S. costatum* from western Florida Bay to be a weaker P-competitor than all of the other four study species.

The results of N-limited competition experiments at 25 °/oo found a pattern of species N-competitiveness from strongest to weakest in all treatments to be: *T. cf. oceanica* ≥ *S. cf. elongatus* ≈ *Synechocystis* sp. > *C. cf. salsugineus*, while the pattern at 15 and 50 °/oo was in general: *S. cf. elongatus* ≈ *Synechocystis* sp. > *T. cf. oceanica* > *C. cf. salsugineus*. Whether the limiting N was delivered semicontinuously (daily pulse) or pulsed (6 day intervals), *C. cf. salsugineus* was largely competitively excluded while *T. cf. oceanica*, *S. cf. elongatus* and *Synechocystis* sp. all co-dominated. Slight shifts in dominance occurred between *T. cf. oceanica* and the cyanobacteria species with changes in ambient salinity.

The form of the limiting N shifted competitive dominance slightly at 25 °/oo, such that NH₄⁺ promoted co-dominance of the cyanobacteria species, while NO₃⁻ resulted in a slight dominance by *T. cf. oceanica*. Paired diatom species competition experiments conducted only at 25 °/oo found *S. costatum* to be a stronger N-competitor than all of the other species.

N and P-sufficient competition experiments verified that the competitive outcomes observed under P and N limitation were the direct result of nutrient-based competitive interactions.

**Introduction**

Phytoplankton species distribution and abundance have been attributed to species-specific reproduction and loss rates in response to a number of environmental and biotic factors. Such factors include light, temperature, pH, stratification, turbulence, sinking, grazing, nutrient availability and salinity (Kilham 1978). Urbanization, deforestation and agriculture have lead to an increase in nutrient input and changes in the magnitude and frequency of the episodic nutrient and freshwater pulses entering estuaries and coastal areas like Florida Bay. Because phytoplankton species exhibit differing nutrient and salinity requirements; the nutrient flux, frequency of input and nutrient ratio of nutrient input events as well as the flux and frequency of freshwater inputs can influence the degree of phytoplankton nutrient competition, coexistence and ultimately the community composition (Tilman 1977, Rhee 1978, Kilham and Kilham 1980, Harrison and Turpin 1982, Sanders et al. 1987, Kilham and Hecky 1988, Rijstenbil 1988). The role that nutrient availability plays in the structuring of phytoplankton communities is dependent upon nutrient limitation of phytoplankton growth and the subsequent competition among phytoplankton species for the limiting nutrient. Whether nutrient limitation occurs *in situ* and whether species of phytoplankton commonly compete for limiting nutrients (resources) *in situ* is still being debated. Nonetheless, given periodic nutrient limitation and the fact that most phytoplankton have similar nutrient requirements and share a common pool of resources, nutrient competition may be inferred (Sommer 1989).

Resource (e.g. nutrient) competition by definition, results in the lowering of a competing species reproductive rate by the consumption of the commonly required limiting resources (Tilman 1981).
Classical ecological theory predicts that under conditions of environmental homogeneity and equal mortality that the species best able to obtain and utilize the limiting nutrient should competitively exclude the other competing species (Tilman 1977). Since Dugdale's (1967) suggestion that differences in phytoplankton species ability to acquire and utilize nutrients may be a potentially important factor in the determination of phytoplankton species composition, numerous investigations have been conducted examining species uptake and growth kinetic parameters. As a result, many measurements have been made of half-saturation constants for uptake ($K_s$) and growth ($K_u$) as well as maximum uptake rate ($V_m$) and maximum growth rate ($U_m$). Several models (Monod, Droop and Dugdale) have been used to describe the patterns of uptake and growth under nutrient limitation as saturation curves which asymptotically approach maximal rates of growth or uptake (Droop 1983). Following Dugdale's (1967) observations that marine diatoms with intersecting Monod curves apparently compete with each other and that the shape of the curves could be used to predict the outcome of nutrient-limited competition, numerous studies have been carried out to examine the validity of these observations (Hecky and Kilham 1988). Although differences in phytoplankton species ability to acquire ($K_s, V_m$) and utilize ($K_u, U_m$) limited resources have been demonstrated and the outcome of competition predicted from the shapes of the Monod curves, no testable resource-based theory of competition existed.

It was not until Tilman's equilibrium theory of competition (Tilman 1982) that the resource-dependent growth kinetics ($K_u, U_m$) could be used to predict a species competitive power in a direct experimental test of phytoplankton resource-based competition (Sommer 1989). In Tilman's equilibrium model of resource competition, a species minimum nutrient requirement at zero net growth rate at equilibrium ($R^*$) is:

$$R^* = U - K_u / (U_m - U)$$

where $U$ is the growth rate, $U_m$ is the maximal growth rate at external nutrient saturating concentrations, and $K_u$ the half-saturation constant is the external nutrient concentration when $U = 0.5 \ U_{max}$. If several species are all limited by the same nutrient and the loss rates are equal, the species with the lowest nutrient requirement ($R^*$) for the limiting nutrient should competitively displace all the others at equilibrium. This occurs when the species with the lowest $R^*$ reduces the concentration of the limiting nutrient to a level below which other species survive (i.e. below the other species $R^*$ values). Coexistence will occur at equilibrium when species have identical $R^*$ values for the limiting nutrient or when potentially competing species are limited by different nutrients or resources (e.g. light). Under these conditions neither species is able to deplete the nutrient limiting to the point where the other species growth rate falls below its $R^*$ value. The rate of competitive displacement will increase as the difference between species $R^*$ values increases for the limiting nutrient.

Phytoplankton ecologists have proposed three strategies that phytoplankton species may utilize to varying degrees, to deal with the inherent variations in ambient nutrient availability. The three basic physiological nutrient strategies are: 1) affinity specialist, 2) storage specialist, and 3) velocity specialist. Affinity specialists have been described as species capable of maintaining growth rates by continually
taking up nutrients at very low ambient concentrations. At the other extreme are storage specialists, which maintain growth rates by utilizing a large intracellular nutrient storage pool accumulated through rapid and excessive uptake of nutrients while exposed to elevated concentrations of nutrients that commonly occur as pulses or patches. The velocity specialists compensate for decreased growth rates during periods of low nutrient supply by utilizing transient pulses or patches of nutrients for very rapid growth (Sommer 1989).

Manipulation of nutrient regimes in competition experiments have demonstrated that the concentration and availability of the single limiting nutrient may be used to select for a species with physiological nutrient strategies that allow them to take advantage of the existing pattern of limiting nutrient availability. The continuous and moderately slow supply of a limiting nutrient will result in a constant and very low limiting nutrient concentration, which will favor species that are affinity specialists, while a temporally variable supply of the limiting nutrient will favor storage and velocity strategists.

Support for the occurrence of interspecific nutrient competition in Florida Bay is found in phytoplankton bioassays in which the bay's phytoplankton community growth rates have been found to be at times alternately limited by nitrogen, phosphorus and/or silica. A spatial pattern of potential nutrient limitation was found in which the growth rates of the phytoplankton communities were primarily P-limited in the eastern region of Florida Bay, primarily N-limited in the western region and alternating between N and P-limitation in the north-central region (Tomas et al. 1999, Brand 2002). Temporal variations in potential nutrient limitation have also been observed (Tomas et al. 1999) although no seasonal pattern in N or P limitation has been described. Although it may be assumed that interspecific nutrient competition has occurred in Florida Bay at least during periods of nutrient limitation, the role that nutrient competition may have played in the subsequent structuring of the phytoplankton communities, in particular the large recurring blooms is not known.

Estuarine phytoplankton species have different salinity requirements and as a result not only has salinity been found to affect phytoplankton growth rates (McLachlan 1961, Smayda 1969, Grant and Horner 1976, Brand 1984, Miller and Kamykowski 1986a,b), but it has been linked to shifts in species composition as well (Sakshaug and Olsen 1986, Rijstenbil 1988). While the influence of static ambient salinities on the growth rates of phytoplankton has been well studied for many years (Braarud 1951, Brand 1984), studies examining the effect of fluctuating ambient salinities on species growth rates are comparatively few (Miller and Kamykowski 1986a, Rijstenbil 1988, Rijstenbil et al. 1989a,b, Flameling and Kromkamp 1994). Similarly while the literature examining the influence of nutrient limitation upon phytoplankton species competitiveness is very extensive, the number of studies that have investigated the influence of salinity on phytoplankton species competitiveness under nutrient limitation are few (Rijstenbil et al. 1989a,b). Relatively few studies have investigated the combined effects of N or P limitation and both static and fluctuating salinities on nutrient based competitive interactions of estuarine phytoplankton. But episodic perturbations such as rainfall and increased surface run-off deliver both nutrients and freshwater simultaneously, and as such the interactive effects of these two factors may also influence phytoplankton community composition. There is evidence for the interaction of salinity and nutrients on species
competitiveness by altering their ammonia metabolism (Rijstenbil et al. 1989a,b). Environmental factors such as temperature have also been shown to influence a phytoplankton species half-saturation constant for growth ($K_u$) as well as the minimum requirement for a limiting nutrient ($R^*$), and thereby affect the species competitive ability for the limiting resource (Tilman 1982).

The recurrence of anomalous cyanobacteria dominated blooms in a sub-region in Florida Bay that experiences very restricted water exchange, hypersaline conditions and alternating P and N limitation along with the finding in Chapter I that the cyanobacteria bloom species (S. cf. elongatus and Synechocystis sp.) are more tolerant of extremes in salinity than the diatom species (C. cf. salsugineus and T. cf. oceanica) led to the formation of a number of hypotheses. The following hypotheses consider how interspecific N- and P-competitive differences and the influence of salinity on those interspecific differences may influence the development of cyanobacteria (S. cf. elongatus and Synechocystis sp.) dominated blooms or the diatom (C. cf. salsugineus and T. cf. oceanica) dominated blooms, in Florida Bay:

- $H_o$: The cyanobacteria species have lower $K_{up}$'s and $R_{P^*}$'s than the diatom species.
- $H_o$: Suboptimal salinities will elevate the species $K_{up}$'s and $R_{P^*}$'s, above their values at more optimal growth salinities.
- $H_o$: The cyanobacteria species are better P-affinity specialists and will dominate and/or exclude the other species under low and steady rates of limiting P supply.
- $H_o$: The diatoms are better N-affinity competitors and will dominate and/or exclude the cyanobacteria species under low and steady rates of limiting N supply.
- $H_o$: Salinity will influence competitive outcomes under N and P limitation.
- $H_o$: The diatoms are better storage and velocity specialists for both N and P and will dominate and/or exclude the cyanobacteria species when the limiting N or P is supplied as infrequent large pulses (6 day interval).
- $H_o$: The form of limiting N and P will alter the pattern of species relative dominance.
- $H_o$: A fluctuating salinity will influence competitive outcomes under N and P limitation.

The objective of Part A in this chapter is in to determine the phosphate-dependent growth kinetics and the predicted ERC-theory-based competitiveness of S. cf. elongatus, Synechocystis sp., C. cf. salsugineus and T. cf. oceanica. The objective in Part B, using competition experiments, was to verify the predictions of ERC-theory from Part A, and to examine the influence of N limitation, P limitation, type of limiting nutrient, patchiness of the limiting nutrient, salinity (both fluctuating and static) as well as the potential interactions between salinity and N or P limitation on the species competitiveness. Interspecific differences in N- and P-competitiveness of the bloom species at various salinities will then be evaluated in light of the prevailing patterns of salinity, N limitation, P limitation and bloom dominance documented for
the bay to determine the roles that N and P limitation and salinity play in determining cyanobacteria and/or diatom bloom dominance in Florida bay.

A. Phosphorus-dependent Growth Kinetics and ERC-theory Competitive Indices

Methods

The relationship between the growth rate and the external PO$_4^{3-}$ concentration was determined for each species using single species short-term batch cultures (Kilham 1978, Tilman 1981, Sommer 1986, and Grover 1989a). Two factors must be minimized when using batch cultures to examine this relationship: 1) the potential effect of intracellular luxury stores on initial rates of cellular growth and 2) the potential effect of decreasing limiting nutrient concentration on rates of cellular growth. Depletion of intracellular phosphate pools is therefore necessary before experimental measurements are taken since phytoplankton are capable of storing surplus phosphorus sufficient for several cell doublings (Oliver and Ganf 2000). Prior to inoculating the experimental flasks, algae were phosphate depleted by batch growth in P-limited media ("f/2", except PO$_4^{3-}$ = 0.3 µM). The cells were judged to be starved of their internal phosphate pools when the population reproduction rate was zero as determined by in vivo fluorescence and cell counts. The potential negative effect that cellular depletion of external ambient PO$_4^{3-}$ may have on the growth rate particularly during the latter stages of the Monod batch culture experiments was minimized by using low initial cell densities, conducting experiments of short duration, terminating growth rate measurements prior to high cell densities (Paasche 1975), and the exclusion of depressed growth rate data near the end of the experiment that appeared to be due to significant depletion of the external ambient PO$_4^{3-}$ concentration.

Initial cell densities ranged from 5 - 74 cells ml$^{-1}$ and experiments were 4-5 days in length. Stationary phase phosphate depleted cells were inoculated into duplicate 1 liter Pyrex flasks containing "f/2" media except PO$_4^{3-}$ which was present at concentrations ranging from 0.03 - 5.0 µM. Concentrations of the limiting nutrient PO$_4^{3-}$ in the experimental flasks were achieved by the addition of appropriate amounts of a freshly prepared stock solution to each experimental flask. The initial soluble reactive phosphorus (SRP) concentrations in flasks with SRP >0.4 µM, were determined with a 100 mm Pyrex cell by the Strickland and Parsons method (1972). Concentrations less than 0.4 µM PO$_4^{3-}$ were determined using the more sensitive MAGIC method, which allows for accurate determinations to approximately 0.01 µM (Karl and Tien 1992) and in this study a minimum level of detection of 0.03 µM. Standard curves were generated for each analysis. On days 0, 1, 2, 3, 4 and 5, 50 ml was removed from each flask for cell counts and on days 0, 2, 4, and 5 100 ml was removed for measurement of SRP.

Cyanobacteria cells were counted using epifluorescence techniques (Booth 1993, MacIsaac and Stockner 1993), while diatom species were preserved in Lugol's, sedimented and counted according to the procedures outlined by Venrick (1978). When possible, a minimum of 400 cells/sample was counted yielding confidence limits of approximately +/- 10% (Venrick 1978). Growth rates (da$^{-1}$) for each flask
were calculated by a linear least squares regression of the log transformed sample cell count vs. time. The kinetic parameters $U_m$ and $K_u$ were estimated by both linear and non-linear methods. Nonlinear regression was used to fit the substrate dependent growth of each species to the Monod model as described by the equation:

$$U = U_m - \left( \frac{S}{K_u + S} \right)$$

where $U$ and $U_m$ are the growth and maximal growth rate; $S$ is the limiting nutrient concentration and $K_u$ is the half saturation constant for growth. Linear transformations of the substrate dependent growth of each species (Dowd and Riggs 1965) were also used to estimate each species nutrient kinetic parameters, $U_m$ and $K_u$. The following three linear transformations commonly used to transform data that follows Michaelis-Menten kinetics were used:

$$U = U_m - K_u \left( \frac{U}{S} \right)$$

$$\left( \frac{S}{U} \right) = \left( \frac{K_u}{U_m} \right) + \left( \frac{1}{U_m} \right) - \left( \frac{S}{U} \right)$$

$$\left( \frac{1}{U} \right) = \left( \frac{1}{U_m} \right) + \left( \frac{K_u}{U_m} \right) - \left( \frac{1}{S} \right)$$

Standard errors were calculated for $U_m$ and $K_u$ from the standard errors of the y-intercept and the slope, respectively.

The index of competitive ability for a limiting resource, $R^*$, as described in the theory of equilibrium resource competition (Tilman, 1982) was calculated using the equation:

$$R^* = \frac{U - K_u}{(U_m - U)}$$

where $R^*$ is the amount of the limiting resource that a species needs to have a reproductive rate equal to its loss rate, and $K_u$, $U_m$, and $U$ are as previously defined. A growth/mortality rate of 0.2 da$^{-1}$ (equivalent to an experimental steady-state dilution rate of 0.2 da$^{-1}$) was used with species $U_m$'s and $K_u$'s to calculate species $R_P^*$ values. $R_P^*$ values were then used to construct zero net growth isocline (ZNGI) plots for each species according to Tilman (1982).

To examine the influence of salinity on $K_u$ and $R_P^*$, short-term batch growth experiments were conducted at salinities of 15, 25, and 50 $\%_0$. This salinity range included both optimal and sub-optimal salinities for growth for each species.

Basal growth media used in the experiments was artificial seawater prepared from deionized water and reagent grade chemicals (Parsons et al. 1984). A combination of autoclaving and sterile filtration was used to sterilize experimental culture vessels, seawater and nutrient stock solutions. The desired salinities were obtained by the addition of deionized water. Aseptic techniques were used throughout. Experiments were conducted in a growth chamber, at 25 $\pm$ 0.5 EC, light-dark cycle of 12L : 12D, and 150 $\mu$m$^{-2}$sec$^{-1}$ provided by cool white fluorescent bulbs. Flasks were swirled twice daily. The potential influence of bacterial biomass was minimized by drawing inocula for experimental use from stock cultures that were transferred frequently to new glassware and maintained at high relative growth rates. A combination of light and epifluorescent microscopy was used to confirm that the bacterial biomass was low.
Results

The growth response of each species as a function of the external \( PO_4^{3-} \) concentration at salinities 15, 25 and 50 \( 0^\circ/00 \) was fit to the Monod model (Figs. 3-2-1, 3-2, 3-3 and 3-4), as well as three different linear transformations. Of the linear transformations, S/U vs. S (equation 3) provided by far the best-fit and most realistic kinetic estimates (\( U_m \) and \( K_{up} \)) (Table 3-1, Figs. 3-1, 3-2, 3-3 and 3-4). Therefore the results of the other two linear transformations (equations 2 and 4) are not presented. The linear derived estimates of \( U_m \) and \( K_{up} \) are presented (Tables 3-2 and 3-3).

As expected, \( U_m \) values were found to differ between species (Fig. 3-5A) and to be influenced by salinity. Significant intraspecific differences in \( U_m \) were found at 15, 25 and 50 \( 0^\circ/00 \) (Fig. 3-5B). \( K_{up} \) values, on the other hand, when detectable, were not found to differ significantly between species at each experimental salinity (Fig. 3-6A), or to be influenced by salinity (Fig. 3-6B).

The \( R_p^* \) values and ranges for each species, calculated using linear derived estimates of \( U_m \) and \( K_{up} \) and a growth/loss rate of 0.2 da\(^{-1}\), revealed no significant interspecific differences in \( R_p^* \) because the \( R_p^* \) ranges of the species overlapped at each experimental salinity (Tables 3-3 and 3-4). Similarly, there were no significant differences in \( R_p^* \) values with salinity, as each species \( R_p^* \) ranges overlapped at all three salinities (Table 3-3 and 3-4).

The absence of a complete ZNGI plots for Synechocystis sp. and S. cf. elongatus (Fig. 3-7) was due to the inability to calculate \( R_p^* \) values, because their \( K_{up} \) values were often below the limit of detection (Table 3-3). For comparative purposes, an estimate of the highest \( R_p^* \) that S. cf. elongatus and Synechocystis sp. could possibly have at 15 and 50 \( 0^\circ/00 \) was calculated using each species respective \( U_m \) and the lowest \( K_{up} \) determined at each salinity (0.003 \( \mu M \) \( PO_4^{3-} \) at 15 \( 0^\circ/00 \) and 0.07 \( \mu M \) \( PO_4^{3-} \) at 50 \( 0^\circ/00 \)). Since the \( K_u's \) for S. cf. elongatus and Synechocystis sp. were lower than the lowest determined \( K_{up} \) (below the limit of detection), the real \( R_p^* \) values for S. cf. elongatus and Synechocystis sp. lie somewhere below the estimated maximum potential experimental values.

The zero net growth isocline plots predict that T. cf. oceanica with the highest \( R^* \) at 15, 25 and 50 \( 0^\circ/00 \) is the least competitive species under P-limitation between the salinities of 15 and 50 \( 0^\circ/00 \). The ZNGI plots predict that S. cf. elongatus is the superior P-competitor and that Synechocystis sp. and C. cf. salsugineus are somewhat intermediate competitors for \( PO_4^{3-} \). The plots also predict that under P-limiting equilibrium conditions, given sufficient time, S. cf. elongatus will competitively exclude all three other species at 25 \( 0^\circ/00 \), with T. cf. oceanica being the first species excluded.

The results of the hypothesis testing can be summarized as follows: the cyanobacteria isolates have lower \( K_{up}''s \) and \( R_p^*''s \) (although only qualitatively) than the diatom species, only the diatom species \( K_{up}''s \) varied with salinity being generally elevated at suboptimal salinities for their growth (again only qualitatively).
Figure 3-1. Phosphorus-dependent algal growth kinetics of *S. cf. elongatus* at (A) 15 °C, (B) 25 °C, (C) 50 °C. Non-linear Monod model (Growth rate vs. [PO₄³⁻]) and linear transformation ([PO₄³⁻] / growth rate vs. [PO₄³⁻]).
Figure 3-2. Phosphorus-dependent algal growth kinetics of *Synechocystis* sp. at (A) 15 °/oo, (B) 25 °/oo, (C) 50 °/oo. Non-linear Monod model (Growth rate vs. [PO₄³⁻]) and linear transformation ([PO₄³⁻] / growth rate vs. [PO₄³⁻]).
Figure 3-3. Phosphorus-dependent algal growth kinetics of T. cf. oceanica at (A) 15\(^0\)/o, (B) 25\(^0\)/o, (C) 50\(^0\)/o. Non-linear Monod model (Growth rate vs. [PO\(_4^{3-}\)]) and linear transformation ([PO\(_4^{3-}\)] / growth rate vs. [PO\(_4^{3-}\)]).
Figure 3-4. Phosphorus-dependent algal growth kinetics of *C. cf. salsugineus* at (A) 15°/00, (B) 25°/00, (C) 50°/00. Non-linear Monod model (Growth rate vs. \([\text{PO}_4^{3-}]\) and linear transformation (\([\text{PO}_4^{3-}] / \text{growth rate vs. [PO}_4^{3-}]\)).
Table 3-1. R squared values of linear (S/U vs. U) and non-linear Monod model (U vs. S) regressions, fit to phosphorus-dependent growth rate data.

<table>
<thead>
<tr>
<th></th>
<th>Linear</th>
<th>Nonlinear</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S=15</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. cf. elongatus</em></td>
<td>0.999</td>
<td>0.26</td>
</tr>
<tr>
<td><em>Synechocystis</em></td>
<td>0.999</td>
<td>0.138</td>
</tr>
<tr>
<td><em>T. cf. oceanica</em></td>
<td>0.999</td>
<td>0.634</td>
</tr>
<tr>
<td><em>C. cf. salsugineus</em></td>
<td>0.996</td>
<td>0.47</td>
</tr>
<tr>
<td><strong>S=25</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. cf. elongatus</em></td>
<td>0.999</td>
<td>0.712</td>
</tr>
<tr>
<td><em>Synechocystis</em></td>
<td>0.999</td>
<td>0.051</td>
</tr>
<tr>
<td><em>T. cf. oceanica</em></td>
<td>0.998</td>
<td>0.658</td>
</tr>
<tr>
<td><em>C. cf. salsugineus</em></td>
<td>0.998</td>
<td>0.768</td>
</tr>
<tr>
<td><strong>S=50</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. cf. elongatus</em></td>
<td>0.999</td>
<td>0.018</td>
</tr>
<tr>
<td><em>Synechocystis</em></td>
<td>0.999</td>
<td>0.341</td>
</tr>
<tr>
<td><em>T. cf. oceanica</em></td>
<td>0.997</td>
<td>0.269</td>
</tr>
<tr>
<td><em>C. cf. salsugineus</em></td>
<td>0.996</td>
<td>0.655</td>
</tr>
</tbody>
</table>
Table 3-2. Phosphorus-dependent growth kinetic parameter $U_m$ (div. da$^{-1}$). Values, standard errors (SE), and ranges as derived from linear (S/U vs. U) analyses. Non-overlapping ranges at each salinity are represented by *.

<table>
<thead>
<tr>
<th></th>
<th>$U_m$</th>
<th>SE</th>
<th>$U_m$ Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S=15</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. cf. elongatus</td>
<td>1.30</td>
<td>0.08</td>
<td>1.22 - 1.38 *</td>
</tr>
<tr>
<td>Synechocystis sp.</td>
<td>1.96</td>
<td>0.02</td>
<td>1.94 - 1.98 *</td>
</tr>
<tr>
<td>C. cf. salsugineus</td>
<td>1.63</td>
<td>0.02</td>
<td>1.61 - 1.65 *</td>
</tr>
<tr>
<td>T. cf. oceanica</td>
<td>1.45</td>
<td>0.05</td>
<td>1.40 - 1.50 *</td>
</tr>
<tr>
<td><strong>S=25</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. cf. elongatus</td>
<td>1.33</td>
<td>0.01</td>
<td>1.32 - 1.34 *</td>
</tr>
<tr>
<td>Synechocystis sp.</td>
<td>1.39</td>
<td>0.02</td>
<td>1.37 - 1.41 *</td>
</tr>
<tr>
<td>C. cf. salsugineus</td>
<td>2.10</td>
<td>0.04</td>
<td>2.06 - 2.14 *</td>
</tr>
<tr>
<td>T. cf. oceanica</td>
<td>3.27</td>
<td>0.06</td>
<td>3.21 - 3.33 *</td>
</tr>
<tr>
<td><strong>S=50</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. cf. elongatus</td>
<td>0.95</td>
<td>0.00</td>
<td>0.94 - 0.95 *</td>
</tr>
<tr>
<td>Synechocystis sp.</td>
<td>0.99</td>
<td>0.02</td>
<td>0.97 - 1.00 *</td>
</tr>
<tr>
<td>C. cf. salsugineus</td>
<td>1.38</td>
<td>0.03</td>
<td>1.35 - 1.41 *</td>
</tr>
<tr>
<td>T. cf. oceanica</td>
<td>0.46</td>
<td>0.02</td>
<td>0.44 - 0.47 *</td>
</tr>
</tbody>
</table>
Table 3-3. Phosphorus-dependent growth kinetic parameter $K_u$ (µM PO$_4^{3-}$). Values, standard errors, and ranges derived from the linear transformation S/U vs. U. Values below the limit of detection are represented by --

<table>
<thead>
<tr>
<th></th>
<th>$K_u$</th>
<th>SE</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S=15</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. cf. elongatus</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Synechocystis sp.</td>
<td>0.003</td>
<td>0.037</td>
<td>≈ 0 - 0.039</td>
</tr>
<tr>
<td>C. cf. salsugineus</td>
<td>0.004</td>
<td>0.043</td>
<td>≈ 0 - 0.047</td>
</tr>
<tr>
<td>T. cf. oceanica</td>
<td>0.066</td>
<td>0.145</td>
<td>≈ 0 - 0.211</td>
</tr>
<tr>
<td><strong>S=25</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. cf. elongatus</td>
<td>0.000</td>
<td>0.048</td>
<td>≈ 0 - 0.048</td>
</tr>
<tr>
<td>Synechocystis sp.</td>
<td>0.007</td>
<td>0.053</td>
<td>≈ 0 - 0.060</td>
</tr>
<tr>
<td>C. cf. salsugineus</td>
<td>0.007</td>
<td>0.087</td>
<td>≈ 0 - 0.094</td>
</tr>
<tr>
<td>T. cf. oceanica</td>
<td>0.065</td>
<td>0.077</td>
<td>≈ 0 - 0.142</td>
</tr>
<tr>
<td><strong>S=50</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. cf. elongatus</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Synechocystis sp.</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>C. cf. salsugineus</td>
<td>0.070</td>
<td>0.115</td>
<td>≈ 0 - 0.185</td>
</tr>
<tr>
<td>T. cf. oceanica</td>
<td>0.192</td>
<td>0.176</td>
<td>≈ 0 - 0.368</td>
</tr>
</tbody>
</table>
Figure 3-5. Estimated $U_m$'s and standard errors (SE) of study species at 15, 25 and 50 °C obtained from linear analyses. (A) Interspecific differences in $U_m$, (B) Intraspecific differences in $U_m$. 
Figure 3-6. Estimated mean $K_u$’s and standard errors (SE) at 15, 25 and 50 $^\circ$C obtained from linear analyses. (A) Interspecific differences in $K_u$, (B) Intraspecific differences in $K_u$. 

Salinity

Ku ($\mu$MPO$_4^-$)

1 S. cf. elongatus
2 Synechocystis sp.
3 C. cf. salsugineus
4 T. cf. oceanica

15 25 50

S. cf. elongatus
Synechocystis sp.
C. cf. salsugineus
T. cf. oceanica

Ku ($\mu$MPO$_4^-$)

15 25 50

1 25 50

Figure 3-6. Estimated mean $K_u$’s and standard errors (SE) at 15, 25 and 50 $^\circ$C obtained from linear analyses. (A) Interspecific differences in $K_u$, (B) Intraspecific differences in $K_u$. 

Salinity

Ku ($\mu$MPO$_4^-$)

15 25 50

S. cf. elongatus
Synechocystis sp.
C. cf. salsugineus
T. cf. oceanica

Ku ($\mu$MPO$_4^-$)

15 25 50

1 25 50
Table 3-4. The calculated index of competitive ability for phosphorus $R^*$ ($\mu$M PO$_4^{3-}$). Values and ranges for each species at each experimental salinity calculated using linear derived $U_m$ and $K_u$ estimates and a hypothetical mortality rate of 0.2 da$^{-1}$. Values below the limit of detection are represented by --.

<table>
<thead>
<tr>
<th>Species</th>
<th>R*</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S=15</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. cf. elongatus</em></td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td><em>Synechocystis sp.</em></td>
<td>0.0005</td>
<td>~0 - 0.0069</td>
</tr>
<tr>
<td><em>C. cf. salsugineus</em></td>
<td>0.0008</td>
<td>~0 - 0.0102</td>
</tr>
<tr>
<td><em>T. cf. oceanica</em></td>
<td>0.0164</td>
<td>~0 - 0.0546</td>
</tr>
<tr>
<td><strong>S=25</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. cf. elongatus</em></td>
<td>0.00005</td>
<td>~0 - 0.0136</td>
</tr>
<tr>
<td><em>Synechocystis sp.</em></td>
<td>0.0019</td>
<td>~0 - 0.0161</td>
</tr>
<tr>
<td><em>C. cf. salsugineus</em></td>
<td>0.0011</td>
<td>~0 - 0.0154</td>
</tr>
<tr>
<td><em>T. cf. oceanica</em></td>
<td>0.0063</td>
<td>~0 - 0.0140</td>
</tr>
<tr>
<td><strong>S=50</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. cf. elongatus</em></td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td><em>Synechocystis sp.</em></td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td><em>C. cf. salsugineus</em></td>
<td>0.0186</td>
<td>~0 - 0.0504</td>
</tr>
<tr>
<td><em>T. cf. oceanica</em></td>
<td>0.3280</td>
<td>0.0243 - 0.7012</td>
</tr>
</tbody>
</table>
Figure 3-7. Zero net growth isoclines (ZNGI’s). $R^*$ calculated using linear derived $U_m$ and $K_u$ values. $S$. cf. elongatus = ▲, Synechocystis sp. = ●, C. cf. salsugineus = ♦, C. cf. oceanica = ■. Hypothetical maximum $R^*$ for $S$. cf. elongatus and Synechocystis sp. at salinities 15 and 50 = ○. Hypothetical maximum ZNGI’s for $S$. cf. elongatus and Synechocystis sp. = ---.

Discussion

The occurrence of large and persistent cyanobacteria dominated blooms, particularly in the north-central region of Florida Bay often coincident with hypersaline waters and either N or P growth rate limiting conditions (Tomas et al. 1999, Brand 2002) suggested that the competitiveness of the dominant bloom species under N and P limitation (i.e. their $U_m$’s, $K_u$’s and $R^*$’s) as well as the influence of salinity upon those kinetic parameters, may be influential in the development of these blooms.

$U_m$

The interspecific differences in $U_m$ and the influence of salinity on $U_m$ determined for the four study species at 15, 25 and 50 $/_{oo}$ using the Monod batch growth method (Fig. 3-5A,B), were similar to those found in earlier light and salinity growth studies (Chapter 2, Fig. 2-1). In both sets of experiments the diatom species $U_m$’s greatly exceeded the cyanobacteria species $U_m$’s at the moderate salinity of 25 $/_{oo}$, while at the lower and higher salinities of 15 and 50 $/_{oo}$, the diatom species $U_m$ values decreased and approached those of the cyanobacteria species. Under saturating light and non-limiting nutrient conditions, the much greater intrinsic $U_m$ of the diatom bloom species at 25 $/_{oo}$ places them at a considerable
advantage over the cyanobacteria bloom species by enabling them to more rapidly exploit the available resources and thereby increase their biomass. But at very low and high salinities ($\leq 5\%_0$ and $>60\%_0$) the $U_m$'s of the cyanobacteria exceed those of the diatoms shifting the competitive advantage to the cyanobacteria and thereby contributing to increased cyanobacteria biomass.

$K_u$

Interspecific differences in $K_{up}$'s are well documented in the literature (Grover 1989a, Sommer 1989) and it was therefore expected that some interspecific differences in $K_{up}$ values would be found in this study. The lack of significant interspecific differences in $K_{up}$'s among the Florida Bay study species may be attributed in part to the large standard errors associated with each $K_{up}$, the similarity of the species $K_{up}$'s, as well as the inability to measure very low $K_{up}$'s (e.g. $S$. cf. elongatus, Table 3-2). The large standard errors commonly associated with $K_u$ values arise primarily from the inherent difficulty of measuring algal growth kinetics. Species $K_u$'s are often so low that algal growth often occurs at nutrient concentrations below those that can accurately be prepared and measured. In these experiments my detection limit was 0.03 µM $PO_4^{3-}$. As a result, the standard errors associated with the experimental $K_{up}$ estimates, although high (Table 3-3), were not totally unexpected and resemble the residual variance encountered by other researchers using this methodology (Grover 1989a). The similarly low $K_{up}$ values between the three species that often dominate the large blooms in the bay ($S$. cf. elongatus, Synechocystis sp. and $C$. cf. salsugineus) was also not unexpected. All three species are apparently well adapted to the low P environment of Florida Bay as evidenced by their bay-wide distribution and their high numerical abundance during blooms.

Even though no significant interspecific differences in $K_{up}$'s were detected, the potential for their existence may be inferred from the general qualitative pattern of $K_{up}$ values calculated at each experimental salinity. The pattern from smallest to largest $K_{up}$ was: $S$. cf. elongatus $<$ Synechocystis sp. $< C$. cf. salsugineus $< T$. cf. oceanica (Fig. 3-6). This general qualitative pattern was most strongly supported by $T$. cf. oceanica which clearly stood apart from the other four study species by having a $K_{up}$ at 15, 25, and 50 $\%_0$ that was consistently higher than all the other species, and by $S$. cf. elongatus which was also was distinctly different from the other four study species by having the lowest $K_{up}$ at 25 $\%_0$, and a $K_{up}$ below the limit of detection at both 15 and 50 $\%_0$. Non-linear Monod estimates, also indicated that $S$. cf. elongatus was uniquely different from the other species with a $K_{up}$ that was consistently below the limit of detection at 15, 25 and 50 $\%_0$ (results not presented). $S$. cf. elongatus's $K_{up}$ was below the limit of detection because it maintained near maximal growth rates at the lowest phosphate concentration used (0.03- 0.04 µM) (Fig. 3-1). Support for the existence of real interspecific differences in $K_{up}$'s, as suggested by the qualitative pattern, was subsequently provided by the outcome of P-limited competition experiments (Chapter IIB).

The $K_{up}$ estimates obtained in this study, ranging from 0.0002 - 0.19 µM $PO_4^{3-}$, are well within the $K_{up}$ literature range of 0.00014 - 1.89 µM $PO_4^{3-}$ reported for microalgal species (Grover 1989a, Sommer
1989, Healy 1985). The low $K_{up}$ value of 0.0002 µM PO$_4$ for S. cf. *elongatus* at 25 °/00 would not be considered to be out of the ordinary for a P-specialist that is well-adapted to a P-limited environment. In fact, the presence in the phytoplankton community of bloom species with very low $K_{up}$'s should not be unexpected in Florida Bay as nutrient bioassays have provided direct evidence that a large portion of the phytoplankton community in the bay consistently experiences P-limitation (Tomas et al. 1999, Brand 2002).

The $K_{up}$ values indicate quite clearly, that under P-limited conditions, of the four species, *T*. cf. *oceanica*, having the highest $K_{up}$ at 15, 25 and 50 °/00 would be expected to grow the least efficiently of all the species and be at a strong competitive disadvantage, while S. cf. *elongatus* with the lowest PO$_4$ $K_{up}$ at 25 °/00 would be predicted to grow the most efficiently and be at a competitive advantage.

Although the effects of physical environmental factors such as temperature and salinity on a species $U_m$ have been well documented, few studies have investigated the influence of environmental factors upon a phytoplankton species $K_u$ (Paasche 1975, Tilman et al. 1981). Although temperature was found to influence $K_u$, such that the silica $K_u$ values for diatom species varied with temperature (Tilman et al. 1981), no studies that I am aware of have investigated the effect of salinity upon a species $K_u$. The $K_u$ for silica exhibited temperature independence at temperatures within or below the optimal temperature range for growth, but increased sharply at temperatures above the optimal temperature range for growth. Consequently, it was hypothesized that outside of the optimal salinity range for growth (i.e. at 15 and 50 °/00 for the diatoms, and 50 °/00 for the cyanobacteria), the $K_{up}$ values of each species would be elevated relative to the $K_{up}$ values nearer the optimal salinities for growth (i.e. 25 °/00 for the diatoms and 15-25 °/00 for the cyanobacteria).

No significant effect of salinity on $K_{up}$ was found for any species between 15 and 50 °/00, which may be due to the absence of a salinity effect, or an inability to detect it, as noted above. Although no significant salinity effect on the species $K_{up}$'s was detectable, a qualitative pattern of $K_{up}$ values with salinity is visible that is largely consistent with the effect that salinity is expected to have on a species $K_u$. The species $K_{up}$ response to salinity was expected to be the inverse of that observed for $U_m$, i.e. at optimal salinities for growth $U_m$ is high and $K_u$ is low, while at suboptimal salinities for growth $U_m$ is reduced and $K_u$ is elevated.

The $K_{up}$'s, of both diatom species were higher at 50 °/00 than they were at 25 °/00 (Fig. 3-6B) which is in agreement with the expectation of elevated $K_{up}$'s at suboptimal salinities for growth, while at 15 °/00 the $K_{up}$'s were not elevated. An explanation for the elevated $K_{up}$'s at 50 °/00 but not at 15 °/00 may be that 50 °/00 is sufficiently outside of their optimal salinity range for growth (see Chapter 1) to influence the $K_{up}$ sufficiently to be detectable, while the influence at 15 °/00 was not sufficient to produce a noticeable change.

The qualitative pattern of the cyanobacteria species $K_{up}$'s responses to salinity was less clear than those of the diatom isolates. The occurrence of a lower $K_{up}$ and higher $U_m$ at 15 °/00 relative to those at 25°/00 were as predicted by the cyanobacteria species optimal salinity range for growth (10 °/00 - 20 °/00).
while the occurrence of very low (undetectable) $K_{up}$'s (Fig. 3-6A) and reduced $U_m$'s (Fig. 3-5B) at 50 $^{0}/_{00}$ was not in complete agreement with the predicted response of their $K_{up}$ to salinity. This apparent contradiction may be explained in part by their euryhaline growth response and broad salinity tolerance, such that 50 $^{0}/_{00}$ was not suboptimal enough, to produce a detectable increase in $K_{up}$.

Indirect support for the existence of a salinity effect on the $K_{up}$ of microalgae may be found in the results of Ikeya et al. (1997) in which an oligotrophic marine *Synechococcus* sp. experienced reduced $PO_4^{3-}$ uptake when exposed to a sudden osmotic shock (0.2 M). They speculated that the *Synechococcus* sp. had an inducible system of periplasmic proteins that functioned in the uptake of $PO_4^{3-}$ under very low $PO_4^{3-}$ concentrations but which were inactivated by osmotic shock. Although the elevated $K_{up}$ values seen at 50 $^{0}/_{00}$ in the diatom species could not be attributed in like manner to the effect of an acute and large osmotic shock, the elevated values may reflect the species acclimated $K_{up}$ response to a sub-optimal salinity that included the inactivation of periplasmic $PO_4^{3-}$ uptake proteins.

Consequently, cyanobacteria and diatom bloom dynamics under P-limitation in Florida Bay may be influenced not only by interspecific differences in $K_{up}$'s but also by the effect of salinity on the diatom species $K_{up}$'s. Qualitative interspecific differences in $K_{up}$'s suggest that between 15 and 50 $^{0}/_{00}$, *S*. cf. *elongatus* by having the lowest $K_{up}$, would be predicted to be the superior competitor, while *T*. cf. *oceanica* by having the highest $K_{up}$ would be predicted to be the most inferior competitor. The observed qualitative influence of salinity on $K_{up}$ suggests that at salinities of 50 $^{0}/_{00}$ and above, the competitiveness of the diatoms is further reduced when their $K_{up}$ is elevated in response to the suboptimal salinity for growth, while at salinities of 15 $^{0}/_{00}$ the competitiveness of the cyanobacteria is enhanced when their $K_{up}$'s are further lowered in response to the more optimal salinity for growth. Whether the qualitative pattern of interspecific differences in species $K_{up}$'s responses to salinity (between 15 and 50 $^{0}/_{00}$) seen in my study reflects real interspecific differences at this time is not known.

*R*

No significant difference was detected between the $R_p*$ values of the four species, but a trend in the interspecific differences in $R_p*$ values was found that was similar to the qualitative pattern of interspecific differences in $K_{up}$ values. *S*. *cf. elongatus* had the lowest $R_p*$ or an undetectably but very low $R_p*$, *T*. *cf. oceanica* had the highest $R_p*$ and *Synechocystis* sp. and *C*. *cf. salsugineus* had $R_p*$'s intermediate in value (Table 3-4, Fig. 3-7). The predictions of ERC-theory are presented graphically for each species as ZNGI's assuming a growth and loss rate of 0.2 da$^{-1}$ (Fig. 3-7). The solid lines are combinations of $PO_4^{3-}$ and salinity for which a species has no net population growth, such that at $PO_4^{3-}$ concentrations above a species ZNGI line the species will experience a net increase in population size, while below the line it will experience a net decrease. The ZNGI's indicates that *S*. cf. *elongatus* with the smallest $R_p*$ at 25 $^{0}/_{00}$ will be the best competitor for phosphorus, and will in time competitively displace all three other species. In contrast, *T*. cf. *oceanica* with the highest $R_p*$ at all three salinities will be the first species to be competitively excluded between 15 and 50 $^{0}/_{00}$. The superior competitor at 15 $^{0}/_{00}$ and 50 $^{0}/_{00}$
could not be predicted with certainty because the $R_p^*$ values of the cyanobacteria could not always be calculated. Instead an estimate of the highest $R_p^*$ that *S. cf. elongatus* and *Synechocystis* sp. could possibly have at 15 and 50 $^{0}/_{00}$ was made using each species respective $U_m$ and the lowest $K_{up}$ determined at each salinity. The real $R_p^*$ values for *S. cf. elongatus* and *Synechocystis* sp. lie somewhere below the estimated maximal values, and illustrate that both cyanobacteria species out-compete both species of diatoms at 15 $^{0}/_{00}$ and most likely out-compete both species of diatoms again at 50 $^{0}/_{00}$, or at the very least out-compete *T. cf. oceanica*. The ZNGI's for *C. cf. salsugineus* and *Synechocystis* sp. predict that they will both be competitively displaced by *S. cf. elongatus* after the displacement of *T. cf. oceanica* because their $R_p^*$ values are intermediate. The validity of these predictions and thus the existence of real interspecific differences in $R_p^*$ values, was subsequently confirmed by P-limited competition experiments (Chapter IIB).

Although environmental factors such as temperature have been found to influence a phytoplankton species $R^*$ (Tilman et al. 1981), and there is evidence for the influence of salinity on species competitiveness for ammonia (Rijstenbil et al. 1989a,b), to date no studies have investigated a dependence of $R^*$ on salinity. In light of the documented influence that salinity has on the $U_m$ of most phytoplankton species, the potential influence of salinity on $R^*$ may be attributed at least in part to the response of $U_m$ to salinity. Consequently, suboptimal salinities for growth that lower a species $U_m$ will lower their competitiveness (seen as an increase in $R^*$). Therefore it was expected that the study species $R^*$ responses to salinity (dependent upon the $U_m$ and $K_u$ responses to salinity) would be similar to the $R^*$ response to suboptimal temperatures described by Tilman et al. (1981). They found the $R^*$ response to temperature of one freshwater diatom to be due solely to the $U_m$ response, as the $K_u$ did not change with temperature, while the $R^*$ response to temperature of another diatom species was due to the responses of both $U_m$ and $K_u$ as they both changed at higher temperatures. A priori, the expectation was that the $R^*$ values of the Florida Bay isolates would be elevated outside of the optimal salinity range for growth as a result of a decrease in $U_m$ and/or an increase in $K_u$. As a result, it was expected that a species would have a lower $R^*$ and be a superior nutrient competitor through the portion of the salinity range which was optimal for growth, while through the portion of the salinity range which was sub-optimal for growth it would have a higher $R^*$ and be an inferior competitor. In this way a species might be a stronger competitor through part of its salinity tolerance range and be a weaker competitor through another portion of its tolerable salinity range as a result of the differential influence of salinity upon $R^*$.

Although salinity was not found to significantly affect $R_p^*$ as indicated by the overlapping intraspecific $R_p^*$ ranges (Table 3-4), the expected qualitative pattern of elevated $R_p^*$ values at suboptimal salinities for growth was seen for *T. cf. oceanica* in which its $R_p^*$ isocline (Fig. 3-7) showed an increase in $R_p^*$ at 15 and 50 $^{0}/_{00}$ relative to the $R^*$ at the more optimal salinity of 25 $^{0}/_{00}$. The expected pattern was in part also seen in *C. cf. salsugineus* which had an elevated $R_p^*$ at the sub-optimal salinity of 50 $^{0}/_{00}$. The elevated $R_p^*$ of the diatom species at 50 $^{0}/_{00}$ relative to that at 25 $^{0}/_{00}$ may be the result of the combined effect of a lowered $U_m$ and an elevated $K_{up}$. The predicted qualitative pattern of elevated $R_p^*$ values at
suboptimal salinities for growth was unclear for the cyanobacteria species, because their $R_p^*$’s could not always be calculated.

**Conclusions**

1) Although no significant interspecific differences in $K_{up}$ and $R_p^*$ values were detected, qualitative interspecific differences in both $K_{up}$ and $R_p^*$ values suggested a relative P-competitive ranking from strongest to weakest as follows: *S. cf. elongatus* > *Synechocystis* sp. > *C. cf. salsugineus* > *T. cf. oceanica*. The ranking suggests that under P limiting equilibrium conditions for an extended period of time between 15 and 50 $\%$, that *S. cf. elongatus* will be the superior competitor and will in time competitively displace first *T. cf. oceanica*, then *C. cf. salsugineus* and finally *Synechocystis* sp..

2) While no significant effect of salinity on $K_{up}$ or $R_p^*$ was detected, qualitative intraspecific differences in both $K_{up}$ and $R_p^*$ values in the diatom species suggested that non-optimal salinities for growth (e.g. $\geq$50 $\%$) may elevate their $K_{up}$’s. No qualitative trend of intraspecific differences was seen for the cyanobacteria species as their $K_{up}$ was often below the limit of detection.

3) The predicted dominance of the cyanobacteria between 15 and 50 $\%$ (lower $R_p^*$'s) under phosphate limitation may be explained in part not only by their generally lower $K_{up}$’s, but by the negative effect that sub-optimal salinities of 15 and 50 $\%$ have on the diatoms $U_m$’s, possibly on their $K_{up}$’s, and consequently on their overall competitiveness for phosphate.

4) If the qualitative interspecific differences between the cyanobacteria and diatom bloom species $K_{up}$’s and $R_p^*$’s are indicative of real differences, then relative bloom dominance by the cyanobacteria and diatom study species in Florida Bay under P limiting conditions may be strongly influenced by these interspecific differences.

**B. P- and N-limited Competition Experiments and the Influence of Salinity**

**Methods**

Three separate sets of competition experiments (Table 3-5) that included mixed species as well as paired diatom species competition experiments were conducted to examine the separate and combined influence of P-limitation, N-limitation, type of limiting nitrogen or phosphorus, frequency of delivery of the limiting nutrient and salinity upon interspecific competition between the following species of Florida Bay: the dominant bloom species *S. cf. elongatus*, *Synechocystis* sp. and *C. cf. salsugineus* and the non-dominant non-bloom species *T. cf. oceanica*. All competition experiments were carried out using semicontinuous dilution that consisted of a single daily manual dilution.
Ideally, continuous dilution culture (e.g. chemostat or cyclostat) is used in competition experiments to confirm the predictions of ERC-theory, as it most closely approximates the steady state equilibrium conditions upon which the predictions are based. Instead, semicontinuous daily dilution was chosen for its simplicity and because it has been found to adequately approximate the steady-state conditions required by competition experiments to test the predictions of ERC-theory (Kilham 1978). Additionally, since the prevailing view is that natural populations of phytoplankton experience temporally and spatially variable nutrient supplies, the temporally variable supply of the limiting nutrient produced by the daily semicontinuous dilution used in my study provided a more natural nutrient environment under which nutrient competition more likely occurs in Florida Bay, than would the artificially low and constant nutrient supply created by continuous dilution. Daily removal of culture and addition of appropriate growth media resulted in approximate steady-state growth conditions. The experiments examined the outcome of interspecific competitive interactions as the relative change in species dominance in terms of total species biovolume over time.

The first set of mixed species competition experiments (Table 3-3) examined the competitive interactions between the four species for three nutrient regimes (P limitation, N limitation and N and P sufficiency) at ambient salinities of 15, 25 and 50 ‰. The 1st set of competition experiments at 15 and 50 ‰ lasted 87 days while those at 25 ‰ lasted only 42 days.

Within each of the three nutrient regimes (P limitation, N limitation and N and P sufficiency) were three different treatments consisting of: (1) a semicontinuous supply of the limiting nutrient (2) a periodic pulse of the limiting nutrient and (3) a semicontinuous supply of the limiting nutrient coupled with a repeating sinusoidal salinity fluctuation which oscillated 2.5 ‰ above and below the ambient salinity with a period of 10 days, and a daily salinity change of approximately 1 ‰. The semicontinuous treatments consisted of the daily addition of media of the appropriate nutrient composition (N-limited, P-limited or N and P sufficient) as part of the once daily manual dilution. The pulsed treatments were diluted once daily as well, but with media lacking the limiting nutrient. Instead the pulsed treatment received the limiting nutrient as a separate single pulse every 6th day. The semicontinuous and pulsed treatments received the same total amount of the limiting nutrient over the course of the experiment, only the temporal distribution of the limiting nutrient varied, from one daily addition to one addition every 6th day. For example the P-limited semicontinuous treatment flasks containing 100 ml of culture received 20 ml of 0.2 µM phosphate media as part of their daily dilution, which elevated the PO$_4^{3-}$ by 0.04 µM daily. Over the course of 6 days the P-limited semicontinuous treatment flasks received a total of 0.024 µmoles of PO$_4^{3-}$. The pulsed treatment flask in turn received a single pulse of 0.024 µmoles of PO$_4^{3-}$ every 6th day. The pulse elevated the phosphate level in the pulse treatment flask containing 100 ml of culture by 0.24 µM PO$_4^{3-}$. Similarly, the N-limited semicontinuous treatment flasks received 20 ml of 3.0 µM NO$_3^-$ media as part of their daily dilution, which elevated the NO$_3^-$ by 0.6 µM daily. Over the course of 6 days the N-limited semi-continuous treatment flasks received a total of 0.36 µmoles of NO$_3^-$. The pulsed treatment flask in turn
received a single pulse of 0.36 µmoles of NO$_3^-$ every 6th day. The pulse elevated the nitrate level in the pulse treatment flask containing 100 ml of culture by 3.6 µM NO$_3^-$. The treatments that consisted of a semicontinuous supply of the limiting nutrient coupled with a repeating sinusoidal salinity fluctuation were identical to the semicontinuous treatments except that the salinity of the media added daily was of the appropriate salinity to produce the sinusoidal salinity fluctuation with approximately a daily 1% change in salinity.

The experimental static salinities of 15, 25 and 50% were chosen to examine the influence of salinity on species competitiveness while under N or P limitation, as these salinities would be comparable to the lower salinities found along the northern boundary, the average bay-wide salinity of the bay and the hypersaline conditions that occur in the north-central region of the bay, respectively (Boyer et al. 1999). The daily fluctuation in salinity of approximately 1% was chosen as it was expected to be within the range that Florida Bay phytoplankton would likely experience in the large bloom basins in the central, eastern and western regions as a result of evaporation and rainfall, and at the same time was believed to be large enough of a daily change over the course of several weeks to produce a detectable change in species relative dominance in the competition experiments.

Experimental flasks (250 ml Pyrex Erlenmeyer) containing 100 ml of the appropriate culture medium were manually diluted each day by the removal of 20 ml of culture followed by the addition of 20 ml of media of the appropriate salinity and nutrient composition. This resulted in a dilution rate of 0.2 da$^{-1}$. At the initiation of the experiments, duplicate experimental flasks of each treatment were inoculated with all four species from exponentially growing stock cultures. The initial inocula of each species in each flask were such that the species initial biomasses were approximately equivalent in terms of total biovolume, and the initial biomass of all four species combined was low enough to allow for a subsequent increase in community biomass. The daily effluent from the semicontinuous dilution of each flask was used for cell counts according to the procedures outlined in Chapter 1. Control flasks, containing only single species were grown adjacent to the experimental flasks to assure that each species was able to survive the experimental conditions. The base for the different media used in the competition experiments was artificial seawater (Parsons et al. 1984). The experimental media contained all nutrients at "f/2" concentrations except the limiting N or P, which were added to experimental flasks at the desired concentrations. Aseptic techniques and sterilization procedures were as described in Chapter 1. Stock unialgal cultures were maintained in exponential growth by semicontinuous dilution, thereby maximizing the physiological health of the algae and minimizing the potential for major bacterial interference in the nutrient-based interspecific competitive interactions of the study species. The ambient experimental conditions for all competition experiments were 25°C, 12:12 light:dark cycle and 150 µEm$^{-2}$sec$^{-1}$.

The 2nd set of mixed species competition experiments (Table 3-3), conducted only at 25% and lasting 62 days, examined the influence of P-limitation, N-limitation, N and P sufficiency, type of N and P (nitrate, ammonia, orthophosphate and glycerophosphate), semicontinuous nutrient delivery (daily dilution), and pulsed limiting nutrient delivery. The 2nd set of experiments was in part conducted to better
determine the relative competitive rankings of the species at 25 °C that were not all determined during the shorter (42 day) 1st set of competition experiments. The 2nd set of competition experiments also differed from the 1st set in that the initial biomass of all four species combined in each flask was near the equilibrium carrying capacity. This resulted in a smaller initial total biomass increase and an earlier onset of limiting nutrient competitive interactions.

The 3rd set of competition experiments consisted of paired diatom species NO$_3^-$-limited and PO$_4^{3-}$-limited competition experiments at 25 °C (Table 3-3). The purpose was to examine in more detail the interspecific N and P competitive differences between the dominant diatom bloom species C. cf. *salsugineus* and the non-dominant non-bloom species *T. cf. oceanica*. An additional diatom species, *Skeletonema costatum*, which was isolated from western Florida Bay, was also included in this set of competition experiments. The Florida Bay *S. costatum* was unavailable at the onset of the study and was therefore not a central part of the study. *S. costatum* is conspicuously absent from most of Florida Bay while being a common and abundant bloom species in other coastal waters of Florida. Its initial inclusion in this study was for comparative purposes. The paired-species competition experiments consisted of single flasks inoculated with either C. cf. *salsugineus* and *T. cf. oceanica*, C. cf. *salsugineus* and *S. costatum*, or *T. cf. oceanica* and *S. costatum*. The experimental methodology employed in the 2nd and 3rd sets of experiments was the same as that described for the first set of experiments.

The experimental concentrations of the limiting nutrients used in this study were similar to ambient N and P concentrations in the Florida Bay. The PO$_4^{3-}$ concentration in the semicontinuous treatment was elevated by 0.04 µM with each daily dilution in the 1st and 2nd sets of competition experiments. The pulsed treatments were elevated by 0.24 µM. Both experimental concentrations which the study species experienced were within the range that phytoplankton in Florida Bay would be expected to experience, as PO$_4^{3-}$ has been found to range from undetectable to 0.3 µM in the bay (Fourqurean et al. 1993). Although the concentration of PO$_4^{3-}$ in Florida Bay has commonly been found to be at or below the limit of detection (0.03 µM PO$_4^{3-}$), it is believed to be rapidly recycled in the water column by grazers and therefore it has been suggested that it may be readily available as elevated patches for use by the phytoplankton (Lavrentyev et al. 1998).

The variation in NO$_3^-$ and NH$_4^+$ concentrations in the semi-continuous and pulsed treatments were within or above the range that phytoplankton in Florida Bay would be expected to commonly experience, which has been found to range from undetectable to ~6 µM for NO$_3^-$, and 0.02 - ~11 µM for NH$_4^+$ (Fourqurean et al. 1993). The experimental NO$_3^-$ and NH$_4^+$ concentrations in the semicontinuous N-limited treatments were each elevated daily by 14.4 and 1.2 µM, respectively, while the pulsed NO$_3^-$ treatment was elevated every 6th day by 86.4 µM NO$_3^-$. 
**Results**

*Mixed species P-limited competition*

The results of the 1st set of mixed species competition experiments under P limitation at 15, 25 and 50 \(^0/00\) are presented in Figures 3-8, 3-9 and 3-10. At 15 \(^0/00\) in all three treatments (semicontinuous, pulsed, and semicontinuous with a salinity fluctuation), *S. cf. elongatus* dominated by biovolume, *T. cf. oceanica* was competitively excluded by day 30 and *C. cf. salsugineus* was competitively excluded by the end of the experiment (Fig. 3-8). Although *Synechocystis* sp. was not excluded, its reduced and decreasing biovolume suggested that given enough time, it too would be competitively excluded.

At both 25 \(^0/00\) and 50 \(^0/00\) in all three treatments, *S. cf. elongatus* dominated by biovolume, *C. cf. salsugineus* and *Synechocystis* sp. were the second and third most abundant species by biovolume and *T. cf. oceanica* was competitively excluded by day 30 of the experiment. The lower and decreasing biovolumes of *C. cf. salsugineus* and *Synechocystis* sp. suggested that given enough time, they too would be competitively excluded by *S. cf. elongatus* (Figs. 3-9 and 3-10).

The results of the 2nd set of mixed species competition experiments conducted only at 25 \(^0/00\) but lasting 62 days, revealed that for all treatments under P limitation *S. cf. elongatus* dominated by biovolume and both diatom species were completely excluded (Fig. 3-11). The competitive outcomes of the PO\(_4^{3-}\)-limited semicontinuous treatment at 25 \(^0/00\) in the longer 2nd set of competition experiments (Fig. 3-11A) confirmed what was found in the 1st set and clarified the relative ranking of species competitiveness for phosphorus at 25 \(^0/00\). The combined results from the 1st and 2nd sets of competition experiments produced a competitive ranking for PO\(_4^{3-}\) from strongest to weakest in which *S. cf. elongatus* > *Synechocystis* sp. > *C. cf. salsugineus* > *T. cf. oceanica*. The 2nd set of competition experiments more closely approached their final competitive outcomes than the 1st set because they were longer in duration and the interspecific competitive interactions began sooner due to an initial total species inocula on day 0 that more closely approximated the total steady state biomass carrying capacity (Fig. 3-11) (for comparison see the experimental duration and the initial biomass surge of *C. cf. salsugineus* following day 0 in the 1st set of experiments (Figs. 3-8, 3-9 and 3-10).

The type of P when supplied semicontinuously as PO\(_4^{3-}\) or as glycerophosphate had no effect on species competitiveness, as the rates of exclusion and patterns of relative dominance were the same for each type of limiting P (Fig. 3-11A,B). The frequency of delivery of the limiting P affected only the competitiveness of *Synechocystis* sp. such that it was not competitively excluded when P was delivered daily by semicontinuous dilution (Fig. 3-11B), while it was excluded when P was delivered as a pulse every sixth day (Fig. 3-11C).

*Mixed species N-limited competition*

The results of the 1st set of mixed species competition experiments at 15, 25 and 50 \(^0/00\) under N limitation are presented in Figures 3-12, 3-13 and 3-14. At 15 \(^0/00\) in all three treatments, *S. cf. elongatus*
and \textit{Synechocystis} sp. were co-dominants in terms of biovolume, while \textit{C. cf. salsugineus} was competitively excluded (Fig. 3-12). \textit{T. cf. oceanica} was the third most abundant species by biovolume at the end of the experiment in both the semicontinuous and pulse treatments while it was competitively excluded in the fluctuating salinity treatment.

At 25\% in all three treatments the very similar final biovolumes of \textit{T. cf. oceanica, S. cf. elongatus} and \textit{Synechocystis} sp. illustrated their similar competitive abilities for NO$_3^-$, while the exclusion of \textit{C. cf. salsugineus} in all three treatments (Fig. 3-13A,B, C) demonstrated its inferior NO$_3^-$ competitiveness.

At 50\% \textit{S. cf. elongatus} and \textit{Synechocystis} sp. were co-dominants in all three treatments by the end of the experiment (Fig. 3-14). In the semicontinuous treatment both \textit{T. cf. oceanica} and \textit{C. cf. salsugineus} were competitively excluded, while in the pulse treatment both diatom species persisted (Fig. 3-14A,B). In the fluctuating salinity treatment, \textit{T. cf. oceanica} persisted with a biovolume below both cyanobacteria species, while \textit{C. cf. salsugineus} was competitively excluded (Fig. 3-14C).

The results of the 2nd set of mixed species competition experiments conducted only at 25\% under N-limitation are presented in Figure 3-15. Whether the limiting N was delivered by semicontinuous dilution (daily pulse), or delivered as a large pulse every 6th day, \textit{C. cf. salsugineus} was competitively excluded and \textit{T. cf. oceanica, S. cf. elongatus} and \textit{Synechocystis} sp. all co-dominated, with the biovolume of \textit{T. cf. oceanica} slightly exceeding that of each cyanobacteria species when the source of N was NO$_3^-$ (Fig. 3-15A,B, C). The form of N altered slightly the competitive outcomes. When N was supplied as NH$_4^+$, \textit{C. cf. salsugineus} was still competitively excluded, but \textit{S. cf. elongatus} and \textit{Synechocystis} sp. became codominants, while the biomass of \textit{T. cf. oceanica} was substantially reduced (Fig. 3-15C).

\textit{Paired diatom species P- and N-limited competition}

The paired diatom species competition experiments at 25\% under P-limitation revealed that \textit{C. cf. salsugineus} was the superior P-competitor excluding both \textit{T. cf. oceanica} and \textit{S. costatum} (Fig. 3-16A,B). \textit{S. costatum} was the least competitive being completely excluded by \textit{C. cf. salsugineus}, and \textit{T. cf. oceanica} by the end of the experiments (Fig. 3-16B,C). The diatom species competitive ranking for PO$_4^{3-}$ from strongest to weakest competitor was \textit{C. cf. salsugineus} > \textit{T. cf. oceanica} > \textit{S. costatum}.

The paired diatom species competition experiments at 25\% under N limitation revealed that \textit{S. costatum} was the superior NO$_3^-$ competitor, completely excluding \textit{T. cf. oceanica} and \textit{C. cf. salsugineus} by day 10 (Fig. 3-17A,B). \textit{C. cf. salsugineus} was the least competitive being completely excluded by \textit{S. costatum} and dominated by and \textit{T. cf. oceanica} (Fig. 3-17B,C). The diatom species competitive ranking under N limitation from strongest to weakest competitor was \textit{S. costatum} > \textit{T. cf. oceanica} > \textit{C. cf. salsugineus}.
Mixed species N and P sufficient conditions

In the 1st and 2nd set of competition experiments at 25°C, for all treatments under N and P sufficient conditions (N:P molar ratio 16:1), no species were excluded (Figs. 3-18 and 3-19). Although the species total biovolumes were not all equivalent at the end of the experiment, the biovolumes appeared to stabilize giving no indication of any pattern of competitive exclusion. But there appeared to be some slight competitive displacement in that for most treatments by the end of the experiment, S. cf. elongatus, had the highest biovolume and T. cf. oceanica had the lowest biovolume.
Table 3-5. Experimental treatments in competition experiment sets #1, 2 and 3. All maintained by semicontinuous daily dilution, d=0.2 da⁻¹. See text for explanation.

<table>
<thead>
<tr>
<th>Condition</th>
<th>N and P Concentrations</th>
<th>N:P Ratio</th>
<th>Limiting Nutrient delivery</th>
<th>Salinity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1st set</strong></td>
<td>-Mixed Species</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-limited</td>
<td>160 µM NO₃⁻ : 0.2 µM PO₄³⁻</td>
<td>800 : 1</td>
<td>semicontinuous</td>
<td>15</td>
</tr>
<tr>
<td>P-limited</td>
<td>160 µM NO₃⁻ : 0.2 µM PO₄³⁻</td>
<td>800 : 1</td>
<td>semicontinuous</td>
<td>25</td>
</tr>
<tr>
<td>P-limited</td>
<td>160 µM NO₃⁻ : 0.2 µM PO₄³⁻</td>
<td>800 : 1</td>
<td>semicontinuous</td>
<td>50</td>
</tr>
<tr>
<td>P-limited</td>
<td>160 µM NO₃⁻ : 0.2 µM PO₄³⁻</td>
<td>800 : 1</td>
<td>pulsed</td>
<td>15</td>
</tr>
<tr>
<td>P-limited</td>
<td>160 µM NO₃⁻ : 0.2 µM PO₄³⁻</td>
<td>800 : 1</td>
<td>pulsed</td>
<td>25</td>
</tr>
<tr>
<td>P-limited</td>
<td>160 µM NO₃⁻ : 0.2 µM PO₄³⁻</td>
<td>800 : 1</td>
<td>pulsed</td>
<td>50</td>
</tr>
<tr>
<td>P-limited</td>
<td>160 µM NO₃⁻ : 0.2 µM PO₄³⁻</td>
<td>800 : 1</td>
<td>salinity fluctuation</td>
<td>15 - 20</td>
</tr>
<tr>
<td>P-limited</td>
<td>160 µM NO₃⁻ : 0.2 µM PO₄³⁻</td>
<td>800 : 1</td>
<td>salinity fluctuation</td>
<td>22.5 - 27.5</td>
</tr>
<tr>
<td>P-limited</td>
<td>160 µM NO₃⁻ : 0.2 µM PO₄³⁻</td>
<td>800 : 1</td>
<td>salinity fluctuation</td>
<td>45 - 50</td>
</tr>
<tr>
<td>N-limited</td>
<td>3 µM NO₃⁻ : 1.5 µMPO₄³⁻</td>
<td>2 : 1</td>
<td>semicontinuous</td>
<td>15</td>
</tr>
<tr>
<td>N-limited</td>
<td>3 µM NO₃⁻ : 1.5 µMPO₄³⁻</td>
<td>2 : 1</td>
<td>semicontinuous</td>
<td>25</td>
</tr>
<tr>
<td>N-limited</td>
<td>3 µM NO₃⁻ : 1.5 µMPO₄³⁻</td>
<td>2 : 1</td>
<td>semicontinuous</td>
<td>50</td>
</tr>
<tr>
<td>N-limited</td>
<td>3 µM NO₃⁻ : 1.5 µMPO₄³⁻</td>
<td>2 : 1</td>
<td>pulsed</td>
<td>15</td>
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<td>N-limited</td>
<td>3 µM NO₃⁻ : 1.5 µMPO₄³⁻</td>
<td>2 : 1</td>
<td>pulsed</td>
<td>25</td>
</tr>
<tr>
<td>N-limited</td>
<td>3 µM NO₃⁻ : 1.5 µMPO₄³⁻</td>
<td>2 : 1</td>
<td>pulsed</td>
<td>50</td>
</tr>
<tr>
<td>N-limited</td>
<td>3 µM NO₃⁻ : 1.5 µMPO₄³⁻</td>
<td>2 : 1</td>
<td>salinity fluctuation</td>
<td>15 - 20</td>
</tr>
<tr>
<td>N-limited</td>
<td>3 µM NO₃⁻ : 1.5 µMPO₄³⁻</td>
<td>2 : 1</td>
<td>salinity fluctuation</td>
<td>22.5 - 27.5</td>
</tr>
<tr>
<td>N-limited</td>
<td>3 µM NO₃⁻ : 1.5 µMPO₄³⁻</td>
<td>2 : 1</td>
<td>salinity fluctuation</td>
<td>45 - 50</td>
</tr>
<tr>
<td>Balanced</td>
<td>3.2 µM NO₃⁻ : 0.2 µMPO₄³⁻</td>
<td>16 : 1</td>
<td>semicontinuous</td>
<td>25</td>
</tr>
<tr>
<td>Balanced</td>
<td>3.2 µM NO₃⁻ : 0.2 µMPO₄³⁻</td>
<td>16 : 1</td>
<td>pulsed</td>
<td>25</td>
</tr>
<tr>
<td>Balanced</td>
<td>3.2 µM NO₃⁻ : 0.2 µMPO₄³⁻</td>
<td>16 : 1</td>
<td>salinity fluctuation</td>
<td>22.5 - 27.5</td>
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Table 3-5 (Continued).

<table>
<thead>
<tr>
<th>Condition</th>
<th>N and P Concentrations</th>
<th>N:P Ratio</th>
<th>Limiting Nutrient delivery</th>
<th>Salinity</th>
</tr>
</thead>
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<tr>
<td><strong>2nd set</strong> - Mixed Species</td>
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<tr>
<td>P-limited</td>
<td>50 :M NO$_3^-$ : 0.2 µMPO$_4^{3-}$</td>
<td>250:1</td>
<td>semicontinuous</td>
<td>25</td>
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<tr>
<td>P-limited</td>
<td>50 :M NO$_3^-$ : 0.2 µM glycerophosphate</td>
<td>250:1</td>
<td>semicontinuous</td>
<td>25</td>
</tr>
<tr>
<td>P-limited</td>
<td>50 :M NO$_3^-$ : 0.2 µM glycerophosphate</td>
<td>250:1</td>
<td>pulsed</td>
<td>25</td>
</tr>
<tr>
<td>N-limited</td>
<td>72 µM NO$_3^-$ : 36 µMPO$_4^{3-}$</td>
<td>2:1</td>
<td>semicontinuous</td>
<td>25</td>
</tr>
<tr>
<td>N-limited</td>
<td>6 µM NH$_4^+$ : 3 µMPO$_4^{3-}$</td>
<td>2:1</td>
<td>semicontinuous</td>
<td>25</td>
</tr>
<tr>
<td>N-limited</td>
<td>72 µM NO$_3^-$ : 36 µMPO$_4^{3-}$</td>
<td>2:1</td>
<td>pulsed</td>
<td>25</td>
</tr>
<tr>
<td>Balanced</td>
<td>3.2 µM NO$_3^-$ : 0.2 µMPO$_4^{3-}$</td>
<td>16:1</td>
<td>semicontinuous</td>
<td>25</td>
</tr>
<tr>
<td>Balanced</td>
<td>3.2 µM NH$_4^+$ : 0.2 µMPO$_4^{3-}$</td>
<td>16:1</td>
<td>semicontinuous</td>
<td>25</td>
</tr>
<tr>
<td>Balanced</td>
<td>3.2 µM NH$_4^+$ : 0.2 µM glycerophosphate</td>
<td>16:1</td>
<td>semicontinuous</td>
<td>25</td>
</tr>
<tr>
<td><strong>3rd set</strong> - Paired Diatom Species</td>
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<tr>
<td>P-limited</td>
<td>880 µM NO$_3^-$ : 1.0 µMPO$_4^{3-}$</td>
<td>880:1</td>
<td>semicontinuous</td>
<td>25</td>
</tr>
<tr>
<td>N-limited</td>
<td>72 µM NO$_3^-$ : 36 µMPO$_4^{3-}$</td>
<td>2:1</td>
<td>semicontinuous</td>
<td>25</td>
</tr>
</tbody>
</table>
Figure 3-8. P-limited competition experiments 1st set, 160 µM NO₃⁻ 0.2 µM PO₄³⁻, at 15 °C (A) semicontinuous nutrient addition, (B) pulsed PO₄³⁻ addition, (C) semicontinuous nutrient addition with salinity fluctuation. Mean value of two flasks with standard error. S. cf. elongatus = ▲, Synechocystis sp. = ●, T. cf. oceanica = ■, C. cf. salsugineus = ◊.
Figure 3-9. P-limited competition experiments 1st set, 160 µM NO$_3^-$ 0.2 µM PO$_4^{3-}$, at 25°C (A) semicontinuous nutrient addition, (B) pulsed PO$_4^{3-}$ addition, (C) semicontinuous nutrient addition with salinity fluctuation. Mean value of two flasks with standard error. *S. cf. elongatus* = ▲, *Synechocystis* sp. = •, *T. cf. oceanica* = ■, *C. cf. salsugineus* = ♦.
Figure 3-10. P-limited competition experiments 1st set, 160 µM NO₃⁻:0.2 µM PO₄³⁻, at 50 °C, (A) semicontinuous nutrient addition, (B) pulsed PO₄³⁻ addition, (C) semicontinuous nutrient addition with salinity fluctuation. Mean value of two flasks with standard error. S. cf. elongatus = ▲, Synechocystis sp. = ●, T. cf. oceanica = ■, C. cf. salsugineus = ★.
Figure 3-11. P-limited semicontinuous competition experiments 2nd set at 25 °C
(A) 50 μM NO₃⁻:0.2 μM PO₄³⁻ (B) 50 μM NO₃⁻:0.2 μM glycerophosphate,
(B) Pulsed glycerophosphate 50 μM NO₃⁻:0.2 μM glycerophosphate.
Mean value of two flasks with standard error. S. cf. elongatus = ▲, Synechocystis sp. = ●,
T. cf. oceanica = ■, C. cf. salsugineus = ●.
Figure 3-12. N-limited competition experiments 1st set, 3 µM NO$_3$:1.5 µM PO$_4$:$^3_-$, at 15 °/o (A) semicontinuous nutrient addition, (B) pulsed NO$_3$ addition, (C) semicontinuous nutrient addition with salinity fluctuation. Mean value of two flasks with standard error. S. cf. elongatus = ▲, Synechocystis sp. = ●, T. cf. oceanica = ■, C. cf. salsugineus = ♦.
Figure 3-13. N-limited competition experiments 1st set, 3 µM NO$_3$:1.5 µM PO$_4$$_3^-$, at 25 °C/0°C (A) semicontinuous nutrient addition, (B) pulsed NO$_3$ addition, (C) semicontinuous nutrient addition with salinity fluctuation. Mean value of two flasks with standard error. S. cf. elongatus = ▲, Synechocystis sp. = ●, T. cf. oceanica = ■, C. cf. salsugineus = ♦.
Figure 3-14. N-limited competition experiments 1st set, 3 µM NO$_3$ :1.5 µM PO$_4^{3-}$, at 50% /% (A) semicontinuous nutrient addition, (B) pulsed NO$_3$ addition, (C) semicontinuous nutrient addition with salinity fluctuation. Mean value of two flasks with standard error. S. cf. elongatus = ▲, Synechocystis sp. = ●, T. cf. oceanica = ■, C. cf. salsugineus = ♦.
Figure 3-15. N-limited semicontinuous competition experiments, 2\textsuperscript{nd} set at 25°C/00.
(A) 72 µM NO\textsubscript{3}\textsuperscript{−}:36 µM PO\textsubscript{4}\textsuperscript{3−}, (B) Pulsed NO\textsubscript{3}\textsuperscript{−}, 72 µM NO\textsubscript{3}\textsuperscript{−}:36 µM PO\textsubscript{4}\textsuperscript{3−}, (C) 6.0 µM NH\textsubscript{4}\textsuperscript{+}:3.0 µM PO\textsubscript{4}\textsuperscript{3−}. Mean value of two flasks with standard error.
S. cf. elongatus = ▲, Synechocystis sp. = ●, T. cf. oceanica = ■, C. cf. salsugineus = ◦.
Figure 3-16. P-limited semicontinuous paired diatom species competition experiments, 3rd set at 25%o. (A), (B) and (C) all at 880 µM NO₃⁻:1.0 µM PO₄³⁻.
Figure 3-17. N-limited semicontinuous paired diatom species competition experiments, 3rd set at 25 °C. (A), (B) and (C) all at 72 µM NO₃:36 µM PO₄³⁻.

Figure 3-18. N & P sufficient competition experiments, 1st set, 3.2 µM NO₃⁻:
0.2 µM PO₄³⁻, at 25 °C. (A) semicontinuous nutrient addition, (B) pulsed N & P
addition, (C) semicontinuous nutrient addition with salinity fluctuation.
Mean value of two flasks with standard error. S. cf. elongatus = ▲, Synechocystis sp. = ●, T. cf. oceanica = ■, C. cf. salsugineus = ♦.
Figure 3-19. N & P sufficient competition experiments, 2\textsuperscript{nd} set at semicontinuous dilution at 25°/00. (A) 3.2 \mu M NO\textsubscript{3}^-:0.2 \mu M PO\textsubscript{4}^{3-}, (B) 3.2 \mu M NH\textsubscript{4}^+:0.2 \mu M PO\textsubscript{4}^{3-}, (C) 3.2 \mu M NH\textsubscript{4}^+:0.2 \mu M glycerophosphate. Mean value of two flasks with standard error. S. cf. elongatus = ▲, Synechocystis sp. = ●, T. cf. oceanica = ■, C. cf. salsugineus = ◊.
Discussion

\textit{P- and N-limited semicontinuous treatments}

The $\text{PO}_4^{3-}$-limited semicontinuous treatments at 15, 25 and 50 $\%_0$ provided an actual test of the ERC-theory predictions made in Chapter IIA for each species $\text{PO}_4^{3-}$-competitiveness under equilibrium competition. The ERC-theory predicted relative competitive ranking for $\text{PO}_4^{3-}$ from strongest to weakest competitor in which: \textit{S. cf. elongatus} $>$ \textit{Synechocystis} sp. $>$ \textit{C. cf. salsugineus} $>$ \textit{T. cf. oceanica}, was in complete agreement with the results of the competition experiments. The competitive outcomes of the $\text{PO}_4^{3-}$-limited semicontinuous treatments at 15, 25 and 50 $\%_0$ in the 1st set of competition experiments were as follows: \textit{S. cf. elongatus} dominated, \textit{T. cf. oceanica} was the first species to be competitively excluded, and the reduced biomass levels of \textit{Synechocystis} sp. and \textit{C. cf. salsugineus} indicated that they would in time also be excluded (Figs. 3-8A, 3-9A, and 3-10A). The competitive outcomes verified that the qualitative interspecific differences in $\text{PO}_4^{3-}$-R* values determined for the study species (Table 3-4) were indeed real.

Although technically the predictions of ERC-theory apply only to competition under equilibrium conditions, the agreement between ERC-theory predictions and the $\text{PO}_4^{3-}$-limited competitive outcomes indicates that the approximate equilibrium conditions attained by semicontinuous dilution were adequate for predicting equilibrium competitive outcomes of this group of four competing species. But because the limiting P was not supplied at a low and continuous rate, but was actually delivered as a small daily pulse during the daily culture dilutions, the experimental competitive outcomes cannot conclusively be considered to be solely the result of interspecific differences in $\text{PO}_4^{3-}$ affinity. As a result, the competitive outcomes of the semicontinuous dilutions (8A, 9A, 10A, 11A) may be due to a combination of interspecific differences in the species abilities to utilize the small (0.04 $\mu$M $\text{PO}_4^{3-}$) daily pulses occurring simultaneously with each daily dilution as well as their ability to utilize the very low $\text{PO}_4^{3-}$ concentrations existing between dilutions. The low $\text{PO}_4^{3-}$ concentrations existing between dilutions would place the superior affinity specialist \textit{S. cf. elongatus} with the lowest $K_{uP}$ at a competitive advantage over the other lesser affinity specialist species having higher $K_{uP}$'s. Because phytoplankton utilize affinity, storage and velocity strategies to varying degrees, species may be viewed as occurring somewhere along a continuum that extends from pure affinity specialists to pure storage specialists. Theoretically the daily pulses occurring simultaneously with each daily dilution in the semicontinuous treatments would select for species with velocity and/or storage specializations capable of taking advantage of this frequency of limiting P-patchiness. Although the nutrient variability introduced by the daily dilution did not alter the overall qualitative competitive outcomes predicted by ERC-theory to occur under equilibrium conditions (continuous dilution), the nutrient variability introduced by daily dilution may have altered the relative rates at which competitors were displaced.

It was expected that the relative ranking of N-competitiveness of the study species from strongest to weakest would be the reverse of the species ranking that was determined for P. This expectation was
Based on a common observation that phytoplankton species which are superior competitors for one major nutrient are more often than not inferior competitors for another nutrient, and implies a strategic “tradeoff” with respect to nutrient competitive abilities (Tilman and Kiesling 1984). Competitive outcomes at 25\(^0\)/\(_{\text{oo}}\) were examined for N and P specialization tradeoffs because this salinity is not suboptimal for any of the species and excludes the influence of suboptimal salinity on competitiveness under N and P limiting conditions, which is addressed later. The relative competitive ranking from strongest to weakest affinity competitor for NO\(_3^-\) was; T. cf. oceanica \(\geq\) Synechocystis sp. \(\approx\) S. cf. elongatus > C. cf. salsugineus (Fig. 3-15A). In contrast, the competitive ranking for PO\(_4^{3-}\) was S. cf. elongatus > Synechocystis sp. > C. cf. salsugineus > T. cf. oceanica. The relative competitive rankings highlight the N-P specialization trade-offs in the diatom species. T. cf. oceanica is an inferior P-competitor but a superior N-competitor, while C. cf. salsugineus is the opposite, an inferior N-competitor and a superior P-competitor. Because the cyanobacteria (particularly S. cf. elongatus) are such excellent P-affinity competitors, it was expected that they would be poor N-affinity competitors. Their decreased N-competitiveness was expected to place them at a competitive disadvantage relative to T. cf. oceanica and lead to their gradual competitive exclusion. But, no comparable N-P tradeoff was seen in the cyanobacteria species as competition experiments determined them to be both superior P-competitors and excellent N-competitors.

Similar to the P-limited semicontinuous treatments, the limiting N was also not delivered continuously, and therefore the co-dominance of T. cf. oceanica and the two cyanobacteria species in the N-limited semicontinuous treatments may be attributable to the combined influence of their NO\(_3^-\) affinity (low K\(_u\)'s), velocity (high V\(_m\)/K\(_s\) ratios) and luxury storage attributes being well suited to the temporal variation of limiting N introduced by the daily semicontinuous dilutions.

**P- and N-limited pulsed treatments**

Results of nutrient limited competition experiments have shown that variability in the supply of the limiting nutrient (e.g. pulses) may allow more species to coexist than could do so at equilibrium (Sommer 1985), delay competitive exclusion (Grover 1988), and result in shifts in species dominance (Turpin and Harrison 1979). The pulse frequency may also be quite selective with shifts in species dominance occurring between daily and weekly pulse intervals (Turpin and Harrison 1979). It was expected that the study species would differ in their P and N velocity and storage attributes and therefore their ability to respond to pulses of PO\(_4^{3-}\) and NO\(_3^-\). This expectation was based largely on distinct differences that were observed between the two taxonomic groups (diatoms and cyanobacteria) growth response curves (lag periods), biovolumes, U\(_m\)'s and K\(_uP\)'s.

It was observed that the lag period in growth, a characteristic of phytoplankton growth response curves that commonly varies in response to changing growth conditions, was consistently shorter for the diatoms than the cyanobacteria species (data not presented). The diatoms shorter lag period coupled with their higher U\(_m\)'s (Chapter 2, Fig. 2-1) suggested that the diatoms would be more successful velocity competitors, being able to convert nutrients to biomass more rapidly than the cyanobacteria when the
limiting nutrient became more available. The results of some nutrient limited competition experiments (Sommer 1985) as well as competition theory (Hsu 1980, Butler et al. 1985) suggest that a temporally variable nutrient supply enhances the competitive ability of species with higher $U_m$'s.

The much greater biovolumes of the diatom species than the cyanobacteria species (Chapter 4, Table 4-1) suggested that they would be better able to utilize the storage strategy, as some larger species have been found to be capable of greater luxury storage than smaller species (Shuter 1978). The qualitatively larger PO$_4^{3-}$ $K_u$'s of the diatom species also suggested that their overall P-competitive strategy would likely include a high P velocity and/or P storage specialization to offset the low P affinity specialization. Nutrient specialization tradeoffs like these have been commonly observed in species of phytoplankton (Tilman 1982).

The interspecific differences just noted, suggested that a taxonomic tradeoff in phosphorus limiting nutrient strategies would be likely, in which the diatom species higher $U_m$'s, decreased lags in growth, larger biovolumes and higher $K_u$'s would place them along an affinity-velocity-storage continuum of nutrient strategy more toward the velocity-storage end while the cyanobacteria species would be more at the affinity end. As velocity and storage specialists, the diatoms were predicted to take better advantage of the infrequent PO$_4^{3-}$ pulses (every 6$^\text{th}$ day) in the pulsed treatments than the cyanobacteria and in time to dominate the pulsed treatments. On the other hand, the low ambient concentrations of phosphate, which would exist for 6 days in the pulsed treatment between experimental pulse additions, would place the diatoms at a competitive disadvantage with respect to the cyanobacteria, which have lower $K_u$'s.

In my study, there was no shift in species relative dominance as a result of limiting PO$_4^{3-}$ pulsing. The nearly identical patterns of dominance, coexistence, displacement and rates of exclusion rates in the pulsed (every 6$^\text{th}$ day) and the semicontinuous treatments (daily pulse) at all three salinities (Figs. 3-8B, 3-9B, 3-10B vs. 3-8A, 3-9A, 3-10A), indicates that neither diatom species nor cyanobacteria species gained a competitive advantage by more effectively utilizing either the small daily pulse or the larger infrequent pulse of PO$_4^{3-}$. If the subdominant PO$_4^{3-}$-competitors C. cf. salsugineus, Synechocystis sp. or T. cf. oceanica had selectively benefited from the daily small or larger ~weekly pulse of PO$_4^{3-}$, elevated biomass levels and/or decreased rates of exclusion would have been visible in either or both of the treatments. The hypothesis that a temporally variable nutrient supply enhances the competitive ability of species with higher $U_m$'s was not supported by my results, as the competitiveness of neither T. cf. oceanica nor C. cf. salsugineus which have higher $U_m$'s than those of the cyanobacteria species were not enhanced. This finding is also in agreement with some other studies (Grover 1989b).

Although the dominance of S. cf. elongatus under pulse intervals of 1 day and 6 days was unexpected, it is not anomalous. There are examples of single species being superior phosphorus competitors under nutrient regimes in which the nutrient is either added continuously, pulsed daily or pulsed at weekly intervals (Smith and Kalff 1983, Sommer 1983, Sommer 1985). The competitive success of a single species over a broad range of nutrient patchiness is also supported by experimental evidence in which species $V_{max}$ values have been found to increase in response to an increase in the availability of
limiting $\text{PO}_4^{3-}$ without a corresponding increase in their $K_s$ or $K_u$ (Rivkin and Swift 1982). Both constancy of $K_s$ with variable cellular nutrient status (Burmaster and Chisholm 1979, Rhee 1973) as well as the dependence of $V_{\text{max}}$ on cellular nutrient status, specifically an enhanced rate in nutrient deficient cells (Fuhs et al. 1972, Rhee 1973, Eppley and Renger 1974, McCarthy and Goldman 1979, Rivkin and Swift 1982) have been well documented. These physiological adaptations may also enable $S. \text{ cf. elongatus}$ to accumulate more limiting N or P during elevated pulses of N or P.

An additional physiological adaptation that may help explain the dominance of $S. \text{ cf. elongatus}$ in both the semicontinuous and pulsed nutrient regimes and presumably under a continuous nutrient regime as well (although not tested using competition experiments, but predicted by ERC-theory P-kinetics) are found in the oceanic $\text{Pyrocystis noctiluca}$ (Rivkin and Swift 1982) and $\text{Synechococcus}$ sp. (Ikeya et al. 1997). The uptake system for orthophosphate in the oceanic dinoflagellate $\text{Pyrocystis noctiluca}$ is multi-phasic in both P-replete and P-depleted cells, such that cells have both a high affinity-low uptake capacity (low $K_s$ - low $V_{\text{max}}$) phase, and a low affinity-high uptake capacity (high $K_s$ - high $V_{\text{max}}$) phase. The first phase optimizes the uptake of orthophosphate in the oceanic environment in which $\text{PO}_4^{3-}$ occurs at continually low concentrations, while the second uptake phase allows for the rapid uptake of orthophosphate when it occurs temporally and spatially in small enriched patches.

Ikeya et al. (1997) documented an oceanic $\text{Synechococcus}$ sp. that was able to alter its $\text{PO}_4^{3-}$ uptake rate by increasing and/or decreasing both its $K_s$ and $V_{\text{max}}$ in response to ambient concentrations of $\text{PO}_4^{3-}$. They speculated that the ability of the oceanic $\text{Synechococcus}$ sp. to grow over a wide range of $\text{PO}_4^{3-}$ concentrations was the result of the induction of a separate system of periplasmic membrane uptake sites under nutrient-depleted conditions, different from the uptake system used under high ambient concentrations of $\text{PO}_4^{3-}$. $S. \text{ cf. elongatus}$ like $\text{P. noctiluca}$ and $\text{Synechococcus}$ sp. flourishes in an environment that has low ambient concentrations of $\text{PO}_4^{3-}$ and presumably elevated patches of $\text{PO}_4^{3-}$ as well. It may also have a similar combination of kinetic uptake phases for $\text{PO}_4^{3-}$, which would allow it to optimize the low ambient $\text{PO}_4^{3-}$ concentrations that characterize much of Florida Bay as well as the patches of elevated $\text{PO}_4^{3-}$ that it would also be expected to experience in the bay.

The influence of N pulsing was similar to that observed for P pulsing. The overall similarity between the competitive outcomes of the N-limited semicontinuous treatments (Figs. 3-12A, 3-13A, 3-14A and 3-15A) and their respective pulsed treatments (6 day intervals) (Figs. 3-12B, 3-13B, 3-14B and 3-15 B) suggested the absence of interspecific differences in the species ability to utilize large and infrequent pulses of limiting N. The relative competitiveness of the species remained unaffected, except for the non-exclusion of both $C. \text{ cf. salsugineus}$ and $T. \text{ cf. oceanica}$ at 50 $\%$ (Fig. 3-14 A,B).

Phytoplankton are capable of forming intracellular storage bodies containing N, P and glycogen which can be used during periods of nutrient limitation to maintain their growth rates. It has even been suggested that phosphorus storage in cyanobacteria may be greater than other algae (Sommer 1985), giving them a distinct competitive advantage under P-limiting conditions when P occurs as pulses. But no such
advantage was observed in the pulsed treatments in my study. In reality, the phosphorus luxury storage capabilities of the cyanobacteria and diatom species were quite similar (Chapter 4, Table 4-3).

Cyanophycin is a unique intracellular storage pool produced by some but not all cyanobacteria (Newman et al. 1987). Both cyanobacteria isolates used in this study have been tested for their ability to produce cyanophycin using the chloramphenicol assay (Allen 1988), and only *Synechocystis* sp. was found to produce cyanophycin (Corbridge 1998). Theoretically this unique nitrogen reserve may place it at a competitive advantage over *S. cf. elongatus* and the two diatom species during periods of N-limitation. A comparison of the pulsed N-limited treatments (Figs. 3-12B, 3-13B, 3-14B, 3-15B) with their respective semicontinuous treatments (Figs. 3-12A, 3-13A, 3-14A, 3-15A) reveals that any competitive advantage that *Synechocystis* sp. may have obtained from its cyanophycin storage pool was not adequate to alter the existing patterns of relative dominance.

**Form of limiting P and N**

It has been suggested that dissolved organic phosphorus (DOP) may be an important source of P for the phytoplankton of Florida Bay as it has been found to be the dominant form of P in the water column, being on average approximately an order of magnitude greater than the dissolved inorganic phosphorus (DIP) concentration (Lavrentyev et al. 1998). DIP concentrations in the bay have been found to range from undetectable (~ 0.03 µM) to ~0.33 µM, while organic phosphorus can range from ~0.04 to 2.03 µM (Fourquarean et al. 1993). Although the most readily utilized form of P by both diatoms and cyanobacteria is generally PO$_4^{3-}$ (Healy 1982), other organic forms of P are utilized as well.

It may be hypothesized that the perpetually low levels of DIP and higher levels of DOP in Florida Bay have led to the selection of phytoplankton species that have physiological nutrient strategies that allow them to thrive under low ambient phosphorus concentrations and or adaptations that also allow them to utilize the more abundant DOP.

Interspecific differences in the study species ability to utilize DOP were considered because interspecific differences in the ability to utilize DOP have been documented for diatoms and cyanobacteria ((*Synechococcus* sp.) (Kuenzler 1970) and any differences have the potential to provide species with a decisive competitive advantage in Florida Bay.

The ability of the study species to utilize DOP was tested directly by supplying P as glycerophosphate. In the glycerophosphate treatments all four species grew well, suggesting the presence of external alkaline phosphatase and efficient hydrolysis of glycerophosphate as well as efficient uptake of the hydrolized PO$_4^{3-}$. Whether the limiting P was supplied as DIP (PO$_4^{3-}$) or DOP (glycerophosphate) made no difference, since the competitive outcomes under both treatments were the same (Fig. 3-11A,B). These results suggest that the relative dominance of the four bloom species in Florida Bay is unlikely to be the result of interspecific differences in the species ability to utilize forms of organic phosphorus via external alkaline phosphatase enzymes. Although the form of the P pulse as PO$_4^{3-}$ or glycerophosphate did not alter the overall pattern of species relative dominance, it did appear that when P was pulsed as glycerophosphate,
it accelerated the competitive exclusion of *Synechocystis* sp., suggesting a slight shift in competitive advantage to *S. cf. elongatus* (Fig. 3-11B,C). The accelerated exclusion of *Synechocystis* sp. in the glycerophosphate pulsed treatment but not in the PO$_4^{3-}$ pulsed treatment suggests a potential synergism of pulsing and limiting P as glycerophosphate on the P-competitive interaction between *Synechocystis* sp. and *S. cf. elongatus*.

The potential influence of ammonium on the development of cyanobacteria dominated blooms particularly in the north-central region of Florida Bay was considered because NH$_4^+$ can be the dominant form of N reaching concentrations as high as ~26 µM (Lavrentyev et al. 1998), and some field and experimental evidence suggests that cyanobacteria growth is favored by NH$_4^+$ while eucaryotic algal growth is favored by NO$_3^-$ (Blomqvist et al. 1994). Additional studies also indicate that growth rates of cyanobacteria and diatoms on NH$_4^+$ and NO$_3^-$ may vary with light intensity (Guerrero and Lara 1987, Ward and Wetzel 1980, Syrett 1981, Thompson et al. 1989), which may at times be limiting in the north-central region of Florida Bay (Phlips et al. 1995). A slight shift in dominance between *T. cf. oceanica* and the cyanobacteria species with the form of N indicated that the cyanobacteria were slightly favored when the source of limiting N was NH$_4^+$, while *T. cf. oceanica* was apparently slightly favored when the source was NO$_3^-$ (Fig. 3-15 A,C).

P- and N-limited salinity treatments

Phytoplankton use several mechanisms to adapt to changes in ambient salinity, which require energy expenditure by the cell that might otherwise be used for cell maintenance or growth (Miller and Kamykowski 1986a). The process of osmoregulation is dependent upon energy and membrane transport, which in turn is regulated by enzymes and proteins. Since the synthesis of ATP, mRNA, enzymes and proteins requires phosphorus and nitrogen, both N and P play essential roles in cellular osmoregulatory processes.

Studies examining the influence of environmental factors upon phytoplankton species cellular processes have found that during unfavorable environmental conditions (e.g. suboptimal temperatures for growth) that the cellular quotas of limiting nutrients are usually higher than the quotas under more optimal environmental conditions for growth (Goldman and Mann 1980). As a result, it was expected that at suboptimal salinities for growth (i.e. at the low and high end of each species salinity tolerance curve), elevated cell quotas for limiting nutrients due to the higher maintenance costs at suboptimal salinities would lower the N- and P-competitiveness of the four study species. It was expected that the osmoregulatory related costs would be greater for the diatoms than the cyanobacteria at 15 and 50 $^0/00$, based on the differences that were seen in their salinity growth response curves (Chapter 2, Fig. 2-1), and that the added costs would reduce the diatom species competitiveness under both N and P limitation. The reduced competitiveness would then be visible as altered competitive outcomes in the N- and P-limited competition experiments at 15 and 50 $^0/00$, relative to the outcomes at 25 $^0/00$. 

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Salinity responses determined for each species (Chapter 2, Fig. 2-1) indicated that ambient static salinities would influence the four study species competitiveness under N limitation and P limitation. But the competitive outcomes at 15, 25 and 50 \%_0/00 with the exception of some isolated responses by *Synechocystis* sp. and *C. cf. salsugineus*, all indicated that ambient static salinities between 15 and 50 \%_0/00 did not significantly alter any of the species P-competitiveness as the competitive outcomes were all equivalent at 15, 25 and 50 \%_0/00 (Figs. 3-8A,B vs. 3-9A,B vs. 3-10A,B). No explanation can be offered for the exclusion of *C. cf. salsugineus* in all three treatments at 15 \%_0/00 while not in any treatment at 25 or 50 \%_0/00, since 15 \%_0/00 was not a suboptimal salinity for its growth (Chapter 2, Table 2-1). Salinity and P limitation were each found to independently affect each species growth, survival, relative dominance and rate of exclusion. The P-limited competitive outcomes (Figs.3-8A,B; 3-9A,B; 3-10A,B) indicated that suboptimal salinities for growth did not act synergistically with semicontinuous or pulsed P limitation to produce an increase or decrease in a species P-competitiveness that was greater than the sum of their individual effects.

The potential role of fluctuating salinities in determining bloom dominance in Florida Bay was considered because the composition of both P- and N-limited mixed natural phytoplankton communities have been found to be altered by an acute 5 \%_0/00 reduction in salinity (Vargo et al. ), and by larger but more gradual decreases in salinity (Rijstenbil 1988). The similar competitive outcomes of the P-limited semicontinuous salinity fluctuation treatments at 15, 25 and 50 \%_0/00 and the semicontinuous treatments at each experimental salinity (Figs. 3-8C, 3-9C, 3-10C vs. 3-8A, 3-9A and 3-10A) indicated that a daily salinity fluctuation of ~1 \%_0/00 did not affect any of the species P-competitiveness. The results also indicated that cellular processes adequately compensated for the energy costs associated with the daily osmoregulatory response without any detectable change in competitiveness for the limiting P.

Under N-limitation, some slight shifts in dominance and co-dominance followed changes in ambient salinity. The larger final steady state biovolumes of the cyanobacteria indicated that they had a small competitive edge over *T. cf. oceanica* at 15 \%_0/00 which increased dramatically at 50 \%_0/00 as indicated by the exclusion of *T. cf. oceanica* (Figs. 3-12A vs. 3-13A vs. 3-14A). On the other hand at 25 \%_0/00, in the 2nd set of experiments, the greater final steady state biovolume of *T. cf. oceanica* suggested a slight competitive advantage over the cyanobacteria species (Fig. 3-15A). The enhanced competitiveness of *T. cf. oceanica* at 25 \%_0/00 and its reduced competitiveness at 15 \%_0/00 and 50 \%_0/00 relative to the cyanobacteria species may be explained by the fact that while 25 \%_0/00 is an optimal salinity for growth, 15 \%_0/00 and especially 50 \%_0/00 are sub-optimal for *T. cf. oceanica* (Chapter 2, Table 2-1). Similar to P limitation, no synergistic effect between N limitation and salinity was observed, as species biomass, relative dominance and rates of exclusion in the competitive outcomes at all three salinities were not dramatically different from the pattern expected from the sum of the individual effects.

Fluctuating salinities also had no effect upon the species N-competitiveness with the exception of *T. cf. oceanica* at 15 and 50 \%_0/00. The similar competitive outcomes of the semicontinuous salinity fluctuation treatments and the semicontinuous treatments at each of the three ambient salinities (Figs. 3-
12C, 3-13C, 3-14C vs. 3-12A, 3-13A, 3-14A) indicated that a daily salinity fluctuation of ~1‰ did not affect any of the species N-competitiveness. The competitive exclusion of *T. cf. oceanica* when exposed to the small daily salinity change of ~1‰ between 15 and 20‰ (Fig. 3-12C), suggested a decrease in N-competitiveness. The decreased competitiveness may be the result of the increased osmoregulatory costs associated with the daily adjustments to the incremental changes of ~1‰ each day, as *T. cf. oceanica* has been found to be capable of growth between 15 and 50‰ when fully acclimated (see Chapter 2, Table 2-1). Support for a decrease in N-competitiveness in *T. cf. oceanica* in response to a fluctuating salinity may be found in the interpretation of other competition experiments with NH$_4^+$ limitation and fluctuating salinities. For example, *Ditylum brightwelli* had a higher affinity for ammonium and became dominant at a salinity of approximately 18‰, while a slight decrease in salinity to approximately 15‰ was believed to reverse the affinities for ammonium and favor *S. costatum* instead (Rijstenbil 1988). On the other hand, the cyclic salinity fluctuation between 45 and 50‰ enhanced the competitiveness of *T. cf. oceanica* such that it was not excluded, as it was at a static salinity of 50‰ in the semicontinuous treatment. This may be explained most simply by the fact that 50‰ is nearer the upper salinity tolerance range for growth for *T. cf. oceanica*, and the fluctuation in salinity reduced the ambient salinity enough to increase its competitiveness sufficiently to prevent its exclusion.

*Paired diatom species P- and N-limited treatments*

Paired diatom species competition experiments determined the competitive ranking among the diatom species from strongest to weakest for P to be: *C. cf. salsugineus* > *T. cf. oceanica* > *S. costatum* (Fig. 3-16), and for N to be: *S. costatum* > *T. cf. oceanica* > *C. cf. salsugineus* (Fig. 3-17). The inverse competitive ranking between N and P, displays a common pattern of nutrient strategy tradeoff that has been documented for many species of freshwater diatoms (Tilman and Kiesling 1984).

The inclusion of *S. costatum* in this study, which is not a dominant bloom species nor a common species in Florida Bay being for the most part conspicuously absent from the majority of the bay (Steidinger et al. 2001), may indirectly provide some insights regarding bloom composition, dominance and phytoplankton community composition in the bay. *S. costatum* is a common and often abundant bloom species in temperate coastal and estuarine waters that are typically more nitrogen than phosphorus limited. It is also a common and often abundant bloom species in Florida's estuarine and coastal waters. Although *S. costatum* has been described as a good N-competitor (Rijstenbil et al. 1989b) capable of overwhelmingly dominating the phytoplankton community when nitrogen is apparently the only limiting nutrient (Kilham and Hecky 1988), the same cannot be said for it’s P-competitiveness. The finding in my study that *S. costatum* was the most inferior P-competitor may help explain the anomalous conspicuous absence of *S. costatum* from the central and eastern regions of Florida Bay (Steidinger et al. 1995). It's phosphorus requirement has been speculated to be relatively high based on its ATP requirements (Lavrentyev et al. 1998), and its conspicuous absence from the eastern and central regions of Florida Bay may be due to its
inability to meet its P requirements in these chronically low and often P-limited regions, as well as its inferior P-competitiveness.

In contrast, the superior N-competitiveness of \textit{S. costatum} determined in my study is in agreement with field observations in which \textit{S. costatum} is a common and frequently abundant bloom species in coastal and estuarine waters that are typically more nitrogen than phosphorus limited. The very limited documented occurrence of \textit{S. costatum} in the more N-limited western region of Florida Bay is still puzzling, but may have to do with its very inferior P-competitiveness. Under P limitation, the consistent and very rapid exclusion of \textit{T. cf. oceanica} by \textit{S. cf. elongatus}, \textit{Synechocystis} sp. and \textit{C. cf. salsugineus} (Figs. 3-8, 3-9 3-10 and 3-11) and the very rapid exclusion of \textit{S. costatum} by \textit{T. cf. oceanica} in the paired diatom species P-limited competition experiments (Fig. 3-16C), indicate a very large disparity in P-competitiveness between \textit{S. costatum} and the other Florida Bay study species. This large disparity in P-competitiveness has the potential to translate into a disproportionally large and rapid loss in biomass, with the loss being dependent upon the duration of the P-limiting conditions and the growth rates of the competing species.

It may be suggested that the P-competitiveness of \textit{S. costatum} is so inferior and the resulting competitive disadvantage so large that the biomass losses accrued under the intermittent P-limiting conditions in the central region of the bay may be large enough to overwhelm the biomass gains accrued under the more favorable intermittent N-limiting conditions. As a result, the large P-N specialization tradeoff of \textit{S. costatum} may restrict its occurrence as a dominant bloom species to regions that are primarily N-limited and if they experience periods of P limitation, the periods of P limitation are of insufficient duration to significantly impact the greater positive influence that longer periods of N-limitation have on the population.

\textit{N & P sufficient treatments}

The coexistence of all four study species under N and P sufficient conditions in all of the experimental treatments at 25 \(^\circ\)/\(^\circ\) (semicontinuous, pulsed, salinity fluctuation and various forms of N and P) (Figs. 3-18 and 3-19), demonstrated not only that all four species were able to survive under each of the experimental treatments but that the species interactions visible in the outcomes of the competition experiments as dominance, coexistence, competitive displacement and ultimately exclusion, were the direct result of interspecific nutrient-based competitive interactions and the influence of salinity on those competitive interactions. In addition, the coexistence of the four study species at saturating irradiances and N and P sufficiency (N:P Redfield ratio) when the P an N sources were DIP (PO\(_4^{3-}\)), DOP (glycerophosphate), NH\(_4^+\) or NO\(_3^-\) indicates that these sources of P and N did not selectively favor either cyanobacteria or diatom dominance (Fig. 3-19). This may be significant as the north-central region recognized as the epicenter of the cyanobacteria dominated blooms, has also been described as having a DIN:TP ratio that approximates the Redfield ratio (Brand 2002). The north-central region also has some of the highest concentrations of NH\(_4^+\) and alkaline phosphatase activity in the bay (Boyer et al. 1999).
Although the final total biovolumes of the species were not all equivalent, the biovolumes appeared to stabilize, suggesting no pattern of any ongoing interspecific competitive exclusion. But there appeared to be some slight competitive displacement, in that for most treatments by the end of the experiment, *S. cf. elongatus*, had the highest biovolume and *T. cf. oceanica* had the lowest.

The large and persistent cyanobacteria dominated blooms in the north-central region resemble an algal chemostat in having: 1) low water turnover rate; 2) similar and fairly constant rates of algal biomass loss and gain; 3) algal biomass that remains at or near a determined carrying capacity for extended periods of time and 4) algal biomass that may be determined for extended periods of time by the concentration of a single (N or P) limiting nutrient. The results of nutrient bioassays have indicated that the standing algal biomass in the north-central bloom region may be determined for extended time periods by a single nutrient and at times be at or near its carrying capacity (Tomas et al 1999). The low water turnover rates of the interior basins are more likely to allow for the development of the near steady state conditions under which nutrient limitation (e.g. N or P) is more likely to result in some nutrient-based interspecific algal competitive interactions. If the analogy is appropriate, then the near steady state P- and N-limited competitive interactions and outcomes seen in the flasks of the competition experiments may at times mirror N- and/or P-limited competitive processes occurring in the near steady state low turnover interior basins of the bay, in which P limitation promotes the dominance of the cyanobacteria clones while N limitation and the ambient salinity together either allow for co-dominance of the cyanobacteria and diatom bloom species or the dominance of the cyanobacteria clones. The low water turnover within the interior basins which typically experience large and persistent cyanobacteria dominated blooms may also play a crucial role in determining cyanobacteria dominance by creating water residence times that are more favorable for the growth of the cyanobacteria bloom species. The longer lag periods of growth that were observed following culture transfers of low inocula densities, suggests that large and/or rapid water turnover rates are less favorable for the growth of the cyanobacteria isolates, which may rely more on "water conditioning" than the diatom isolates.

Bloom dominance in Florida Bay may also in part be regulated by Si limitation, since bioassays have indicated that the phytoplankton community growth rates in Florida Bay are at times N-, P- and/or Si-limited (Tomas et al. 1999). Because Si is an essential nutrient only for diatoms, Si-limitation would allow the cyanobacteria to more fully exploit the available P or N supplies without having their rate of N or P consumption restrained by a Si-limited rate of growth. Although Si-limitation has the potential to exert a very strong influence on the broad taxonomic composition of algal blooms in Florida Bay, it's limited occurrence according to nutrient bioassays (Tomas et al. 1999) predicts that it will be a contributing factor, but not the primary factor determining cyanobacteria or diatom species bloom dominance in Florida Bay.

**Conclusions**

1) P-limited competition experiments confirmed intraspecific and interspecific differences in $U_m$, $K_{up}$
and $R_P^*$ that were determined or inferred from earlier phosphate kinetic studies (Chapter IIA).

Interspecific differences in $U_m$'s, $R_P^*$'s and $R_N^*$'s, indicated that salinity may influence the diatom species competitiveness under P- and N-limitation through their influence on their $U_m$'s.

2) *S. cf. elongatus* is the superior phosphate competitor, *T. cf. oceanica* the most inferior P-competitor while *C. cf. salsugineus* and *Synechocystis* sp. are intermediate competitors between 15 and 50 $\%_o$, at 25°C, under near-equilibrium conditions (semicontinuous dilution), as well as under non-equilibrium conditions when the limiting P is pulsed at 6 day intervals.

3) *T. cf. oceanica, S. cf. elongatus* and *Synechocystis* sp. all have similar N-competitive abilities, while *C. cf. salsugineus* is the most inferior N-competitor between 15 and 50 $\%_o$, at 25°C, under both near-equilibrium conditions and non-equilibrium (pulsed) conditions.

4) At 25 $\%_o$ and 25°C *S. costatum* is the most inferior P-competitor and the superior N-competitor. The relative ranking of the species PO$_4^{3-}$ affinity-competitiveness at 25 $\%_o$, from strongest to weakest was *S. cf. elongatus > Synechocystis* sp. > *C. cf. salsugineus > T. cf. oceanica > S. costatum*, while that for N is almost the reverse being *S. costatum > T. cf. oceanica ≈ S. cf. elongatus ≈ Synechocystis* sp. > *C. cf. salsugineus*.

5) Neither ambient salinity (15, 25 or 50 $\%_o$) nor fluctuations in salinity (~1 $\%_o$ da$^{-1}$) influenced species relative dominance patterns under P limitation. Under N-limitation, the slight shift in dominance from *T. cf. oceanica* at 25 $\%_o$ to the cyanobacteria species at 15 and 50 $\%_o$ may be best explained by the fact that 25 $\%_o$ is an optimal growth salinity while 15 and 50 $\%_o$ are both suboptimal growth salinities for *T. cf. oceanica*.

6) The form of the limiting P as PO$_4^{3-}$ or glycerophosphate did not influence species relative dominance. In contrast, limiting N as NO$_3^-$ shifted the dominance slightly to *T. cf. oceanica* while NH$_4^+$ shifted dominance slightly to the cyanobacteria species.

7) The frequently observed dominance of the phytoplankton community in Florida Bay by *S. cf. elongatus* and *Synechocystis* sp. may be in part due to extended periods of P-limitation, while the co-dominance of the phytoplankton community in Florida Bay by *T. cf. oceanica, S. cf. elongatus* and *Synechocystis* sp. may be promoted by periods of N limitation. Prolonged low ($\leq$15 $\%_o$) and high ($\geq$50 $\%_o$) salinities under N-limitation will promote the dominance of *S. cf. elongatus* and *Synechocystis* sp. over *T. cf. oceanica*, while more moderate salinities ($\sim$25 $\%_o$) will promote co-dominance and/or the slight dominance of *T. cf. oceanica*. The fact that *T. cf. oceanica* is not a common bloom species that dominates the phytoplankton community may in part be attributed to its relatively poor P-competitiveness and its narrower optimum salinity range for growth.

8) The conspicuous absence of *S. costatum* from most of Florida Bay may be best explained by its very poor P-competitiveness, the chronically low levels of available phosphate found throughout most of the bay, and the absence of extended periods of N-limitation, which would favor its superior N-competitive abilities.

9) The ability of *S. cf. elongatus* to dominate blooms in the north-central region where P-limitation
alternates with N-limitation, suggests that the long-term cumulative net increase in biomass during P-limitation as a result of its vastly superior P-competitiveness at all salinities overwhelms the cumulative slight loss in biomass that may occur during N-limitation at 25 \(^{0}/_{100}\) to the slightly superior \(T. \text{cf. oceanica}\). The predicted slight to moderate biomass gains accrued during N-limitation at very low (\(\leq 15 \(^{0}/_{100}\) ) and high (\(\geq 50 \(^{0}/_{100}\) ) salinities by the two cyanobacteria clones over the diatom clones would only add to the biomass accrued during periods of P limitation. Alternate periods of N and P limitation do not appear to severely impact the overall relative competitiveness of \(S. \text{cf. elongatus}\). In contrast, when P limitation switches to N limitation, \(C. \text{cf. salsugineus}\) experiences a drastic reduction in competitiveness as do \(S. \text{costatum}\) and \(T. \text{cf. oceanica}\) when N limitation switches to P limitation. The competitive advantages that \(S. \text{cf. elongatus}\) has over the diatom species during P-limitation at all salinities and N-limitation at low and high salinities, as well as under Si-limitation may all contribute to its dominance if the community in the north-central region where P-, N- and Si-limitation as well as extremes in salinity occur.

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CHAPTER 4
CELL QUOTAS AND CHEMICAL COMPOSITION OF SYNECHOCoccus CF. ELONGATUS,
SYNECHOCYSTIS SP., THALASSIOSIRA CF. OCEANICA, AND CHAETOCEROS CF.
SALSUGINEUS

Abstract

Cell quotas, chemical composition and nutrient strategies of the two cyanobacteria S. cf. elongatus and Synechocystis sp., and the two diatoms T. cf. oceanica and C. cf. salsugineus from Florida Bay were examined at various salinities in an effort to better understand how nutrient requirements and nutrient strategies may contribute to the broad spatial patterns of abundance and bloom dominance that have been observed for these dominant bloom species in Florida Bay. Phosphorus and nitrogen minimum cell quotas (Q_oP and Q_oN) and volume-specific minimum cell quotas (Q_VoP and Q_VoN) varied between species and with salinity. The Q_oP’s, Q_oN’s, Q_VoP’s and Q_VoN’s of the cyanobacteria species were not influenced by salinity, while those of the diatoms, particularly T. cf. oceanica, were elevated at 15 %00 and 50 %00 relative to their respective values at 25 %00. The elevated cell quotas of T. cf. oceanica may be explained by the proximity of the experimental salinities of 15 %00 and 50 %00 to T. cf. oceanica’s lower and upper salinity tolerance limits. The results indicate that suboptimal salinities for growth may increase a species minimum N or P cell quota, which may decrease their competitiveness under N or P limitation. The absence of interspecific differences between the study species Q_VoP’s and Q_VoN’s indicates that the relative success of the four study species is not due to interspecific differences in the efficiency of phosphorus or nitrogen utilization in biomass.

The coefficient of luxury phosphorus consumption (R_mP) for S. cf. elongatus, Synechocystis sp., T. cf. oceanica and C. cf. salsugineus of 10.9, 15.1, 8.1 and 29 respectively, indicate that C. cf. salsugineus has by far the greatest phosphorus luxury storage capability and that under conditions of P-limited patchiness, the competitiveness of C. cf. salsugineus should be enhanced significantly more than the other species. The coefficients of luxury nitrogen consumption (R_mN) for S. cf. elongatus, Synechocystis sp., T. cf. oceanica and C. cf. salsugineus of 2.6, 2.9, 9.1 and 8.2 indicates that the diatoms have greater nitrogen luxury storage capabilities than the cyanobacteria species and their greater N-storage capabilities predict enhanced competitiveness in a N-limited patchy environment.
Optimal molar N:P ratios of 68 and 93 for the cyanobacteria isolates and 12 and 28 for the diatom isolates indicate not only interspecific differences but also a substantial difference in the optimal N:P ratio between the two taxonomic groups.

Similar intracellular ratios of C, N, P, and Chl a at high relative growth rates (U_{rel}) and nutrient replete conditions indicate that the nutrient requirements of the study species are comparable. Intracellular N:P molar ratios at high U_{rel} and under nutrient-replete conditions for S. cf. elongatus, Synechocystis sp., T. cf. oceanica and C. cf. salsugineus of 12, 12, 10 and 11 respectively, are all within the range of 10-20 that is typical for the majority of marine algae (Goldman et al. 1979). The similar chemical composition of the study species suggests that bloom dominance by these species in Florida Bay under light saturated and nutrient-replete conditions cannot be attributed to substantial interspecific differences in the species N, P or C requirements.

**Introduction**

In general, phytoplankton require the elements N and P in relatively fixed proportions in order to reproduce (Hecky and Kilham 1988). But interspecific differences exist in the absolute requirements for N and P, as well as their relative proportions (Terry 1980, Tilman 1982). A cell’s minimum requirement for a nutrient is that amount required by the cell in order to survive and is referred to as the minimum cell quota (Q_o). Normally cells have more intracellular N and P than their minimum cellular requirements, and in fact most phytoplankton are capable of storing N and P in considerable excess of their minimum requirements. A measure of a cell’s ability to store a nutrient in excess of that required for survival is the coefficient of luxury consumption (R). R is the ratio of the cell quota of a nutrient when it is not limiting, to the cell quota of the nutrient when it is limiting at a given steady state growth rate. R is at a maximum (R_{m}) near zero growth (Droop 1974).

Phytoplankton species also differ in the proportions at which they consume N and P. The ratio at which a species consumes N and P is referred to as its optimum or critical N:P ratio and it is at this ratio that nutrient limitation switches between N limitation and P limitation. Optimum nutrient ratios have been found to be species specific and may vary widely between species (Rhee and Gotham 1980). Theory and experiment have defined the optimum N:P ratio to be the ratio of the minimum cellular quotas for N and P (i.e. Q_{oN}:Q_{oP}) (Rhee and Gotham 1980, Kilham and Hecky 1988), as well as the ratio of the half-saturation constants for growth for N and P (i.e. K_{uN}:K_{uP}) (Tilman 1982). Interspecific differences in optimum ratios are believed to influence phytoplankton community composition (Kilham and Kilham 1984). Laboratory studies have demonstrated that nutrient supply ratios can influence phytoplankton community structure (Tilman 1977, Tilman et al. 1986, Kilham 1986) with shifts in phytoplankton community composition following changes in N:P supply ratios (Suttle and Harrison 1988, Sommer 1989). Manipulations of the supply rates of N and P in natural lakes have shown that the N:P supply ratio can select for or against N\textsubscript{2}-fixing cyanobacteria (Barica et al. 1980, Smith 1983, Schindler 1977). It has been suggested that species
relative dominance in nature may reflect ambient nutrient supply ratio gradients, such that species are most abundant at ambient ratios that approximate their optimal ratios (Tilman 1982).

In aquatic environments where either N or P may limit growth, vary in concentration in space or time or occur at a particular supply ratio, phytoplankton have evolved nutrient strategies which contribute to their competitive success. These include strategies that allow species to: easily meet their cellular nutrient requirements (i.e. low Q_{oN} or Q_{oP}); optimize the use of a patchy nutrient supply (i.e. high luxury storage capacity for N or P) and utilize the nutrient supply ratios most efficiently by consuming nutrients in the proportion that they are supplied (i.e. optimum N:P ratio ≈ the ambient N:P supply ratio).

The large and recurring blooms in Florida Bay, typically dominated by the cyanobacteria *S. cf. elongatus* and *Synechocystis* sp., but also occasionally dominated by diatoms including *C. cf. salsugineus* and *T. cf. oceanica* suggest that these species have nutrient strategies that are well suited to the prevailing nutrient conditions in Florida Bay. The prevailing nutrient conditions in Florida Bay that were considered in this chapter included the following: the very low ambient levels of SRP found through much of the bay; the west to east spatial gradient of decreasing water column DIN:DIP and the west to east spatial gradient of decreasing water column N:P supply ratio that has been inferred from the results of phytoplankton nutrient bioassays (Fourquarean et al. 1993, Boyer et al. 1999, Brand 2002). These patterns of N and P availability in Florida Bay led to the formation of the following hypotheses that consider how nutrient requirements and nutrient strategies of the dominant bloom species may contribute to the broad spatial patterns of abundance and bloom dominance that have been observed in the bay.

- **H_{o1}:** The cyanobacteria have lower N and P volume-specific Q's than the diatoms.
- **H_{o2}:** The cyanobacteria and diatom species Q's for N and P will be elevated at sub-optimal salinities for growth relative to their corresponding values optimal growth salinities.
- **H_{o3}:** The cyanobacteria and diatom species will differ in their P luxury storage (R_{Pm}) and N luxury storage (R_{Nm}) capabilities.
- **H_{o4}:** The cyanobacteria will have higher optimal N:P ratios than the diatoms.
- **H_{o5}:** The cyanobacteria will have substantially different intracellular ratios of C, N P and chl a from those of the diatom species under nutrient replete light saturated growth conditions.

**Methods**

Phosphorus and nitrogen cell quotas were determined for *S. cf. elongatus, Synechocystis* sp., *C. cf. salsugineus*, and *T. cf. oceanica* at 15, 25 and 50 °/00 using single species batch and semicontinuous culture techniques. Single species cultures were fully acclimated to the experimental conditions of salinity, 25EC, 150 μEm⁻²sec⁻¹, and a 12:12 light:dark cycle before measurements were made. The three experimental enrichment media used consisted of a base of artificial seawater (Parsons et al. 1984) enriched to "f/2"
(Guillard and Ryther 1962), artificial seawater enriched to "f/2" concentrations except N and P which were adjusted to produce a P-limited media (250 µM NO$_3^-$: 1.0 µM PO$_4^{3-}$), and artificial seawater enriched to "f/2" concentrations except N and P which were adjusted to produce an N-limited media (40 µM NO$_3^-$: 20 µM PO$_4^{3-}$).

**Batch culture**

Minimum cell quotas were determined for nitrogen (Q$_{oN}$) and phosphorus (Q$_{oP}$) using batch cultures grown to stationary phase and sampled following 2 consecutive days of zero growth as indicated by constant in vivo fluorescence and cell count values. Volume-specific Q$_{oN}$ and Q$_{oP}$ were calculated for each species by dividing mean values of Q$_{oN}$ and Q$_{oP}$ by mean cell biovolumes. The optimum N:P (atomic) ratio at which N limitation changes over to P limitation, defined by Rhee and Gotham (1980) as Q$_{oN}$: Q$_{oP}$, was calculated for each species.

Cell quotas of nitrogen (Q$_N$) and phosphorus (Q$_P$) were determined for exponentially growing cultures of each species at 25 $^\circ$C under non-limiting nutrient conditions using "f/2" media and batch culture. Intracellular C:N:P ratios (Q$_C$:Q$_N$:Q$_P$) were also obtained for each species under nutrient saturated high relative growth rate conditions.

**Semicontinuous culture**

Q$_N$ and Q$_P$ for each species were also determined at selected steady state growth rates using semi-continuous culture at 25 $^\circ$C. Single-species cultures of 100 ml volume in 250 ml Erlenmeyer flasks were grown at four separate dilution rates in both P-limiting and N-limiting growth media. Dilution rates of 0.1, 0.2, 0.3, and 0.6 da$^{-1}$ were maintained by manually removing 10, 20, 30, and 60 ml of culture respectively, and replacing it with an equal volume of the appropriate medium each day. Culture growth was monitored daily, using in vivo fluorescence. Steady state culture conditions were considered achieved when the daily fluctuation in biomass as measured by in vivo fluorescence was less than 10% (Grover 1989). After steady state conditions had been attained and maintained for at least one week, cultures of each species under P-limitation and N-limitation were sampled at each of the four steady state growth rates for determination of both Q$_N$ and Q$_P$.

Cell quotas (Q) for P and N and steady state growth rates for each species were then fit to the linearized form of the Droop equation:

$$\frac{DQ}{D_m} = Q - Q_o$$  \hspace{1cm} (1)

where D is the dilution rate or steady state growth rate (U), D$_m$ is the maximal dilution rate or growth rate (U$_m^*$) when Q is infinite, Q is the cell quota, and Q$_o$ is the minimum cell quota (Q, when U=0). Estimates of Q$_{oN}$, Q$_{oP}$ and U$_m^*$ were then obtained for each species from the equation.

The coefficient of luxury consumption (R), defined as the ratio of the cell quota of a nutrient when it is not limiting, to the cell quota of the nutrient when it is limiting at a given steady state growth rate (Droop 1974), was determined for both nitrogen and phosphorus, for each species at each steady-state
growth rate. The maximum coefficient of luxury consumption \((R_m)\) of both nitrogen \((R_{mN})\) and phosphorus \((R_{mP})\) for each species was determined by a least squares regression of \(R\) vs. relative growth rate \((U/U_m)\) (Droop 1974, Kilham 1978).

Optimum N:P ratios, defined by Rhee and Gotham (1980) as \(Q_o^N:Q_o^P\), were also determined for each species using the values obtained from the N-limited and P-limited semicontinuous cultures.

Particulate carbon and nitrogen determinations were made on duplicate culture samples analyzed on a Carlo Erba Model 1106 CHN analyzer. Samples were filtered onto pre-combusted (500 °C) GF/F filters, rinsed with 5 ml of 0.22 µm filtered seawater, frozen, lyophilized and stored frozen over desiccant until analyzed. Filter blanks were treated as samples except 5 ml of the appropriate sterile experimental culture media was substituted in place of the culture sample. Cellular nitrogen quotas were calculated using sample particulate nitrogen values, filter blank nitrogen values, an average nitrogen standard value, sample volumes filtered, and cell densities.

Particulate phosphate was determined from duplicate culture samples following the procedures of Solórzano and Sharp (1980). Filter blanks were treated as samples except 5 ml of the appropriate sterile experimental culture media was substituted in place of the culture sample. Cellular phosphate quotas were calculated using sample particulate phosphate concentrations, filter blank phosphate values, an average phosphate standard value, sample volume filtered and cell densities.

Chl \(a\) determinations were made in duplicate by methanol extraction (Holm-Hansen and Reimann 1978). Cell densities were determined following the recommendations of Venrick (1978). Epifluorescence microscopy was used to determine cyanobacteria cell densities (Booth 1993, MacIsaac and Stockner 1993), while light microscopy was used to determine diatom densities as well as cellular dimensions of both cyanobacteria and diatom species. Estimates of species cellular biovolumes were calculated for each experimental treatment by measurements at 400 - 1000X using a minimum of 10 cells. The volumes of standard geometrical shapes were used to estimate each species mean biovolume. A sphere was used to estimate the biovolume of \(Synechocystis\) sp., while a cylinder was used to estimate the biovolume of \(S.\ cf.\ elongatus\), \(C.\ cf.\ salsugineus\) and \(T.\ cf.\ oceanica\). Only the biovolumes of the diatom species were corrected for non-cytoplasmic volume using the equation of Smayda (1978) and a cytoplasm thickness of 1.0 µm Hitchcock (1983). Mean values and their standard errors were determined following standard statistical methods, while the absolute error was determined for the biovolume-specific cell quotas and intracellular chemical ratios using the quotient rule for indeterminate errors.

**Results**

The study species \(Q_{op}\)’s and \(Q_{on}\)’s determined by batch growth at 15, 25 and 50 \(^0/\_0\) and by semicontinuous culture at 25 \(^0/\_0\) are presented in Tables 4-1 and 4-2 and Figures 4-1, 4-2, 4-3 and 4-4. The estimated \(Q_{op}\)’s and \(Q_{on}\)’s at 25 \(^0/\_0\) determined by the application of the linearized Droop equation to the
cellular N and P quotas at various semicontinuous culture dilution rates were for the most part equivalent to those obtained at 25 \(^{0}/_{00}\) using batch culture.

Minimum phosphorus and nitrogen cell quotas obtained from batch cultures and semicontinuous cultures both displayed a pattern of interspecific differences at each of the experimental salinities in which \(Q_o\)'s from smallest to largest were: \textit{S. cf. elongatus} \(<\) \textit{Synechocystis} sp. \(<\) \textit{T. cf. oceanica} \(<\) \textit{C. cf. salsugineus} (Figs. 4-5A,B and 4-6A,B). No \(Q_{ON}\) value was obtained for \textit{C. cf. salsugineus} using the semicontinuous culture technique. The interspecific pattern of increasing \(Q_{OP}\)'s and \(Q_{ON}\)'s paralleled the increase in cell size, in which cell biovolume at each of the experimental salinities from smallest to largest were: \textit{S. cf. elongatus} \(<\) \textit{Synechocystis} sp. \(<\) \textit{T. cf. oceanica} \(<\) \textit{C. cf. salsugineus} (Tables 4-1 and 4-2).

Biovolumes not only varied widely between species but also varied within species at the different experimental salinities, with the largest differences recorded in the diatom species (Tables 4-1 and 4-2). Although the diatom species biovolumes in particular varied across salinity, no consistent effect of salinity on any of the four study species biovolume was apparent (Tables 4-1 and 4-2).

When each species \(Q_{OP}\) and \(Q_{ON}\) are examined at each of the three experimental salinities and cell biovolumes are not considered, the species responses appear to follow taxonomic lines. Salinity for the most part did not appear to influence the \(Q_{OP}\) or the \(Q_{ON}\) of either cyanobacteria species, as the \(Q_{OP}\) and the \(Q_{ON}\) of each isolate were approximately equivalent at 15, 25 and 50 \(^{0}/_{00}\) (Figs. 4-5A and 4-6A). In contrast salinity appeared to influence the \(Q_{OP}\)'s and the \(Q_{ON}\)'s of both diatom species such that they differed at all three salinities, with the \(Q_{OP}\)'s and the \(Q_{ON}\)'s for each species being lower at 25 \(^{0}/_{00}\) than at 15 or 50 \(^{0}/_{00}\) (Fig. 4-5A and 4-6A). No \(Q_{ON}\) was obtained for \textit{C. cf. salsugineus} at 50 \(^{0}/_{00}\).

Normalization of the species \(Q_{OP}\)'s and \(Q_{ON}\)'s to volume-specific \(Q_{OP}\)'s and \(Q_{ON}\)'s made possible not only comparisons between species that differed greatly in size, but also allowed for an examination of the influence of salinity on each species \(Q_{OP}\) and \(Q_{ON}\) without the potentially confounding influence of variable cellular volume. Estimations of plasma volumes for the N-starved and P-starved batch cultured diatom species revealed that plasma volume was equivalent to cell volume or cell biovolume at all salinities, with the exception of the large sized \textit{C. cf. salsugineus} cells at 15\(^{0}/_{00}\). The N-starved and P-starved batch cultured biovolumes of 748 \(\mu\text{m}^3\) and 503 \(\mu\text{m}^3\) respectively for \textit{C. cf. salsugineus} (Tables 4-1 and 4-2) were reduced to plasma volumes of 531 \(\mu\text{m}^3\) and 404 \(\mu\text{m}^3\), respectively.

The considerable scatter and overlap visible in the volume-specific \(Q_{OP}\)'s and \(Q_{ON}\)'s of the four isolates (Figs. 4-5C and 4-6C), suggested the absence of interspecific differences in \(Q_{OP}\) and \(Q_{ON}\) when interspecific differences in species biovolumes are accounted for. The volume-specific \(Q_{OP}\)'s and \(Q_{ON}\)'s of the cyanobacteria displayed the same pattern that was seen in their \(Q_{OP}\)'s and \(Q_{ON}\)'s on a per cell basis (Figs. 4-5A and 4-6A) and revealed no influence of salinity on their minimum N and P cell quotas. In contrast to the cyanobacteria and \textit{C. cf. salsugineus}, the volume-specific \(Q_{OP}\)'s and \(Q_{ON}\)'s of \textit{T. cf. oceanica} varied widely in response to salinity. The recurring pattern seen among both diatom species on a per cell basis in which \(Q_o\) was lowest at 25 \(^{0}/_{00}\) and higher at 15 and 50 \(^{0}/_{00}\), was seen most clearly in the volume-specific
Q_{ON}'s and Q_{OP}'s in *T. cf. oceanica* (Figs 4-5C and 4-6C) and to a much lesser degree in the volume-specific Q_{ON} of *C. cf. salsugineus*, when the biovolume corrected plasma volume was used (Fig. 4-6C).

The maximum coefficients of luxury consumption for nitrogen (R_{mN}) and phosphorus (R_{mP}) were determined using semicontinuous culture. The R_{mP}'s for *S. cf. elongatus*, *Synechocystis* sp., *T. cf. oceanica* and *C. cf. salsugineus* were; 10.9, 15.1, 8.1 and 28.7 (Figs. 4-7 and 4-8) and their R_{mN}'s were 2.6, 2.9, and 9.2, respectively (Figs. 4-9 and 4-10). No estimate of R_{mN} was obtained for *C. cf. salsugineus* by semicontinuous culture. Instead an R_{mN} value of 8.2 was estimated using values of Q_N and Q_{ON} from batch growth experiments (Table 4-3). This is possible because in theory, the R_{m} obtained using semicontinuous culture is comparable to a Q_{m}:Q_{OP} ratio determined using batch growth values as long as Q_{m} is determined at a low relative growth rate (Tilman and Kilham 1976).

The optimal N:P molar ratios (Q_{ON}:Q_{OP}) determined for each species from batch and semicontinuous cultures were reasonably comparable and when the values determined from each method were combined they produced average optimal N:P molar ratios for *S. cf. elongatus*, *Synechocystis* sp., *T. cf. oceanica* and *C. cf. salsugineus* of 68, 93, 28 and 12, respectively (Table 4-4).

The intracellular chemical composition (C, N, P, Chl a) of all four species during exponential growth at high relative growth rates under non-limiting nutrient conditions at 25 °C are given in Table 4-5.
Table 4-1. Phosphorus minimum cell quotas (Q_o), cell biovolumes and biovolume-specific cell quotas at 15, 25 and 50 %_00 determined using batch and semicontinuous culture technique. Batch mean value and semicontinuous estimate in fmol cell^{-1}, with standard error of mean and standard error of estimate (SE), respectively. Biovolume-specific Q_o with estimated absolute error (±).

<table>
<thead>
<tr>
<th>Species</th>
<th>Salinity</th>
<th>Batch Cell</th>
<th>Biovolume-specific</th>
<th>Semi-continuous Cell</th>
<th>Biovolume-specific</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Q_o (fmol cell^{-1})</td>
<td>Biovolume (µm^3)</td>
<td>Q_o (fmol µm^3)</td>
<td>Q_o (fmol cell^{-1})</td>
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<tr>
<td><em>S. cf. elongatus</em></td>
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<td>0.026</td>
<td>1.41</td>
<td>0.018</td>
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<td></td>
<td></td>
<td>(0.001)</td>
<td>(0.08)</td>
<td>(0.002)</td>
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<td></td>
<td>25</td>
<td>0.026</td>
<td>1.87</td>
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<td>0.022</td>
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<td></td>
<td></td>
<td>(0.002)</td>
<td>(0.08)</td>
<td>(0.002)</td>
<td>(.006)</td>
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<td></td>
<td>50</td>
<td>0.024</td>
<td>1.45</td>
<td>0.017</td>
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<td></td>
<td></td>
<td>(0.001)</td>
<td>(0.08)</td>
<td>(0.002)</td>
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<tr>
<td><em>Synechocystis sp.</em></td>
<td>15</td>
<td>0.133</td>
<td>10.2</td>
<td>0.013</td>
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<td></td>
<td></td>
<td>(0.006)</td>
<td>(0.66)</td>
<td>(0.001)</td>
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<td></td>
<td>25</td>
<td>0.13</td>
<td>10.27</td>
<td>0.013</td>
<td>0.124</td>
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<td></td>
<td></td>
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<td>(0.66)</td>
<td>(0.003)</td>
<td>(.004)</td>
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<td></td>
<td>50</td>
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<td></td>
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<td>(0.0002)</td>
<td>(0.60)</td>
<td>(0.001)</td>
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<td><em>T. cf. oceanica</em></td>
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<td>2.77</td>
<td>116</td>
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<td></td>
<td></td>
<td>(0.005)</td>
<td>(4.2)</td>
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<td></td>
<td>25</td>
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<td>207</td>
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<td>(0.126)</td>
<td>(14.4)</td>
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<td></td>
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<td>(0.018)</td>
<td>(19.9)</td>
<td>(0.003)</td>
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<td><em>C. cf. salsugineus</em></td>
<td>15</td>
<td>5.68</td>
<td>503</td>
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<td>(0.035)</td>
<td>(11.7)</td>
<td>(0.003)</td>
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<td></td>
<td>25</td>
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<td>(28)</td>
<td>(0.003)</td>
<td>(0.86)</td>
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<td>4.19</td>
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<td>(0.030)</td>
<td>(24)</td>
<td>(0.001)</td>
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Table 4-2. Nitrogen minimum cell quotas ($Q_0$), cell biovolumes and biovolume-specific cell quotas at 15, 25 and 50 °C determined using batch and semicontinuous culture technique. Batch mean value and semicontinuous estimate in fmol cell$^{-1}$, with standard error of mean and standard error of estimate (SE), respectively. Biovolume-specific $Q_0$ with estimated absolute error (±).

<table>
<thead>
<tr>
<th>Species</th>
<th>Salinity</th>
<th>Batch Cell $Q_0$ (fmol cell$^{-1}$)</th>
<th>Batch Biovolume (µm$^3$)</th>
<th>Semi-continuous Cell $Q_0$ (fmol cell$^{-1}$)</th>
<th>Semi-continuous Biovolume (µm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. cf. elongatus</td>
<td>15</td>
<td>1.7 (0.005)</td>
<td>1.39 (0.05)</td>
<td>1.6 (0.065)</td>
<td>1.46 (0.04)</td>
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<tr>
<td></td>
<td>25</td>
<td>1.6 (0.065)</td>
<td>1.52 (0.04)</td>
<td>1.08 (0.06)</td>
<td>0.88 (0.09)</td>
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<tr>
<td></td>
<td>50</td>
<td>-----</td>
<td>1.64</td>
<td>-----</td>
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<tr>
<td>Synechocystis sp.</td>
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<td>15.9 (0.1)</td>
<td>11.1 (0.92)</td>
<td>11.1 (2.0)</td>
<td>11.2 (0.95)</td>
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<td>25</td>
<td>12.5 (2.0)</td>
<td>10.7 (0.95)</td>
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<td>50</td>
<td>8.8 (0.56)</td>
<td>9.9 (1.2)</td>
<td>8.9 (1.2)</td>
<td>8.7 (1.2)</td>
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<td>T. cf. oceanica</td>
<td>15</td>
<td>147 (13)</td>
<td>200 (14)</td>
<td>43.4 (5)</td>
<td>255 (3.7)</td>
</tr>
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<td></td>
<td>25</td>
<td>43 (5)</td>
<td>128 (0.07)</td>
<td>43.4 (3.7)</td>
<td>255 (0.07)</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>164 (8)</td>
<td>121 (0.22)</td>
<td>136 (8)</td>
<td>136 (0.22)</td>
</tr>
<tr>
<td>C. cf. salsugineus</td>
<td>15</td>
<td>411 (8)</td>
<td>748 (8)</td>
<td>-----</td>
<td>320 (40)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>46 (4)</td>
<td>79 (10)</td>
<td>-----</td>
<td>320 (40)</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
</tbody>
</table>

115
Figure 4-1. Phosphorus cell quota as a function of phosphate uptake rate in semicontinuous culture. Regression line expresses the linearized Droop equation. $Q_0 =$ minimum cell quota. (A) *S. cf. elongatus*, (B) *Synechocystis* sp..
Figure 4-2. Phosphorus cell quota as a function of phosphate uptake rate in semicontinuous culture. Regression line expresses the linearized Droop equation. $Q_o =$ minimum cell quota (A) *T.* cf. *oceanica*, (B) *C.* cf. *salsugineus.*
Figure 4-3. Nitrogen cell quota as a function of nitrate uptake rate in semicontinuous culture. Regression line expresses the linearized Droop equation. $Q_0 =$ minimum cell quota. (A) *S. cf. elongatus*, (B) *Synechocystis* sp.
Figure 4-4. Nitrogen cell quota as a function of nitrate uptake rate in semicontinuous culture. Regression line expresses the linearized Droop equation. $Q_o = 43.35$ 

$Q_o$ = minimum cell quota for *T. cf. oceanica*.
Figure 4-5. Batch growth phosphorus cell quotas. ▲ = 15\%_00, ● = 25\%_00, and ■ = 50\%_00.
(A) Cyanobacteria minimum cell quotas with standard error, (B) Diatom minimum cell quotas with standard error, (C) Biovolume-specific minimum cell quotas with absolute error. Estimated plasma volume at 15\%_00 = Δ, see discussion for explanation.
Figure 4-6. Batch growth nitrogen cell quotas ▲ = 15 %/00, ● = 25 %/00, and ■ = 50 %/00. (A) Cyanobacteria minimum cell quotas with standard error, (B) Diatom minimum cell quotas with standard error, (C) Biovolume-specific minimum cell quotas with absolute error. Estimated plasma volume at 15 %/00 = △, see discussion for explanation.
Figure 4-7. Phosphorus coefficient of luxury consumption as a function of relative growth rate (U/U_m) in semicontinuous culture. R_{max} = maximum coefficient of luxury consumption. (A) S. cf. elongatus, (B) Synechocystis sp.
Figure 4-8. Phosphorus coefficient of luxury consumption as a function of relative growth rate \((\frac{U}{U_m})\) in semicontinuous culture. \(R_{\text{max}}\) = maximum coefficient of luxury consumption. (A) \(T.\ cf.\ oceanica\), (B) \(C.\ cf.\ salsugineus\).
Figure 4-9. Nitrogen coefficient of luxury consumption as a function of relative growth rate ($U/U_m$) in semicontinuous culture. $R_{max}$ = maximum coefficient of luxury consumption. (A) *S. cf. elongatus*, (B) *Synechocystis* sp..
Figure 4-10. Nitrogen coefficient of luxury consumption as a function of relative growth rate \((U/U_m)\) in semicontinuous culture. \(R_{max}\) = maximum coefficient of luxury consumption for \(T. cf\ oceanica\).
Table 4-3. Cellular nitrogen and phosphorus minimum ($Q_o$) cell quotas as fmol cell$^{-1}$ estimated by linearized regression of Droop equation with standard errors of estimates (SE). Coefficient of luxury storage $R_{max}$ with standard error of estimate (SE). $Q_m$ as fmol cell$^{-1}$ with absolute error (±) calculated from $R_{max}$ and $Q_o$. All values obtained using semicontinuous culture technique at 25°C.

**Phosphorus**

<table>
<thead>
<tr>
<th>Species</th>
<th>$Q_m$</th>
<th>$Q_o$</th>
<th>$R_{max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S. cf. elongatus</strong></td>
<td>0.24</td>
<td>0.022</td>
<td>10.96</td>
</tr>
<tr>
<td></td>
<td>(0.11)</td>
<td>(0.006)</td>
<td>(1.93)</td>
</tr>
<tr>
<td><strong>Synechocystis sp.</strong></td>
<td>1.87</td>
<td>0.124</td>
<td>15.07</td>
</tr>
<tr>
<td></td>
<td>(0.23)</td>
<td>(0.004)</td>
<td>(1.37)</td>
</tr>
<tr>
<td><strong>T. cf. oceanica</strong></td>
<td>10.66</td>
<td>1.41</td>
<td>7.56</td>
</tr>
<tr>
<td></td>
<td>(1.04)</td>
<td>(0.71)</td>
<td>(1.47)</td>
</tr>
<tr>
<td><strong>C. cf. salsugineus</strong></td>
<td>55</td>
<td>2.28</td>
<td>24.1</td>
</tr>
<tr>
<td></td>
<td>(4.3)</td>
<td>(0.86)</td>
<td>(5.0)</td>
</tr>
</tbody>
</table>

**Nitrogen**

<table>
<thead>
<tr>
<th>Species</th>
<th>$Q_m$</th>
<th>$Q_o$</th>
<th>$R_{max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S. cf. elongatus</strong></td>
<td>4.092</td>
<td>1.55</td>
<td>2.64</td>
</tr>
<tr>
<td></td>
<td>(0.37)</td>
<td>(0.33)</td>
<td>(0.414)</td>
</tr>
<tr>
<td><strong>Synechocystis sp.</strong></td>
<td>32.2</td>
<td>11.05</td>
<td>2.91</td>
</tr>
<tr>
<td></td>
<td>(.17)</td>
<td>(1.21)</td>
<td>(0.167)</td>
</tr>
<tr>
<td><strong>T. cf. oceanica</strong></td>
<td>398.9</td>
<td>43.4</td>
<td>9.19</td>
</tr>
<tr>
<td></td>
<td>(.37)</td>
<td>(3.7)</td>
<td>(2.66)</td>
</tr>
<tr>
<td><strong>C. cf. salsugineus</strong></td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
</tbody>
</table>

$^1$ Single values determined from batch growth culture.
<table>
<thead>
<tr>
<th>Species</th>
<th>Batch</th>
<th>Semicontinuous</th>
<th>Batch</th>
<th>Semicontinuous</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Q&lt;sub&gt;oN&lt;/sub&gt;</td>
<td>Q&lt;sub&gt;oP&lt;/sub&gt;</td>
<td>Q&lt;sub&gt;oN&lt;/sub&gt;</td>
<td>Q&lt;sub&gt;oP&lt;/sub&gt;</td>
<td>Q&lt;sub&gt;oN&lt;/sub&gt;:Q&lt;sub&gt;oP&lt;/sub&gt;</td>
</tr>
<tr>
<td><em>S. cf. elongatus</em></td>
<td>1.6 (0.07)</td>
<td>0.026 (0.002)</td>
<td>1.6 (0.3)</td>
<td>0.022 (0.006)</td>
<td>62 (7)</td>
</tr>
<tr>
<td><em>Synechocystis</em> sp.</td>
<td>12.5 (2.0)</td>
<td>0.13 (0.02)</td>
<td>11.1 (1.2)</td>
<td>0.124 (0.004)</td>
<td>96 (30)</td>
</tr>
<tr>
<td><em>T. cf. oceanica</em></td>
<td>42.6 (5.0)</td>
<td>1.8 (0.13)</td>
<td>43.4 (3.7)</td>
<td>1.41 (0.71)</td>
<td>24 (5)</td>
</tr>
<tr>
<td><em>C. cf. salsugineus</em></td>
<td>45.5 (3.6)</td>
<td>3.9 (0.11)</td>
<td>---- ----</td>
<td>2.28 (0.86)</td>
<td>12 ----</td>
</tr>
</tbody>
</table>

<sup>a</sup> Not mean, but single batch growth value.
Table 4-5. Cellular relative growth rates, chemical composition, and ratios obtained from batch culture growth during exponential growth near $U_m$. Mean values with standard errors (SE), ratios with estimated absolute errors (±). Relative growth rates determined using $U_m$ values from Chapter 2, Table 2-1.

<table>
<thead>
<tr>
<th></th>
<th>$U_{rel}$</th>
<th>C</th>
<th>N</th>
<th>P</th>
<th>C:N</th>
<th>C:P</th>
<th>N:P</th>
<th>Chl$\text{a}$</th>
<th>C</th>
<th>N</th>
<th>P</th>
<th>PP: Chl$\text{a}$</th>
<th>C: Chl$\text{a}$</th>
<th>Weight</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S. cf. elongatus</strong></td>
<td>1.31</td>
<td>28 (3)</td>
<td>4 (0.3)</td>
<td>0.29 (0.01)</td>
<td>8 (1.6)</td>
<td>100 (10)</td>
<td>12.4 (1.2)</td>
<td>0.005 (0.0004)</td>
<td>0.34 (0.04)</td>
<td>0.050 (0.005)</td>
<td>0.009 (0.0002)</td>
<td>1.8 (0.2)</td>
<td>69 (6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Synechocystis sp.</strong></td>
<td>0.94</td>
<td>201 (20)</td>
<td>37.4 (3.9)</td>
<td>3 (0.2)</td>
<td>67 (1.1)</td>
<td>12.4 (12)</td>
<td>0.037 (2.1)</td>
<td>2.42 (0.001)</td>
<td>0.520 (0.05)</td>
<td>0.094 (0.007)</td>
<td>2.5 (0.2)</td>
<td>55 (6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>T. cf. oceanica</strong></td>
<td>0.72</td>
<td>1134 (124)</td>
<td>170 (3.7)</td>
<td>17.6 (2.2)</td>
<td>67 (0.9)</td>
<td>10 (16)</td>
<td>0.406 (1.5)</td>
<td>13.6 (0.012)</td>
<td>2.380 (1.5)</td>
<td>0.545 (0.05)</td>
<td>1.6 (0.3)</td>
<td>33 (5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>C. cf. salsugineus</strong></td>
<td>0.67</td>
<td>1371 (245)</td>
<td>176 (39)</td>
<td>16.1 (0.9)</td>
<td>84 (3.2)</td>
<td>11 (20)</td>
<td>0.346 (3.1)</td>
<td>16.5 (0.118)</td>
<td>2.460 (2.9)</td>
<td>0.498 (0.028)</td>
<td>2.1 (0.8)</td>
<td>60 (31)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>S. costatum</strong></td>
<td>0.78</td>
<td>1240 (167)</td>
<td>156 (29)</td>
<td>17.8 (2.0)</td>
<td>8.2 (2.6)</td>
<td>66 (16)</td>
<td>7.5 (2.3)</td>
<td>0.207 (0.111)</td>
<td>97 (50)</td>
<td>14.9 (7.8)</td>
<td>(3.5) (1.77)</td>
<td>2.4 (0.9)</td>
<td>63 (26)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Cellular quotas

The results of this study were in general agreement with those of other similar studies. The positive linear relationship between cell size and minimum cell quota (Q\text{op} and Q\text{on}) that was observed for the study species has been documented for numerous other eucaryotic and procaryotic microalgae (Shuter 1978). This was expected as larger cells with a larger plasma volume generally have larger minimum cellular requirements for N and P. The estimated Q\text{op} and the Q\text{on} values of the study species were also comparable to available literature values of similar species. For example, the Q\text{op} values (fmol cell\textsuperscript{-1}) at 25 °/00 of 0.026 for S. cf. elongatus and 1.8 for T. cf. oceanica, are comparable to 0.024 for the marine cyanobacteria Synechococcus sp. (Ikeya et al. 1997), and 0.9 for the marine diatom Cyclotella nana (Fuhs 1969). Likewise, the Q\text{on}(fmol cell\textsuperscript{-1}) at 25 °/00 of 42.6 for T. cf. oceanica, is very similar to literature values of 36 and 47 for Thalassiosira pseudonana (Goldman and McCarthy 1978, Eppley and Renger 1974).

The results of this study supported the hypothesis that salinity may alter species Q\text{op}’s and Q\text{on}’s. This was not unexpected, as phytoplankton cell quotas have been found to vary with environmental conditions. Elevated cell quotas (Q and Q\text{op}) have been recorded under suboptimum temperatures and suboptimum irradiances relative to cell quotas under more optimum growth conditions (Rhee and Gotham 1981a b, Goldman 1979, Healy 1985). The accepted explanation is that more of the limiting nutrient is needed by the cell to maintain a given growth rate under non-optimum growth conditions than under optimum growth conditions (Rhee and Gotham 1981a).

For the most part, the influence of salinity on cell quota followed taxonomic lines. Salinity had no detectable effect on the Q\text{op}’s, Q\text{on}’s, Q\text{op}'s, or Q\text{on}'s of the cyanobacteria, while salinity was found to influence the Q\text{op}’s, Q\text{on}’s, Q\text{op}'s, and Q\text{on}'s of the diatom species to varying degrees. Although all four of the study species would be considered euryhaline species based on their salinity-growth response curves, the cyanobacteria species had broader salinity tolerances and broader optimal salinity ranges for growth than the diatom species (Chapter 2, Fig. 2-1). The absence of a salinity effect on the Q\text{op}’s, Q\text{on}’s, Q\text{op}'s, and Q\text{on}'s of the cyanobacteria may be explained by their very broad optimum salinity growth range. Salinities of 15 °/00 and 50 °/00 were not sufficiently outside of their broad optimum salinity growth range to produce a detectable increase in their subsistence nutrient requirements for either phosphorus or nitrogen (Figs. 4-5C and 4-6C). In contrast, the elevated Q\text{op}’s, Q\text{on}’s, Q\text{op}'s, and Q\text{on}'s of T. cf. oceanica at 15 °/00 and 50 °/00 relative to those at 25 °/00 may be explained by the fact that 15 °/00 and 50 °/00 are both sufficiently outside of its optimum salinity range for growth to produce a detectable increase above the Q\text{op}’s, Q\text{on}’s, Q\text{op}'s, and Q\text{on}'s at 25 °/00, which is nearer its optimal salinity for growth. T. cf. oceanica's N and P cell quotas were more affected by salinity than any of the other four study species, and T. cf. oceanica was also the species with the narrowest salinity tolerance and optimum salinity growth range.
The mixed response of C. cf. salsugineus’s N and P cell quotas to salinity (Figs. 4-5C and 4-6C), may be explained by its moderately broad tolerance and optimum salinity growth ranges (intermediate to the cyanobacteria species and T. cf. oceanica) (Chapter 2 Fig. 2-1).

Suboptimal salinities for growth are significant in that they may influence species competitiveness. Earlier laboratory salinity growth rate experiments under nutrient-replete conditions indicated that salinity alone might influence microalgal bloom species composition in the bay. The diatom species competitiveness, based on \( U_{\text{m}} \), dropped sharply relative to those of the cyanobacteria below 15 \(^{\circ}/_{00}\) and above 50 \(^{\circ}/_{00}\) (Chapter 2, Fig. 2-1). The results of this study indicate that suboptimal salinities for growth may also influence species competitiveness under N or P limitation through their influence on species minimum N or P requirements. For example T. cf. oceanica ’s increased minimum requirements for N and P (elevated \( Q_{\text{oN}} \) or \( Q_{\text{oP}} \)) at suboptimal salinities for growth would be expected to lower its competitiveness relative to the cyanobacteria species when either N or P becomes limiting and the ambient salinity is below 15 \(^{\circ}/_{00}\) or above 50 \(^{\circ}/_{00}\). Consequently hyposaline or hypersaline conditions when combined with P-limiting or N-limiting conditions in Florida Bay may contribute to the development of S. costatum and Synechocystis sp. dominated blooms over C. cf. salsugineus and T. cf. oceanica dominated blooms, by lowering the diatoms \( U_{\text{m}} \)'s and elevating their \( Q_{\text{op}} \)'s or \( Q_{\text{oN}} \)'s, thereby decreasing their overall competitiveness.

The influence of salinity on \( Q_{\text{o}} \) was found not only to vary with species but also with nutrient type. Suboptimal growth salinities of 15 \(^{\circ}/_{00}\) and 50 \(^{\circ}/_{00}\) had a proportionately larger influence on \( Q_{\text{oN}} \) than on \( Q_{\text{op}} \). For example, the diatom species \( Q_{\text{oN}} \)'s were elevated on average 3-9 fold while their \( Q_{\text{op}} \)'s were elevated on average only 1-2 fold above their respective values at 25 \(^{\circ}/_{00}\) (Tables 4-1 and 4-2). The proportionally larger increase in \( Q_{\text{oN}} \) than \( Q_{\text{op}} \) required for cellular maintenance at suboptimal salinities may be explained by a greater demand for N than P for the synthesis of RNA, enzymes, proteins, amino acids, and ATP, all of which play roles in phytoplankton maintenance processes including osmoregulation. Rhee and Gotham (1981a) also found a proportionally greater increase in \( Q_{\text{oN}} \) than \( Q_{\text{op}} \) at suboptimal temperatures, with \( Q_{\text{oN}} \) increasing as much as 5 fold and \( Q_{\text{op}} \) increasing approximately 1.8 fold. They attributed the greater increase in N to the greater demand for additional RNA for protein synthesis as the efficiency of protein synthesis per unit RNA decreases at low temperatures. The higher \( Q_{\text{oN}} \) of T. cf. oceanica at the suboptimal salinity of 50\(^{\circ}/_{00}\) may also be due in part to an increased use of amino acids as osmotica, similar to the increase in intracellular amino acid pools that occurred in S. costatum with an increase in salinity (Rijstenbil 1988).

Another hypothesis of this study was that the dominant bloom species in Florida Bay might owe their dominance in part to a more efficient use of the limiting P or N. This hypothesis was considered because Grover (1989), in a study of the phosphorus kinetics of freshwater algae found a correlation between \( \text{PO}_4^{3-}K_a \) and \( Q_{\text{vp}} \), that qualitatively suggested that competitive ability resulted from a more efficient use of phosphorus in biomass, although the relationship was later found to be insignificant. The overall absence of interspecific differences in \( Q_{\text{vp}} \)'s and \( Q_{\text{oN}} \)'s, at each experimental salinity, indicates a
lack of interspecific differences in the efficiency of phosphorus and nitrogen use in biomass (Figs. 4-5C and 4-6C). As a result, the competitive superiority of S. cf. elongatus in competition experiments under P-limitation as well as the slight superiority of T. cf. oceanica in competition experiments under N-limitation at 25%_00 (Chapter IIB), cannot be attributed to any competitive advantage resulting from a more efficient utilization of phosphorus or nitrogen in biomass than any of the other species.

Phytoplankton cell volume has been found to be both dependent and independent of various abiotic factors. Suboptimal temperatures have been found to increase phytoplankton cell volume (Rhee and Gotham 1981a), while suboptimal salinities have been found to both increase cell volume (Miller and Kamkykowski 1986a) and not affect cell volume (Rijstenbil and Sinke 1989, Tsuruta et al. 1985). In my study, salinity did not influence cell volume, as biovolumes varied independent of salinity (Table 4-1).

**Luxury consumption**

The interspecific differences in luxury N and P storage (R_mN and R_mP) indicate differences in nutrient competitive strategies between the dominant bloom species of Florida Bay. The four species may be ranked according to their relative competitive storage abilities (R_mN’s and R_mP’s) from strongest to weakest, with the ranking for P storage being: C. cf. salsugineus > Synechocystis sp. ≈ S. cf. elongatus ≈ T. cf. oceanica and the ranking for N storage being: T. cf. oceanica ≈ C. cf. salsugineus > Synechocystis sp. ≈ S. cf. elongatus. This ranking suggests that under conditions of P-limited patchiness, the high R_mP of C. cf. salsugineus will give it a competitive advantage over the other species. Similarly, the greater R_mN’s of both diatoms would be predicted to give them a competitive advantage over the cyanobacteria in an N-limited patchy environment. The generally greater luxury storage capacities of the diatom species were not entirely unexpected, as diatoms possess vacuoles that provide them with added storage capabilities (Smayda 1978).

Although C. cf. salsugineus’s R_mP predicted that it would be the superior competitor in a P-limited patchy environment, competition experiments demonstrated however, that whatever competitive advantage C. cf. salsugineus may have over the other species in terms of P-storage by being able to store ~29 times its Q_oP, the advantage was not sufficient in the P-limited patchy environment to prevent it from being competitively excluded (Chapter 3B, Figs. 3-9 and 3-11). Similarly, although C. cf. salsugineus was predicted to be a better competitor than the cyanobacteria in an N-limited patchy environment, based on its higher R_mN, the results of competition experiments indicated that whatever competitive advantage C. cf. salsugineus may have over the cyanobacteria species in terms of N-storage, the advantage was not sufficient to prevent its rapid exclusion. In contrast, the very good performance of T. cf. oceanica in the N-limited patchy environment and the poor performance of T. cf. oceanica in the P-limited patchy environment (Chapter IIB) is in agreement with their estimated superior luxury N storage capability (R_mN) and relatively low luxury P storage capability (R_mP), respectively.

Even though R_m values are species-specific attributes that reflect nutrient competitive strategies such that R_m values of similar or related species may or may not be comparable, the R_mN and R_mP values of
the study species are comparable to available literature values of some species of *Synechococcus* and small centric diatoms. For example, the average $R_{mP}$ and $R_{mN}$ values for *S. cf. elongatus* of 10.9 and 2.6, respectively are quite similar to the freshwater cyanobacteria, *Synechococcus linearis* $R_{mP}$ of ~12 and $R_{mN}$ of ~2 (Healy 1985). In contrast, the $R_{mP}$ of the oceanic marine *Synechococcus* sp. of approximately 3 (Ikeya et al. 1997) is substantially lower than the $R_{mP}$ of *S. cf. elongatus*. This difference is due to the much greater $Q_{mP}$ of *S. cf. elongatus* which is ~0.25 fmol cell$^{-1}$ compared to the 0.07 fmol cell$^{-1}$ of the oceanic *Synechococcus* sp., as both species $Q_{oP}$'s are approximately 0.024 fmol cell$^{-1}$. An explanation for the smaller $R_{mP}$ of the oceanic *Synechococcus* species compared to the nearshore *S. cf. elongatus* Florida Bay clone may be that each species utilizes a luxury nutrient storage strategy according to the nutrient availability patterns of their environment. The larger $R_{mP}$ of *S. cf. elongatus* may be viewed as being advantageous in an estuarine environment in which P would be expected to be highly variable, while the smaller $R_{mP}$ of the oceanic *Synechococcus* species may be considered to be well suited to an oceanic environment in which for the most part, P levels remain typically low and do not vary as widely as they do in nearshore environments. The luxury storage capabilities of the diatom study species were comparable to literature values with the $R_{mN}$'s and $R_{mP}$'s of 9.1 and 8.1 for *T. cf. oceanica* being similar to the $R_{mN}$ of ~4 and ~5 for *T. pseudonana* (Eppley and Renger 1974, Goldman and McCarthy 1978) and the $R_{mP}$ of 12 and $R_{mN}$ of 12 for *Skeletonema costatum* (Sakshaug 1978).

Interspecific differences in storage capabilities of phytoplankton have also been described in terms of the r-K continuum with R-strategists characteristically having high storage capacities ($Q_{m} >> Q_{o}$) and K-strategists with low storage capacities ($Q_{m} \approx Q_{o}$) (Kilham and Hecky 1988). The higher storage capability of the *S. cf. elongatus* Florida Bay clone would make it more of an r-strategist than the more K-strategist oceanic *Synechococcus* species described by Ikeya et al. (1997). For example, the strongly r-selected storage strategist diatom *Asterionella formosa* with an $R_{mP}$ of 82 is considered to have a distinct advantage under conditions of P-limited patchiness over the more intermediate r-strategist *Cyclotella meneghiniana* with a $R_{mP}$ of 6.6 (Tilman and Kilham 1976). The $R_{mP}$'s and $R_{mN}$'s of the study species ranging from 8.1 to 29 and 2.6 to 9.1 respectively, suggests that none of the four species appear to be extreme r-selected or K-selected species, instead being more intermediate r-K strategists with respect to their P and N luxury storage capabilities.

Although the luxury storage capabilities were not determined for the Florida Bay *S. costatum* clone, the $R_{mP}$ and $R_{mN}$ of a temperate population of *S. costatum* were determined to be both 12 (Sakshaug 1978). If an $R_{mP}$ and $R_{mN}$ of 12 are representative of the storage capabilities of the Florida Bay *S. costatum* clone, then the predicted species relative competitive ranking for the ability to utilize pulses of P, including *S. costatum* is: *C. cf. salsugineus* > *Synechocystis* sp. ~ *S. cf. elongatus* ~ *S. costatum* ~ *T. cf. oceanica*, while the ranking for N is: *S. costatum* ~ *T. cf. oceanica* ~ *C. cf. salsugineus* > *Synechocystis* sp. ~*S. cf. elongatus*.

The larger $R_{mN}$ of *S. costatum* relative to the cyanobacteria study species in particular, predicts that *S. costatum* will be a superior competitor under conditions of N-patchiness. This is in general agreement
with the outcomes of the N-limited competition experiments in which \textit{S. costatum} was determined to be the superior N-competitor in the semicontinuous dilution (daily pulses) (Chapter IIB). The greater N-storage competitiveness of \textit{S. costatum} also is in agreement with its common and often dominant presence in more N-limited marine coastal environments where nutrients are presumed to commonly occur as patches and fluctuate widely.

These results suggest that the abundance and frequent dominance of the cyanobacteria species in Florida Bay are not the result of a competitive advantage that they have over the other species in a P-limited or N-limited patchy environment as a result of their luxury storage capabilities. To the contrary, their success in Florida Bay under either N or P limitation must be due largely to physiological nutrient adaptations other than N or P luxury storage.

\textit{Optimum N:P ratios}

Available literature values indicate that phytoplankton optimum N: P ratios are species specific and may vary widely between species. The following ranges of optimal N:P ratios have been reported for marine and freshwater algae: 20-50 (Geider and La Roche 2002), 7-30 (Rhee and Gotham 1980), 7-87 (Hecky and Kilham 1988) and 51-88 (Fugimoto et al. 1997). The optimum N:P ratios of the diatoms (12 and 28) found in this study fall well within the range of reported literature values, while those of the cyanobacteria (68 and 93) are clearly at the upper range of the reported values. Although the N:P ratios found for the cyanobacteria isolates (68 and 93) in this study are distinctly high, they resemble the high values of 88 (Fugimoto et al. 1997) and 70 (Healy 1985) that have been reported for other species of cyanobacteria.

Although the optimal N:P ratios of the cyanobacteria isolates may be regarded as abnormally high and therefore suspect, several factors lend support to their validity. These include the fact that two different experimental culture methods (batch and semicontinuous) yielded similar optimum N: P ratio estimates (Table 4-4) and the experimentally determined \(Q_{oN}\) and \(Q_{oP}\) values used to calculate the optimum N:P ratio were individually similar to available literature values of similar species. In addition, qualitative estimates of each study species optimal N:P ratio using their \(K_{uN}\) and \(K_{uP}\) values (Chapter IIA) yield a similarly large difference in optimal N:P ratios between the cyanobacteria and the diatom species. In theory a species \(K_{uN}:K_{uP}\) is equivalent to its \(Q_{oN}:Q_{oP}\) ratio (Tilman 1982). Although only the \(K_{uP}\)'s were experimentally determined in my study, estimates of the study species \(K_{uN}\)'s relative to each other may be inferred from the outcomes of the N-limited competition experiments. For example, the \(K_{uN}\)'s of \textit{T. cf. oceanica}, \textit{Synechocystis} sp. and \textit{S. cf. elongatus} may be inferred to be very similar, based upon their co-dominance under approximate steady state N-limited equilibrium conditions (Chapter IIB). When the much smaller experimentally determined \(K_{uP}\) of \textit{S. cf. elongatus} and \textit{Synechocystis} sp. and the higher \(K_{uP}\) of \textit{T. cf. oceanica} are coupled with a \(K_{uN}\) of similar value, the resulting optimal N:P ratios (\(K_{uN}:K_{uP}\)) of the cyanobacteria species are qualitatively much higher than the optimal N:P ratio (\(K_{uN}:K_{uP}\)) for \textit{T. cf. oceanica}.\footnote{133}
Similarly, a moderately low optimum N:P ratio may be estimated for *C. cf. salsugineus* from its' experimentally determined moderately low $K_{\text{up}}$ and its experimentally inferred very high $K_{\text{un}}$.

But the possibility that the optimum N:P ratios of the cyanobacteria in particular, may be erroneously high should also be considered. Phytoplankton species optimal ratios were initially considered to be invariant (Rhee 1978, Rhee and Gotham 1980), but a number of studies (Healy 1985, Terry et al. 1985, Elfiri and Turpin 1985, Tett et al. 1985) have shown that phytoplankton species optimal ratios may vary with factors such as relative growth rate and irradiance, such that the optimal N:P ratio may increase with increasing light limitation as well as with decreasing relative growth rate. Although the study species optimum ratios were calculated from $Q_{\text{oN}}$ and $Q_{\text{oP}}$ that were determined at ambient saturating irradiances, high biomass levels particularly in the cyanobacteria cultures may have resulted in some degree of light limitation as a result of self-shading. In addition, cyanobacteria have also been found to have high N requirements for their phycobilisomes (Raven 1984). This increased requirement for N at reduced irradiances may have contributed to erroneously elevated N:P optimal ratio estimates for the Florida Bay cyanobacteria isolates. The methodology in which low growth rates ($d = 0.1 - 0.6 \text{ da}^{-1}$) are used to determine $Q_{\text{oN}}$ and $Q_{\text{oP}}$ may also have contributed to the high optimal N:P ratio estimates of the study species. Lastly, the highest N:P ratio (93) found in the Florida Bay *Synechocystis* sp. isolate may be in part due to luxury storage of N as cyanophycin. The ability of *Synechocystis* sp. to store excess nitrogen, as cyanophycin, (Corbridge 1998) is not found in any of the other study species. But even if the optimum N:P ratios of all of the four species are erroneously higher than their true values, and possibly more so for the cyanobacteria species, a substantial difference in optimal N:P ratios between the cyanobacteria and the diatoms would still likely remain, indicative of the existence of real differences in optimal N:P ratios between the two groups.

The high optimum N:P ratios of the Florida Bay cyanobacteria isolates suggests that they are uniquely well adapted to high N:P supply ratios, while the lower ratios of the diatoms indicate that like the majority of marine algae they are better suited to lower N:P supply ratios. Although the two cyanobacteria isolates differed in their optimal N:P ratio as did the two diatom species, the most dramatic and potentially significant difference in optimum N:P ratios was seen between the two taxonomic groups. The substantial difference in optimum N:P ratios found between the cyanobacteria and the diatoms suggests that optimal N:P ratios may play a significant role in the development of group dominance (i.e. diatom vs. cyanobacteria) in the phytoplankton blooms of Florida Bay. The large interspecific differences in optimal N:P ratios between the cyanobacteria and the diatom study species coupled with the increasing N:P supply ratio gradient that runs from west to east across Florida Bay provided an ideal opportunity for examining the ability of the resource ratio hypothesis to explain the spatial patterns of distribution and abundance of these species in Florida Bay.

A decreasing N:P supply gradient along a broad west to east transect across the bay has been inferred from phytoplankton nutrient bioassays which have found the western region to be predominantly N-limited, the central region to be alternately N- and P-limited and the eastern region to be predominantly 134
P-limited. The increasing N:P supply gradient across the bay has also been inferred from N and P concentrations in: sediment; sediment pore waters; water column; seston and seagrass tissue (Yarbro and Carlson 1998, Boyer et al. 1999, Fourquarean et al.1992). For example, median water column DIN:SRP molar ratio over an eight year period in the western, central and eastern regions was respectively 52, 121 and 153 and a similar pattern of TN:TP ratios in the same regions was 56, 132 and 184 (Boyer et al. 1999). It has been argued that DIN:SRP and TN:TP may not accurately reflect N:P supply ratios. Brand (2002) suggests using the DIN:TP water column ratio as an indicator of the N:P supply ratio, because it excludes the DON fraction that may not be readily available to the phytoplankton while including the more readily available DOP fraction. Brand's DIN:TP ratios result in N:P ratios that are <5 in the western region, 10-30 in the central region and >30 in the eastern region. Regardless of the forms of N and P used to estimate the N:P supply ratios, they all suggest that an increasing N:P supply gradient exists from west to east across Florida Bay.

The resource ratio hypothesis predicts that under approximate steady state conditions along a N:P supply ratio gradient when the only limiting nutrients are N and P, that the species with higher optimal N:P ratios will be found in greater relative abundance at the higher N:P supply ratios while species with lower optimal N:P ratios will be found in greater relative abundance at lower N:P supply ratios. Using the study species optimal N:P ratios, the resource ratio hypothesis predicts that the relative dominance of the species along an increasing N:P gradient from west to east across Florida Bay, would be S. costatum -- C. cf., salsugineus -- T. cf. oceanica -- S. cf. elongatus -- Synechocystis sp.. The results of competition experiments under P and N limitation in which the N:P supply ratios were 2:1, 250:1, and 800:1, found species relative dominance along the increasing N:P supply gradient to be: S. costatum -- T. cf. oceanica -- C. cf., salsugineus -- Synechocystis sp -- S. cf. elongatus --. Although the pattern of species relative abundance predicted by the resource ratio hypothesis is not identical to the pattern obtained from N- and P-limited competition experiments, the overall pattern of diatom dominance, particularly S. costatum, at low N:P ratios and cyanobacteria dominance at high N:P ratios predicted by the resource ratio hypothesis is in general agreement with the outcomes of the competition experiments at various N:P supply ratios.

The overall patterns of species abundance and relative dominance predicted by the resource ratio hypothesis and the outcomes of competition experiments are also both in general agreement with some of the broad spatial patterns of species relative abundance and dominance documented in field studies of the phytoplankton of Florida Bay. Although the ambient N:P (DIN:SRP) ratio does not consistently increase in a gradual and steady progression across Florida Bay from west to east but is variable due to the heterogeneous rates of N and P supply, there still exists a broad overall decreasing N:P gradient that runs west to east across the bay. Similarly, although the physical boundaries of species spatial abundance patterns are variable and not precisely defined due to the spatial and temporal heterogeneity of their abundances and distributions, broad overall patterns of their relative abundances which display some degree of spatial separation are visible along a west to east transect across the bay. For example, the absence of S. costatum in particular from the eastern and central regions of Florida Bay may be in part
explained in terms of its very low optimum N:P ratio and the inferred high N:P supply rates in those
regions. Haigh et al. (1992) explained the dominance of *S. costatum* in the Sechelt Inlet in British
Columbia to be due to the favorable water column N:P ratio, which was well below the Redfield ratio
suggesting N limitation, but which essentially matched the optimum N:P ratio for *S. costatum* of 8.1. Other
optimal molar N:P ratios for *S. costatum* include ~5 (Mykelstad 1977) and 12 (Kilham and Hecky 1988).
Likewise the overall higher N:P supply ratios found consistently in the eastern and regularly in the central
regions of Florida Bay would be expected to favor the species with highest optimal N:P ratios, such as the
cyanobacteria bloom study species. The optimal ratios of 68 and 93 of the cyanobacteria isolates are closer
to the 8 year median DIN:SRP of 121 for the north-central region of Florida Bay, the region considered to
be the epicenter of the cyanobacteria dominated blooms, than the diatom study species optimal ratios of 12
and 28. Although the high optimum N:P ratios of the cyanobacteria and the low optimum N:P ratio of
*S. costatum* may be a contributing factor to their relative high abundance and absence in the eastern and
central regions respectively, their R*_P*’s and R*_N*’s will be more influential in determining their relative
abundance at a given N:P supply ratio.

Another ecological significance of interspecific differences in optimal N:P ratios lies in the fact
that under a given environmental N:P supply ratio, some species will be P-limited while others are N-
limited. Competition theory has proposed that nutrient limitation by different nutrients may serve to
promote the coexistence of otherwise potentially competing phytoplankton species. For example, using the
study species optimal N:P ratios, at N:P supply ratios >93 all of the species are P-limited, at N:P supply
ratios <12 all of the species are N-limited, while at N:P supply ratios >28 and <68, both cyanobacteria
isolates will be N-limited while both diatom isolates will be P-limited. Although the interspecific
differences in optimal N:P ratios will not by themselves determine competitive outcomes under equilibrium
conditions as competitive outcomes are determined primarily by interspecific differences in species R*
values, optimal N:P ratios will determine the N:P ratio at which two competing species will be able to
coexist.

Resource supply ratios have also been used to explain the successional patterns of phytoplankton
communities. Dissolved N:P ratios when used as indicators of N:P supply ratios have been successfully
used to explain in part the successional patterns in lakes leading to dominance by N2-fixing cyanobacteria
as the ambient N:P ratio decreases (Tilman and Kiesling 1984). No obvious seasonal pattern in DIN:SRP
ratios has been found in either the western or the eastern regions of Florida Bay. But a seasonal trend in the
central region in which the median DIN:SRP is lowest (<50) in July and August, increases during the fall
and remains high (>100) for most of the remaining months has been observed (Boyer et al. 1999). If the
DIN:SRP ratio is used as a proxy for the N:P supply ratio, then the relatively constant annual DIN:SRP
ratios in the eastern and western regions suggest that seasonal changes in bloom dominance in the eastern
and western region are not likely to be driven by changes in the N:P supply ratio. In contrast, shifts in
species bloom composition and relative dominance that occur in the central region during the late summer
may be due to the decrease in the N:P supply ratio. The optimal N:P ratios determined in this study
indicating that the overall high N:P supply ratios found in central region for much or most of the year will favor cyanobacteria bloom dominance. The reduced N:P ratio in late summer that is approximately midway between the optimal ratios of the diatom and cyanobacteria species will promote the development of mixed diatom and cyanobacteria blooms. The general pattern of central region blooms dominated by cyanobacteria or co-dominated by cyanobacteria and diatoms during the summer, fall and early winter leading to reduced dominance of the cyanobacteria in late winter cannot be clearly explained by interspecific differences in optimal N:P ratios. This is not surprising as competition theory states that although optimal ratios may contribute to patterns of species dominance, interspecific competitive differences (R* values) play much greater roles.

Seasonal bloom initiation, growth and decline in the central region of the bay cannot be adequately explained by ambient N:P supply ratios, and interspecific differences in optimum N:P ratios and N-and P-competitiveness. Although the high DIN:SRP ratios in the fall and the superior P-competitiveness of the cyanobacteria species may together be used to explain in part their frequent dominance of the phytoplankton community during this period of major bloom growth, bloom decline and retraction in the central region during the winter-spring season during periods of high DIN:SRP ratios, which are predicted to favor the growth of the cyanobacteria species cannot be explained in terms of the ambient DIN:SRP ratios. Other biological and environmental factors are surely influential in seasonal bloom initiation, growth and decline. For example, cyanobacteria typically dominate lake communities during increased summer water temperatures that characterize late summer (Tilman and Kiesling 1984), while diatoms typically dominate lake communities when temperatures are at their seasonal low (Tilman and Kiesling 1984).

Nutrient replete N:P ratios

Under non-limiting nutrient conditions when phytoplankton species are growing near their maximal rates of growth, their intracellular N:P ratios are normally between 10 and 20 (Goldman et al. 1979, Goldman 1980), while their estimated optimal N:P ratios are typically higher (Geider and LaRoche 2002). This was also found to be the case for the intracellular N:P molar ratios of the Florida Bay study species.

Ratios of 12, 12, 10 and 11 under nutrient-replete conditions for: S. cf. elongatus, Synechocystis sp., T. cf. oceanica and C. cf. salsugineus, respectively (Table 4-5) were lower than their respective optimum N:P ratios of 68, 93 28 and 12 (Table 4-4). Substantial differences have been found between species optimum N:P and their cellular N:P ratios. For example, Fragilaria crotonensis has an optimum N:P ratio of 25 and a cellular N:P ratio under nutrient replete near maximal growth conditions of 8 (Rhee and Gotham 1981b).

Although the Redfield N:P ratio of 16 is usually considered to be the ratio on average that the nutrients N and P are depleted during phytoplankton growth and therefore the average optimal N:P ratio for phytoplankton growth, studies suggest that there is no ‘optimal’ N:P ratio that applies to all phytoplankton
(Ryther and Dunstan 1971). In fact, other average optimal N:P ratios include 17 (Rhee and Gotham 1980), 10 (Ryther and Dunstan 1971) and 30 (Geider and La Roche 2002). In theory, intracellular N:P ratios at maximal growth rates \( (U_m) \) would be expected to be nearly equivalent to the ratio of the minimum N and minimum P cell quotas \( (Q_{oN}:Q_{oP}) \), (since cell growth at \( U_m \) approximates the balanced growth that occurs when nutrient uptake and growth are in balance. But species cellular N:P ratios at \( U_m \) are generally lower than the \( Q_{oN}:Q_{oP} \) ratio. The lower N:P ratio at \( U_m \) has been attributed to the disproportionately greater amount of P stored in the cell than N, because phytoplankton are typically capable of storing more phosphorus than nitrogen (Rhee and Gotham 1980). As a result, at near maximal rates of growth, after a balance has been achieved between uptake and utilization, the intracellular phosphorus storage products (e.g. polyphosphates) will be greater than the intracellular nitrogen storage products (e.g. proteins, amino acids), such that the N:P ratio at \( U_{\text{max}} \) is lower than what would be expected, based on minimum phosphorus and nitrogen subsistence values.

The similarity of the study species nutrient replete N:P ratios at high relative growth rates indicates that under the experimental growth conditions used in my study the intracellular requirements of the four bloom study species are quite similar. This suggests that bloom dominance in Florida Bay under nutrient replete light saturated conditions is not determined by interspecific differences in the species N and P utilization or consumption ratios. The interspecific similarities of intracellular C, N, P and Chl \( a \) ratios among the study species (Table 4-5), all indicate that while the competitive abilities for N and P are quite different, the two cyanobacteria bloom species do not differ dramatically from the diatom bloom species in these basic intracellular chemical constituents.

Conclusions

1) The cyanobacteria species \( Q_{oP}'s \) and \( Q_{oN}'s \) were unaffected by salinity while the diatom species response was typified by \( T. \text{ cf. } oceanica \) was characterized by elevated \( Q_{oP}'s \) and \( Q_{oN}'s \) at suboptimal salinities for growth (15 \( \% \)/\( \text{o} \) and 50 \( \% \)/\( \text{o} \)) relative to those at 25 \( \% \)/\( \text{o} \). The elevated \( Q_{oP}'s \) and \( Q_{oN}'s \) may place the diatoms at a competitive disadvantage with respect to the cyanobacteria study species when either P or N limiting conditions co-occur with either very low or very high salinities. As a result, hyposaline and hypersaline conditions in Florida Bay may contribute to the increased relative dominance of the cyanobacteria species by not only differentially lowering the diatoms \( U_m \)'s (Chapter I), but by acting in concert with either N or P limiting conditions to elevate the diatoms minimum cellular quotas of the limiting N or P thereby further lowering their N- or P-competitiveness.

2) The species did not differ in their efficiency of phosphorus or nitrogen use per unit biomass as evidenced by the absence of interspecific differences in the study species volume-specific \( Q_{oP}'s \) and \( Q_{oN}'s \) at each experimental salinity. Consequently, species dominance in the competition experiments and in Florida Bay cannot be explained by any competitive advantage resulting from
a more efficient utilization of limiting phosphorus or nitrogen in biomass.

3) The much larger $R_{mP}$ of *C. cf. salsugineus* predicts that it will be the superior competitor under conditions of P-limited patchiness, while the larger $R_{mN}$'s of the diatom species predict that they will be better competitors than the cyanobacteria in an N-limited patchy environment. Consequently, the frequent cyanobacteria dominated blooms in Florida Bay must be due largely to cyanobacteria physiological nutrient adaptations other than luxury storage of either N or P. In contrast, dominance by the diatom study species may be enhanced by either patchy P- or N-limited conditions, both of which often characterize well-mixed shallow water marine environments like Florida Bay.

4) Although interspecific differences in optimum N:P ratios were found between all four species, the magnitude of difference in optimum N:P ratios between the cyanobacteria and the diatoms suggests that optimum N:P ratios may play a significant role in the development of group dominance (i.e. diatom vs. cyanobacteria) in the phytoplankton blooms of Florida Bay.

5) The resource ratio hypothesis predicts that from west to east along the decreasing N:P gradient in Florida Bay that the study species maximum abundances will occur in the following spatial sequence: *S. costatum* -- *C. cf. salsugineus* -- *T. cf. oceanica* -- *S. cf. elongatus* -- *Synechocystis* sp.. This pattern is in general agreement with the broad spatial patterns of relative abundance and dominance that have been observed for these species in Florida Bay. *S. costatum*'s peak abundance west of the peak abundances of the cyanobacteria species and the conspicuous absence of *S. costatum* from the majority of Florida Bay may be due to its much lower optimal N:P ratio in addition to its inferior P-competitiveness. In contrast, the frequent dominance of the cyanobacteria species in the north-central region that is typically characterized by high ambient DIN:SRP ratios (inferring high N:P supply ratios) may be explained by their superior P-competitiveness and their high optimal N:P ratios. Heterogeneous rates of N and P supply within Florida Bay may create highly variable N:P supply ratios, which may in turn erase any N:P ratio driven patterns of species distribution and relative abundance. But the long-term influence of the broad and persistent N:P supply gradient on the species relative abundances across the bay is still apparent in their general distribution and relative abundances, particularly the species at each end of the gradient *S. costatum* and *S. cf. elongatus*.

6) Species intracellular N:P ratios were as expected, both lower than their respective optimum N:P ratios yet within the typical range of N:P ratios for marine phytoplankton. The similarity of the four bloom study species nutrient replete ratios of intracellular chemical constituents (C, N, P, Chl a) at high relative growth rates are indicative of their similar intracellular requirements and suggest that bloom dominance in Florida Bay under nutrient replete light saturated conditions is not determined by interspecific differences in the species C, N or P utilization ratios.
References


CHAPTER 5
INVESTIGATION OF NITROGEN FIXATION, ALLELOCHEMICAL INTERACTIONS
AND SEDIMENT RESUSPENSION AS POTENTIAL REGULATORY FACTORS
INFLUENCING THE INTERSPECIFIC COMPETITIVENESS OF SYNECHOCOCUS CF.
ELONGATUS, SYNECHOCYSTIS SP., THALASSIOSIRA CF. OCEANICA, CHAETOCEROS
CF. SALSUGINEUS AND SKELETONEMA COSTATUM

Abstract

The occurrence of large and persistent algal blooms frequently dominated by species of
Synechococcus in regions of Florida Bay that experience large and frequent sediment resuspension events,
periodic N-limitation, and long basin water residence times led to the formation of several hypotheses that
associated these environmental conditions with potential ecophysiological attributes particular to
Synechococcus species that may give them a competitive advantage over other phytoplankton species,
thereby contributing to their frequent dominance. Laboratory experiments were conducted to test these
hypotheses using clonal isolates of the following dominant bloom species from Florida Bay:
Synechococcus cf. elongatus, Synechocystis sp. and Chaetoceros cf. salsugineus and the non-dominant
Florida Bay species Thallassiosira cf. oceanica and Skeletonema costatum.

N-deficient growth studies demonstrated that neither cyanobacteria species S. cf. elongatus nor
Synechocystis sp. was able to aerobically fix nitrogen. Although the phytoplankton community in the
region of these large blooms has been shown to be at times N-limited, the results of this study indicate that
the frequent dominance of S. cf. elongatus and Synechocystis sp. cannot be attributed to an ability to
aerobically fix nitrogen during periods of N limitation.

Experiments designed to test for allelochemical interactions indicated that S. cf. elongatus produced
allelochemicals that selectively depressed the final yields of the commonly co-occurring Florida Bay
diatom species C. cf. salsugineus and T. cf. oceanica as well as the uncommon Florida Bay diatom S.
costatum. The dense, near monospecific blooms of S. cf. elongatus (up to 5 million cells ml\(^{-1}\)) in Florida
Bay, coupled with long basin water residence times, particularly in the north-central region of the bay, may
together provide the conditions under which allelochemical production by S. cf. elongatus may contribute
to its increased relative bloom dominance.

Laboratory experiments indicated that Florida Bay sediment resuspension did not selectively
enhance the growth of S. cf. elongatus and/or diminish the growth of any of the following co-occurring
potential diatom competitors: *C. cf. salsugineus*, *T. cf. oceanica* or *S. costatum*. This suggests that the frequent dominance of the phytoplankton community in Florida Bay by *S. cf. elongatus* in areas that commonly experience large and frequent sediment resuspension is not related to any cell-sediment interactions that are uniquely beneficial to *S. cf. elongatus* nor to any cell-sediment interactions that are selectively detrimental to the co-occurring diatom study species.

**Introduction**

*Nitrogen fixation*

When sufficient light is present, phytoplankton biomass in estuaries and lagoons is often limited by the availability of nitrogen and/or phosphorus. Beginning in 1991, large and persistent planktonic algal blooms began to occur in Florida Bay, which had been historically characterized as having a clear water column supporting a low phytoplankton biomass. A prerequisite for the development of these new large blooms in the bay was a substantial increase in the supply of new N and/or P.

Potential sources of new nitrogen necessary to support the large phytoplankton blooms in Florida Bay that have been considered include: terrestrial runoff from the Everglades (Rudnick et al. 1999), remineralization of below-ground seagrass tissue following seagrass die-offs (Boyer et al. 1999), advection from surrounding water masses (Boyer et al. 1999), atmospheric precipitation (Rudnick et al. 1999), groundwater (Lapointe and Clark 1992), sediment N\(_2\)-fixation (Cornwell et al. 2001) and N\(_2\)-fixation by bloom forming planktonic cyanobacteria (Phlips and Badylak 1996). Although N\(_2\)-fixation has been documented to occur in Florida Bay sediments (Cornwell et al. 2001), aerobic N\(_2\)-fixation by the dominant Florida Bay cyanobacteria water column bloom species has as yet, not been examined. The dominance of the cyanobacteria in the phytoplankton community has even been speculated to have been due in part to the potential competitive advantage that they would have under N limiting conditions if they were capable of aerobically fixing nitrogen and thereby have exclusive access to an unlimited supply of nitrogen.

**Allelochemic interactions**

There has been considerable debate over the factors believed to regulate phytoplankton blooms in Florida Bay, in particular the factors that have led to the development of the large cyanobacterial dominated blooms. The recurrence and persistence of these large phytoplankton blooms often dominated by *S. cf. elongatus* led to numerous hypotheses attempting to explain the dominance of *S. cf. elongatus* in terms of the population losses from grazing and the population gains from growth processes commonly referred to as top-down and bottom-up regulatory processes. An early top-down hypothesis was that *S. cf. elongatus* dominance was the result of reduced predation due to its distasteful and/or toxic characteristics. An early bottom-up hypothesis was that *S. cf. elongatus* dominance was the result of allelopathic interactions in which *S. cf. elongatus* allelochemical production selectively enhanced its own growth and/or inhibited the growth of the other competing phytoplankton species.
Considerable evidence exists indicating that phytoplankton produce allelochemicals that influence the behavior of the species that produce it, other species of phytoplankton, and predators. Numerous studies have documented the production of allelochemicals by phytoplankton as a defense against predation (Smayda, 1997). Cyanobacteria are also known to produce toxins as well as compounds that make them distasteful to predators (DeMott and Moxter 1991). Although many cyanobacteria, including some species of *Synechococcus* have been found to produce harmful toxins (Mitsui et al. 1989), to date the Florida Bay *Synechococcus* blooms have given no outward sign (e.g. fish or invertebrate mass mortalities) that they produce a toxin harmful to animals (Butler et al. 1995). The hypothesis that *S. cf. elongatus* frequently dominates Florida Bay algal blooms because they produce a distasteful or toxic compound, as a defense against predation has not received support. A variety of short-term grazing and community composition studies have indicated that the *Synechococcus* cells in the bloom waters are effectively grazed by benthic populations of filter and suspension feeders (Vargo et al. 1996), the zooplankton community (Brenner and Dagg 2001) and by microzooplankton (Lavrentiev et al. 1998). Laboratory grazing studies using the same Florida Bay *S. cf. elongatus* isolate that was used in my study, found that benthic populations of filter and suspension feeders effectively grazed the cells with no indication of avoidance or harmful effects (Vargo et al. 1996).

Although the top-down hypothesis that *Synechococcus*-produced allelochemicals selectively inhibit their consumption by predators has found no support, the bottom-up hypothesis that *Synechococcus*-produced allelochemicals contribute to *Synechococcus* dominated blooms by selectively inhibiting the growth of other phytoplankton species had to date not been investigated.

**Sediment resuspension**

Another factor that may have particular relevance in Florida Bay is sediment resuspension. Large recurring phytoplankton blooms often dominated by *Synechococcus* sp., have appeared to regularly initiate, grow, and persist in Florida Bay particularly in the north-central region and to a lesser extent in the transitional zone between the north-central and western zones (Philips et al. 1999, Steidinger et al. 2001) where massive seagrass mortality events beginning in 1987 (Robblee et al. 1991), have left large regions of sediment surface exposed and subject to frequent resuspension (Boyer et al. 1999). The sediments in these regions are well-developed carbonate muds with high organic content (up to 15%) (Prager and Halley 1998). The spatial coincidence of organic rich carbonate sediment resuspension and *Synechococcus* dominated bloom initiation and growth led to several hypotheses on the role that sediment resuspension in the north-central and western regions may play in both the formation of phytoplankton blooms and the relative dominance of cyanobacteria and/or diatoms in those blooms.

Sediments have been found to be a significant source of recycled nutrients, particularly in shallow water systems (Kemp and Boynton 1984). In shallow water systems subjected to sediment resuspension, phytoplankton growth may be both stimulated and/or inhibited by sediment resuspension, as sediment resuspension typically introduces nutrients into the water column and reduces light penetration in the water
column, respectively. But the composition of the sediments in Florida Bay may cause sediment resuspension to act as a mechanism of nutrient removal as well. The sediments are composed mostly of biogenic carbonates that are known to readily adsorb phosphate (DeKanel & Morse 1978), and as such, it has been inferred that the carbonate sediments of Florida Bay act largely as a phosphate removal mechanism (Fourquarean et al. 1993). The very low levels of phosphorus found in freshwater entering the bay from the Everglades as well as the low levels of soluble reactive phosphate (SRP) throughout most of the bay have been attributed to a combination of biological uptake and chemical scavenging by carbonates in the substrate/sediment (Boyer et al. 1999). The resuspension of carbonate dominated sediment particles in the water column would be expected to accelerate the carbonate adsorption rate of water column dissolved inorganic phosphorus (DIP) by temporarily increasing the total reactive surface area of the carbonate particles exposed to the water. Support for the role of resuspended sediments as efficient DIP scavengers in Florida Bay has been documented in laboratory experiments (Yarbro and Carlson 1996).

Because Florida Bay waters have been found in general to be P limited (Fourquarean et al. 1993) and nutrient bioassays have found phosphorus, at times, to be the nutrient limiting the growth of phytoplankton in Florida Bay (Tomás et al. 1999, Brand 2002), carbonate sediment resuspension through its potential to remove phosphate from the water column, may be considered to play a role in the regulation of Florida Bay species dominance and bloom development through its influence on the availability of phosphorus in the water column. In addition to acting as a sink, sedimentary carbonate may also act as a potential source of phosphate as the role of desorption in the resupply of phosphate to Florida Bay waters has not been quantified under field conditions. Carbonate sediments may adsorb and desorb not only phosphate but phosphorus containing organic compounds as well. Laboratory extractions of Florida Bay sediments suggest that organic phosphorus containing compounds are loosely bound to carbonate sediments (Yarbro and Carlson 1996), and as such, carbonate sediments may also act as a reservoir of organic phosphorus.

The primary objectives of the preliminary experiments in this chapter were to determine if either Florida Bay cyanobacteria isolate was capable of aerobically fixing nitrogen, whether S. cf. elongatus produced allelochemic compounds that enhanced its own growth and/or inhibited the growth of selected other commonly co-occurring microalgae and if sediment resuspension enhanced the growth of the cyanobacteria isolates and/or inhibited the growth of other selected commonly co-occurring microalgae. The hypotheses that were tested included:

- **H₀:** S. cf. elongatus and Synechocystis sp. are capable of aerobic N₂-fixation.
- **H₁:** S. cf. elongatus produces allelochemic compound(s) that selectively enhance its own growth relative to the growth of other commonly co-occurring species.
- **H₂:** S. cf. elongatus produces allelochemic compound(s) that selectively inhibit the growth of other commonly co-occurring phytoplankton species.
• $H_0$: Sediment resuspension enhances the growth of $S. \text{ cf. }$ elongatus significantly more than it does for the diatom study species.
• $H_a$: Sediment resuspension inhibits the growth of the diatom study species.

The hypotheses stated above were investigated using a series of experiments that were designed to serve simply as preliminary investigations whose results could then be used to justify more thorough subsequent investigations.

Methods

Nitrogen fixation

$S. \text{ cf. }$ elongatus and Synechocystis sp. isolates from Florida Bay were examined for their ability to aerobically fix nitrogen. The two Florida Bay cyanobacteria isolates as well as a marine unicellular cyanobacteria of the genus Cyanothece, known to aerobically fix nitrogen, were grown as single species batch cultures in ASNIII media (Rippka et al. 1979) without combined nitrogen (ASNIII-N) as well as ASNIII media containing combined nitrogen (ASNIII+N) added as 1 g $\text{NO}_3^-\text{l}^{-1}$. The axenic Cyanothece strain obtained from the American Type Culture Collection (ATCC#51472) was originally isolated from intertidal waters near Port Aransas, Texas. The Cyanothece strain was included as part of the experiment to serve as a control, insuring that the experimental growth conditions used to test for nitrogen fixation were actually suitable for aerobic $\text{N}_2$-fixation by unicellular cyanobacteria.

Growth media and stocks were prepared using NANOpure deionized water. Single species experimental cultures consisted of 100 ml of culture in 250 ml Erlenmeyer flasks. Duplicate flasks of each species in ASNIII-N and ASNIII+N were shaken on an orbital shaker at ~100 rpm. The gentle swirling water motion produced by the shaker maintained the Cyanothece cells in suspension which otherwise would have adhered to the bottom of the experimental flasks. Single species stock cultures maintained in ASNIII-N and ASNIII+N were used to inoculate the ASNIII-N and ASNIII+N experimental flasks, respectively. Because repeated preliminary trials found that neither of the Florida Bay cyanobacteria clones could be maintained in ASNIII-N (as well as other media lacking combined nitrogen), the stock cultures used to inoculate the $S. \text{ cf. }$ elongatus ASNIII-N and the Synechocystis sp. ASNIII-N experimental flasks were maintained in media containing combined nitrogen. In order to minimize both the amount of dissolved combined nitrogen that would accompany the inoculums of $S. \text{ cf. }$ elongatus and the Synechocystis sp. cells placed in the ASNIII-N experimental flask, as well as the intracellular surplus stores of nitrogen within the inocula cells, the stock culture used to inoculate the ASNIII-N experimental flasks was first grown to near stationary phase in N-limited ASNIII media ($300 \text{ µM NO}_3^-:147 \text{ µM PO}_4^{3-}$). Additional experimental flasks with the Florida Bay clones in both ASNIII-N and ASNIII+N media were grown adjacent to the flasks on the shaker to determine whether the constant mixing had an adverse effect their growth.
Both stock and experimental cultures were maintained at 25 °C, 12:12 light:dark cycle and 75 µEm sec⁻¹. Population growth was measured using in vivo fluorescence, cell counts and extracted Chl a. The methods used for in vivo fluorescence, extracted Chl a, and cell count determinations were those described in Chapter 1 with the exception that Cyanothece were also enumerated using an improved Neubauer hemacytometer. Experimental culture flasks were sampled daily for in vivo fluorescence, every other day for cell counts, and every third day for extracted Chl a, for the duration of the 12 day experiment.

Two different clones of S. cf. elongatus from Florida Bay were also prepared for an analysis of their N₂-fixing capabilities, by the acetylene reduction assay. Stock cultures were grown in "f/2" media with limiting nitrogen (NO₃⁻ at 50 µM) in flasks at 25 °C, 12:12 light:dark cycle and 75 µEm sec⁻¹. The two clonal N-limited cultures were grown to near stationary phase and then used to inoculate experimental cultures containing "f/2" media lacking nitrogen. Population growth was monitored using in vivo fluorescence and after 2 days of zero or negative growth, the cultures were made available for acetylene reduction activity measurements.

Exponentially growing cultures of S. cf. elongatus and Synechocystis sp. were also made available for analysis of their genome for the presence of the nitrogen fixation (nif) genes, indicative of a genetic potential to fix nitrogen.

Allelochemic interactions

Clonal cultures of S. cf. elongatus, Synechocystis sp., C. cf. salsugineus T. cf. oceanica, and Skeletonema costatum were maintained in "f" medium at 25 °C, 100 µEm sec⁻¹, on a 12:12 light:dark cycle in exponential growth using semicontinuous batch culture. S. cf. elongatus culture filtrate was obtained by gently filtering the dense exponentially growing culture (4 million cells ml⁻¹) through a Whatman GFF filter and subsequently through a polycarbonate 0.22 µm filter under low vacuum (<5 mm Hg). Experimental growth media was prepared by combining various proportions of the 0.22 µm S. cf. elongatus filtrate and sterile offshore natural seawater (NSW), adjusted to 25 °C using deionized water and enriched to "f" nutrient levels. Three different media types were prepared consisting of 0% filtrate (= 100% NSW, controls), 50% filtrate (50% filtrate and 50% NSW) and 100% filtrate. Media was dispensed as 25 ml aliquots into 25 x 125-mm borosilicate culture tubes. Single species batch cultures were established in duplicate tubes of each media type by inoculating the tubes with S. cf. elongatus, Synechocystis sp., C. cf. salsugineus, T. cf. oceanica and S. costatum.

Growth rates of duplicate test tubes of single species cultures in 0%, 50% and 100% filtrate were monitored using the in vivo fluorescence methodology described in Chapter 1.

Sediment resuspension

Clonal cultures of S. cf. elongatus, C. cf. salsugineus, T. cf. oceanica and S. costatum were maintained at 25 °C, 50 µEm sec⁻¹, on a 12:12 light:dark cycle in "f" medium except PO₄³⁻ (1.0 µM). The populations were maintained in exponential growth using semicontinuous batch culture and allowed to
reach P-depleted stationary phase before being used to inoculate the one-liter experimental Erlenmeyer flasks. Single species batch cultures were established at each experimental treatment by inoculating a single species into each experimental treatment flask. The two treatments consisted of 300 ml of "f" medium except PO$_4^{3-}$ (1.0 µM), and 300 ml of "f" medium except PO$_4^{3-}$ (1.0 µM) and 1 gram (wet weight) of sediment. The sediment was taken from the upper layer of a sediment core collected in Rabbitt Key basin in Florida Bay, stored in the dark at 5 °C, and prior to adding to the experimental flasks was autoclaved for 15 minutes at 121 °C and allowed to cool overnight. A flask containing only media and one gram of sediment was included as a control.

Growth in each single-species experimental flask was monitored using *in vivo* fluorescence, cell counts and extracted Chl $a$ following the procedures described in Chapter 1. Flasks were swirled twice daily, prior to sampling for *in vivo* fluorescence and extracted Chl $a$. The experimental growth conditions were 25 °/oo, 50 µEm$^{-2}$sec$^{-1}$, and a 12:12 light:dark cycle. Following the end of the experiment, phosphorus was added to the experimental flasks, both with and without sediment, to confirm that the nutrient limiting growth and biomass throughout the experiment was P.

**Results**

**Nitrogen fixation**

Growth curves based on *in vivo* fluorescence, cell counts and extracted Chl $a$ for all three cyanobacteria clones grown with and without combined nitrogen are shown in Figures 5-1, 5-2 and 5-3. The *Cyanothece* strain (ATCC#51472) grew well in media lacking combined nitrogen, and grew even faster and attained a higher cell density in media containing combined nitrogen (Fig. 5-1), as was also observed by Reddy et al. (1993). In media lacking combined nitrogen, *in vivo* fluorescence measurements indicated no growth for the duration of the experiment for *S. cf. elongatus* (Fig. 5-2A) and *Synechocystis* sp. (Fig. 5-3A), while cell counts indicated that population growth, as cell division, continued up to day 4 for *S. cf. elongatus* and up to day 8 for *Synechocystis* sp. (Figs. 5-2B and 5-3B). Extracted Chl $a$ values revealed no increase for *S. cf. elongatus* (Fig. 5-2B), while values for *Synechocystis* sp. increased only up to day 3, after which no increase was seen (Fig. 5-3B). Gentle mixing on the orbital shaker was not found to inhibit or enhance the growth rate of either Florida Bay cyanobacteria clone when grown in media with or without combined nitrogen (results not presented).

**Allelochemic interactions**

Figures 5-4 and 5-5 show the *in vivo* fluorescence growth responses of *S. cf. elongatus*, *C. cf. salsugineus*, *T. cf. oceanica*, and *S. costatum* grown in media containing different amounts of *S. cf. elongatus* culture filtrate. The growth of *S. cf. elongatus* in the 50% filtrate medium showed no initial lag in growth and the final yield on day 10 was equivalent to the control, while in the 100% filtrate medium there was a slight lag in growth and the final yield was 90% of the control which was 0% filtrate (100%
natural seawater based medium) (Fig. 5-4A). In contrast, the initial growth rates of *C. cf. salsugineus, T. cf. oceanica*, and *S. costatum* in the 50% filtrate media were slightly reduced, while a more substantial lag in growth was seen for all three of the diatom species in the 100% filtrate media (Figs. 5-4B and 5-5). Following their initial lags in growth, the exponential growth rates for all species in the 50% and 100% filtrate media approximated the exponential growth rates seen in their respective controls (Figs. 5-4 and 5-5).

The final yield in the 50% filtrate for *T. cf. oceanica*, was 78% of its control while the final yields of *C. cf. salsugineus* and *S. costatum* were approximately equivalent to their controls (Figs. 5-4B and 5-5). The final yields in the 100% filtrate media of *T. cf. oceanica, C. cf. salsugineus*, and *S. costatum* were all reduced to 68%, 77%, and 88% of their controls, respectively (Figs. 5-4B and 5-5).

**Sediment resuspension**

The *in vivo* fluorescence and Chl *a* response of *S. cf. elongatus, T. cf. oceanica, C. cf. salsugineus, and S. costatum* to sediment resuspension under P limitation is shown in Figures 5-6 and 5-7. Both *in vivo* fluorescence and Chl *a* values increased for all flasks except the control flask, (containing only growth media and the sediment addition) which remained constant. The growth responses (initial and exponential) of each species in the flask containing the sediment addition were comparable to those seen in the flasks without the sediment addition. The final yields of *S. cf. elongatus* were equivalent for flasks with and without sediment, while the final yields for *T. cf. oceanica, C. cf. salsugineus, and S. costatum* were slightly greater in the sediment addition flasks than in the flasks containing only media. The addition of $\text{PO}_4^{3-}$ to the experimental flasks following the end of the experiment confirmed in all cases that P remained the nutrient limiting growth and biomass throughout the experiment.

The results can be summarized as follows: neither cyanobacteria species is capable of aerobic $\text{N}_2$ fixation; *S. cf. elongatus* does not produce an allelochemical that enhances its own growth while it does produce an allelochemical that selectively inhibits the growth of some co-occurring diatom species; and sediment resuspension did not enhance the growth of the cyanobacteria and/or selectively inhibit the growth of the diatom species.
Figure 5-1. Growth response of *Cyanothece* in media with and without nitrogen. (A) *In vivo* fluorescence: -N = ▲, +N = Δ; (B) Cell number and chlorophyll *a*: -N cell number = □, +N cell number = ■, -N Chl *a* = ○, +N Chl *a* = ●. Mean values and standard errors.
Figure 5-2. Growth response of *S. cf. elongatus* in media with and without nitrogen. (A) *In vivo* fluorescence: -N = △, +N = Δ; (B) Cell number and chlorophyll *a*: -N cell number = □, +N cell number = ■, -N Chl *a* = ○, +N Chl *a* = ●. Mean values and standard errors.
Figure 5-3. Growth response of *Synechocystis* in media with and without nitrogen. (A) *In vivo* fluorescence: -N = ▲, +N = △; (B) Cell number and chlorophyll a: -N cell number = □, +N cell number = ■, -N Chl a = ○, +N Chl a = ●. Mean values and standard errors.
Figure 5-4. Single species growth response in various concentrations of enriched *S. cf. elongatus* culture filtrate. (A) *S. cf. elongatus* in 0% filtrate = ○, 50% filtrate = ●, 100% filtrate = ■; (B) *T. cf. oceanica* in 0% filtrate = ○, 50% filtrate = ●, 100% filtrate = ■. Mean values and standard errors.
Figure 5-5. Single species growth response in various concentrations of enriched *S. cf. elongatus* culture filtrate. (A) *C. cf. salsugineus* in 0% filtrate = ○, 50% filtrate = ●, 100% filtrate = ■; (B) *S. costatum* in 0% filtrate = ○, 50% filtrate = ●, 100% filtrate = ■. Mean values and standard errors.
Figure 5-7. Chl a values of single species batch cultures under P limitation with and without sediment addition/resuspension. *S. cf. elongatus* = Δ, *S. cf. elongatus* + sediment = ▲, *T. cf. oceanica* = □, *T. cf. oceanica* + sediment = ■, Control (sediment + media) = +.
Nitrogen fixation

The two Florida Bay cyanobacteria clones were logical candidates for aerobic N₂-fixation, since marine species of both *Synechococcus* and *Synechocystis* have been found to aerobically fix nitrogen (Rippka et al. 1979, Reddy et al. 1993). Unicellular aerobic nitrogen fixation has also been documented for a spherical *Synechococcus* sp. (not *S. cf. elongatus*) from the Florida Keys (Phlips et al. 1989). The simple test for the ability to aerobically fix nitrogen in which growth is measured in an enriched growth medium lacking reduced nitrogen, revealed that neither Florida Bay *S. cf. elongatus* nor *Synechocystis* sp. isolate was able to grow in an enriched growth medium lacking combined nitrogen, suggesting an inability to fix nitrogen. Confirmation that the experimental conditions used in this study were adequate for aerobic nitrogen fixation was demonstrated by the experimental control, in which *Cyanothece* sp., a known unicellular N₂-fixer, grew in the media lacking combined N (Figs. 5-1, 5-2 and 5-3).

Although the cyanobacteria isolates were incapable of growth in ASNIII-N media for the duration of the experiment, they experienced limited growth in the ASNIII-N media during the initial days of the experiment, which warrants explanation. While constant *in vivo* and extracted Chl a values for *S. cf. elongatus* in ASNIII-N indicated that the population was not growing, the cell counts indicated some net growth (Fig. 5-2). Similarly in the ASNIII-N media, *Synechocystis* sp. *in vivo* values showed no growth while cell division occurred through day 8 and Chl a synthesis through day 3 (Fig. 5-3). The growth *S. cf. elongatus* and *Synechocystis* sp. in the ASNIII-N media during the early days of the experiment may be attributed to both DIN brought over with the inocula and cellular luxury stores of nitrogen. In addition, the *Synechocystis* sp. isolate is also a known producer of cyanophycin (Corbridge 1998), which has been speculated to serve as a nitrogen reserve for the cell during periods of very low nitrogen availability. The pattern of initial growth for several days followed by a lack of growth for the remainder of the experiment by the Florida Bay cyanobacteria isolates in ASNIII-N media was also repeatedly observed during attempts to establish cultures of each isolate in ASNIII-N media. Consequently, the inability of the Florida Bay cyanobacteria isolates to sustain growth in ASNIII-N media for an extended period of time is interpreted to reflect their inability to aerobically fix nitrogen.

The results of these ASNIII-N growth experiments have also been confirmed by acetylene reduction assays which indicated no nitrogenase activity for the Florida Bay *S. cf. elongatus* clone (Dr. Paul Carlson, Florida Marine Research Institute, pers. comm.). The possibility that the cyanobacteria clones *S. cf. elongatus* and *Synechocystis* sp. have the genetic capacity to fix nitrogen but require different environmental conditions from those used in this experimental study to express their genetic potential, can be dismissed because preliminary genetic analyses have confirmed that neither clone has the nitrogenase reductase (Nif) genes necessary for nitrogen fixation and therefore lack the genetic potential for N₂-fixation (Dr. Wayne Litaker, University of North Carolina at Chapel Hill, pers. comm.).
Lastly, although the cyanobacteria study clones inability to fix nitrogen may not be representative of all of the *Synechococcus* and *Synechocystis* strains in the bay, it remains unlikely that nitrogen fixation by strains of *Synechococcus* and *Synechocystis* play a significant role in the development of cyanobacteria dominated blooms in the north-central region. Although the phytoplankton community has at times been found to be N-limited in the north-central region, considered to be the epicenter or source of the recurring cyanobacteria dominated blooms, this region characteristically has elevated levels of water column ammonia (median 7.3 µM) that are on average twofold greater than that of the eastern bay and sevenfold higher than the western bay (Boyer et al 1999). These high levels of ammonia would provide no selective advantage to N$_2$-fixing cyanobacteria and would for the most part inhibit N$_2$-fixation in the water column.

*Allelochemic interactions*

Phytoplankton allelochemical interactions may be described as being: mutually advantageous, mutually exclusive, selectively inhibiting, selectively stimulating and indifferent (Smayda 1997). Support for the hypothesized production of allelochemicals by *S. cf. elongatus* is found in the fact that cyanobacteria as a group are known producers of bioactive substances (Carmichael 1992, Carmichael 1994, Nagle and Paul 1999), and studies have demonstrated that diatom growth rates can be inhibited by cyanobacteria (Keating 1978, 1997). A priori, it was hypothesized that *S. cf. elongatus* produced an allelochemical that stimulated its own growth and/or inhibited the growth of the other study species and that the effect of this allelochemical would be visible in the study species growth responses (i.e. lag phase, growth rate, yield, and survival).

The extended lags in growth, reduced growth rates and reduced final yields in the experimental treatments (50% and 100% filtrate media) relative to their controls (100% NSW) (Figs. 5-4 and 5-5), indicated that *S. cf. elongatus* did not produce a growth stimulating compound, but instead produced a selective growth inhibiting substance. Although all species except *S. cf. elongatus* experienced extended lags in growth in the experimental treatments, the fact that the experimental treatments eventually reached exponential rates of growth comparable to their controls, suggests that the inhibitory effect on growth rate was temporary. Allelochemic action on final yield was species-specific and concentration-dependent. While yields were reduced for all species in the 100% filtrate, being greatest in *T. cf. oceanica* (33% reduction) and least in *S. cf. elongatus* (10% reduction), the only reduction in yield in the 50% filtrate was seen in *T. cf. oceanica* (22% reduction).

The 100% filtrate media may be considered to be too concentrated as evidenced by the autoinhibitory effect observed for *S. cf. elongatus* (9% yield reduction). It is possible that the 9% reduction was the result of metabolites whose primary function is not allelochemic, but nonetheless at very high concentrations is capable of inhibiting the final yield. A conservative estimate of the % yield reduction attributable to the apparent action of *S. cf. elongatus* produced allelochemicals (excluding what might be considered to be non-species specific non-allelochemic metabolites) may be obtained by subtracting the 9% yield reduction of *S. cf. elongatus* in the 100% filtrate media from each species % yield reduction in 100%
filtrate media. After corrections, substantial reductions in yield still remained for *C. cf. salsugineus* and *T. cf. oceanica* in 100% filtrate media.

The simple experimental design that was used in this study to test for allelochemic interactions was not without some of the weaknesses that are inherent to many studies that have attempted to examine allelopathy in phytoplankton. Allelopathic interactions have long been suspected to influence phytoplankton succession and competition, but inadequately defined laboratory conditions (e.g. phytoplankton physiological state, use of abnormally dense cultures, non-axenic experimental cultures, etc.) permitted only the inference of allelopathic interactions (Maestrini and Bonin 1981). But evidence in support of phytoplankton allelochemic interactions has increased as more recent studies have successfully isolated, characterized and, through the use of bioassays, demonstrated the inhibitory action of phytoplankton allelochemicals at concentrations that are more representative of natural conditions (Gentien, 1985, Gentien and Arzul, 1990).

Although the design of my study shares some of the weaknesses common to earlier studies including the use of non-axenic cultures and the absence of conclusive evidence of allelochemic action, efforts were made to minimize some of the other common experimental weaknesses that would interfere with the detection of real allelochemic interactions. This included the use of exponentially growing populations in experimental cultures, filtrate from an exponentially growing population of *S. cf. elongatus* and cellular densities comparable to those observed for field populations. This was done to minimize the growth inhibiting effects that usually arise from non-allelochemic metabolite accumulation and overall water quality degradation that commonly occurs as cultures age. The density of the *S. cf. elongatus* culture used to obtain the filtrate was 4 million cells ml\(^{-1}\), which is comparable to the documented cyanobacteria dominated bloom densities in Florida Bay which may exceed 5 million *Synechococcus* cells ml\(^{-1}\) (Phlips et al. 1999). Nonetheless, it was still only possible in this study to infer that the observed species responses to the *S. cf. elongatus* filtrate were due to the action of *S. cf. elongatus* produced allelochemicals.

The goal of this study was to conduct a brief and simple preliminary screening for the potential presence of stimulatory and/or inhibitory compounds, which would then justify the more detailed studies under various environmental and physiological conditions that are necessary to demonstrate the real production of allelochemic compounds by *S. cf. elongatus*. Although definitive evidence of allelochemic interactions requiring the isolation of the chemical compound(s) and bioassays using the compound(s) were not carried out, the similar pattern of selective inhibition of the initial growth rates and yield reductions of the diatom species in both the 50% and 100% filtrate media, all lend support to the inference that *S. cf. elongatus* produces an allelochemical that may contribute to its dominance in Florida Bay phytoplankton blooms.

The natural bloom conditions that include high *S. cf. elongatus* cell densities, low basin water turnover rates, long bloom durations and periodic bloom recurrence are all favorable for *S. cf. elongatus* produced allelochemic interactions playing a potential regulatory role in Florida Bay phytoplankton community structure.
The hypothesis that sediment resuspension contributes to the dominance of *S. cf. elongatus*, through enhanced removal of phosphorus from the water column by carbonate adsorption, was not supported by the experimental results. Sediment resuspension was predicted to lower the growth rates and final yields of all of the species relative to their controls except *S. cf. elongatus*, through the removal of most or all of the available growth limiting DIP (≈ 1 µM PO$_4^{3-}$) in the experimental media. *S. cf. elongatus*, with the lowest K$_{up}$’s and a capability of maintaining near maximal growth rates at undetectable concentrations of phosphate (see Chapter IIA) was predicted to experience little or no decrease in growth rate. In contrast, the diatoms with higher K$_{up}$’s, in particular *T. cf. oceanica* (see Chapter IIA), were expected to have decreased growth rates and final yields in the sediment resuspension treatments. In complete opposition to the predicted results, all of the study species had higher final yields and equivalent growth rates in the sediment treatments relative to their controls, except for *S. cf. elongatus* whose yield and growth rate was unaffected (Figs. 5-5 and 5-6). Explanations for the greater yield in the sediment treatments include: sedimentary inorganic and organic phosphorus in excess of the sediment carbonate particle adsorption capacity; sedimentary organic phosphorus not readily adsorbed by the carbonate sediment particles; and inorganic and organic phosphorus that was released by the sterilization process and not readsorbed. This suggests that although the dominance of *S. cf. elongatus* in the phytoplankton blooms in the areas subject to large and frequent sediment resuspension may be in part the result of interspecific competition under sustained P-limiting conditions, the ambient P-limited conditions do not appear to be entirely brought about by, enhanced or maintained by the action of sediment resuspension. To the contrary, these results highlight the potential for Florida Bay sediments high in carbonate and organic content to act as a source of readily utilizable phosphorus that can be efficiently used by the phytoplankton.

Some marine cyanobacteria are known to play a role in physical/chemical processes that lead to the formation of carbonate precipitates known as whitings (Robbins and Blackwelder 1992). This led to the consideration that *S. cf. elongatus* might also develop close physical/chemical associations with carbonate particles in Florida Bay. The hypothesis that sediment resuspension contributes to the dominance of *S. cf. elongatus* by providing increased opportunities for *S. cf. elongatus* to establish a close physical association between its phycosphere and the resuspended carbonate sediment particles during which time it is able to extract phosphorus from the surface of the suspended sediment particles was also not supported by the results. The results indicate that *S. cf. elongatus* does not possesses any unique ability to extract P from suspended sediment particles, as its final yield and growth rate in the sediment treatment was not enhanced relative to it's control (Figs. 5-6 and 5-7). It is also possible that the hypothesized P-extraction capability of *S. cf. elongatus* may have gone undetected, being smaller and overwhelmed by the much larger growth resulting from phosphorus that was released by the sediments. The results suggest that the dominance of *S. cf. elongatus* in Florida Bay is not due to any unique ability to harvest bound P from resuspended carbonate particles through the development of a close association with those particles.
The hypothesis that sediment resuspension contributes to the dominance of a particular species or taxonomic group, by the action of a selective growth stimulating and/or inhibiting factor whose supply to the overlying water column is enhanced by sediment resuspension was also not supported by the results. Although the growth of all of the species was enhanced by sediment resuspension, no species or group appeared to be selectively favored (Figs. 5-6 and 5-7). Evidence for the release of a selective inhibitory factor from the sediment that favors *Synechococcus* spp. over other planktonic algae is found in the study of Sorokin et al. (1996). They found that a large bloom dominated by species of *Synechococcus* in the shallow water lagoons of Comacchio, in the Adriatic Sea was selectively enhanced by a bloom-sediment interaction. When non-grazed cyanobacterial bloom cells sedimented, the algal biomass fueled sulfide production in the sediments. The sulfides and free H\(_2\)S entered the water column and created a toxic sulfur environment that inhibited the growth of eucaryotic algae through cytochrome poisoning, while the cyanobacteria *Synechococcus* spp. remained largely unaffected. *Synechococcus* spp. are known to tolerate sulfides and anoxia (Schmidt 1988) as well as other conditions, which are normally unfavorable for the growth of other eucaryotic phytoplankton species (Stockner 1988). Florida Bay sediments in seagrass die-off areas, which are in part spatially coincident with the recurring large cyanobacteria dominated blooms also have been found at times to have very high levels of sulfides in pore waters (Carlson et al. 1994). Since some species of *Synechococcus* have been found to have a benthic stage (Thompson et al. 1990) it may be hypothesized that the Florida Bay *Synechococcus* bloom species may be able to populate the higher sulfide exposed sediment surface and flourish under conditions that would be unfavorable for the other competing eucaryotic species.

Trace nutrients and unidentified growth promoting compounds (e.g. metals, vitamins and chelators) found in the organic matter of soil and sediments have been shown to selectively enhance the growth of many species of phytoplankton in culture. The frequent spatial coincidence of the cyanobacterial blooms, organic-rich sediment resuspension and high concentrations of POM and DOM in the north central region suggest that organic growth promoting compounds may play a role in the dominance of *Synechococcus* spp. in this region. But the results gave no indication that a phytoplankton stimulatory factor was released from the sediment. The possibility that an inhibitory or stimulatory factor was inactivated by the sterilization process or that it was not in the field sediment sample used in the experiment due to seasonal variability cannot be discounted. Future bioassays carried out using site water and sediment samples collected over larger spatial and temporal scales, sterilized using ultraviolet light and then spiked with clonal cultures of the study species should be considered.

The experimental results also did not support the hypothesis that sediment resuspension and subsequent sedimentation under P-limiting conditions contributes to the taxonomic dominance of cyanobacteria by the selective removal of other co-occurring phytoplankton species when they co-flocculate with settling resuspended sediments. In this study, *T. cf. oceanica* was found to produce excessive extracellular mucopolysaccharides under extended P-limitation, causing them to adhere to the glass culture flasks. The production of extracellular polysaccharides during P-limitation has also been
observed with other taxa (Avnimelech et al. 1982, Soballe and Threlkeld 1988), as has co-flocculation of phytoplankton with sediment particles introduced into the water column and their subsequent settling out of the photic zone (Burkholder and Cuker 1991). It was therefore hypothesized that phytoplankton such as *T. cf. oceanica* when subjected to P limitation and sediment resuspension are selectively removed from the water column with the sedimenting particles. But, *T. cf. oceanica* showed no sign of being selectively removed from the water by co-flocculation with the sediment as Chl *a* and *in vivo* measurements, were not lower but actually higher in the sediment treatments (Figs. 5-6 and 5-7). This indicates that overall population growth exceeded any loss that may have occurred as a result of co-flocculation. Consequently, the relative dominance of *S. cf. elongatus* in blooms under P-limitation does not appear to be significantly enhanced through selective removal of other co-occurring species (such as *T. cf. oceanica*) that form extracellular mucopolysaccharides under P-limiting conditions and are therefore susceptible to co-flocculation with resuspended sediment particles.

**Conclusions**

1) The success of *S. cf. elongatus* and *Synechocystis* sp. in Florida Bay cannot be attributed to an ability to aerobically fix nitrogen under N-limiting conditions.

2) At very high cellular densities *S. cf. elongatus* may promote its dominance via allelochemic reduction of the yields of the co-occurring competing diatom species *C. cf. salsugineus*, *T. cf. oceanica* and *S. costatum*.

3) The dominance of *S. cf. elongatus* in the blooms in the regions which experience frequent sediment resuspension, does not appear to be the result of any of the following: sediment resuspension enhanced P-limited conditions favoring the superior P-competitiveness of *S. cf. elongatus*; a unique sediment-cell physical association between *S. cf. elongatus* and resuspended sediment particles in which *S. cf. elongatus* is able to extract phosphorus from the resuspended particles; an unidentified selective inhibitory or stimulatory compound released from the sediment; or the selective loss of other co-occurring species such as *T. cf. oceanica* as a result of co-flocculation with resuspended sediment particles under P-limited conditions.

**References**


Rudnick, D., S. Kelley and C. Donovan. 2001 Patterns of inorganic nitrogen flux from northern Florida Bay sediments. Florida Bay Science Conference. Key Largo, FL.


CONCLUSIONS

1) The broader salinity tolerances of the cyanobacteria species will favor the development of cyanobacteria (*S. cf. elongatus* and *Synechocystis* sp.) dominated blooms over diatom dominated blooms (*C. cf. salsugineus* and *T. cf. oceanica*) above salinities of 50 \%/oo, as well as below salinities of 10 \%/oo. The development and persistence of cyanobacteria dominated blooms predominantly in the north-central region which has a history of recurring hypersaline conditions, may be due in part to the cyanobacteria species much greater tolerance of hypersaline conditions.

2) At moderate salinities (10 \%/oo - 50 \%/oo) the diatom species much larger *U* m’s, and larger *α* g’s places them at a competitive advantage over the cyanobacteria species under both saturating and light limiting conditions. The prevalence of saturating irradiances throughout the majority of Florida Bay favors the development of diatom (*C. cf. salsugineus* and *T. cf. oceanica*) dominated blooms over cyanobacteria (*S. cf. elongatus* and *Synechocystis* sp.) dominated blooms.

3) The cyanobacteria species *S. cf. elongatus* and to a lesser degree *Synechocystis* sp. are superior P-competitors to the diatoms *C. cf. salsugineus* and *T. cf. oceanica*.

4) The superior P-competitiveness of the cyanobacteria may be explained in part not only by their generally lower *K* uP’s, but by the minimal negative influence that suboptimal growth salinities have on their *U* m’s and possibly on their *K* uP’s. Similarly, the inferior P-competitiveness of the diatoms may be explained in part not only by their generally higher *K* uP’s, but by the negative influence that sub-optimal growth salinities have on their *U* m’s and possibly on their *K* uP’s.

5) *S. costatum* is the superior N-competitor and *C. cf. salsugineus* is the most inferior N-competitor. *T. cf. oceanica*, *S. cf. elongatus* and *Synechocystis* sp. all have similar N-competitive abilities.

6) The following conditions did not influence species relative dominance: non-equilibrium conditions of P or N pulsing every 6th day, ambient salinity (15, 25 or 50 \%/oo) under P limitation, fluctuations in salinity (~1 \%/oo da⁻¹) and the form of limiting P; whereas under N limitation both the form of limiting N and the ambient salinity had a slight influence on species relative dominance.

7) The frequently observed dominance of the phytoplankton community in the north-central region of Florida Bay by *S. cf. elongatus* and *Synechocystis* sp. may be due in part to extended
periods of P limitation, while the less frequent co-dominance by *T. cf. oceanica*, *S. cf. elongatus* and *Synechocystis* sp. may be explained in part by extended periods of N limitation.

8) The frequent dominance of blooms in the north-central region by *S. cf. elongatus* where P limitation alternates with N limitation, may also be explained by the magnitude of interspecific differences in species R$_N$’s and R$_P$’s. A long-term cumulative net increase in cyanobacteria biomass could be achieved under alternating periods of N and P limitation of by: (1) the rapid displacement of other competing species by the cyanobacteria under P limitation due to their much lower R$_P$’s, and (2) the minimal interspecific competitive displacement under N limitation due to the similarity of the competing species R$_N$’s. As a result, the competitive advantages that the cyanobacteria have over the diatom species under P limitation, coupled with the species similar competitiveness under N limitation, may both contribute to cyanobacteria dominance of the community in the north-central region where P and N limitation periodically occur.

9) The conspicuous absence of *S. costatum* from most of Florida Bay may be best explained by its very poor P-competitiveness and the P limiting conditions that exist throughout much of the bay.

10) The proportionally greater elevations of the diatoms Q$_{oP}$’s and Q$_{oN}$’s at suboptimal salinities for growth than those of the cyanobacteria may place the diatoms at a competitive disadvantage when either P or N limiting conditions co-occur with either very low or very high salinities. As a result, hyposaline and hypersaline conditions in Florida Bay may promote cyanobacteria dominance by not only differentially lowering the diatoms U$_m$’s and possibly elevating their K$_w$’s, but also by elevating their Q$_{oP}$’s and Q$_{oN}$’s.

11) The frequent dominance of cyanobacteria in Florida Bay blooms is not due to any of the following factors: (1) a more efficient utilization of limiting phosphorus or nitrogen per unit biomass (2) greater luxury storage capacities for either N or P (3) interspecific differences in the species C, N or P utilization ratios (4) an ability to aerobically fix nitrogen under N-limiting conditions (5) conditions brought about by sediment resuspension that selectively enhance cyanobacteria growth and/or inhibit diatom growth.

12) The large differences in optimum N:P ratios between the cyanobacteria and the diatoms suggests that N:P supply ratios may play a role in the development of group dominance (i.e. diatom vs. cyanobacteria) in the phytoplankton blooms of Florida Bay.

13) At very high cellular densities, *S. cf. elongatus* may promote its dominance via allelochemic reduction of the yields of the co-occurring competing diatom species *C. cf. salsugineus*, *T. cf. oceanica* and *S. costatum*. 
APPENDICES
### APPENDIX 1. Species growth rates at experimental salinities.

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at 15°/00, 1st set, S. cf. elongatus.

**Competition at S=15**

**P-limited**

**Semicontinuous treatment**

**S. cf. elongatus**

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180
APPENDIX 5. Batch growth nitrogen and phosphorus cell quotas.

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APPENDIX 6. Semicontinuous dilution nitrogen and phosphorus cell quotas.

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<th>( \text{Synechocystis sp.} ) P-limited Flask</th>
<th>( \text{Synechocystis sp.} ) N-limited Flask</th>
<th>( T. \text{ cf. oceanica} ) P-limited Flask</th>
<th>( T. \text{ cf. oceanica} ) N-limited Flask</th>
<th>( C. \text{ cf. salsugineus} ) P-limited Flask</th>
<th>( C. \text{ cf. salsugineus} ) N-limited Flask</th>
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<th>( \text{Synechocystis sp.} ) N-limited Flask</th>
<th>( \text{Synechocystis sp.} ) P-limited Flask</th>
<th>( T. \text{ cf. oceanica} ) N-limited Flask</th>
<th>( T. \text{ cf. oceanica} ) P-limited Flask</th>
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APPENDIX 7. Study rationale.

Study Rationale

- Hypersalinity
- Turbidity
- P limitation
- N limitation
- Cyano blooms
- Long Water Residence Time

Is cyanobacterial bloom dominance influenced by:
- Salinity
- Light
- P limitation
- N limitation
- Form of N or P
- Frequency of N or P supply
- N:P supply ratio
- N₂-fixation
- Allelochernic interactions
- Sediment resuspension
APPENDIX 8. Study species.

Study Species

Cyanobacteria

*Synechococcus cf. elongatus*
*Synechosystis sp.*

Diatoms

*Chaetoceros cf. salsugineus*
*Thalassiosira cf. oceanica*

*Skeletonema costatum*
APPENDIX 9. Predicted group dominance along a salinity gradient.

![Predicted Dominance Along A Salinity Gradient](image)

Salinity (‰) 5 15 45 60
APPENDIX 10. Predicted group bloom dominance during reduced salinities.

Predicted Group Bloom Dominance During Reduced Salinities

Salinity isopleths October 18-20, 1999, redrawn from South Florida Ecosystem Research and Monitoring Program: http://mpo.rsmas.miami.edu/flabey/foe.html
APPENDIX 11. Predicted group bloom dominance during hypersaline conditions.

Predicted Group Bloom Dominance During Hypersaline Conditions

Salinity isopleths June 1989 – August 1990, redrawn from Fourquarean et al. (1993)
APPENDIX 12. Predicted group dominance along gradients of salinity and light.
APPENDIX 13. Predicted group bloom dominance in response to light.

Predicted Group Bloom Dominance Response to Light

Periodically Light limited regions
APPENDIX 14. Regions of nutrient limitation in Florida Bay.
APPENDIX 15. Species relative competitive ranking for phosphorus and nitrogen.

P & N Competitive Ranking

P-limitation

[S. cf. elongatus] > Synechosystis sp. > C. cf. salsugineus > T. cf. oceanica > S. costatum

Cyanobacteria

Winners

N-limitation


Diatoms & Cyanobacteria

Winners
APPENDIX 16. Predicted group dominance along gradients of nutrient limitation and salinity (excluding *S. costatum*).
APPENDIX 17. Predicted group dominance under P limiting conditions along a salinity gradient.

**Predicted Group Dominance Under P-limiting Conditions Along A Salinity Gradient**

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<td>&gt; D</td>
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APPENDIX 18. Predicted group bloom dominance under P limiting conditions.
APPENDIX 19. Predicted species relative dominance under P limiting conditions.
APPENDIX 20. Predicted group dominance under P limiting conditions along a salinity gradient.

**Predicted Group Dominance Under P-limiting Conditions Along A Salinity Gradient**

Cyanobacteria

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APPENDIX 21. Predicted species relative dominance along an N:P supply gradient.
APPENDIX 22. Predicted species relative dominance when the central region is alternately P-limited and N-limited.
About the Author

Ralph Richardson received a Bachelor of Science in Biology from Oregon State University in 1972. In 1982 he earned a Masters of Science in Entomology at Pennsylvania State University. In 1989 he enrolled in the University of South Florida’s College of Marine Science. In 1993 he began his current position with the Florida Fish and Wildlife Conservation Commission, where he is researching harmful algal blooms. Dramatic ecological changes were observed in Florida Bay in the late 1980’s. These changes included the appearance of large, persistent and recurring algal blooms frequently dominated by cyanobacteria. Efforts were been made to determine the factors that regulated the initiation, growth, development, persistence and decline of the blooms. Factors that may have contributed to the frequent dominance of these blooms by cyanobacteria became the focus of his dissertation.