EFFECTS OF THE VERTICAL STRUCTURE OF THE WATER COLUMN ON THE PHYTOPLANKTON IN A SHALLOW, LAGOONAL ESTUARY

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ABSTRACT

NATHAN SAMUEL HALL: Effects of the vertical structure of the water column on the phytoplankton in a shallow, lagoonal estuary (under the direction of Hans W. Paerl)

Phytoplankton community composition is an important determinant of the effects of eutrophication in coastal systems. However, the system specific attributes governing the phytoplankton response to anthropogenic nutrient loading are still poorly resolved. The mixing regime of the Neuse River Estuary is an unusual attribute of the ecosystem. Unlike lakes and open ocean systems, stratification is primarily determined by salinity rather than temperature. Unlike most estuaries where tidal straining is the dominant mixing process, astronomical tides are negligible in the Neuse River Estuary. As a result, riverine discharge and wind stress determine oscillations between well-mixed and poorly mixed conditions on time scales of hours to days rather than the seasonal time scales of lakes and oceans or the daily time scales of most estuaries.

The purpose of this study was to determine how this unusual pattern of mixing affects the phytoplankton community. The observed negative correlation between diatom biomass and stratification intensity indicates that settling losses significantly impact diatom biomass. Wind energy for mixing is linked to the maintenance of the diatoms through increases in the eddy diffusivity of the upper layer, deepening of the pycnocline, and resuspension from the sediments. When mixing is weak, growth limiting nutrients often accumulate in the bottom waters. The depth of the euphotic zone approximates the depth of the pycnocline creating a
strong tendency for vertically separated light and nutrient resources. Under these conditions flagellates, particularly dinoflagellates and cryptophytes, use vertical migrations to access light and nutrients for growth. Water column stability does not appear to have an effect on flagellate biomass. However, as diatom biomass decreases under stratified conditions, flagellates dominate.

The linkage between water column stability and community composition helps explain previously observed spatial and temporal distributions of community composition within the Neuse River Estuary. Additionally, average intensities of wind induced mixing may explain why the Neuse River Estuary is dominated by flagellates while tidally forced estuaries, such as Chesapeake Bay and San Francisco Bay, are dominated by diatoms. Eutrophication models may be improved by separately simulating the ecologically distinct diatom and flagellate groups.
This dissertation is dedicated…. 

In honor of my mom and dad, 
Mary Elizabeth Anderson Hall and Stephen Lane Hall

Thank you for your endless love and support.
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# TABLE OF CONTENTS

LIST OF TABLES……………………………………………………………………………………...xii

LIST OF FIGURES……………………………………………………………………………………xiii

LIST OF ABBREVIATIONS AND SYMBOLS…………………………………………………xvii

CHAPTER

1 Background, goals of this study, and overview of the chapters..............................1

1.1 Background.............................................................................................................2

1.1.1 The eutrophication problem..............................................................................2

1.1.2 System specific attributes modulate eutrophication effects.........................2

1.1.3 Phytoplankton structure modulates effects of eutrophication.....................3

1.1.4 Study site, the Neuse River Estuary.................................................................5

1.1.5 Phytoplankton biomass in the Neuse River Estuary......................................7

1.1.6 Nutrient limitation............................................................................................8

1.1.7 Phytoplankton community composition.....................................................8

1.2 Goals of this study...............................................................................................11

1.3 Overview of the chapters.....................................................................................11

1.4 Literature Cited....................................................................................................18

2 Diel patterns of phytoplankton depth distributions in relation to light and nutrient availability: Results from three diel studies.........................................................24

2.1 Introduction..........................................................................................................27

2.2 Methods and materials........................................................................................30
2.2.1 Study site.................................................................30
2.2.2 Sample collection.......................................................31
2.2.3 Physical data..........................................................32
2.2.4 Nutrient and chlorophyll \( a \) analyses..............................32
2.2.5 Primary productivity and nutrient uptake..........................33
2.2.6 Cell counts............................................................35
2.2.7 Analysis of phytoplankton profiles.................................35
2.2.8 Comparison of conditions with long-term monitoring data......36
2.3 Results......................................................................37
  2.3.1 Weather conditions...................................................37
  2.3.2 Temperature and salinity..........................................38
  2.3.3 Nutrients and nutrient uptake ..................................38
  2.3.4 Light and productivity profiles ..................................40
  2.3.5 Vertical distribution of phytoplankton in May .................41
  2.3.6 Vertical distribution of phytoplankton in June ...............42
  2.3.7 Vertical distribution of phytoplankton in July ...............43
  2.3.8 Diel patterns of depth distribution ..............................45
  2.3.9 Comparison of conditions with long-term monitoring data ..46
2.4 Discussion...............................................................48
2.5 Literature Cited..........................................................76

3 Dominant modes of variability in phytoplankton depth distributions in relation to light and nutrient availability: Results from bi-hourly, decimeter-scale profiles of phytoplankton biomass ..............................................................................81
  3.1 Introduction..................................................................84
3.2 Methods and materials

3.2.1 Study sites

3.2.2 Data collection

3.2.3 Data processing

3.2.4 Data analyses

3.2.5 Phytoplankton photophysiology

3.3 Results

3.3.1 Temperature, salinity, and degree of stratification

3.3.2 Fluorescence

3.3.3 Nutrients

3.3.4 Light availability

3.3.5 Representative data from the AVP

3.3.5.1 April 2002

3.3.5.2 June 2003

3.3.5.3 November 2003

3.3.6 Influence of degree of stratification on vertical patchiness of phytoplankton

3.3.7 Local wavelet spectra of CV_{chl}, Z_{max}, and Z_{cent}

3.3.8 Global wavelet and Fourier spectra of CV_{chl}, Z_{max}, and Z_{cent}

3.3.9 Local diel power and phase

3.3.10 Diurnal signal strength during different stratification intensity and temperature regimes

3.3.11 Influence of Z_{pyc} and H on Z_{max} and Z_{cent}

3.3.12 Global wavelet and Fourier spectra of Z_{pyc}, H, and Δρ
5 Environmental factors contributing to the development and demise of a toxic dinoflagellate (*Karlodinium veneficum*) bloom in a shallow, eutrophic, lagoonal estuary

5.1 Introduction

5.2 Methods and materials

5.2.1 Study site, sample collection, and chemical analyses

5.2.2 Cell counts

5.2.3 Physical data

5.2.4 Photopigment, DNA, and toxin analyses

5.3 Results

5.3.1 Bloom characteristics

5.3.2 Bloom chronology

5.3.3 Fish kills

5.4 Discussion

5.4.1 Summary

5.4.2 Bloom initiation

5.4.3 Growth phase of the bloom

5.4.4 Bloom termination

5.4.5 Association with fish kills

5.5 Conclusion

5.6 Acknowledgments
6 Summary, conclusions, and management implications………………………………………307

6.1 Summary……………………………………………………………………………………308
6.2 Conclusions………………………………………………………………………………309
6.3 Management implications………………………………………………………………311
   6.3.1 Improved eutrophication models………………………………………………311
   6.3.2 Phytoplankton as indicators…………………………………………………..314
6.4 Literature Cited…………………………………………………………………………316
LIST OF TABLES

Table 2.1 Diffuse light attenuation coefficient, the depth of the euphotic zone, and depth of the pycnocline........................................................................................................52

Table 2.2 NH$_4^+$ concentrations, minimum uptake rates, and maximum turn over times for the NH$_4^+$ pool ........................................................................................................53

Table 3.1 Select physical and chemical properties of the AVP sampling stations during the study period ........................................................................................................................................124

Table 3.2 Least squares estimates of the diel signal in Z$_\text{max}$ and Z$_\text{cent}$ from NR 120 and NR 180 under different stratification and temperature regimes ................................................. 125

Table 3.3 Correlations between physical parameters and phytoplankton depth distribution indices........................................................................................................................................126

Table 4.1 Summary of the dominant phytoplankton groups and their biovolumes.........205

Table 4.2 Mean contribution of each phytoplankton group to total phytoplankton abundance and biovolume.......................................................................................................................... 206

Table 4.3 Spearman’s rank correlations between $\Delta \rho$ and measures of phytoplankton biomass (abundance, biovolume, chlorophyll $a$) at the community and class/group levels ..........207

Table 4.4 Results of step-wise multiple regression analyses for physical and chemical parameters on each phytoplankton group........................................................................................................208

Table 5.1 Summary of surface and bottom Karlodinium veneficum abundance, particulate organic carbon, particulate nitrogen, and photopigment data from the bloom maximum at station 26 on 19 October 2006.........................................................................................................................276

Table 5.2 Average abundance and apparent growth rates of dominant phytoplankton and microzooplankton species within the bloom region (ModMon stations 19, 26, 28, 37, and 43) during the bloom growth (3-19 October) and collapse (19-30 October) phases……………….278

Table 5.3 Regression equations and statistical results of HPLC chlorophyll $a$ on in vivo fluorescence and toxin concentration and DNA copy # on Karlodinium veneficum cell abundance ........................................................................................................................................280

Table 5.4 Summary of fish kill investigations following the observed Karlodinium veneficum bloom ........................................................................................................................................281
LIST OF FIGURES

Figure 1.1  Map of the NRE showing sampling stations for the Neuse River Estuary Modeling and Monitoring Program ................................................................. 14

Figure 1.2  Average class-level community composition of the NRE, the Chesapeake Bay, and San Francisco Bay ................................................................. 16

Figure 2.1  Map of the Neuse River Estuary and sampling stations for Chapters 2-4 ...... 54

Figure 2.2  Average temperature and salinity profiles during three diel observations .... 56

Figure 2.3  Depth profiles of PAR, chlorophyll a, primary productivity, and assimilation during mid-day productivity assays ....................................................... 58

Figure 2.4  Time series of depth profiles of DIN and PO$_4$ during three diel studies .... 60

Figure 2.5  Phytoplankton vertical distribution patterns during the 2-3 May 2001 diel study ........................................................................................................ 62

Figure 2.6  Phytoplankton vertical distribution patterns during the 12-13 June 2001 diel study ........................................................................................................ 64

Figure 2.7  Vertical distribution patterns of the cell abundance of common flagellate species during the June and July diel studies ....................................................... 66

Figure 2.8  Phytoplankton vertical distribution patterns during the 17-18 July 2001 diel study ........................................................................................................ 68

Figure 2.9  Diel patterns in vertical distribution of several phytoplankton species or groups ............................................................................................................ 70

Figure 2.10  Diel patterns in vertical distribution of several phytoplankton classes and total phytoplankton biomass ................................................................. 72

Figure 2.11  Long term median values of the down estuary distributions of surface and bottom water DIN, chlorophyll a, Z$_{euph}$, Z$_{pyc}$, and $\Delta\rho$ during warm and cold seasons ......................................................... 74

Figure 3.1  Time series of average water column temperature, salinity, fluorescence, and $\Delta\rho$ from the AVP deployments ................................................................. 127

Figure 3.2  Time series of surface and near bottom DIN and PO$_4$ concentrations during the AVP deployments ................................................................. 129
Figure 3.3  Time series of $Z_{euph}$ and $Z_{pyc}$ during the AVP deployments …………………131

Figure 3.4  Example AVP chlorophyll and density data from 8-14 April 2002 at NR 120: a time period of dense aggregation of phytoplankton biomass at the pycnocline …………..133

Figure 3.5  Vertical profiles of *Prorocentrum minimum*, small (<20 μm diameter) centric diatoms, and PAR………………………………………………………………………………..135

Figure 3.6  Photosynthesis versus irradiance plot for *Prorocentrum minimum*………………..137

Figure 3.7  Example AVP fluorescence and density data from 25 June to 2 July 2003 at NR 180…………………………………………………………………………………………………….139

Figure 3.8  Vertical profiles of *Scrippsiella trochoidea*, small (<20 μm diameter) centric diatoms, and PAR………………………………………………………………………………..141

Figure 3.9  Photosynthesis versus irradiance plot for *Scrippsiella trochoidea*………………..143

Figure 3.10  Example AVP fluorescence and density data from 3-19 November 2003 at NR 180…………………………………………………………………………………………………………145

Figure 3.11  CV _chl_ versus $\Delta \rho$ during the AVP deployments ……………………………147

Figure 3.12  Time localized wavelet power spectra of CV _chl_, $Z_{max}$, and $Z_{cent}$ for the AVP data ………………………………………………………………………………………….149

Figure 3.13  Time averaged wavelet power spectra and Fourier power spectra of CV _chl_, $Z_{max}$, and $Z_{cent}$ for the AVP data …………………………………………………………………………………..151

Figure 3.14  Power and phase of the diel component of $Z_{max}$ and $Z_{cent}$ from the AVP data ………..153

Figure 3.15  Time averaged wavelet power spectra of H, $Z_{pyc}$ and $\Delta \rho$ for the AVP data…..155

Figure 3.16  Average profiles of day-time and night-time fluorescence and density gradient for the AVP data……………………………………………………………………………………………………157

Figure 4.1  Margalef’s Mandela………………………………………………………………………………209

Figure 4.2  Relationship between the variance of $Z_{pyc}$ and $\Delta \rho$……………………………………211

Figure 4.3  Time series of average water column temperature, salinity, turbidity, and $\Delta \rho$…213

Figure 4.4  Relationships between key parameters describing the physical structure of the water column………………………………………………………………………………………………215

Figure 4.5  Time series of surface and bottom water DIN and PO₄⁻³ and light attenuation………………217
Figure 4.6  Correlations between $\Delta \rho$ and the abundance and biovolume of diatoms and dinoflagellates. 219

Figure 4.7  Correlations between $\Delta \rho$ and the cell abundance of three size classes of diatoms. 221

Figure 4.8  Correlations between $\Delta \rho$ and the cell abundance and biovolume of centric and pennate diatoms and the fraction of total diatom cell abundance and biovolume represented by pennate diatoms. 223

Figure 4.9  $\Delta \rho$ versus the fractions of the total phytoplankton biovolume composed of diatoms and dinoflagellates. 225

Figure 4.10  Vertical profiles of diatom and dinoflagellate abundance and biovolume. 227

Figure 4.11  Comparison of vertically averaged versus surface diatom and dinoflagellate abundance and biovolume. 229

Figure 4.12  Centric and pennate diatom abundance on the sediment surface from a cross-river transect of the lower NRE. 231

Figure 4.13  Resuspension events during the spring of 2004 at NR180. 233

Figure 4.14  Power spectra of the occurrence of super-critical wave orbital velocities. 235

Figure 4.15  Annual cycle in the NE-SW wind velocity component for the NRE. 237

Figure 4.16  Seasonal and down estuary relationship between diatom abundance and water column stability. 239

Figure 5.1  Map of the Neuse River Estuary showing locations of sampling stations and documented fish kill events. 283

Figure 5.2  Toxin analysis from the bloom sample collected on 19 October 2006 at station 26. 285

Figure 5.3  Downstream distribution of surface water *Karlodinium veneficum* cell abundance and DNA copy number on five sampling dates spanning the bloom period. 287

Figure 5.4  Time series of riverine discharge and salinity. 289

Figure 5.5  Time series of environmental data from channel marker CM 11 for the bloom period, October 2006. 291
Figure 5.6  Estuarine conditions in the downstream and vertical dimensions on 3 October 2006…………………………………………………………………………………………293

Figure 5.7  Estuarine conditions in the downstream and vertical dimensions on 19 October 2006…………………………………………………………………………………………295

Figure 5.8  Surface water abundance of the numerically dominant dinoflagellates and diatoms at the three stations (19, 26, and 28) encompassing the frontal region on 19 October 2006…………………………………………………………………………………………297

Figure 5.9  Downstream and vertical distribution of chlorophyll a within the bloom region on 26 October 2006…………………………………………………………………………………………299
# LIST OF ABBREVIATIONS AND SYMBOLS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVP</td>
<td>autonomous vertical profiler</td>
</tr>
<tr>
<td>Chl-a</td>
<td>chlorophyll $a$</td>
</tr>
<tr>
<td>CV$_{chl}$</td>
<td>coefficient of variation of a fluorescence profile</td>
</tr>
<tr>
<td>$\Delta \rho$</td>
<td>difference in density between bottom and surface of water column</td>
</tr>
<tr>
<td>$\bar{\Delta \rho}$</td>
<td>average $\Delta \rho$ for the week prior to phytoplankton sample collection</td>
</tr>
<tr>
<td>DIN</td>
<td>dissolved inorganic nitrogen</td>
</tr>
<tr>
<td>DVM</td>
<td>diel vertical migration</td>
</tr>
<tr>
<td>H</td>
<td>water column depth</td>
</tr>
<tr>
<td>$K_d$</td>
<td>diffuse light attenuation coefficient</td>
</tr>
<tr>
<td>NR</td>
<td>Neuse River</td>
</tr>
<tr>
<td>NRE</td>
<td>Neuse River Estuary</td>
</tr>
<tr>
<td>PAR</td>
<td>photosynthetically active radiation</td>
</tr>
<tr>
<td>$Z_{cent}$</td>
<td>depth of the center of mass of a fluorescence profile</td>
</tr>
<tr>
<td>$Z_{euph}$</td>
<td>depth of the euphotic zone</td>
</tr>
<tr>
<td>$Z_{max}$</td>
<td>depth of the fluorescence maximum of a fluorescence profile</td>
</tr>
<tr>
<td>$Z_{pyc}$</td>
<td>depth of the pycnocline</td>
</tr>
<tr>
<td>$Z_{UML}$</td>
<td>depth of the upper mixed layer</td>
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Chapter 1

BACKGROUND, GOALS OF THIS STUDY,
AND OVERVIEW OF THE CHAPTERS
1.1. Background

1.1.1. The eutrophication problem—Eutrophication is defined as the increase in the rate of supply of organic matter to an ecosystem (Nixon 1995). In coastal and estuarine systems, eutrophication is principally due to enhancement of phytoplankton organic matter production via anthropogenic increases in loads of mineral nutrients that enhance their growth (Nixon 1995). Nitrogen and phosphorous from point and non-point sources such as agriculture, municipal waste water treatment, combustion of fossil fuels, etc. enter estuaries via surface runoff, groundwater discharge, and atmospheric deposition (Paerl 1997). Often eutrophication of estuarine and coastal waters is accompanied by detrimental changes to the ecosystem. Common symptoms are periodic or seasonal hypoxia of bottom waters, elevated baseline levels of phytoplankton biomass, changes in the composition of the phytoplankton community, and increased frequency of algal blooms all of which can have dramatic cascading effects through the ecosystem (Paerl 1988; Baird and Ulanowicz 1989; Sellner et al. 2001). As a result, the National Research Council considers coastal eutrophication to be the largest environmental problem impacting the coastal zone of the United States (National Research Council 2000).

1.1.2. System-specific attributes modulate eutrophication effects—Increasingly, the nutrient loading to estuaries around the world is a function of the rapidly expanding human populations and the activities of those populations within the water sheds (Peierls et al. 1991). However, for a given level of nutrient loading, estuaries display dramatic differences in their degree of susceptibility to eutrophication and its associated symptoms (NRC 2000;
Cloern 2001). System specific attributes such as basin morphology and the physical
circulation regimes within an estuary often play a role that is as important or even more
important than biological factors in determining a systems eutrophication potential (NRC
2001; Cloern 2001).

Estuaries that are well flushed by tides or river flow nearly always display lower
phytoplankton biomass than their poorly flushed counterparts (Monbet 1992; Cloern 2001). In
large part, this is due to dilution. However, nutrient concentrations within macrotidal
estuaries are also affected by dilution and the observation that microtidal estuaries generally
have much higher phytoplankton biomass at the same ambient nutrient concentrations
suggests that some other aspect of tidal mixing affects the efficiency of transferring nutrient
inputs into phytoplankton biomass (Monbet 1992). Macrotidal estuaries typically have much
higher concentrations of suspended sediment and more strongly mixed water columns. As a
result of the combination of these factors, phytoplankton growth is often light limited and
regardless of the nutrient load, phytoplankton biomass is generally low (Cloern 1999;
Monbet 1992). In contrast, in microtidal estuaries, dilution represents a smaller loss factor to
the phytoplankton community and suspended sediment concentrations are usually an order of
magnitude lower (Monbet 1992). As a result, the degree of light limitation within microtidal
estuaries depends more strongly on water column depth (Cloern 1999), the degree of mixing
in the water column (Cloern 1999; Monbet 1992), and attenuation of light by dissolved
substances and the phytoplankton themselves (Biber et al. 2008).

1.1.3. Phytoplankton structure modulates effects of eutrophication—For a given level of
phytoplankton organic matter enrichment, the manner in which the organic matter is
packaged, i.e. the species that are present, is a principle determinant of how the symptoms of eutrophication are manifested within an estuary. Larger phytoplankton have greater settling velocities (Fogg 1991) and are more likely to settle to the sediments contributing to sediment oxygen demand. Some phytoplankton can swim and thus live cells may not settle at all. Smaller phytoplankton are less prone to sedimentation, but require many more cells per an equivalent unit of large cell organic matter. Since scattering and absorption of light are proportional to the cross sectional area of the particles and biomass is proportional to particle volume, small phytoplankton with a higher surface to volume ration are likely to have an impact on light attenuation that is disproportionate to their biomass (Kirk 1975).

It is now widely recognized that the diet of zooplankton, the principle consumers of phytoplankton biomass in most estuaries, is selective and based on the size (Hansen et al. 1994), shape (Nielsen 1991), and taste (Poulet and Marsot 1978; Koehl 1993) of the various phytoplankton species. As a result, some phytoplankton species are more readily grazed upon than others. This surely becomes a very important part of the competitive strategies of coexisting estuarine phytoplankton species. However, it also has very important consequences for the efficiency of trophic transfer and may be the principle reason for the observed lack of correlation between phytoplankton production and metazooplankton production within nutrient enriched marine pelagic foodwebs (Micheli 1999). Some phytoplankton produce toxins or have morphological attributes such as spines or mucilage that deter ingestion by grazers and allow these phytoplankton to reach very high levels of biomass (Sunda et al. 2006). For other species, grazing is deterred indirectly by affecting the reproduction of zooplankton (Ban et al. 1997; Kleppel and Hazzard 2000). Phytoplankton which are poorly grazed tend to accumulate in the water column or in the sediments where
their “extra algal biomass” (Smetacek et al. 1991) may exacerbate light attenuation and bottom water hypoxia.

1.1.4. Study Site, the Neuse River Estuary—The Neuse River Estuary (NRE) is located along the central Atlantic seaboard of the U.S. and is a major tributary estuary of the Albemarle-Pamlico Sound estuarine system (Paerl et al. 1997). The few narrow inlets in the Outer Banks of North Carolina restrict exchange with shelf waters, making the Albemarle-Pamlico Sound the largest lagoonal estuary in the U.S. Astronomical tides in the NRE are negligible (Luettich et al. 2002) and as a consequence the estuary is poorly flushed with residence times varying from a few weeks up to 3 months (Pietrafesa et al. 1986). Riverine discharge and wind are the primary drivers of circulation, producing a partially mixed estuary where periods of strong salinity-based stratification are common (Luettich et al. 2000). Maximum depths along the axis of the estuary generally increase from approximately 4 m at the head of the estuary to near 7 m where the estuary empties into Pamlico Sound. Average depth for the entire estuary is only 2.3 m due to extensive shelves and shoals that rim the estuary. As a consequence of its long residence time, and shallow depths, nutrient inputs are efficiently assimilated by the phytoplankton within the estuary (Pinckney et al. 1997; Boyer et al. 1994) and the system is highly fertile with areal primary production exceeding 300 g C m\(^{-2}\) y\(^{-1}\) (Mallin and Paerl 1994). High fertility of the phytoplankton fuels productivity at higher trophic levels (Mallin and Paerl 1994). As a consequence, the NRE and Pamlico Sound provide a rich supply of valuable fisheries resources (Stanley 1992; Mallin et al. 2000).

The high residence time and shallow depths that make the estuary so fertile also make it extremely sensitive to nutrient over-enrichment from a variety of point and non-point sources
within its watershed and airshed (Paerl et al. 2004). Harmful algal blooms and summer time hypoxia/anoxia of the bottom waters are currently the two most troublesome water quality problems (Paerl et al. 1997; Buzzelli et al. 2002) and have been linked to eutrophication of the estuary (Paerl et al. 1997).

Episodic fish kills associated with hypoxia (Paerl et al. 1997; Reynolds-Fleming and Luettich 2004) and/or toxic algal blooms (Glasgow et al. 2001; Hall et al. 2008) create an obvious visual and olfactory expression of the wide range of cascading impacts that eutrophication has on the food web. Even moderate hypoxia can have a direct negative affect on growth rates of demersal estuarine fin fish (Del Toro-Silva et al. 2008). Sessile benthic invertebrates can suffer mass mortality during hypoxia/ anoxia events (Eby et al. 2005; Lenihan et al. 2001; Luettich et al. 2000). Motile organisms often migrate to escape hypoxic waters and as a result, available habitat space is reduced and densities of motile organisms are increased (Lenihan et al. 2001; Eby et al. 2005; Taylor et al. 2007). High densities of predators reduce available prey and prey limitation negatively impacts the growth rate of individuals and populations of ecologically and commercially valuable fin fish (Eby et al. 2005; Taylor et al. 2007).

Because of these symptoms of eutrophication, the Neuse River was declared one of America’s top 20 most threatened rivers (American River Foundation 1997). In response, the NRE was placed on the United States Environmental Protection Agency’s 303d list for impaired aquatic systems. This designation mandated the development of a Total Maximum Daily Load (TMDL) nutrient load reduction strategy (North Carolina Department of Environment and Natural Resources 2001).
1.1.5. *Phytoplankton biomass in the Neuse River Estuary*—Since the early 1980’s, research within the NRE and its receiving body the Pamlico Sound has provided a wealth of information on the structure and function of the phytoplankton community within the estuarine system. In particular, since 1994, the Neuse River Modeling and Monitoring Program (ModMon) has provided monthly to bi-weekly monitoring of water quality conditions from the head waters of the estuary to near where the estuary empties into the Pamlico Sound (Fig. 1.1).

Phytoplankton community biomass, as measured by chlorophyll $a$, is strongly tied to river flow which fuels phytoplankton biomass through nutrient delivery but can also, at times, wash out resident phytoplankton communities. In the upper regions of the estuary, phytoplankton biomass displays a negative correlation with river flow because, during high flow, advective losses override phytoplankton growth (Pinckney et al. 1997). The larger volume of the lower estuary increases the residence time and within this region phytoplankton biomass typically exhibits a positive relationship with enhanced nutrient loading that accompanies flow (Pinckney et al. 1997; Valdes-Weaver et al. 2006; Arhonditsis et al. 2007). As a result, the estuary can conceptually be divided (sensu Boynton et al. 1982) into an upper river dominated system and a lower estuary that includes Pamlico Sound that has more lagoonal characteristics. A well defined maximum in phytoplankton biomass is often observed within the transition region between the two systems (Wagener 2006).

Geographically, the transition zone between river dominance and lagoonal usually occurs somewhere between ModMon station 50 and 100 but the location of the transition is highly dependent on river flow with higher flows pushing this transition zone further downstream in the estuary (Wagener 2006). In general, the region below the bend in the river at Cherry
Branch typically has a more lagoonal character than further upstream. However, according to Boynton et al. (1982) lagoonal systems should be well mixed and this is not always the case in the lower NRE (Luettich et al. 2000; Buzzelli et al. 2002).

1.1.6. Nutrient limitation—Several studies employing nutrient limitation bioassays in the NRE have shown that, as in many coastal waters, nitrogen is the primary limiting nutrient (Rudek et al. 1991; Pael et al. 1995; Pinckney et al. 1999; Richardson et al. 2001; Piehler et al. 2002; Pael et al. 2004). Monitoring data corroborate these findings, showing that bloom occurrence is often preceded by large riverine discharges of dissolved inorganic nitrogen (DIN) (Pinckney et al. 1997, 1998). However, based on considerations of Redfield stoichiometry and the ambient available inorganic nutrient pools, P limitation is possible during the cooler months (Rudek et al. 1991). Bioassay experiments agree with a stronger potential for P limitation during the colder months (Rudek et al. 1991; Richardson et al. 2001) but also demonstrate that stoichiometry is not always a reliable indicator of the limiting nutrient (Piehler et al. 2002). This research on nutrient limitation was instrumental in the development of the TMDL for the Neuse River Estuary which mandated a 30 percent nitrogen load reduction to the estuary (North Carolina Department of Environment and Natural Resources 2001).

1.1.7. Phytoplankton community composition—The phytoplankton community of the NRE is generally a mixed assemblage of diatoms, cryptophytes, dinoflagellates, chlorophytes, and cyanobacteria and each have their own characteristic periodicities of prominence within the community assemblage (Pinckney et al. 1998). Cyanobacteria, dominated by *Synechococcus*...
like coccoid picoplankton (Gaulke 2009) and thin non-heterocystous filamentous forms (Mallin et al. 1991) achieve peak biomass during the summer months and at times may represent up to forty percent of the total chlorophyll within the phytoplankton community (Mallin et al. 1991; Pinckney et al. 1998). In contrast, diatom and chlorophyte abundances generally display maxima in the winter-spring months; temporally coincident with the highest seasonal riverine nutrient load (Mallin et al. 1991; Pinckney et al. 1998; Valdes-Weaver 2006). Dinoflagellates generally form blooms during the winter months which are composed of *Heterocapsa triquetra*, *Prorocentrum minimum*, and *Heterocapsa rotundata* (Pinckney et al. 1998; Mallin et al. 1991). However, high concentrations of dinoflagellates persist throughout the year (Mallin et al. 1991) and can reach bloom concentrations during any season (Pinckney et al. 1998). Cryptophytes seem to show no perceptible seasonality but can form blooms and dominate biomass of the phytoplankton community (Pinckney et al. 1998).

Along the continuum from the fresh head waters of the NRE to its saltier receiving waters, Pamlico Sound, the relative abundance of diatoms generally increases downstream within the Neuse – Pamlico Sound system (Valdes-Weaver et al. 2006). In contrast, the relative abundance of dinoflagellates reaches a maximum within the lower NRE and exhibits much lower relative abundance within the Pamlico Sound (Valdes-Weaver et al. 2006). Class level relative abundance of cyanobacteria, chlorophytes, and cryptophytes do not exhibit strong spatial patterns along the estuarine continuum (Valdes-Weaver et al. 2006).

While these studies have determined major forcing features that control phytoplankton biomass accumulation and have identified temporal and spatial patterns of community composition, they have been relatively unsuccessful at determining the suite of
environmental controls that regulate community composition. In particular, it is unclear why phytoplankton blooms that occur within the estuary are dominated by dinoflagellates and cryptophytes. In testimony to the ecosystem level importance of these blooms, up to half of the annual primary production of the estuary is contributed by late winter-early spring dinoflagellates blooms (Paerl et al. 1995).

Light and nutrients are the primary determinants of phytoplankton growth at the cellular level (Cloern 1999), and based solely on light and nutrient availability diatoms should always dominate over dinoflagellates. Maximum growth rates of diatoms nearly always exceed those of dinoflagellates (Banse 1982, Smayda 1997). Diatoms have faster nutrient uptake across the range of naturally occurring nutrient concentrations (Lomas and Glibert 1982) and require lower light levels for growth (Richardson 1983, Smayda 1997). In mixed culture competition experiments, diatoms nearly always outcompete flagellates (Sommer 1988). In other estuarine systems diatoms assume the role of the dominant primary producer (Fig. 1.2). For example, in San Francisco Bay diatoms comprise greater than eighty percent of the phytoplankton biomass (Cloern and Dufford 2005). Similarly, in the Chesapeake Bay, diatoms comprise greater than half of phytoplankton biomass (Adolf et al. 2006) and the spring bloom of diatoms contributes greater than half of the annual production (D’Elia et al. 1983). However, in a tributary estuary of the Chesapeake, the Patapsco River, dinoflagellates are dominant (Sellner et al. 2001). What suite of system specific attributes leads to diatom dominance of some systems and flagellate dominance of others? Since community composition is an important determinant of the effects of eutrophication, understanding the factors that shape the structure of the phytoplankton community will aid
development of current nutrient management strategies for the NRE and increase our ability to predict eutrophication potential between estuarine systems (Cloern 2001).

1.2. Goals of this study

This body of work aims to determine the important system-specific attributes of the NRE that produce a flagellate dominated phytoplankton community. For all phytoplankton, the vertical organization of light and nutrients and the degree of vertical mixing within the water column are principal determinants of population growth (Cloern 1999). The vertical light gradient is always a negative function of depth and by its influence on phytoplankton uptake plays a great role in organization of the vertical nutrient distribution in aquatic systems (Margalef 1978). Vertical nutrient gradients can form in the water column as long as the time scales associated with vertical mixing are slow compared to the time scales associated with advection and the biological processes of uptake and remineralization. Diatoms are generally negatively buoyant and thus require turbulent mixing to remain in the water column. Flagellates are also generally negatively buoyant but their swimming ability allows them to migrate vertically in poorly mixed water columns and effectively exploit gradients of light and nutrients (Margalef 1978). We hypothesize that vertical light and nutrient gradients and the degree of vertical mixing act along the sink or swim life modes of diatoms and dinoflagellates, respectively, to select for flagellate forms over diatoms in the NRE.

1.3. Overview of the chapters
Chapter 2 describes a series of field observations on the vertical distribution of phytoplankton in relation to the vertical physical and chemical structure of the water column in the NRE. The dominant patterns in the depth distributions of phytoplankton biomass and community composition were compared to the depth distributions of growth limiting light and nutrient resources. In particular, we were interested in determining whether dinoflagellates and cryptophytes displayed patterns of vertical migration that would allow them to exploit gradients of light and nutrients when the water column was poorly mixed.

Margalef’s Mandela (1978) is a classic conceptual model of phytoplankton competition and succession based upon nutrient availability and degree of mixing of the water column. This conceptual model nicely accounts for changes in phytoplankton community composition across a wide range of aquatic systems where mixing and nutrient availability are linked and where changes in turbulent mixing occur over seasonal time scales. Because astronomical tides are negligible in the lower NRE, wind is the principle driver of vertical mixing (Luettich et al. 2000). In Chapter 3, we used the wind induced alternations between stratified and mixed conditions in the NRE as a natural experiment to evaluate the applicability of Margalef’s (1978) Mandela in this shallow eutrophic system. If phytoplankton community composition varies according to the tenets of the Mandela, diatoms should be favored by more mixed conditions and flagellates should be favored under higher degrees of water column stability.

Hurricanes and tropical storms are increasingly an important part of the ecology of the NRE and Pamlico Sound system (Paerl et al. 2006). Following the flood pulse associated with Tropical Storm Ernesto, a dense bloom of the toxic dinoflagellate *Karlodinium*
veneficum developed within the lower NRE. Chapter 4 documents the suite of environmental conditions that fostered the development of this dinoflagellate bloom. The vertical structure of the water column played key roles in the initiation, growth phase, and likely in the collapse of the bloom. This study is also published in Hall et al. (2008).

In Chapter 5 we review the conclusions of Chapters 2 through 4 that demonstrate linkages between the vertical structure of the water column and phytoplankton community composition. We comment on how this new knowledge can be used to improve current mechanistic models of eutrophication for the Neuse River Estuary and briefly discuss potential effects of impending climate change on phytoplankton community composition.
Figure 1.1. Map of the Neuse River Estuary showing sampling locations of the Neuse River Estuary Modeling and Monitoring Program.
Figure 1.2. Average class-level community composition of the Neuse River Estuary, the Chesapeake Bay, and San Francisco Bay. The pie chart for San Francisco Bay was redrawn from Cloern and Dufford (2005). The chart for Chesapeake Bay was redrawn from Adolf et al. (2006). The chart for the Neuse River Estuary was redrawn from Pinckney et al. (1998).
Diatoms

Dinoflagellates

Crytophytes

Other

San Francisco Bay

Chesapeake Bay

Neuse River Estuary

Diatoms

Dinoflagellates

Crytophytes

Other
1.4. LITERATURE CITED


Chapter 2

DIEL PATTERNS OF PHYTOPLANKTON DEPTH DISTRIBUTIONS IN RELATION TO LIGHT AND NUTRIENT AVAILABILITY: RESULTS FROM THREE DIEL STUDIES
ABSTRACT

Physical and chemical vertical gradients in estuaries can be very steep and small changes in the depth distribution of phytoplankton can result in drastic changes in light and nutrient availability over short time (s to d) and spatial (cm to m) scales. At these seldom studied scales, interactions between light and nutrient conditions, phytoplankton physiology, currents and turbulence, and the grazer community are primary determinants of phytoplankton growth at the individual and population levels. We investigated the vertical distribution of phytoplankton biomass and community composition, light, and nutrients within the lower Neuse River Estuary to determine how patterns of vertical distribution were likely to affect the structure of the phytoplankton community. In particular, we were interested in determining whether flagellates that dominate the biomass of the Neuse River Estuary displayed migration patterns that would allow them to exploit gradients of light and nutrients. Depth profiles of phytoplankton biomass and community composition, light, and nutrients were collected during three diel studies. During the diel studies, primary productivity and nutrient uptake were measured to investigate the linkage between vertical light and nutrient gradients and the functional aspects of phytoplankton growth.

Light and nutrient profiles revealed a tendency for the vertical separation of these growth limiting resources with growth limiting light levels below an often well established pycnocline and potentially growth limiting nutrient concentrations in the surface waters. Cryptophytes and dinoflagellates, the dominant flagellates, displayed diel changes in depth distribution indicative of diel vertical migration into the surface waters during the day and into the bottom waters during the night. The observed migration behaviors likely alleviate potential light and nutrient limitation when light and nutrient availability are vertically
separated within the water column. Long-term monitoring data suggest that this is normally the case during the warmer months.
2.1. Introduction

Phytoplankton vertical distribution is of central interest to phytoplankton ecologists because vertical gradients of light, nutrient concentrations, current velocities, and turbulence are much steeper than horizontal gradients and greatly affect phytoplankton growth and survival strategies (Margalef 1978; Fogg 1991). Among marine systems, some of the steepest vertical physical and chemical gradients are found in estuaries which can be strongly salinity stratified. Phytoplankton depth distributions are controlled by a number of interacting factors including internally and environmentally cued swimming and buoyancy regulation, vertical advection due to up/downwelling, and turbulent mixing (Margalef 1978). Changes in the depth distribution of phytoplankton coupled with the steep physical and chemical gradients in estuaries can result in drastic changes in light and nutrient availability over short time (s to d) and spatial (cm to m) scales.

At these seldom studied scales, interactions between environmental conditions and phytoplankton physiology are primary determinants of individual cell growth (Litaker et al. 1987, Fogg 1991, Kononen 1992, Waters and Mitchell 2002). At the population level, coupling between the depth distribution of phytoplankton and vertical gradients of current velocities may decrease advective losses (Anderson and Stolzenbach 1985, Crawford and Purdie 1992, Chang and Carpenter 1985). Additionally, mismatches in the vertical localization of phytoplankton and zooplankton patches can decrease grazing losses (Jones 1991; Salonen and Rosenberg 2000).

Phytoplankton vary in their ability to regulate their depth distribution in the water column. For example, the lower Neuse River Estuary is usually a mixed assemblage with
diatoms, chlorophytes, cyanobacteria, dinoflagellates, and cryptophytes being the major classes (Mallin et al. 1991; Pinckney et al. 1998). Diatoms lack motility and have only a weak ability for depth regulation by reducing settling velocity via changing the lipid and ionic content of storage vacuoles (Sournia 1982). Some cyanobacteria can effectively use gas vacuoles to regulate their depths (Paerl 1988). However, the picoplanktonic *Synechococcus* cyanobacteria that dominate the cyanobacteria of the lower NRE (Gaulke 2009) lack gas vacuoles and are unlikely to effectively regulate depth (Sournia 1982). The dinoflagellates, cryptophytes, and some of the chlorophytes, however, are flagellated with swimming speeds that allow for effective depth regulation when turbulence levels are low enough to make their swimming ability effective. Thus, the physical and chemical gradients of the water column that confer advantages on species more adept at depth regulation may be important determinants of phytoplankton community structure (Margalef 1978; Fogg 1991).

Given the potential importance of phytoplankton depth distribution to phytoplankton production, biomass, and community structure, the goals of this study were: 1) To document the vertical chemical and physical structure of the water column with respect to those features that are likely to affect phytoplankton biomass and community composition. Most importantly, this included understanding vertical gradients of light and nutrient availability. 2) To determine the dominant depth distribution patterns of the phytoplankton and relate these patterns to the vertical distribution of light and nutrients. Inspired by the earlier PULSE project carried out in the adjacent Newport River Estuary (Litaker et al. 1987, 1993), we assumed that phytoplankton depth distributions would be significantly related to identifiable external factors such as the degree of stratification, pycnocline depth, and the daily solar cycle.
Several observations from the NRE as well as from similar systems revealed key modes of phytoplankton distribution variability that may be important. A preliminary set of nighttime chlorophyll \( a \) profiles from the Neuse River Estuary revealed higher concentrations of chlorophyll \( a \) below the pycnocline than at the surface (data not shown). This finding contradicted the pattern of higher chlorophyll in the surface waters documented through 2 years of bi-weekly daytime sampling in the NRE (Pinckney et al. 1998) and suggested that diel vertical migration (DVM) by flagellates may be an important determinant of phytoplankton vertical distribution. DVM is a migration pattern of flagellated phytoplankton (particularly dinoflagellates) whereby phytoplankton swim to the well-lit surface waters during the day and into deeper waters at night that are usually more nutrient rich. Aggregation at the pycnocline is another commonly observed vertical distribution pattern of motile organisms in stratified environments (Tyler and Seliger 1978; Waters and Mitchell 2002; Kononen et al. 2003).

Analysis of phytoplankton depth patterns is often highly subjective and vertical migration patterns are often described with little regard given to other mechanisms that may have produced the observed changes in phytoplankton depth distribution (Jones 1988). For example, a dense aggregation of phytoplankton along a pycnocline may be cyclically transported up and down in the water column by the passage of internal waves (Denman 1977). Thus, a passive response to changes in the physical structure of the water may masquerade as an active vertical migration pattern.

In the NRE, basin morphology, characteristic frequencies of wind energy, and typical stratification regimes produce significant short time scale, hours to days, oscillations in important components of the physical water column structure such as density, water depth,
degree of stratification, and pycnocline depth. In particular, there is considerable energy in
the power spectra of these physical water column parameters in the semi-diurnal to diurnal
time scales (Luettich et al. 2002; Reynolds-Fleming et al. 2004; Whipple et al. 2006). The
ability to detect active vertical migration patterns, such as DVM, in the presence of similarly
time-scaled passive responses to changes in the physical structure of the water column is a
recognized need for understanding phytoplankton depth distributions (Jones 1988). By
comparing vertical distributions of phytoplankton to the vertical density structure, we aimed
to determine whether observed patterns in phytoplankton depth distribution were produced
actively by migration patterns of the phytoplankton or passively in response to changes in the
physical structure of the water column.

This chapter describes three diel observations performed during the summer 2001.
Profiles of light, nutrients, and phytoplankton biomass were made at 3-6 h intervals to
identify diel patterns in the vertical distribution of phytoplankton and relate these patterns to
water column structure. Because this study includes vertical profiles of microscopically
determined, cell abundance, differences in patterns of phytoplankton distribution within
taxonomic levels from class to species are elucidated. Additionally, primary productivity and
nutrient uptake studies were performed to establish the linkage between vertical structure of
the water column and the functional aspects of phytoplankton growth.

2.2. Methods and Materials

2.2.1. Study Site—The three diel observations described in this chapter were performed at
station NR 120 (34° N 57.132’, 76° W 48.779’) on 2-3 May, 12-13 June, and 17-18 July
2001. This sampling station is located near the bend in the estuary (Fig. 2.1), has a depth of approximately 5 m, is mesohaline, and is mesotrophic to eutrophic (Boyer et al. 1994). Salinity is highly variable and largely dependent on riverine discharge (Christian et al. 1991). This site is monitored on a bi-weekly basis as part of the Neuse River Estuary Modeling and Monitoring (ModMon) Program (Luettich et al. 2000) that has collected environmental data within the NRE since 1994 (http://www.unc.edu/ims/neuse/modmon).

2.2.2. Sample Collection—All water samples were collected using a 2 L Van Dorn sampler and immediately poured into 2-4 L polyethylene bottles. Water was collected from 0.5 m, 2 m, and 4 m depths during the May 2001 observation. During the June and July 2001 observations, water was collected from 0.1 m, 0.5 m, 1 m, 2 m, 3.5 m, and 5 m depths. A primary goal of this study was to investigate diel patterns of phytoplankton abundance and activity (production and nutrient uptake) so sampling efforts were focused at roughly six hour intervals to capture the early morning, midday, afternoon, and midnight periods. During the June and July observations, additional collection of samples for phytoplankton biomass occurred at the midpoint of the 6 hour intervals. All times reported are Eastern Standard Time (EST).

Within minutes of collection, 150 mL of each sample was poured into amber polyethylene bottles and fixed with Lugol’s solution at 1% final concentration for microscopic enumeration of the phytoplankton. Chlorophyll $a$ and nutrient measurements were made at all three depths of the May observation and at 0.5 m, 2 m, and 5 m during the June and July observations. For chlorophyll $a$ measurements, three aliquots of 50 mL were filtered separately through Whatman 25 mm GFF filters (nominal pore-size 0.7 μm). Filters
were folded in half (content side faced inward), blotted with a paper towel to remove excess water, wrapped in aluminum foil, and stored on ice in plastic bags for the duration of each 24 hour observation. The filtrate from the three filters was poured into acid washed and sample rinsed 150 mL polyethylene bottles and stored on ice for the duration of each 24 hour observation. Once back at the laboratory, chlorophyll $a$ and nutrient samples were frozen at $-20^\circ$C.

2.2.3. Physical Data—Profiles of temperature, salinity, pH, dissolved oxygen, turbidity, and fluorescence were collected using a YSI 6600 multiparameter water quality instrument (Yellow Springs Inc, Yellow Springs, Ohio). For the May and June observations, discrete measurements were made at 0.5 m depth intervals. For the July observation, the YSI 6600 continuously collected data at 1 Hz and was lowered through the water column slowly to achieve a depth resolution of approximately 10 cm. Profiles of photosynthetically active radiation (PAR) were collected using a Li-Cor 192S $4\pi$ spherical PAR sensor (Li-Cor Biosciences, Lincoln, Nebraska) and diffuse light attenuation coefficients ($K_d$) were calculated from least squares fits of the exponential decay of PAR profiles. Throughout the diel studies qualitative descriptions of weather conditions were recorded. These observations included estimates of wind speed and direction, cloud cover, and wave height.

2.2.4. Nutrient and Chlorophyll $a$ Analyses—For nutrient analyses, the frozen nutrient samples were quick thawed and $\text{NO}_2^- + \text{NO}_3^-$ (reported as $\text{NO}_3^-$), $\text{NH}_4^+$, $\text{PO}_4^{3-}$ and dissolved silica (DSi) concentrations immediately determined using a Lachat Quick-chem 8000 auto-analyzer (Lachat, Milwaukee, Wisconsin) (Lachat Quik-chem methods 31-107-04-3-B, 31-
107-04-1-C, 31-107-06-1-B, and 31-114-27-1-A respectively). Detection limits of NO$_3^-$, NH$_4^+$, PO$_4^{3-}$, and DSi were 0.26 μM, 0.34 μM, 0.024 μM, and 1.24 μM, respectively. Chlorophyll $a$ samples were analyzed within a week of each diel observation and nutrient analysis was performed within 4 weeks of each diel observation. For chlorophyll $a$ analysis, filters were extracted using a tissue grinder in 90% acetone (EPA method 445.0). Chlorophyll $a$ concentration was measured on the extracted samples using the non-acidification method of Welshmeyer (1994) on a Turner TD-700 fluorometer.

2.2.5. *Primary Productivity and Nutrient Uptake*—$^{14}$C productivity and $^{15}$N nitrogen uptake measurements were conducted on water samples collected from all three depths sampled during the May observation and from 0.1 m, 2 m, and 5 m during the June and July observations. Timing of incubations of productivity and nitrogen uptake assays was set to capture rates for the early morning, midday, afternoon, and midnight periods. Midnight productivity assays were not performed. For $^{15}$N measurements, whole water samples were added to 150 mL polyethelene terephthalate glycol (PETG) bottles and were inoculated with $^{15}$N tracer in the form of ammonium chloride ($^{15}$NH$_4$Cl), and potassium nitrate (K$^{15}$NO$_3$) (Sigma Chemicals, St Louis, Missouri). Ambient NO$_3^-$ concentration was below the limits of detection for all but 4 of 36 NO$_3^-$ measurements made during the three diel studies. As such, measurement of in situ uptake of NO$_3^-$ was not possible and these data are not shown. Tracers were added at an initial concentration of 0.1 μM. For NH$_4^+$, $^{15}$NH$_4^+$ represented a small portion of the total NH$_4^+$ pool (range 1.4-18.7%; average 9.4%) so it seems unlikely that significant stimulation of uptake via increased substrate availability occurred. Triplicate samples were incubated in the water at the depth from which they were collected by
attaching the bottles to a rope at the appropriate depth. The rope had a weight at one end and a float on the other to insure that the bottles were maintained at the appropriate depth level throughout the incubation. Shading by the vessel was prevented by allowing the incubation rope to drift on a tether approximately 5 m from the vessel. Incubations lasted from 2 to 5.1 h and were terminated by filtration onto pre-combusted (500 °C, 16 h) Whatman 25 mm GFF filters (nominal pore-size 0.7 μm). Filters were placed in small Gelman Petri dishes, wrapped in plastic bags, and stored on ice for the duration of each 24 hour observation. Upon return to the laboratory, filters were dried at 60 °C. For 15N analysis and calculation of uptake rates, we followed the procedures described in Twomey et al. (2005).

Primary productivity was determined using the 14C method adapted for in situ estuarine conditions by Paerl (1987). Triplicate light and single dark 150 ml PTFE bottles were filled with water collected from each depth level followed by the addition of 0.3 ml of 14C NaHCO3 with an activity of 7.5 μCi mL⁻¹, (ICN Pharmaceuticals Inc.). Incubations were conducted at the depth from which they came in the same manner as the 15N incubations. Incubation duration varied from 3.1 to 5.5 h. After incubation, a 50 mL subsample of each bottle was filtered onto a Whatman 25 mm GFF filter (nominal pore-size 0.7 μm). Filters were placed in small Gelman Petri dishes, wrapped in plastic bags, and stored on ice for the duration of each 24 hour observation. Immediately upon return to the laboratory, filters were fumed in an HCl saturated atmosphere to remove inorganic 14C and air dried. 14C incorporation was measured using a Beckman TD-5000 liquid scintillation spectrometer. Dissolved inorganic carbon was determined by acidifying the samples and measuring the evolved CO2 gas with a Beckman 865 infrared gas analyzer calibrated with sodium carbonate.
standards (Paerl 1987). Primary productivity was calculated according to the formula of Wetzel and Likens (1991).

2.2.6. **Cell Counts**—Lugol’s-preserved phytoplankton samples were stored in the dark at approximately 22 °C. Cell counts were performed using the inverted microscope technique of Utermöhl (1958) with a Thomas Scientific microscope under phase contrast at 400X magnification. A 24 h settling time was not sufficient to ensure that all of the smaller phytoplankton had settled so smaller phytoplankton are likely underrepresented in the counts. However, consistent settling time and use of the same size settling chamber throughout the study (30.8 mL, 10 cm height) provide an adequate basis for comparing samples. For each sample, 65-255 fields were counted, providing between 34 and 1,973 counts of the most abundant of 15-27 cell types. Autotrophic cells with clearly visible cytoplasmic contents (no empty diatom frustules or dinoflagellate thecae were counted) were identified to the lowest possible taxonomic level. However, we were unable to identify many cell types to the genera level. In particular, Dinoflagellate A was initially identified as *Gyrodinium estuariale* based on the description by Campbell (1973). However, at the time of the cell counts we were unable to distinguish between *Gyrodinium estuariale* and *Karlodinium veneficum* and it is likely that Dinoflagellate A contains both species.

2.2.7. **Analysis of Phytoplankton Profiles**—Two indices of phytoplankton depth distribution were constructed from each profile of phytoplankton biomass: the depth of the biomass maximum within each profile, and the center of mass of the biomass depth distribution of each profile. Denman (1977) found these metrics of depth distribution to be useful for
investigating phytoplankton depth distributions in Delaware Bay. The depth of the center of biomass of each profile \( (Z_{\text{cent}}) \) was calculated as

\[
Z_{\text{cent}} = \frac{\sum_{z=0}^{z=H} C_z Z}{\sum_{z=0}^{z=H} C_z}
\]

where \( Z \) is the depth in meters of each bin containing a biomass value, \( C_z \) is the cell abundance, chlorophyll \( a \), or in vivo fluorescence at depth \( Z \), and \( H \) is the maximum depth of the water column. These metrics serve to identify changes in the vertical distribution of each population in a manner that is independent of the absolute concentrations between profiles. By subtracting these depths from the depth of the pycnocline, erroneously perceived changes in vertical distribution brought about by changes in the depth of the pycnocline were avoided. Pycnocline depth for each profile was calculated as the average of the two depths over which the largest change in density was measured. Density was calculated from temperature and salinity profiles according to the equation of state found in Pond (1983).

To investigate day versus nighttime changes in the vertical distribution of phytoplankton biomass, these metrics were grouped by day or night. Data for the day/night category were those collected between 08:00 and 16:00/20:00 and 04:00, respectively. Two-sample t-tests were then used to compare the mean vertical distribution metrics of each phytoplankton group between the day and night categories.

2.2.8. Comparison of Conditions with Long-Term Monitoring Data—To determine if the vertical structure of the water column over the course of the diel observations were representative of typical conditions in the lower NRE, we investigated the long term data
record (1 January 1994 through 1 January 2007) collected by the ModMon program. Median values of water column depth, euphotic zone depth, pycnocline depth, degree of water column stratification, surface chlorophyll a, and surface and bottom water DIN concentrations were calculated for each station along the axis of the estuary. To determine if the average water column structure varied by season, the data were further broken down into a warm and cold season based on whether water temperatures were above or below the long term median (18.89 °C). The methods that we used during the diel studies for collection of physical data, nutrient and chlorophyll a analyses are identical to those from the ModMon program. However, for the ModMon program, collection of discrete water samples for nutrient and chlorophyll a analyses, surface waters were collected from 0 m and bottom water samples were collected 0.5 m above the bottom.

2.3. Results

2.3.1. Weather Conditions—During all three diel observations, winds were out of the south to southwest with general speeds of ~2-5 m s\(^{-1}\). Waves were generally less than 0.3 m. The exception to these generalities occurred during the afternoon of the June observation when wind speeds increased briefly to ~ 8 m s\(^{-1}\) and wave height increased to between 0.3 and 0.6 m. Skies were clear during the May and July observations but were partly cloudy in June.
2.3.2. *Temperature and Salinity*—During all three diel studies, the water column remained highly stratified with average differences in salinity of 4-8 psu and average temperature differences of 2-3 °C from the surface to the bottom of the water column (Fig. 2.2). Within any one of the diel studies, differences in salinity and temperature between profiles were minor compared to differences with depth (Fig. 2.2). From the May to the July observation, salinity and temperature throughout the water column increased (Fig. 2.2). The pycnocline depth varied but was generally around 2 m throughout all three diel observations (Fig 2.2. Table 2.1).

2.3.3. *Nutrients and Nutrient Uptake*—Dissolved inorganic nitrogen (DIN) was principally in the form of NH$_4^+$ throughout the three diel observations. NO$_3^-$ was below detection for all but 4 samples and was never greater than 0.5 μM. DIN in the surface waters (0.5 m) generally ranged between 0.5 and 1 μM for all three diel observations (Fig. 2.4). DIN concentrations at 2 m, approximately the pycnocline depth of most profiles, closely matched those at the surface for each profile. However, for 6 of the 12 DIN profiles, concentrations at 2 m were less than DIN concentrations from the surface. Bottom water DIN was higher than at the surface for all but one nutrient profile (3 May 2001 05:00) and ranged from 1 to 7 μM. During the June diel observation bottom water DIN was consistently 4 to 7 times higher than at the surface or near pycnocline depths. The only consistent temporal pattern observed for DIN between the three diel observations was an increase in DIN at all depths from the early morning to midday samples. Surface water PO$_4^{3-}$ ranged from 0.1 to 0.8 μM. Bottom water P was always higher than at the surface and ranged from 0.12 to greater than 3 μM. The
highest observed DIN:DIP ratio during the diel observations was 8.2, so it is unlikely that P limited phytoplankton growth.

The $^{15}$N uptake method that we used did not account for isotopic dilution and there is good evidence that isotopic dilution was a major factor affecting calculated uptake rates. For example, there was a strong negative correlation ($R^2 = 0.45$) between calculated N specific uptake rates and incubation duration. Additionally, during most of the incubations greater than fifty percent of the added $^{15}$N was recovered in the particulate fraction and the measured uptake rates were often high enough to completely remove the ambient NH$_4^+$ pool over the duration of the incubation. As a result, the values for NH$_4^+$ uptake are considered minimum uptake rates (Glibert et al. 1982) and we make no attempt to identify diel or vertical patterns of NH$_4^+$ uptake from this data. However, the data can be used to establish a maximum turnover time for the ambient NH$_4^+$ pool in the water column by dividing the ambient NH$_4^+$ concentration by the minimum uptake rate. For all the incubations performed at the near surface (0.5 m) and near pycnocline (2 m), the turn over time of the NH$_4^+$ pool could have been no more than 7 h (Table 2.2). For the bottom water incubations, maximum turn over time varied greatly but was much higher than nearer the surface due to generally lower minimum uptake rates and higher NH$_4^+$ concentrations (Table 2.2).

Our estimates of maximum turn over times for the NH$_4^+$ pool in the surface waters of the lower NRE (3 to 7 h) agree well with the results of previous studies. Christian et al. (1991) and Boyer et al. (1994) found that mean NH$_4^+$ concentrations for the lower NRE were 2 to 3 $\mu$M and median uptake was $\sim 0.5$ $\mu$M h$^{-1}$. This gives a turn over time of 4 to 6 h. Similarly, data from Twomey et al. (2005) suggest turnover times of a few hours for the surface waters of the lower NRE. It should be noted that neither of these studies accounted for isotopic
dilution and as a result their data also are likely to overestimate the turn over time of the NH₄⁺ pool.

With low DIN pool sizes and high DIN demand, remineralization and uptake must be near steady state to maintain a relatively constant DIN pool in the surface waters. The fact that NH₄⁺ concentrations were often slightly lower at the pycnocline than at either the surface or bottom suggests that uptake within the region of the pycnocline must at times be more important than mixing between the surface and bottom waters in determining the NH₄⁺ concentration within the pycnocline. Thus, it seems likely that much of the diffusive flux of NH₄⁺ from the bottom waters is stripped within the region of the pycnocline before it enters the surface waters. In the bottom waters, lower NH₄⁺ uptake, high inputs from the sediments, and weak mixing into the surface waters due to salinity based stratification lead to NH₄⁺ concentrations that were generally 50 to 600 percent higher than near the surface.

2.3.4. Light and Productivity Profiles—Light attenuation among and between the three diel studies was very similar with mean values for each diel observation ranging from 1.32 to 1.56 m⁻¹. This corresponds to euphotic depths ranging from 3 to 3.5 m (Table 2.1). The pycnocline depth was approximately 2 to 2.5 m so most of the water column below the pycnocline was dysphotic and light at the depth of the pycnocline was between 1-5 % of incident irradiance.

Throughout the three diel studies, chlorophyll a normalized productivity, assimilation, closely followed light availability with highest values of assimilation found closer to the surface (2-6 μg C μg Chl-a⁻¹ h⁻¹) and very low values (~0.32-0.36 μg C μg Chl-a⁻¹ h⁻¹) at 4 or 5 m (Fig. 2.3). While assimilation always tracked light availability, during the midday
productivity assay on 2 May 2001, aggregation of cells along the pycnocline resulted in maximum productivity occurring at 2 m. Chlorophyll $a$ concentration was 19.8 $\mu$g L$^{-1}$ at 2 m but was only 7.3 $\mu$g L$^{-1}$ at the near surface (0.5 m) sampling depth. PAR flux at 2 m was ~50 $\mu$mol photons m$^{-2}$ s$^{-2}$.

Within the range of irradiances (600-1100 $\mu$mol photons m$^{-2}$ s$^{-1}$) observed at 0.5 m during the midday productivity assays, photosynthetic rates of phytoplankton in the NRE are saturated and not photoinhibited (Bergmann et al. 2002). Hence, these values are similar to $P_{b_{\text{max}}}$ from a photosynthesis versus irradiance analysis. Phytoplankton respiration rates are generally 10-30 percent of $P_{b_{\text{max}}}$ (Cole et al. 1992; Falkowski and Raven 1997; Geider and Osborne 1989). Assimilation in the bottom waters were 6, 10, and 23 percent of $P_{b_{\text{max}}}$ for the May, June, and July midday productivity assays. These considerations lead to the conclusion that light levels in the bottom waters of the NRE are unlikely to be sufficient for net growth. The accumulation of ammonium and phosphate at subpycnocline depths is also consistent with this view.

2.3.5. Vertical distribution of phytoplankton in May—Near surface (0.5 m) dinoflagellate and cryptophyte abundance was generally less than deeper in the water column (Fig. 2.5). During the midday (10:45) and early morning (06:00), dinoflagellates had highest abundance, ~700 and ~500 cells mL$^{-1}$ respectively, at a depth very close to the depth of the pycnocline (~2 m). In contrast, during the late afternoon and night profiles, the highest concentrations of dinoflagellates, ~800-1000 cells mL$^{-1}$, were found at depths below the pycnocline (4 m). Cryptophytes also displayed a biomass maximum below the pycnocline (4 m) at night. Diatom concentrations were generally low (< 100 cells mL$^{-1}$) during the May
sampling period. For all profiles but the early morning (06:00) profile, diatom abundance was greatest below the pycnocline.

In vivo fluorescence from the 10:45 and 15:32 profiles showed maxima above the pycnocline at ~1 m depth. During the late afternoon (17:48) and nighttime (22:55) profiles, the fluorescence maxima were found deeper in the water column at ~2.5 m depth. The early morning profile showed a large fluorescence maximum near the bottom with a smaller local maximum near the pycnocline (~2 m depth). The 09:12 profile showed a large fluorescence maximum just above the pycnocline at ~2 m depth. The May diel observation of the vertical distribution of dinoflagellates, cryptophytes and fluorescence was consistent with a pattern of DVM. However, poor temporal and depth resolution produced an unconvincing data set.

2.3.6. Vertical distribution of phytoplankton in June—Increased vertical and temporal resolution during the June diel observation provided a much more detailed documentation of the vertical distribution of the phytoplankton (Fig. 2.6). As during May, dinoflagellates and cryptophytes dominated the community assemblage throughout the June observation. During the late afternoon (18:00), dinoflagellates displayed a pronounced maximum along a very well defined pycnocline. During the 22:04 and 00:57 profiles, the dinoflagellate maximum was found below the pycnocline at 3.5 m. Fluorescence profiles reflected the change in dinoflagellate distribution with corresponding fluorescence peaks at 3.5 m during the 22:04 and 00:57 profiles. From 04:00 through 15:50, the dinoflagellate biomass maximum was again found at or above the pycnocline. Fluorescence profiles generally tracked the vertical distribution of the dinoflagellates (Fig. 2.6).
The observed patterns in dinoflagellate abundance were largely due to changes in the depth distribution of the dinoflagellate, *Scrippsiella trochoidea* (Fig. 2.7). Due to its abundance and large size (~30 μm diameter) it was an important biomass constituent of the phytoplankton community and was the dominant species observed within nighttime fluorescence maxima at 3.5 m depth (Fig. 2.7). The other common dinoflagellate during the June diel study, Dinoflagellate A displayed a different pattern of DVM. With the exception of the 09:50 profile, highest cell abundances of Dinoflagellate A were observed in the sample closest to the depth of the pycnocline (Fig. 2.7). However, during the day abundance from the surface to the pycnocline was higher than during the nighttime. Also in contrast to *S. trochoidea*, high concentrations of Dinoflagellate A were never found below the pycnocline at the 3.5 m and 5 m depths. Cell abundance of cryptophytes was highly dominated by a nanoplancktonic species (cell volume ~ 45 μm³), *cf. Chroomonas minuta* (Campbell 1973). As a result, depth profiles of cryptophytes closely corresponded to depth profiles of *cf. C. minuta* (Fig. 2.6, 2.7). *cf. C. minuta* generally displayed maximum abundance at depths close to the pycnocline. However, during the late morning and early evening distinct bimodal distributions of cells were observed with one peak near the surface and another along the pycnocline. Generally, diatom abundance was higher in the surface waters than in the bottom waters and often showed maxima near the pycnocline (Fig. 2.6). There were no apparent patterns of diel changes in the depth distribution of diatoms.

2.3.7. *Vertical distribution of phytoplankton in July*—Results from the July diel observation (Fig. 2.7) were very similar to those of June (Fig. 2.6). Dinoflagellates and cryptophytes dominated the community assemblage throughout the July diel observation. During the late
afternoon, both dinoflagellates and cryptophytes displayed a pronounced maximum along a very well defined pycnocline. By 21:26 the maxima of dinoflagellates and cryptophytes diminished and abundance below the pycnocline increased. As in June, cryptophyte abundance was dominated by the nanoplankter, *cf. Chroomonas minuta* (Fig. 2.7, 2.8). The 00:38 profile revealed high concentrations of both dinoflagellates and cryptophytes below the pycnocline at 3.5 m depth. This contrasts with the results from the June observation (Fig. 2.7) during which *cf. Chroomonas minuta* maintained population maxima near or above the pycnocline. The dinoflagellate maximum remained at 3.5 m through the 03:07 profile while the cryptophyte maximum returned to near the pycnocline by 03:07.

Increased sampling frequency during the YSI casts provided greater depth resolution and showed the nocturnal subpycnocline maxima were actually bimodal. We speculate that the individual peaks in fluorescence within the broad peak from 2.5- 4 m during the 00:38 and 03:07 profiles are due to different flagellate species. Unfortunately, the poor resolution of the discrete preserved phytoplankton samples precluded making this determination.

Similar to the June observation period, from early morning (07:09) through mid-afternoon (16:28), the dinoflagellate and cryptophyte maxima were found at or above the pycnocline. *Scrippsiella trochoidea* was again a prominent component of the phytoplankton community and displayed a clear pattern of DVM (Fig. 2.7). The larger colonial dinoflagellate *Pheopolykrikos hartmanii* generally had cell abundances of less that 200 cells mL$^{-1}$ but achieved cell concentrations of $>1600$ cells mL$^{-1}$ within the nocturnal subpycnocline dinoflagellate maximum at 3.5 m, 03:07 (Fig. 2.7, 2.8). Generally, diatom abundance was higher near the pycnocline and there were no apparent diel patterns to changes in the diatom depth distribution (Fig. 2.8). As in June, fluorescence profiles
generally tracked the vertical distribution of the dinoflagellates throughout the July diel study (Fig. 2.8).

These diel studies have underscored the high degree of variability in phytoplankton biomass that can occur over very short (10’s of cm) vertical distances. It seems likely that the actual vertical profiles of cell abundances are aliased by the low vertical resolution (0.5-1.5 m) of the discrete samples collected for phytoplankton enumeration. As a consequence, some species seem to virtually disappear from the water column and then reappear a few hours later as large peaks in cell abundance at a single depth. The profiles of *P. hartmanii* from the July diel study provide an excellent example. During intensely stratified periods, as was the case during these diel studies, adequate assessment of the populations of motile algae likely require at least decimeter scale sampling resolution.

2.3.8. *Diel patterns of depth distributions*—We used the depth distribution metrics, the depth of the biomass maximum and depth of the center of biomass, to describe diel changes in the vertical distribution of eleven groups of phytoplankton that were abundant throughout the three diel observations. For both vertical distribution metrics, the dinoflagellates, *Polykrikos hartmanii* and *Scrippsiella trochoidea* and the cryptophyte, *Cryptomonas sp.*, showed statistically significant elevations above the pycnocline during the daytime versus nighttime (Fig. 2.9). The other two common dinoflagellates, *Prorocentrum minimum* and Dinoflagellate A displayed diel changes in depth distribution indicative of DVM but these day/night changes in depth distribution were not statistically significant. Despite displaying a pattern of DVM during the July diel observation, when the data was grouped for the three diel studies, no statistically significant DVM pattern was identified for the numerically
dominant, nanoplanktonic cryptophyte, *cf. Chroomonas minuta*. None of the other phytoplankton groups (centric diatoms, pennate diatoms, *Ankistrodesmus sp.*, *Eutreptia sp.*) showed significant differences in vertical distribution between the daytime and nighttime profiles (Fig. 2.9).

In the same manner as for the previous 11 phytoplankton groups, we analyzed cell counts grouped by taxonomic class along with chlorophyll *a* and in vivo fluorescence profiles. Of the dinoflagellates, cryptophytes, chlorophytes (included euglenophytes), and diatoms, only the dinoflagellates showed a statistically significant pattern of DVM (Fig. 2.10). The day/night vertical distribution pattern of chlorophyll *a* showed a pattern indicative of DVM. However, the limited number of daytime and nighttime chlorophyll profiles precluded statistical significance. In vivo fluorescence strongly captured the DVM pattern. Every observed daytime fluorescence maximum was higher in the water column than every nighttime fluorescence maximum. The center of mass of the fluorescence vertical distribution similarly revealed the DVM pattern of phytoplankton biomass produced by the flagellates.

2.3.9. *Comparison of conditions with long-term monitoring data*—The conditions during the diel observations were well suited to shipboard observations and this was no stroke of luck. The dates were chosen based on forecasts of fair weather and on two occasions planned diel observations were canceled due to deteriorating weather. Hence, these observations are classic cases of “fair weather oceanography” and may not represent normal conditions of even the summer season within the lower NRE. However, the long-term median values from the long-term ModMon database showed that the conditions during the diel studies were
typical. During the warm season (Fig. 2.12) and for entire lower NRE, stratified conditions are common with an approximate 3 kg m\(^{-3}\) difference in density between the surface and bottom waters. The depth of the euphotic zone closely approximates the pycnocline. Surface water concentrations are about 1 \(\mu\)M and bottom water DIN is generally 2-3 times greater.

The long-term median values of DIN and chlorophyll \(a\) (Fig. 2.11) also clearly demonstrate the downstream transition of the importance of riverine versus regenerated nutrient sources that fuel growth of the phytoplankton. Riverine derived DIN, dominated by NO\(_3^\)\(^-\), is largely stripped by algal uptake within the upper regions of the estuary (Boyer et al. 1994; Twomey et al. 2005), 19-30 km downstream. Within this region, phytoplankton growth is rapid (Pinckney et al. 1999; Waggener 2006; Paerl et al. 2004) and chlorophyll \(a\) concentrations increase dramatically forming a poorly defined chlorophyll \(a\) maximum at approximately 15-43 km downstream. Within and downstream of the high chlorophyll zone, NH\(_4^+\) is the primary form of DIN and remineralization within the water column and release of NH\(_4^+\) from the sediments are the dominant sources (Boyer et al. 1994; Twomey et al. 2005). This is clearly shown by the reversal of the DIN depth gradient that occurs within the high chlorophyll zone.

During the warmer months, the transition zone between river versus remineralization dominance of DIN inputs occurs further upstream than during the colder months. During the warmer months, DIN decreases rapidly from 9 to 26 km downstream and over this interval surface water DIN is higher than bottom water DIN. During the cold season, the distance over which DIN decreased extended from about 15 to 50 km downstream (Fig. 2.11) and the maximum phytoplankton abundance was also located further downstream at approximately 28-43 km downstream. As during the warm months, past this point surface water DIN was
about 1 μM but there was no tendency toward increased DIN concentrations in the bottom water. For all stations, the degree of stratification was lower during the cold season relative to the warm season.

2.4. Discussion

Data from the three diel observations as well as the long term monitoring data suggest that light and nutrient availability for phytoplankton growth are often vertically separated within this shallow estuarine system. This situation is typical of most aquatic systems when phytoplankton have enough time for uptake of nutrient inputs within the euphotic zone and the primary nutrient source comes from below (Margalef 1978). Within the vertical light and nutrient gradients of the lower NRE, the prominence of flagellated phytoplankton (Pinckney et al. 1998) capable of vertical migration is not surprising. This is fully consistent with the view of Harris (1980), that within the constraints imposed by light limitation, the community composition of the phytoplankton changes to efficiently exploit the temporal and spatial patterns of nutrient availability.

Through DVM, flagellates are able to temporally and spatially separate their acquisition of C and nutrients (Fraga et al. 1992). Phytoplankton cells only require a short period of nutrient supply to accumulate internal nutrient concentrations that are sufficient for division (Harris 1980) and noctural nutrient uptake at depth can supply sufficient nutrients for continued growth of flagellate populations when surface waters are nutrient deplete (Harrison 1976; Amano et al. 1998). Near surface aggregation during the day provides light levels
sufficient for photosynthetic production of carbon substrates while avoiding damaging high irradiance conditions found at the very surface (Passow 1991; Ault 2000).

In mesocosm experiments, phytoplankton assemblages from the NRE were shown to be susceptible to photosystem II damage due to natural surface irradiances (Bergman et al. 2002). However, profiles of flagellate biomass from this field study show that the majority of flagellate biomass is typically found at subsurface depths and likely avoids deleterious mid-day surface irradiances. In accord with Passow (1991), it seems that the primary mechanism for light adaptation of flagellates in poorly mixed systems is through motility rather than photophysiology.

While this study documented obvious patterns of DVM, particularly for several of the dominant, summertime, bloom-forming species *Scrippsiella trochoidea*, *Pheopolykrikos hartmanii*, and *Cryptomonas sp.* (Mallin et al. 1991), DVM is probably only one of several vertical migration behaviors that were performed by the flagellates during this study. Even during the daytime, many of the flagellates displayed peak abundances within the region of the pycnocline which coincided with the nutricline during this study. Within the pycnocline, light levels were low but sufficient for net photosynthesis and diffusion across the pycnocline would provide a steady supply of nutrients. In this situation, aggregation along the pycnocline enhances growth due to enhancement of combined light and nutrient availability (Klausmeir 2000).

Recently, Ralston et al. (2006) have suggested that bimodal vertical distributions of motile cells, as was observed for *cf. Chroomonas minuta* during the June diel study, are likely the product of vertical migrations that are asynchronous with the diel solar cycle. Ascent and descent phases of asynchronous vertical migrations are thought to be linked to
cellular demands for photosynthates and nutrients. If cellular growth is limited by photosynthate, cells swims upwards and arrest migration at depth levels that satisfy light requirements. If growth is nutrient limited, cells swim downward and arrest migration at the nutricline. Thus, for a population of cells containing a mix of nutritional statuses ranging from carbon to nutrient limitation the population will be split between a large aggregation near the surface, a large aggregation near the nutricline, and a diffuse, actively-migrating fraction in between (Ralston et al. 2006).

The observation that the nanoplanktonic cryptophyte *cf Chroomonas minuta* displayed depth distribution patterns indicative of DVM and asynchronous vertical migration is consistent with previous studies that have shown flexibility in migration strategies (Cullen and Horrigan 1981). Changes in vertical migration strategies emerge as growth conditions change and this provides a strong link between nutritional state and fluctuations in the light and nutrient environment (Cullen and Horrigan 1981; Ralston et al. 2006).

We are unaware of previous accounts of nanoflagellate DVM within a marine system. Nanoflagellates, such as *cf. Chroomonas minuta*, typically have maximum swimming velocities of only a few tens of cm hr⁻¹ (Sommer 1988; Crawford 1992). In most marine systems, light attenuation is low enough and mixing is sufficient to preclude formation of significant vertical gradients of light and nutrients at length scales small enough to allow nanoflagellates to make the round trip migration from well lit to nutrient rich waters within 24 hours (Sommer 1988). Thus, in most marine systems, nanoflagellate motility is viewed as little use for traversing large gradients in light and nutrients and accounts of nanoflagellate migrations have been restricted to steeply thermally stratified lakes (Sommer 1982). As such, Goldman (1984) hypothesized that the primary advantage of motility for marine
nanoplankton is for exploitation of nutrient micro-patches such as might be found within or near detrital aggregates (i.e., “marine snow”). However, in a system like the NRE with such steep light and nutrient gradients even these smallest, slow-swimming flagellates may effectively use motility as means to maintain access to light and nutrients (Sommer 1988).

As mixing zones, estuaries are characterized by complex spatial gradients and thus would hardly be considered isotropic (Lewis and Platt 1982). Additionally, high degrees of temporal variability in salinity, nutrient inputs, mixing, turbidity etc. occur on time scales that approximate the generation times of phytoplankton (Lewis and Platt 1982; Litaker et al. 1987; Cloern and Jassby 2008). This temporal variability probably dictates that estuarine phytoplankton communities are most often in disequilibrium with respect to interspecific competition (Hutchinson 1961). It appears as no coincidence that Hutchinson (1961) restricted his arguments of the “paradox of the plankton” to the “turbulent open water” of oceans and large lakes where more isotropic and temporally stable conditions are more commonly found.

During periods of intense stratification as was observed during these diel studies, the vertical gradients of light and nutrients create intense habitat complexity and a plethora of potential niches. Within these gradients interspecific variation in vertical migration strategies affords niche diversification for competing flagellate species (Blasco 1978; Cullen 1985; Olli et al. 1998). This coupling between water column structure and vertical migrations likely contributes to the diversity of the phytoplankton community and the ability to simultaneously maintain high concentrations of several species of flagellated algae without competitive exclusion (Sommer 1982; Olli et al 1998).
Table 2.1. Diffuse light attenuation coefficient, the depth of the euphotic zone, and depth of the pycnocline. Values are means (standard deviations).

<table>
<thead>
<tr>
<th>Date</th>
<th>Light Attenuation (m⁻¹)</th>
<th>Euphotic Depth (m)</th>
<th>Pycnocline Depth (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-3 May 2001</td>
<td>1.56 (0.08)</td>
<td>2.95 (0.15)</td>
<td>2.47 (0.51)</td>
</tr>
<tr>
<td>12-13 June 2001</td>
<td>1.44 (0.12)</td>
<td>3.22 (0.28)</td>
<td>1.97 (0.38)</td>
</tr>
<tr>
<td>17-18 July 2001</td>
<td>1.32 (0.07)</td>
<td>3.48 (0.19)</td>
<td>2.11 (0.72)</td>
</tr>
</tbody>
</table>
Table 2.2. $\text{NH}_4^+$ concentrations, minimum uptake rates, and maximum turn over times for the $\text{NH}_4^+$ pool. Values are means (standard deviations) from near surface, near pycnocline, and subpycnocline depths.

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>$[\text{NH}_4^+]$ (μM)</th>
<th>$\text{NH}_4^+$ Uptake (μM h$^{-1}$)</th>
<th>Turnover Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.85 (0.25)</td>
<td>0.22 (0.075)</td>
<td>3.95 (0.85)</td>
</tr>
<tr>
<td>2</td>
<td>0.87 (0.37)</td>
<td>0.21 (0.091)</td>
<td>4.23 (1.0)</td>
</tr>
<tr>
<td>4 or 5</td>
<td>2.84 (2.26)</td>
<td>0.15 (0.069)</td>
<td>30.6 (36)</td>
</tr>
</tbody>
</table>
Figure 2.1. Map of the Neuse River Estuary and sampling stations for Chapters 2 and 3. Open circles indicate sediment core locations used to determine the benthic diatom community composition in Chapter 3.
Figure 2.2. Average temperature and salinity profiles during three diel studies. Average salinity (solid black line) and temperature (solid red line) at each 0.5 m depth interval over the course of the three diel observations. Dotted lines represent the standard deviation of the mean values of each 0.5 m depth interval.
Figure 2.3. Depth profiles of PAR, chlorophyll \(a\), primary productivity, and assimilation during mid-day productivity assays.
Figure 2.4. Time series of depth profiles of DIN and PO$_4^{3-}$ during three diel studies. Sampling events occurred during the late afternoon, midnight, early morning and midday sampling events for the three diel observations. Note that the order in which the May time points appear has been arranged so that the ordering of the period of the day is consistent with the June and July diel observations. The 10:45 sample collection was actually the first set of samples collected during the May observation.
Figure 2.5. Phytoplankton vertical distribution patterns during the 2-3 May 2001 diel study. The top three rows represent dinoflagellate, cryptophyte, and diatom cell abundance. The bottom row shows the vertical distribution of in vivo fluorescence (solid green line) and salinity (solid black line). Red lines across figure panels indicate the depth of the pycnocline. Times of sample collection are indicated above each column.
Figure 2.6. Phytoplankton vertical distribution patterns during the 12-13 June 2001 diel study. The top three rows represent dinoflagellate, cryptophyte, and diatom cell abundance. The bottom row shows the vertical distribution of in vivo fluorescence (solid green line) and salinity (solid black line). Red lines across figure panels indicate the depth of the pycnocline. Times of sample collection are indicated above each column.
Dinoflagellate Abundance (cells mL⁻¹)
Cryptophyte Abundance (cells mL⁻¹)
Diatom Abundance (cells mL⁻¹)
Salinity (psu)
Fluorescence (AU)
Figure 2.7. Vertical distribution patterns of the cell abundance of common flagellate species during the June and July diel studies. C. min. = cf. Chroomonas minuta. S. troc. = Scrippsiella trochoidea. Dino A = Dinoflagellate A. P. hart. = Pheopolykrikos hartmanii. Note the change of scale for each flagellate species indicated on the first panel of each row. Dotted lines across figure panels indicate the depth of the pycnocline. Times of sample collection are indicated above each column.
Figure 2.8. Vertical distribution patterns of phytoplankton biomass during the 17-18 July 2001 diel study. The top three rows represent dinoflagellate, cryptophyte, and diatom cell abundance. The bottom row shows the vertical distribution of in vivo fluorescence (solid green line) and salinity (solid black line). Red lines across figure panels indicate the depth of the pycnocline. Times of sample collection are indicated above each column.
Figure 2.9. Diel patterns in vertical distribution of several phytoplankton species or groups. Day versus nighttime differences in the depth of the biomass maximum with respect to the depth of the pycnocline (top panel) and depth of the center of mass with respect to the depth of the pycnocline (bottom panel) for several phytoplankton species or groups commonly observed throughout the three diel observations. Values above each day/night column of depth values indicate the p-value associated with a two sample t-test of the mean day versus nighttime depth distribution.
Figure 2.10. Diel patterns in vertical distribution of several phytoplankton classes and total phytoplankton biomass. Day versus nighttime differences in the depth of the biomass maximum with respect to the depth of the pycnocline (top panel) and depth of the center of mass with respect to the depth of the pycnocline (bottom panel) for the dominant algal classes and total phytoplankton biomass indicators, chlorophyll a and in vivo fluorescence. Figure configuration is identical to figure 7. The chlorophyte group includes euglenoids.
Figure 2.11. Long term median values of the down estuary distributions of surface and bottom water DIN, chlorophyll \( a \), \( Z_{euph} \), \( Z_{pyc} \), and \( \Delta \rho \) during warm and cold seasons. Data used were collected from 1 January 1994 through 1 January 2007. The two columns represent data collected during the warm and cold seasons (water temperature > and < 18.89 °C).
2.5.  LITERATURE CITED


80
Chapter 3

DOMINANT MODES OF VARIABILITY IN PHYTOPLANKTON DEPTH DISTRIBUTIONS IN RELATION TO LIGHT AND NUTRIENT AVAILABILITY: RESULTS FROM BI-HOURLY, DECIMETER-SCALE PROFILES OF PHYTOPLANKTON BIOMASS
ABSTRACT

Using semi-hourly, decimeter scale profiles of in vivo fluorescence, temperature, and salinity we tracked the vertical distribution of phytoplankton biomass and physical water column structure over an annual cycle at each of two stations in the lower Neuse River Estuary. Biweekly measurements of light and nutrients showed a strong tendency toward accumulation of nutrients in the poorly lit hypolimnion and much lower nutrient concentrations in the well lit surface waters. A variety of time series analysis techniques were used to determine dominant modes of variability in phytoplankton depth distributions. The coherent variability in phytoplankton depth distributions was dominated by two vertical migration patterns of flagellates, diel vertical migration and aggregation at the pycnocline. Vertical mixing, however, was an over-riding factor leading to homogenous phytoplankton distributions during wind events.

Seasonal and inter-site differences in the occurrence of the two main vertical migration patterns revealed strong linkages between behavior of the phytoplankton community and the physical and chemical structure of the water column. At both stations diel vertical migration occurred intermittently throughout the year except during the late winter early spring period when sediment nutrient loading was at an annual minimum due to low water temperatures. Aggregation at the pycnocline was more common at the upstream station where the pycnocline was usually within the euphotic zone while diel vertical migration was more common at the downstream station where the pycnocline was usually well below the euphotic depth.
These observations strongly corroborate prior observational and modeling studies of phytoplankton strategies for accessing vertically separated light and nutrients. Enhanced light and nutrient access coupled with reduced advective losses afforded by vertical migration are likely principle reasons why phytoplankton blooms in the lower Neuse River Estuary are dominated by dinoflagellates and cryptophytes. We emphasize that sediment derived nutrients in this shallow stratified estuary are directly available to fuel flagellate blooms. This may prolong the time required for the current nutrient management strategy to achieve its goal of reducing phytoplankton biomass and production within the estuary. Additionally, the regular occurrence of diel vertical migration by flagellates suggests that diel vertical migration may represent a significant vector for transporting sediment derived nutrients from the hypolimnion into the upper mixed layer.
3.1. Introduction

We do not, therefore, always know until we have had a great deal of empirical experience, whether a given example of structure is very extraordinary, or a more trivial expression of something which we may learn to expect all the time (Hutchinson 1953).

This chapter builds on Chapter 2 by examining two circum-annual records of semi-hourly, decimeter scale, vertical profiles of phytoplankton vertical distribution and density structure of the NRE collected remotely by an Autonomous Vertical Profiler buoy. These records document the phytoplankton distribution and water column structure across a broad spectrum of environmental conditions and allow testing of specific hypotheses on the relationship between water column structure and phytoplankton distribution.

To gain a greater understanding of phytoplankton vertical distribution patterns of the lower NRE, we performed two circum-annual studies of phytoplankton vertical distribution using high resolution profiles of temperature, salinity, and in vivo fluorescence as a measure of phytoplankton biomass. Our goals were: 1) Determine the frequency with which vertical migration patterns occur. 2) Understand the suite of physical and chemical conditions that lead to vertically migrating flagellate communities. 3) Understand the relative importance of physical versus biological mechanisms for controlling phytoplankton depth distributions.

Intuitively, the frequency and intensity of the occurrence of an event or process would directly bear on its ecological significance within an ecosystem. For example, intense events such as the flooding associated with hurricanes have can load enough allochthonous carbon into the estuary to drive the whole system to hypoxia (Paerl et al. 1997). Obviously, these infrequent, but intense events are of relevance to our understanding of the functioning of the
estuary. However, it is the persistent though less intense supply of autochthonous carbon from the phytoplankton that produces the characteristic seasonal hypoxia of the bottom waters (Paerl et al. 1997). Vertical migration of the resident phytoplankton would appear to be another small scale, low intensity, process and as such would require a high frequency of occurrence in order for it be an important process governing the structure and function of the estuary.

Using semi-hourly, decimeter-scale, profiles collected by an autonomous vertical profiler (AVP), we tracked the vertical distribution of phytoplankton by *in vivo* fluorescence and the physical properties, temperature and salinity. AVP deployments of > 1 yr duration were made at two representative sites in the lower NRE. Photosynthetically active radiation (PAR) and dissolved inorganic nutrient data were also collected with weekly to bi-weekly resolution during the two deployments to place observed phytoplankton depth distributions into the context of the vertical distribution of primary growth limiting resources.

The wide range of light and nutrient conditions that occurred during the two AVP deployments provided an opportunity to investigate how site specific and seasonal water column characteristics affect dominant depth distribution patterns. Specifically, we address the hypothesis that the occurrence of a vertically migrating phytoplankton community is a response to vertically separated light and nutrient resources. Because we were not able to measure light and nutrients with the same frequency as the vertical distribution of phytoplankton biomass, we also investigated the relationship between the phytoplankton vertical distribution and properties of the water column, temperature and degree of stratification, that are major drivers of the vertical separation of light and nutrients. When the water is warmer, nutrient uptake in the surface waters of the lower NRE is greater (Boyer
et al. 1994; Twomey et al. 2005). Warmer temperatures also increase the rates of remineralization of organic matter in the sediments and thus increase the flux of NH$_4^+$ from the sediments into the bottom waters (Rizzo and Christian 1996; Fear 2003). Under higher degrees of stratification, mixing between the surface and bottom layers is reduced (Buzzelli et al. 2002) and should allow nutrients released from the sediments to accumulate in the bottom waters.

Due to decreased mixing within the pycnocline (Condie 1999), increased water density relative to cell density (Bienfang et al. 1982; Condie 1999), and potentially more favorable environments for growth (Bienfang et al. 1982; Davey and Heaney 1989), even non-motile cells often display higher abundance along pycnoclines. However, the cells observed to aggregate at the pycnocline during this study were principally flagellates and presumably could have swum to other areas of the water column. As such, and in accordance with Ji and Franks (2007), aggregation along the pycnocline is described as a vertical migration pattern even though changes in depth distributions due to aggregation at the pycnocline are passive.

3.2. Methods and Materials

3.2.1. Study sites—Data from this chapter were obtained from two sites on the NRE (Fig. 2.1), NR 120 (34° N 57.132’, 76° W 48.779’) and NR 180 (35° N 3.848’, 76° W 31.561’). NR 120 is described in chapter 2. NR180 is located near the mouth of the NRE where it empties into Pamlico Sound. This site has a depth of approximately 7 m, is mesohaline and is mesotrophic (Boyer et al. 1994). At both sites, salinity is highly variable and largely dependent on riverine discharge (Christian et al. 1991). Both of these sites are monitored on
a bi-weekly basis as part of the Neuse River Estuary Modeling and Monitoring (ModMon) Program (Luettich et al. 2000) that has collected environmental data within the NRE since 1994 (http://www.unc.edu/ims/neuse/modmon).

3.2.2. Data collection—For chemical and phytoplankton community analysis, samples were collected from the surface and from 0.5 m above the bottom on a weekly to biweekly basis using a Van Dorn sampler and dispensed into 2 L polyethylene bottles. Samples were transported in a dark cooler at ambient temperature to the laboratory within 4 hr of collection. Nutrient analyses and PAR profiles were performed as in Chapter 2, Section 1. Profiles of PAR and calculation of light attenuation were performed during each sampling trip in the manner described in Chapter 2, Section 1. The depth of the euphotic zone ($Z_{euph}$) was defined as the depth of 1% of surface PAR. Samples for phytoplankton identification were collected with a Van Dorn sampler at 1 m depth intervals at both stations intermittently throughout this study and preserved in 1% Lugol’s solution. Cell counts were performed as in Chapter 2, Section 1.

High temporal and spatial resolution profiles of temperature, salinity, dissolved oxygen, 

*in vivo* fluorescence, and turbidity were collected using an autonomous vertical profiler (AVP) system instrumented with a YSI 6600 multiparameter water quality sonde (Yellow Springs Inc, Yellow Springs, Ohio). For a complete description of the AVP system, see Reynolds-Fleming et al. (2002). The AVP consists of a moored floating platform housing a computer controlled winch mechanism that casts the water quality instrument at programmable time intervals. Prior to each cast, a sonic fathometer measured the water depth, ensuring a full cast of the water column with the exception of buffer regions at the
surface (~ upper 0.5 m at both stations) and bottom (lower 0.5-1 m at NR 120 and 0.3-0.5 m at NR 180) to prevent the instruments from exiting the water or striking the bottom. Eastern Standard Time was used throughout the deployments.

Deployments were made from 19 November 2001 through 26 December 2002 at station NR 120 and from 25 May 2003 through 26 August 2004 at station NR 180. Equipment malfunctions and storm damage to the AVP resulted in episodic data gaps. The AVP was programmed to make casts every 20 min for the data collected at station NR 120 from Nov, 19 through Dec. 31, 2001. Beginning 1 January 2002 at NR 120, and for all the data collected at NR 180 casts were made every 30 min. For the data collected at NR 120, the winch speed and sampling frequency were set to achieve a depth resolution of 25 cm. For the data collected at NR 180, the winch speed and sampling frequency of the YSI 6600 were set to achieve a depth resolution of 10 cm.

Instruments were exchanged with cleaned and calibrated instruments on a weekly basis at NR 120 and at least biweekly at NR 180. Conductivity was calibrated using a one point calibration of KCl solution according to the YSI User’s manual. Precision of the fluorescence probes was ensured by a two point calibration of deionized water and a 0.1 g L⁻¹ rhodamine-b solution according to the YSI User’s Manual. Fluorescence measurements from these instruments have been shown to accurately portray changes in the vertical distribution of phytoplankton within the NRE (Chapter 2) and generally agree well with chlorophyll \(a\) measurements made by extraction of filtered samples and analysis by high performance liquid chromatography (Hall et al. 2008). Since we were only interested in the relative depth distribution of chlorophyll \(a\), no post calibration of the fluorescence data was performed and the fluorescence data are reported in arbitrary units. The YSI instrument was programmed to
automatically wipe the optical sensors (fluorescence and turbidity) at hourly intervals to prevent fouling.

While the factors that control vertical stratification were not a primary focus of this study, interpretation of example data from the AVP is aided by showing hourly wind data from the National Data Buoy Center’s Coastal Automated Marine Network station CLKN7 located (34°37.30' N 76°31.50 W) 45 km south of NR 180 (Fig. 2.1).

3.2.3. Data processing—Data from the AVP were gridded using 20 min by 0.25 m bins for data collected in 2001 at NR 120, and 30 min by 0.25 m bins for NR 120 data collected after 1 January 2002. Bin sizes of 30 min by 0.10 m were used to grid all the data collected during the NR 180 deployment. When a bin contained more than one parameter value, the bin was filled with the mean of the bin values. Within each cast, empty bins were filled by linear interpolation from nearest neighbors. The 2001 data from NR 120 were linearly interpolated across time to create a constant sampling interval of 30 min in the NR 120 time series. No interpolation over time was performed to fill in missing casts.

Density was calculated from salinity and temperature using the equation of state found in Pond (1983) and the depth of the pycnocline \((Z_{pyc})\) was calculated as the average of the two depths over which the largest change in density occurred. The degree of stratification \((\Delta \rho)\) was calculated as the difference between the density at the shallowest depth measured and the density at the deepest depth measured and is reported in kg m\(^{-3}\).

The coefficient of variation of each fluorescence profile \((CV_{chl})\) was used as a measure of the overall vertical phytoplankton patchiness that is independent of total phytoplankton biomass in the water column. The \(CV_{chl}\) expected due to instrument level noise is \(~0.025\).
This was determined by calculating the coefficients of variation from fluorescence measurements of a bucket of surface water (NR 180; 29 July 2002) made over 17 half hour periods at a sampling rate of 1 Hz. We compared $\text{CV}_{\text{chl}}$ to $\Delta \rho$ to test the hypothesis that stronger stratification leads to a greater degree of vertical phytoplankton patchiness. To resolve the dominant modes of variability in the vertical location of phytoplankton, we used the two indices of phytoplankton depth distribution from Chapter 2, Section 1, depth of the biomass maximum ($Z_{\text{max}}$) and depth of the center of mass ($Z_{\text{cent}}$) of each fluorescence profile.

3.2.4. Data analyses—Traditional time series analysis techniques, such as Fourier analysis and least squares harmonic analysis, are based on the assumption of a stationary time series and generate average spectral estimates of amplitude and phase for each frequency component. Averaging of the spectral estimates over the length of the time series or sections of the time series may obscure episodic signals in the data (Luettich et al 2002), that are important in understanding phytoplankton depth distributions. Attempts to use Fourier spectral analysis on segments of data from this study revealed a high degree of non-stationarity. Given the episodic nature of meteorological forcing features, phytoplankton biomass, and composition of phytoplankton communities, we used wavelet analysis as well as Fourier analysis to investigate the power spectra of the data. Wavelet analysis is a relatively new method that provides estimates of periodic signals localized in both the time and frequency domains making it particular useful for the analysis of non-stationary serial data (Torrence and Compo 1998).

In the NRE and the adjacent Newport River Estuary, much of the variability in physical and biological properties of the water column occur in the semidiurnal to diurnal band, or in
the synoptic scale (2-7 d band) (Reynolds-Fleming and Luettich 2004, Litaker et al. 1987). Based on these findings, we chose a range of wavelet scales representing periods from 1/24 d up to 14 d to encompass the band likely to be most important for structuring depth distributions of the phytoplankton community.

Prior to all spectral analyses, time series were demeaned and data gaps were filled with zeros. All aspects of the wavelet analysis were performed using the software of Torrence and Compo (1998) written for Matlab. Both time localized (local) and time averaged (global) wavelet power spectra were calculated for the CV$_{chl}$, $Z_{\text{max}}$ and $Z_{\text{cent}}$ records. A Morlet wavelet (wavenumber = 6) was chosen because it is complex, retaining both the amplitude and phase information of signal components within the wavelet transform. For the local wavelet power spectra, regions of artificially increased or decreased power are produced by convolution of the Morlet wavelet at the edges of each data series as well as those produced by each data gap (filled with zeros). These edge effects increase linearly with wavelet scale (period) producing a cup shaped region (cone of influence) of corrupted power estimates when contour plotted on time versus log (period) axes. For aesthetic reasons, we do not show the cone of influence on the contour plots as suggested by Torrence and Compo (1998). However, the data edges and gaps are indicated in each figure. Power estimates near these data edges must be interpreted with caution, particularly in the lower frequency bands where the cone of influence becomes increasingly wider. At a period of 1 d, the cone of influence is narrow (~ 3 d width) and interpretation of time localization of power is less tenuous.

The 95% significance levels for the global wavelet power spectra were calculated by multiplying a theoretical white spectrum (variance equal to the variance of the data series) by $\chi^2_2/2$ (according to Torrence and Compo 1998, equation 17). $\chi^2_2$ is the 95th percentile value.
of the chi square distribution with 2 degrees of freedom. 95% confidence intervals were calculated for the global wavelet power spectra based on a $\chi^2$ distribution with the degrees of freedom calculated according to equation 23 of Torrence and Compo (1998). For statistical rigor, power is considered significant when the lower 95% confidence interval is greater than the 95% significance level. Because the frequency resolution of the Morlet wavelet transform decreases with increasing frequency (Torrence and Compo 1998), we also calculated standard Fourier power spectra using a 1344 point (28 d) Hamming window with 50 percent overlap.

The phase of the wavelet transform, $\theta_{W_n(s)}$, is given by

$$\theta_{W_n(s)} = \tan^{-1} \left[ \Im \{ W_n(s) \} / \Re \{ W_n(s) \} \right]$$

where $\Im \{ W_n(s) \}$ and $\Re \{ W_n(s) \}$ are the imaginary and real parts of the wavelet transform, $W$, at wavelet scale, $s$, and time point, $n$.

We calculated the wavelet phase for both the observed diurnal signal and the diurnal solar cycle represented by a cosine wave starting at 00:00 midnight. The phase shift between the two signals was found by subtracting the wavelet phases of the two signals with a phase shift of zero hours indicating that the phase of the diurnal signal is perfectly synchronized with the solar cycle (closest to the surface at noon and closest to the bottom at midnight).

To investigate the importance of temperature and degree of stratification on the occurrence of DVM, we first divided the $Z_{cent}$ and $Z_{max}$ records into segments corresponding to three different levels of average water column temperature (<10 °C, 10-20 °C, and > 20 °C) and three different levels of degree of stratification (< 2 kg m$^{-3}$, 2-4 kg m$^{-3}$, and > 4 kg m$^{-3}$). We then used least squares harmonic analysis to estimate the amplitude of the diel frequency component of each data segment at zero phase shift (perfect synchronicity with the
solar cycle). Comparison of the amplitude estimates provides a measure of the strength of the DVM signal under the temperature and stratification regimes. The variability in each data segment explained by the DVM signal is given by the square of the Pearson correlation coefficient ($R^2$) for the fit between the observed data segments and the modeled signal from the least squares procedure. In data segments where DVM plays a large role in determining $Z_{\text{cent}}$ and $Z_{\text{max}}$, both the amplitude estimate and $R^2$ should be comparatively higher than in segments where DVM plays a lesser role. For testing the significance of all correlations reported in this study, autocorrelation was accounted for by determining the equivalent degrees of freedom according to Emery and Thomson (1997, p. 263).

We used the serial correlation of $H$ and $Z_{\text{pyc}}$ with $Z_{\text{max}}$ and $Z_{\text{cent}}$ and the power spectra of $H$ and $Z_{\text{pyc}}$ to investigate the influence of physical processes on phytoplankton depth distributions and to determine the likelihood of physical processes producing DVM-like signals in $Z_{\text{cent}}$ and $Z_{\text{max}}$. Changes in $Z_{\text{pyc}}$ could greatly affect $Z_{\text{cent}}$ and particularly $Z_{\text{max}}$ when phytoplankton aggregate at the pycnocline (Denman 1977). Changes in $H$ could have drastic effects on $Z_{\text{cent}}$. For example, in a water column with a homogenous fluorescence distribution, if $H$ changes from 4 to 5 m, $Z_{\text{cent}}$ would change from 2 to 2.5 m though no real change in phytoplankton depth distribution had occurred. Additionally, global wavelet spectra were calculated for the $Z_{\text{pyc}}$ and $H$ records in the same manner as for the $CV_{\text{chl}}$, $Z_{\text{max}}$ and $Z_{\text{cent}}$ records.

3.2.5. Phytoplankton photophysiology—Photosynthesis versus irradiance studies were performed on two of the most common vertically migrating species to determine how position within the ambient light gradient was likely to affect photosynthesis. *Scrippsiella*
*trochoidea* (CMAS-1) was procured from Dr. Carmelo Tomas at the University of North Carolina at Wilmington’s Center for Marine Science and was originally isolated by Tomas from the nearby New River Estuary, NC. *S. trochoidea* was grown in batch culture on F/2 – Si media at 25°C under a 12:12 light dark cycle with a PAR flux of 250 μmol photons m⁻² s⁻¹ supplied by fluorescent lighting (Sylvania GroLux). Cultures were transferred biweekly and photosynthesis versus irradiance measurements were made in the middle of the light period during the log growth phase. For both the *S. trochoidea* culture and *Prorocentrum minimum* bloom waters (described below), measurements of photosynthesis under a range of irradiances were performed using the photosynthetron method exactly as described in Bergman et al. (2002). *S. trochoidea* cell counts were made to express productivity on a per cell basis.

On 19 November 2001, a dense surface bloom of the dinoflagellate *Prorocentrum minimum* was discovered near New Bern, NC upstream of the AVP deployment locations. A detailed description of the progression of the bloom is provided by Springer et al. (2005). On the morning of 21 November 2001, two surface water samples were collected from areas of obvious discoloration into 20 L polyethylene carboys. One sample was collected at the confluence of the Trent and Neuse rivers by the old highway 70 draw bridge (35° 6.091’ N, 77° 2.227’ W). The other was collected upstream from Union Point Park by the rail road trestles (35° 7.214’ N, 77° 2.468’ W). Water was immediately returned to the UNC-Chapel Hill Institute of Marine Sciences in Morehead City and photosynthesis versus irradiance measurements were made at midday. The bloom samples were nearly monospecific with > 50,000 cells mL⁻¹ of *P. minimum* so it is unlikely that other phytoplankton contributed significantly to the shape of the photosynthesis versus irradiance profiles. Unfortunately,
dissolved inorganic carbon samples were not collected from the bloom water to determine the magnitude of the rate of carbon fixation so production at each light level is expressed as the percent of the maximum observed counts per minute from the scintillation counter.

3.3. Results

3.3.1. Temperature, salinity and degree of stratification— The average water column temperature shows the annual cycle with minimum water column temperatures (~ 5 - 7 °C) occurring during the late winter to early spring and maximum temperatures (~ 30 °C) in late summer to early fall (Fig. 2.11). Weather events are evident in the data as weekly to bi-weekly variations of 2 - 4 °C in both time series.

Effects of inter-annual differences in riverine discharge are clearly evident from the two salinity records of the two AVP deployments. Average water column salinity was ~ 5 ppt higher upstream at NR 120 than downstream at NR 180 (Fig. 2.11; Table 2.3) reflecting drought conditions during the 2001-2002 deployment at NR 120. NR 120 was only slightly more stratified on average than NR 180 during this study period with an average density difference between the surface and bottom of ~ 3 kg m⁻³ at both stations (Fig. 2.11; Table 2.3). The relative importance of salinity and temperature in stabilizing the water column was calculated according to Gowen (1995). Temperature accounted for less than 7% of the variability in density at both stations, indicating that stratification was almost completely salinity based.

Δρ was highly variable, ranging from 0 to > 10 kg m⁻³ at both stations (Fig. 2.11). At station NR 120, the highest Δρ occurred in the late winter through early spring period with
lower Δρ from late spring through early winter (Fig. 2.11). At NR 180 Δρ was generally higher during the warmer months and lowest during the late winter period from mid-January through February (Fig. 2.11). At both stations, periods of increased Δρ were interrupted at frequent intervals by short lived periods of mixing.

3.3.2. Fluorescence—Average water column fluorescence at NR120 revealed a late winter to early spring bloom that was subsequently shown to be due to the dinoflagellate *Prorocentrum minimum* (this study; Springer et al. 2005). Fluorescence values during this period were as high as 280 compared to normal values around 10 (Fig. 2.11). Similarly, higher average water column fluorescence was observed at NR180 during the winter and spring compared to the summer and fall (Fig. 2.11).

3.3.3. Nutrients—DIN in surface waters was generally low, in the 0.5 – 2 μM range (Fig. 2.12; Table 2.3), at both stations and in three instances was below detection. An exceptionally high surface water DIN value (29.8 μM) occurred on 17 January 2002 at NR 120 (Fig. 2.12). Salinity profiles revealed that the unusually high value was due to a brief (~3 d long) period of direct riverine input. Surface salinity was 13.5 ppt on 28 January 2002, fell to 2.4 ppt on Jan 29 (the day of sampling) and returned to 14.2 ppt by 31 January. This single event increased the mean surface water DIN at NR 120 by a factor of two and the standard deviation by more than a factor of five (Table 2.3). In general, the dominant DIN form was NH₄⁺. However, the large pulse on 29 January 2002 at NR 120 was 98% NO₃⁻ and there were two time periods in the fall of 2002 at NR 120 and the fall of 2003 at NR 180 when NO₃⁻ comprised greater than 25% of the DIN pool.
Surface water PO$_4^{3-}$ varied seasonally at both stations from less than 0.5 μM in the cooler months to greater than 1 μM during the warmer months (Fig. 2.12). There were also three instances during the winter of 2004 at NR 180 when PO$_4^{3-}$ was below detection. Excluding data points where the ratio could not be calculated due to values below limits of detection, mean DIN:DIP ratios at both stations were ~ 8 and stoichiometric considerations of the Redfield ratio would suggest that nitrogen was generally the limiting nutrient for phytoplankton growth. However, on 5 sampling dates at NR 120 and 7 sampling dates at NR 180, DIN:DIP ratios were greater than 16 indicating the potential for phosphorous limitation. All of the high DIN:DIP ratios occurred during the winter period of low DIP concentrations. This is in agreement with numerous studies of nutrient limitation within the mesohaline region of the NRE that have shown that N is the primary limiting nutrient but phosphorous may become limiting during the cooler months (Pinkney et al. 1999; Richardson et al. 2001; Piehler et al. 2002; Paerl et al. 2004).

A comparison of surface and bottom water DIN at the two stations reveals that on most of the sampling dates surface and bottom water DIN were nearly equal or bottom water DIN was higher (Fig. 2.12). Bottom water DIN was higher than at the surface for 24 of 35 (69 %) and 18 of 30 (60 %) sampling dates at stations NR 120 and NR 180, respectively. Bottom water DIN appeared to be particularly higher during the warmer months from April through November. PO$_4^{3-}$ followed a pattern similar to NH$_4^+$ with concentrations often being higher in the bottom waters (Fig. 2.12; Table 2.3).

3.3.4. Light availability—In all but 7 of 35 cases at NR 120 and in all 30 cases at NR 180, there was some portion of the water column below the 1% irradiance depth (H>Zeuph) (Fig.
2.13). In general, a much larger portion of the water column was euphotic at NR 120 than at NR 180. At NR 120 and NR 180, $Z_{\text{euph}}$ averaged 3.62 m and 4.34 m, respectively (Table 2.3). Water depth ($H$) varied by more than 0.5 m at both stations (Fig. 2.13), with averages of 4.71 m and 7.06 m at stations NR 120 and NR 180, respectively (Table 2.3).

The average pycnocline depths at NR 120 and NR 180 were 2.92 m and 5.14 m, respectively (Table 2.3). $Z_{\text{pyc}}$ was less than $Z_{\text{euph}}$ in 27 of 35 dates where light profiles were made at NR 120 (Fig. 2.13). At NR 180, there were only 8 instances out of 30 observations where $Z_{\text{pyc}}$ was shallower than $Z_{\text{euph}}$ (Fig. 2.13). Average values for $Z_{\text{pyc}}$ and $Z_{\text{euph}}$ also show that at NR 120 the average pycnocline depth was above the average depth of the euphotic zone while at NR 180 the average pycnocline depth was below the euphotic zone (Table 2.3).

3.3.5. Representative data from the AVP—The time series ($Z_{\text{max}}$, $Z_{\text{cent}}$, CV$_{\text{chl}}$, $Z_{\text{pyc}}$, $H$, and $\Delta \rho$) used to investigate patterns in the vertical distribution of phytoplankton and vertical water column structure displayed a high degree of variability throughout the study. Due to the data density and record length, little useful information can be conveyed by showing time series plots of the entire records. Rather, we have chosen to show three sets of example data (~1 week in length) that demonstrate the behavior of these time series under varying conditions of phytoplankton vertical distributions and water column structure.

3.3.5.1. April 2002—From 8-14 April 2002 at NR 120, the AVP data revealed an aggregation of phytoplankton biomass confined to a thin layer 0.5 - 1 m thick, centered at $Z_{\text{pyc}}$ (Fig. 2.14). Phytoplankton samples collected at 10:30 on 12 April 2002 showed that the aggregation of phytoplankton biomass along the pycnocline was due to the dinoflagellate, *Prorocentrum*
minimum. Cell abundance reached a maximum of ~ 40,000 cells mL\(^{-1}\) at a depth of 3 m (Fig. 2.15), coinciding with \(Z_{\text{pyc}}\) at the time of sampling (Fig. 2.14). For comparison to non-motile phytoplankton, small centric diatoms (<20 μm diameter) showed a much more even vertical distribution with only a slight increase in the surface waters (Fig. 2.15). Winds were generally light (<6 m s\(^{-1}\)) (Fig. 2.14) with the exception of the wind event on the afternoon of the 9 April during which the aggregation was mixed throughout the water column. Strong stratification occurred throughout most of the time period with \(\Delta\rho\) values generally exceeding 4 kg m\(^{-3}\) (Fig. 2.14). Unfortunately, the maximum depth sampled during this period left a full meter of water unsampled. It seems likely that the few low \(\Delta\rho\) values that were interspersed within this record were due to the fact that the true pycnocline was occasionally lower than the deepest depth sampled. CV\(_{\text{chl}}\) was ~ 1 and was generally lowest when \(\Delta\rho\) was at a minimum (Fig. 2.14). Both \(Z_{\text{max}}\) and \(Z_{\text{cent}}\) track the aggregation of cells at the pycnocline. The correlation between \(Z_{\text{pyc}}\) and \(Z_{\text{max}}\) was strong during this time period and explained 29 % of the variability in \(Z_{\text{max}}\). Based on average water column fluorescence (Fig. 2.11), it appears that the pycnocline bloom of \textit{Prorocentrum minimum} lasted from about 5 March through 19 April 2002. During this time span, \(Z_{\text{pyc}}\) explained 28 % of the variability in \(Z_{\text{max}}\) (Pearson’s correlation).

PAR flux at the depth maximum of \textit{P. minimum} on 8 April 2002 was only 8 μmol photons m\(^{-2}\) s\(^{-1}\) (Fig. 2.15). Bottom water DIN concentration (1.4 μM) was more than two fold higher than the surface water (0.6 μM). Photosynthesis versus irradiance profiles determined a few months earlier from the same bloom event showed that these irradiance levels are far below levels that saturate photosynthesis (Fig. 2.16).
3.3.5.2. June 2003—During the period 25 June-02 July 2003 fluorescence profiles showed an obvious pattern of DVM (Fig. 2.17). At night, fluorescence was highest in the region at or below the pycnocline. In early morning, fluorescence diminished in the bottom water becoming diffuse through the water column. By late morning, fluorescence was highest in the surface waters and remained so throughout the day. During the late afternoon to early evening, the fluorescence maximum descended forming a subsurface aggregation at or below the pycnocline at night. Both $Z_{\text{max}}$ and $Z_{\text{cent}}$ track the DVM pattern well during this period. The variability in $Z_{\text{cent}}$ is much less with a higher tendency toward the center showing that there was a substantial portion of the phytoplankton community that was not migrating. Wind during this period was generally light ($<6 \text{ m s}^{-1}$) and the water column was stratified with a $\Delta \rho$ of approximately 3-4 kg m$^{-3}$ and a pycnocline well delineated by $Z_{\text{pyc}}$. $CV_{\text{chl}}$ was lower than during the *Prorocentrum minimum* pycnocline bloom at NR 120 (Fig. 2.14; Fig. 2.17) and generally highest during mid-day when migrating cells aggregated near the surface. However, nocturnal aggregation at depth and the tendency for a more diffuse distribution during the migration between surface and bottom resulted in an additional cycle in $CV_{\text{chl}}$ with a period of 0.5 d.

Cell counts from discrete samples collected at 1 m intervals at 10:18 on 30 June, 2003 showed that the dinoflagellate, *Scrippsiella trochoidea*, was the dominant flagellated organism. The peak in *S. trochoidea* abundance at 1 m implicates this dinoflagellate as the organism responsible for producing the DVM fluorescence pattern (Fig. 2.18). Small ($<20 \mu\text{m}$ diameter), centric diatoms show a more homogenous vertical distribution (Fig. 7) decreasing only slightly with depth. *S. trochoidea* was found to be a prominent DVM species during the three diel observations from Chapter 2, Section 1. PAR at 1m depth was
~300 μmol photons m\(^{-2}\) s\(^{-1}\) (Fig. 2.18). This light level is very close to the minimum light level required for saturation of the cultured \(S.\ trochoidea\) and is consistent with regulation of depth to optimize photosynthesis (Fig. 2.19). However, it is also possible that this is merely an artifact of the conditions under which the cultures were grown prior to analysis of photophysiology using the photosynthetron. The PAR flux in the culture incubator was ~250 μmol photons m\(^{-2}\) s\(^{-1}\).

3.3.5.3. November 2003—From 3-19 November 2003 at NR 180, there was a strong pattern of DVM interrupted by two short-lived wind mixing events that vertically homogenized the density and fluorescence distributions (Fig. 2.20). For the first 2 d, the water column was weakly stratified despite weak (<3 m s\(^{-1}\)) south winds. \(Z_{\text{pyc}}\) was poorly defined during this period and ranged from near the surface to near the bottom. Despite indications of mixing, the fluorescence data show a clear pattern of DVM similar to that seen from 25 June- 02 July 2003 (Fig. 2.17). Both \(Z_{\text{max}}\) and \(Z_{\text{cent}}\) track the pattern of DVM well. DIN was slightly elevated in the bottom waters (2.6 μM) compared to the surface (2.4 μM) (Fig. 2.12). Cell counts from a surface sample showed that the phytoplankton assemblage was a mix of diatoms, cyanobacteria, dinoflagellates, and cryptophytes. The dinoflagellates, \(Akashiwo sanguinea\), \(Scrippsiella trochoidea\), and \(Polykrikos hartmannii\) and the cryptophyte, \(Teleaulax amphioxeia\), were present at concentrations of 155, 68, 55, and 88 cells mL\(^{-1}\), respectively.

Stratification increased from mid-day on 6 November to mid-day on 7 November and \(Z_{\text{pyc}}\) clearly delineated the well defined pycnocline. Winds shifted to the north-east and increased up to ~12 m s\(^{-1}\) starting on the evening of 7 November. Mixing from this wind
event homogenized both the phytoplankton and density distributions. $Z_{\text{max}}$ and $Z_{\text{pyc}}$ were noisy and $Z_{\text{cent}}$ remained very close to the center of the water column. On 10 November, the winds relaxed, the water column restratified, and the DVM pattern in fluorescence resumed. The DVM pattern was clear until on 13 November when another south west wind even mixed the water column again. The data record for 14 November was missing due to malfunctioning of the AVP. On 15 November, the water column was stratified and the pattern of DVM reappeared. The depth of the nocturnal descent shallowed from 15-19 November as the depth of the pycnocline rose from approximately 5 to 2 m from the surface. On 17 November, DIN of the surface waters (4.7 $\mu$M) was nearly two fold higher than the bottom water (2.6 $\mu$M) (Fig. 2.12). Cell counts of a surface sample from 17 November showed that the cryptophyte, *Teleaulax amphioxeia*, and the dinoflagellates, *Akashiwo sanguinea*, *Scrippsiella trochoidea*, and *Heterocapsa rotundata* were present at concentrations of 386, 35, 33, and 85 cells mL$^{-1}$, respectively.

Throughout this period, variability in CV$_{\text{chl}}$ at small temporal scales (hr to d) was related to the migration patterns of phytoplankton (Fig. 2.20). As in the June 2003 record, there was a diel cycle due to a higher degree of aggregation at the surface during the day and a cycle with a half day period due to the reduced aggregation during morning and evening migrations. On larger temporal scales, variability in CV$_{\text{chl}}$ was due to variations in the strength of vertical mixing due to wind events.

### 3.3.6. Influence of degree of stratification on vertical patchiness of phytoplankton

There was a weak but highly significant positive correlation between the vertical patchiness of phytoplankton (CV$_{\text{chl}}$) and the degree of stratification of the water column ($\Delta \rho$) at both
stations (Fig. 2.21). Mean CV_{chl} binned by 0.2 \Delta \rho units showed that below a \Delta \rho of about 1 kg m\(^{-3}\), mean CV_{chl} dropped precipitously to values that were only about a factor of two greater than those that would be expected due to instrument noise. 23 and 37 percent of observed \Delta \rho values were below 1 kg m\(^{-3}\) at NR120 and NR180, respectively.

3.3.7. Local wavelet spectra of CV_{chl}, Z_{max}, and Z_{cent} — The local wavelet spectra of CV_{chl} at NR120 and NR180 are dominated by low frequency variability with periods of 4 to 14 d. However, at NR180 there is a weak but perceptible band of power at a period of 1 d. The local wavelet power spectrum for Z_{max} at station NR 120 (Fig. 2.22) is characteristically red with intermittent peaks in power in the 4-14 d band. The intermittent band of power at a period of 1 d occurred from May through December but was largely absent from January through April. This period where the DVM pattern was largely absent coincides with the Prorocentrum minimum bloom (Fig. 2.11; Fig. 2.14) which remained aggregated along the pycnocline. The local power spectrum of Z_{cent} at station NR 120 (Fig. 2.22) is redder in nature than the Z_{max} spectrum and unlike the spectra of Z_{max} there were events of concentrated power in the 4-8 d band. These were present throughout the year but were most prominent in the January through April period. The diurnal band of power is less prominent than in the Z_{max} record but showed the same pattern of time localization, occurring intermittently from May through early December. A weak semi-diurnal signal is evident during times when the diurnal signal was strong in both the Z_{max} and Z_{cent} records.

The diurnal band dominates the local spectrum of Z_{max} at station NR 180 (Fig. 2.22). The peak is very strong through December, intermittent through mid-January, largely absent from late January through early March and very strong again from late March through August.
Water column stability was persistently low during the late winter early spring at NR 180 (Fig. 2.11). It appears that the minimum in diel power from late January through early March at NR 180 reflects the well mixed conditions that prevailed during this period. Power at longer wavelengths is also high with episodes of increased power in the 2-14 d band. As was the case at NR 120, the diurnal band of power in the \( Z_{\text{cent}} \) spectrum at NR 180 (Fig. 2.22) is less pronounced than in the \( Z_{\text{max}} \) spectrum and the pattern of time localization of the diurnal signal was similar between \( Z_{\text{max}} \) and \( Z_{\text{cent}} \). As at NR 120, a weak semi-diurnal signal is evident during times when the diurnal signal was strong in both the \( Z_{\text{max}} \) and \( Z_{\text{cent}} \) records.

The spectrum of \( Z_{\text{cent}} \) at NR 180 is also redder in nature than the \( Z_{\text{max}} \) spectra showing episodes of concentrated power at periods of roughly 2 d, and intermittently throughout the 4-14 d band.

3.3.8. Global wavelet and Fourier spectra of \( CV_{\text{chl}}, Z_{\text{max}}, \) and \( Z_{\text{cent}} \) — At station NR 120, the global or time averaged wavelet power spectrum (Fig. 2.23) of \( CV_{\text{chl}} \) is characteristically red with a small peak in power at a 3-4 d period that shows up more prominently in the Fourier spectra. Both the global wavelet and Fourier spectra of \( Z_{\text{max}} \) show a significant peak in power at a period of 1 d and then a gradual increase in power at longer periods. In the Fourier spectra the semi-diurnal signal is evident. For \( Z_{\text{cent}} \) at NR 120 (Fig. 2.23), the average power of both the global wavelet and Fourier spectra is two orders of magnitude lower than for \( Z_{\text{max}} \) but the distribution of power is very similar with a peak at 1 d and a much smaller peak at 0.5 d. The main difference between the spectra of \( Z_{\text{max}} \) and \( Z_{\text{cent}} \) is the peak in power in the 4-8 d period in \( Z_{\text{cent}} \) that is absent from the \( Z_{\text{max}} \) spectrum.
Both the global wavelet and Fourier power spectra of CV$_{chl}$ at NR 180 show a characteristically red spectrum but also have significant peaks in power at 1 d and 0.5 d that were not apparent in the spectra of CV$_{chl}$ at NR 120. For $Z_{\text{max}}$ and $Z_{\text{cent}}$ at NR 180 (Fig. 2.23), the power spectra are dominated by the diurnal signal and power increases almost monotonically at periods longer than 1 d. There are also small peaks in power at 0.5 d that are evident in the Fourier spectra. As at NR 120, the spectra of $Z_{\text{cent}}$ at NR 180 (2.23), also has a peak at a period of 4-8 d.

The average power of the NR 180 $Z_{\text{max}}$ and $Z_{\text{cent}}$ records was higher than for the NR 120 records, reflecting the higher variance of the NR 180 data as a result of greater water column depth. However, normalization of the wavelet power spectra by the variance of each data series (according to Torrence and Compo 1998) showed that the greater power of the diel peak at NR 180 compared to NR 120 was not just an effect of the increased variance in $Z_{\text{max}}$ and $Z_{\text{cent}}$ at NR 180 due to greater water depth. Rather, the greater time averaged diurnal power at NR 180 was the result of the more consistent occurrence of the diurnal signal through time.

3.3.9. Local diel power and phase—Power and phase of the diel signal in $Z_{\text{max}}$ and $Z_{\text{cent}}$ are shown for the time periods when the diel power was statistically significant (Fig. 2.24). At both stations, the phase of the $Z_{\text{max}}$ and $Z_{\text{cent}}$ records was very close to a zero hr phase shift (Fig. 2.24) during periods of increased power at the diurnal frequency. This indicates that when DVM occurred the phase of the DVM cycle was closely synchronized with the solar cycle (depth minimum at noon, depth maximum at midnight). During periods of reduced
power, such as the period from late January through March, the phase was highly variable at both stations (Fig. 2.24).

3.3.10. Diurnal signal strength during different stratification intensity and temperature regimes—At both stations, the diel signal in $Z_{\text{max}}$ and $Z_{\text{cent}}$ showed clear trends of increased amplitude with higher water temperatures (Table 2.4). Additionally, at both stations, the diel signal amplitude in $Z_{\text{max}}$ was highest under moderate ($1 < \Delta \rho < 3 \text{ kg m}^{-3}$) stratification. The causes of this are probably two fold. First, at the lowest level of stratification intensity it appears that turbulence levels preclude the capacity to regulate depth through swimming. This is evidenced by the steep descent in $\text{CV}_{\text{chl}}$ when $\Delta \rho$ is less than 1 kg m-3 (Fig. 2.21). Secondly, at both stations, there was a strong tendency toward a shallower pycnocline under higher stratification intensities (Table 2.5). During DVM patches of fluorescence were commonly noted to halt their nocturnal descents at depth levels at or just below the pycnocline (Fig. 2.7; Fig. 2.17; Fig. 2.20). Thus a shallower pycnocline would decrease the migration amplitude necessary to traverse between the surface and bottom waters and may have decreased the observed amplitude of $Z_{\text{max}}$ under the highest stratification intensities.

The diel signal in $Z_{\text{cent}}$, at both stations, showed a trend of increased amplitude with increased degree of stratification though the effect was not as pronounced as with temperature (Table 2.4). The observation that the amplitude of the $Z_{\text{max}}$ signal decreased from moderate to high stratification while the amplitude of the $Z_{\text{cent}}$ signal increased indicates that the biomass of phytoplankton performing DVM (relative to the non migrating phytoplankton) must have been higher under the highest levels of stratification intensity. The phase estimates for the
diel signals of each data segment were all less than 1 hr, indicating close synchronization with the solar cycle.

The fit of the least squares signal estimate to the data segments (R) generally followed the same pattern as the amplitude (Table 2.4). Much more of the variability in \( Z_{\text{max}} \) and \( Z_{\text{cent}} \) was explained by the diel signal at NR 180 than at NR 120. The diel signal estimate explained a significant 23% of the variability in the complete \( Z_{\text{max}} \) record at NR 180 \((R^2 = 0.23)\) and a significant 37% of the variability in \( Z_{\text{max}} \) at NR 180 under the highest temperature category. Though statistically significant, at NR 120 the estimates explained only 5% of the variability in the complete \( Z_{\text{max}} \) record and 10% of the variability in \( Z_{\text{max}} \) under the highest temperature category. The least squared estimates explained much more of the variability of \( Z_{\text{max}} \) than of \( Z_{\text{cent}} \) for both stations.

3.3.11. **Influence of \( Z_{\text{pyc}} \) and \( H \) on \( Z_{\text{max}} \) and \( Z_{\text{cent}} \)**—Serial correlations of \( Z_{\text{max}} \) and \( Z_{\text{cent}} \) with \( Z_{\text{pyc}} \) and \( H \) reveal a degree of control of the phytoplankton community depth indices by physical processes (Table 2.5). As expected, there was a significant correlation between \( H \) and \( Z_{\text{cent}} \) at both stations because \( Z_{\text{cent}} \) tends toward the center of the water column. The only affect that \( H \) directly has on \( Z_{\text{max}} \) is by limiting the range of possible \( Z_{\text{max}} \) values. There was no significant correlation between \( H \) and \( Z_{\text{max}} \) at either station. \( Z_{\text{pyc}} \) was much more strongly correlated with \( Z_{\text{max}} \) and \( Z_{\text{cent}} \) at NR 120 than at NR 180. \( Z_{\text{pyc}} \) had a significant correlation with both \( Z_{\text{max}} \) and \( Z_{\text{cent}} \) at NR 120, explaining 6% and 12% of the variability in the two records, respectively. \( Z_{\text{pyc}} \) correlated significantly with \( Z_{\text{max}} \) at station NR 180 but explained only 2% of the variability. The influence of \( Z_{\text{pyc}} \) on \( Z_{\text{max}} \) is consistent with the observation of episodic dense aggregations of fluorescence at the pycnocline such as was seen in Fig. 4. At
NR 120 aggregation along the pycnocline was more common than at NR 180. Even if the time period of the spring *Prorocentrum minimum* bloom (9 March through 19 April 2002) is removed from the NR120 record, the correlation between $Z_{\text{max}}$ and $Z_{\text{pyc}}$ is still stronger at NR120 ($R = 0.21$) than at NR180 ($R = 0.15$). It seems likely that much of the low frequency variability (d to wk periods) in $CV_{\text{chl}}$, $Z_{\text{max}}$, and $Z_{\text{cent}}$ is due to physical processes while vertical migration patterns are responsible for the coherent variability at smaller time scales.

3.3.12. *Global wavelet and Fourier spectra of $Z_{\text{pyc}}$, $H$, and $\Delta \rho$*—In contrast to the spectra of $Z_{\text{max}}$, $Z_{\text{cent}}$ and $CV_{\text{chl}}$ (particularly at NR 180) that showed high power in the semi-diurnal and diurnal frequencies (Fig. 2.23), the spectra of $Z_{\text{pyc}}$, $H$, and $\Delta \rho$ contain very weak power at these frequencies. The global wavelet and Fourier power spectra for $Z_{\text{pyc}}$, $H$, and $\Delta \rho$ (Fig. 2.25) show near monotonic increases in power with increased period at both stations with only slight indications of peaks in power at 3-10 d periods. There is a single peak in power at about 10 d in the global wavelet spectrum of $Z_{\text{pyc}}$ at NR 120 but this peak is absent from the Fourier spectrum. Weak peaks in power occur at periods of ~ 3 d and 4-8 d in both the wavelet and Fourier spectra of $Z_{\text{pyc}}$ at NR 180 (13 d). There is a broad peak in power at ~ 8 d in $H$ at NR 120 that is present in both the wavelet and Fourier spectra and a hint of elevated power at ~ 8 d in the wavelet spectra of $H$ at NR180. For $\Delta \rho$ at NR120, there is a peak in power at ~8 d at NR 120 in the wavelet power spectra but not the Fourier spectra. For $\Delta \rho$ at NR 180, there was elevated power at 4-8 d in the wavelet power spectra but this peak was also absent from the Fourier spectra.
3.3.13. *Average fluorescence profiles over the study period*—Fluorescence and density data from each depth were averaged over time to generate average profiles of fluorescence and density gradient \((\Delta \rho \Delta z^{-1})\) for the study period at each station. In addition, the mean fluorescence value at each depth of profiles taken from 9:00 A.M. through 3:00 P.M. and from 9:00 P.M. through 3:00 A.M. were used to produce average daytime and nighttime fluorescence profiles, respectively.

The average fluorescence profile for NR 120 shows a broad subsurface maximum at a depth of 2-3.25 m (Fig. 2.26). This depth generally coincides with the average \(Z_{\text{pyc}}\) as revealed by the vertical density gradient. A comparison of average daytime and nighttime fluorescence profiles reveals no obvious pattern of DVM at NR 120.

At NR 180, the average fluorescence profile is fairly homogenous with depth only slightly increasing just above the bottom (Fig. 2.26). A comparison of the average daytime and nighttime fluorescence profiles reveals the influence of DVM. Near the surface, the daytime fluorescence profile is about 1 fluorescence unit greater than the average profile, reaching a maximum at about 1.5 m. Near the surface the nighttime profile is about 1 unit less than the average profile. At depth, the daytime profile is 1 unit less than the average and the nighttime profile is 1 unit greater than average reaching a maxima just below the average depth of the pycnocline.

3.3.14. *Other potential sources of the diel signal in phytoplankton depth distribution*—Daily cycles in phytoplankton biomass due to the balance between phytoplankton growth and mortality are known to occur (Sournia 1974, Litaker et al. 1988). This cycle is produced when daytime phytoplankton growth exceeds grazing and nighttime grazing exceeds growth.
This cycle should be more prominent higher in the water column where greater light availability can lead to rapid growth. If such a cycle were active in the surface waters of the Neuse River Estuary, it could produce a daily oscillation in $Z_{\text{cent}}$ and $Z_{\text{max}}$. However, a daily cycle in the balance between growth and grazing cannot explain nocturnal accumulation of biomass in the bottom waters.

*In vivo* fluorescence, as a measure of chlorophyll concentration, is confounded by changes in the photo-physiology of phytoplankton cells in natural waters. In particular, non-photochemical quenching is the result of photo-damage to photosystem II that reduces the fluorescence emission per unit chlorophyll $a$ and is probably the largest source of error in measurements of chlorophyll $a$ by *in vivo* fluorescence (Kiefer 1973). Given the rapid light attenuation with the NRE, only the upper 2 m of the water column receive irradiance in excess of the ~100 W m$^{-2}$ (Kiefer 1973) threshold necessary to induce non-photochemical quenching. Nevertheless, non-photochemical quenching could produce a daily cycle in both $Z_{\text{max}}$ and $Z_{\text{cent}}$ by depressing fluorescence at the surface during the day where cells are exposed to higher irradiance. This explanation is rejected because the phase of the signal produced by non-photochemical quenching would be nearly 180 degrees or 12 hours out of phase with the observed signal. Non-photochemical quenching could also produce subsurface maxima as observed commonly at NR 120. However, recovery from light induced non-photochemical quenching generally occurs over the time course of minutes up to 1 hr so night time measurements of chlorophyll by *in vivo* fluorescence are not affected by NPQ (Falkowski and Kiefer 1985). The average nighttime profile which is not affected by non-photochemical quenching also displayed the subsurface maxima in the region of the pycnocline. Additionally, during periods where the fluorescence profiles displayed
aggregation at the pycnocline, profiles of cell counts confirmed higher cell numbers near the pycnocline (Fig. 2.14; Fig. 2.15). There was no diel cycle in either $Z_{\text{pyc}}$ or H that could have driven the diel signal in $Z_{\text{max}}$ and $Z_{\text{cent}}$.

Though non-photochemical quenching could not have produced the diurnal signal, it is possible that NPQ produced the semi-diurnal signal by depressing fluorescence at the surface, driving $Z_{\text{max}}$ and $Z_{\text{cent}}$ deeper in the water column during mid-day. The signal could also have been produced by a secondary vertical migration away from photoinhibiting irradiances near the surface at mid-day. Yet another possibility is that the semi-diurnal signal is an even harmonic of the diurnal signal created by Gibb’s phenomena and is just an artifact of the analysis. The temporal coincidence of strength of the semi-diurnal signal with the strength of the diurnal signal would support this explanation but may also support a true semi-diurnal migration since the presence of the diurnal signal indicates a population of migratory cells.

3.4. Discussion

3.4.1. Effects of vertical water column structure on the vertical distribution of phytoplankton-Throughout the two circum-annual study periods within the lower Neuse River Estuary, fluorescence profiles revealed patterns of vertical phytoplankton patchiness related to two vertical migration patterns, DVM and aggregation at the pycnocline. These vertical migration patterns were detectable most of the time; the exception being when the water column was fully mixed from surface to bottom which occurred about a quarter and a third of the time at NR120 and NR180, respectively. This means that not only were
vertically migrating phytoplankton commonly present in the water column but also that the concentrations of the flagellates were high enough relative to their non-migrating counterparts to affect the vertical distribution of fluorescence. Bottom waters within the estuary were generally dysphotic and contained higher concentrations of DIN and PO$_4$$^{-3}$ than the surface waters. Exploitation of vertically separated light and nutrient resources is a primary component of the successful life history strategy of the flagellate community of this shallow lagoonal estuary.

About the only time periods when vertical migration patterns were not detected was when the water column was well mixed from surface to bottom. This was revealed by the precipitous decrease in CV$_{chl}$ when the difference between bottom and surface density fell below about 1 kg m$^{-3}$. Since mixing in this tideless estuary is driven primarily through wind energy (Luettich et al. 2000), this implies that when wind generated levels of turbulence are high enough to fully mix the water column, swimming ability of the phytoplankton becomes ineffective against the large grained turbulence.

DVM by definition affects the vertical location of phytoplankton biomass in the water column. However, we found that DVM was also responsible for both diurnal and semidiurnal cycles in the overall patchiness of phytoplankton in the water column. Typically, patchiness was greatest at midday when phytoplankton were aggregated higher in the water column. However, patchiness was also higher during nighttime aggregations at depth and patchiness was minimal during morning and evening ascents and descents, respectively. Similarly, Waters and Mitchell (2002) used fractal dimension analysis to demonstrate diurnal and semidiurnal cycles of vertical patchiness due to DVM.
Algal blooms in the middle and lower part of the NRE are largely due to dinoflagellates and occasionally cryptophytes (Pinckney et al. 1998) which were also the organisms responsible for the vertical migration patterns observed in this study. On average, the spatial maximum of dinoflagellate biomass along the estuarine continuum from the headwaters of the NRE to its receiving body, the Pamlico Sound, occurs in the middle to lower NRE (Valdes-Weaver et al. 2006). Within this region of the estuary, access to growth limiting light and nutrients are typically vertically separated by only a few meters. With typical swimming speeds of 1-2 m h\(^{-1}\) (Sommer 1988; Crawford 1992), dinoflagellates and cryptophytes can traverse the entire water column in 4-8 h. Typically, fluorescence profiles from our study showed maximum migration amplitudes of only 4-5 m between the near surface and near bottom of the water column and the migration between layers appeared to last less than 4 h. Obviously, in such a shallow system, flagellates performing DVM can make their transits and still have ample time for nocturnal nutrient uptake and daytime photosynthate production. Thus via their vertical migrations, it appears that the dinoflagellates and cryptophytes are well suited to the average light and nutrient gradients of the lower NRE.

Even if nutrient concentrations were homogeneously distributed throughout the water column, movement would enhance nutrient uptake by reducing the diffusive boundary layer of nutrient deplete water surrounding the cell (Sommer 1988). For the size (10-50 \(\mu\)M equivalent spherical diameter) and speed (~1 m h\(^{-1}\)) of the flagellates observed in this study, swimming would increase nutrient uptake by about fifty percent over a stationary existence. Additionally, even without a concentration gradient, vertical migration to depths would increase the available volume of water for capturing nutrients.
Enhancement of nutrient uptake and growth by vertically migrating into waters of higher nutrient concentration is widely discussed in the literature (Margalef 1978; Ganf and Oliver 1982; Lieberman et al 1994; Smayda 1997; Amano et al. 1998). However, due to the wide range of ecophysiological characteristics, many of which are poorly constrained, it is difficult to estimate the magnitude of growth enhancement that vertical migration affords flagellates of the lower NRE. First, the magnitude of growth enhancement is dependent on the species specific capacity for daytime versus nighttime uptake of nutrients. For seven species of dinoflagellates, including *Scrippsiella trochoidea* and *Prorocentrum minimum*, the ratio of light to dark NH$_4^+$ uptake ranged only from ~1-2.5 (Paasche 1984). Thus it appears that most dinoflagellates are physiologically capable of significant nocturnal uptake of NH$_4^+$.

The magnitude of growth enhancement is also highly dependent on the degree of nutrient limitation imposed on the vertically migrating flagellates by nutrient concentrations within the surface waters. For most of the year, N limits phytoplankton growth throughout the NRE (Paerl et al. 2004). Riverine inputs of NO$_3^-$ are generally stripped from the surface waters prior to entering the lower NRE and as a result NH$_4^+$ is the dominant form of DIN in the lower NRE. High rates of NH$_4^+$ inputs from remineralization maintain the high level of productivity within the lower NRE, but as a result uptake rates generally balance inputs and residual concentrations are low, typically ~1 μM. Regardless of input rates, this is the instantaneous concentration experienced by the phytoplankton in competition for the limiting nutrient in the surface waters. If this concentration is higher than concentrations that saturate growth of the flagellates, then there should be no apparent growth advantage gained by vertically migrating into more nutrient rich waters.
Available data on the steady state NH$_4^+$ uptake kinetics of dinoflagellates suggest that 1 μM is substantially less than the NH$_4^+$ necessary to saturate uptake. However, the capacity for enhanced uptake under N stressed conditions and reduction of the intracellular N quota are well established and lead to significant departures in observed growth rates compared to what would be expected based on steady state nutrient uptake kinetics (Morel 1987). For several diatom species and a picoplanktonic chlorophyte, these acclimation responses to nutrient stress have been demonstrated to allow growth to proceed at rates comparable to steady state nutrient saturated conditions at NH$_4^+$ concentrations that are an order of magnitude lower than would be possible based on the concentration that saturates steady state NH$_4^+$ uptake (Goldman and Glibert 1982; Morel 1987). Sunda and Hardison (2007) found that two species of coastal diatoms, and three picoplanktonic species displayed growth limitation at very low NH$_4^+$ concentrations; ~0.1 μM for the diatoms and ~0.03-0.04 for the picoplanktonic species.

Unfortunately, coastal dinoflagellate species have not been represented in these studies and further research is necessary to determine the degree to which steady state kinetics and acclimation to nutrient stress affect their growth rates. However, if we assume that the ratio of half saturation for uptake to the half saturation for growth is approximately 10, as was shown for other taxa, (Goldman and Glibert 1982; Morel 1987), we can make some inference as to the likely growth limitation that would be imposed on the dinoflagellates of the lower NRE if they were confined to the surface waters. From Smayda (1997), the mean half saturation coefficient for NH$_4^+$ uptake of 7 dinoflagellate species was 6.4 μM and with the above assumption would suggest that ~1.3 μM would saturate growth. Only about one third of the observed surface NH$_4^+$ values were greater than 1.3 μM. It appears that within the
average nutrient gradients of the estuary, nocturnal migration into the bottom waters or intercepting the DIN supply as it is mixed across the pycnocline should lead to a modest enhancement of growth relative to staying in the surface waters.

A recent model developed by Ji and Franks (2007) investigated how various water column structure scenarios would affect the growth of phytoplankton performing these two types of vertical migration patterns. Vertical migration was cued internally based on the cellular N:C ratio and nutrients increased with depth. The growth optimizing vertical migration strategy was controlled by two principal factors, the depth of the nutricline and the depth of the euphotic zone. In cases where the nutricline depth was equal to or shallower than the euphotic depth, aggregation at the pycnocline was the growth optimizing strategy. Subsurface chlorophyll maxima at the pycnocline are often observed when the pycnocline/nutricline occurs within the euphotic zone (Bjørnsen et al. 1993, Bjørnsen and Nielsen 1991, Estrada et al. 1993, Strass and Woods 1991, Kononen et al 1998). On average this situation typified NR120 during this study. Another case explored was where the nutricline was deeper than the euphotic zone but the distance between the nutricline and the well lit surface waters was less than a half day swim. This situation is representative of the average conditions at NR180. Under this situation, aggregation at the pycnocline would lead to chronic light limitation and DVM was shown to be the growth optimizing vertical migration strategy (Ji and Franks 2007). Thus the model results indicate that the observed dominant vertical migration strategies at the two stations are consistent with the use of depth regulation to optimize access to the average light and nutrient availability within the lower NRE.

It also appears that the close synchrony between DVM and the solar cycle observed in this study may be a feature that was selected for by the average water column conditions.
Variability in the phase relationship between the DVM pattern and the solar cycle is well documented and has been ascribed to the interactions of endogenous rhythms, physiological status, and differences in water column structure (Kamykowski 1981). Similar to our observations, Kiefer and Lasker (1975) documented a closely synchronized DVM pattern for a *Gymnodinium splendens* bloom in the Bahia Concepcion, Gulf of California. Using a model of dinoflagellate growth, Kamykowski (1981) showed that under the observed turbid water conditions in Bahia Concepcion, the close synchrony of DVM with the solar cycle observed by Kiefer and Lasker (1975) should result in higher growth than other phase relationships of DVM with the solar cycle. Under greater or lesser water column turbidity this was not always the case. The potential light field experienced by phytoplankton migrating from 1.5 m (~18 % incoming irradiance) to 6 m (~0.3 % incoming irradiance) in the NRE is very similar to the light field experienced by the modeled dinoflagellates in Kamykowski’s (1981) high turbidity scenario (22 % incoming irradiance at 5 m to 0.6% incoming irradiance at 15.5 m).

While it appears that the patterns of vertical migration observed are well atuned to the average condition of the estuary, at any given time within the study observed vertical migration patterns may or may not have been explainable based on optimization of light and nutrients. For example, on 30 June 2003, the observed maximum in *S. trochoidea* abundance at light levels of ~300 μmol photons m⁻² s⁻¹ is consistent with previous findings that dinoflagellates can vertically migrate to depths that maximize photosynthetic yield (Ault 2000). However, on 8 April 2002, the pycnocline bloom of *Prorocentrum minimum* during the spring of 2002 was observed to remain at light limiting depths. Based on the increased nutrient concentrations at depth and rapid light attenuation present during this time,
optimization of light and nutrients would prescribe a pattern of DVM. Tyler and Seliger
(1981) clearly demonstrated that aggregations of *P. minimum* within the pycnocline of
Chesapeake Bay were strongly light limited and strong light limitation has been observed for
pycnocline aggregations of the dinoflagellates *Heterocapsa triquetra* (Kononen et al. 2003),
*Ceratium tripos* (Malone 1978), and *Alexandrium tamarense* (Rasmussen and Richardson
1989). Additionally, on 17 November 2003 at NR180, a clear pattern of DVM was apparent
despite surface water DIN being nearly two fold higher than bottom water concentrations.
Optimization of light and nutrient acquisition would require the cells to remain in the higher
nutrient surface waters.

The migratory behavior of every motile phytoplankter appears to be governed by a set of
rules that links the surrounding environmental conditions including gradients of light and
nutrient but also gravity, and the physiological status of the cell to its vertical migration
behaviors. These rules appear to be highly species specific. For example, Kamykowski et al.
(1998) found that five dinoflagellate species used five different combinations of
positive/negative phototaxis/geotaxis to synchronize their migration to the light/dark cycle.
To some degree, the rules provide flexibility of behavioral response as the environment
changes. For example, *Akashiwo sanguinea* can switch from a pattern of DVM to
aggregation along a nutricline on time scales of a couple of days (Cullen and Horrigan 1981).
However, the ability of some dinoflagellates to migrate through the pycnocline is impeded
when temperature is below a critical value (Heaney and Eppley 1981, Tyler and Seliger
1981) and other dinoflagellates have been observed to continue to expend energy performing
DVM even when no light source was present during the daylight hours (Eppley et al. 1968).
Obviously, the rules simply do not allow enough flexibility of behavioral response to take advantage of every short term fluctuation in the environment.

While light and nutrient gradients are highly relevant environmental parameters for cellular growth, vertical gradients of current velocities have been shown to be very important for determining the effect of a vertical migration pattern at the population level. Due to their slow growth rates (Smayda 1997), dinoflagellates would be expected to be more susceptible to advective losses within an estuary than faster growing groups (Valdes-Weaver et al. 2006). By splitting time between the opposing flows of the surface and bottom layers (DVM) or remaining in the zone of minimum transport (aggregation at the pycnocline), migration behaviors are likely to minimize net advective losses of flagellate populations from the estuary (Anderson and Stolzenbach 1986, Crawford and Purdie 1992, Tyler and Seliger 1978, Chang and Carpenter 1985). Reduction of advective losses may be an important population growth advantage gained through vertical migration in the lower NRE. Low advective losses are consistent with flagellate blooms persisting in stationary positions in the NRE for weeks to months (Pinckney et al 1998) despite average downstream freshwater transit velocities of 2-3 km d\(^{-1}\) (Sweet 2000). This may be particularly important within the region of the chlorophyll \(a\) maximum where residence times are shorter compared to further downstream and yet flagellates commonly dominate the phytoplankton assemblage (Pinckney et al. 1998; Chapter 5).

Interspecies differences among the rules governing migration diversifies the potential migration patterns and distributions of cells within the water column (Blasco 1978; Cullen 1985). On time scales of many generation times (weeks) those organisms whose rules of migration and physiology are better adapted to the specifics of the environment may rise to
dominance over cooccurring species. However, it seems likely that the environmental fluctuations within the estuary occur at rates faster than the rate of competitive displacement. If this is true, then the selection of the dominant phytoplankton within the system would largely be based on the average conditions that occur over seasonal time scales of weeks to months (Hutchinson 1953) and it is not surprising that the observed vertical migration patterns reflect adaptation to average conditions.

Vertical migration is just one aspect of the population growth strategy of flagellates. Mixotrophy, grazing deterrence, and cyst formation are also likely to be vital to the success of estuarine dinoflagellates (Smayda 1997; Cloern and Dufford 2005). The dinoflagellates that were common during this study are mixotrophic and can significantly enhance growth rates by feeding on a wide range of prey species (Jeong et al. 2005). When conditions become unfavorable for growth, many dinoflagellates form cysts which settle to the sediments and lie dormant until environmental cues trigger excystment (Ishikawa and Taniguchi 1997). From year to year, the sediments serve as a reservoir of the dominant species and can provide a large inoculum when conditions are again favorable for growth of the vegetative cells (Walker and Steidinger 1979). Like vertical migration patterns the rules that govern encystment and excystment may not always lead to cellular growth optimization. For example, the optimum temperature for growth of the dinoflagellate, *Heterocapsa triquetra*, is \( \sim 20 \, ^\circ C \) and *H. triquetra* excysts in early winter as water temperatures fall below \( 12 \, ^\circ C \) (Litaker et al. 2002, references therein). This timing of excystment ensures that the newly vegetative cells will experience suboptimal temperatures for growth. However, suboptimal temperatures are often accompanied by high nutrient availability and reduced grazing pressures that allow *H. triquetra* to reach bloom concentrations (Litaker et al. 2002).
In a similar manner, during the winter in the Chesapeake Bay, *P. minimum* accumulates along the pycnocline at depths that are far below optimum light levels for growth. However, this short term period of severe light limitation is part of a longer term strategy of upstream transport that eventually seeds nutrient rich surface waters during the spring when warmer temperatures allow for rapid growth (Tyler and Seliger 1981). Hence, vertical migration patterns must be considered an integral component of a complex life history strategy for maintenance of vegetative cells in regions favorable for growth (Donaghay and Osborne 1997).

3.4.2. Feedback of DVM on the vertical structure of the water column - The vertical structure of the water column shapes the vertical distribution of the phytoplankton. Conversely, through shading and nutrient uptake, the vertical distribution of phytoplankton can shape the light and nutrient gradients of the estuary. Phytoplankton account for greater than a third of the light attenuation within the lower NRE (Woodruff 1993). As such, the large population of flagellates within the estuary substantially decreases light availability.

The capacity of vertically migrating phytoplankton to redistribute nutrients in the water column is increasingly well recognized across a diverse range of aquatic systems from small lakes (James et al 1992, Salonen and Rosenberg 2000, Ganf and Oliver 1982), large estuaries (Fraga et al. 1992), to the open ocean (Villareal et al. 1993). The amount of nutrients taken up at depth and transported into the surface waters can be significant. The nutrient flux due to nutrient retrieval by *Rhizosolenia* represents 2-27% of the turbulent diffusive flux across the thermocline of the north Pacific gyre and up to 50% of the phytoplankton demand for new nitrogen in the upper mixed layer (Villareal et al. 1993). The fate of nutrients retrieved
from the bottom waters depends on the suite of factors (grazing, viral lysis, advection, sedimentation) that govern population losses of the vertically migrating phytoplankton. It has been suggested that nutrient inputs from the regeneration of DVM phytoplankton biomass in the surface waters may be an important source of nutrients for non-migrating phytoplankton and thus stimulate the productivity of the entire phytoplankton community (Prego 1992; Olli et al. 1998).

Here we present a rough calculation to demonstrate the potential significance of nocturnal nutrient retrieval from the bottom waters of the lower NRE. We assume a cell concentration of $8.0 \times 10^8$ cells m$^{-3}$ occupying the bottom meter of the water column for a ten hour period at night. This is roughly the maximum cell concentration of *Scrippsiella trochoidea* observed on 30 June 2003. Assuming a cellular *S. trochoidea* N quota of $0.2 \times 10^{-5}$ μmol N cell$^{-1}$ (Lirdwitayaprasit et al 1990) and a maximum specific dark N uptake for NH$_4^+$ of 0.02 hr$^{-1}$ (Paasche et al 1984), we obtain a maximum cellular uptake of $\sim 4.3 \times 10^{-6}$ μmol NH$_4^+$ cell$^{-1}$ over the 10 hr dark period in the bottom waters. At a concentration of $8.0 \times 10^8$ cells m$^{-3}$, the *S. trochoidea* population could take up $\sim 3,400$ μmol NH$_4^+$ m$^{-3}$ during its nocturnal residence in the bottom waters. This represents $\sim$140% of the estimated 2,400 μmol m$^{-2}$ d$^{-1}$ NH$_4^+$ flux (Rizzo and Christian 1996) from the sediments into the water column. Half saturation concentrations for NH$_4^+$ uptake by dinoflagellates are generally greater than 2 μM (Smayda 1997) meaning that maximum uptake rates would only occasionally be achieved in the bottom waters of the NRE. This would significantly reduce the 140% estimate of daily uptake of NH$_4^+$ released from the sediments by DVM dinoflagellates. However, it could also be argued that without significant uptake of bottom water NH$_4^+$ by DVM phytoplankton, NH$_4^+$ may reach saturating concentrations. To truly understand the importance of DVM as a
“nutrient pump” in the NRE, nutrient fluxes due to nocturnal nutrient retrieval must be quantified and compared to the diffusive flux into the euphotic zone.

3.4.3. Management implications The strong linkage between sediment nutrient loading and growth of the dominant bloom forming flagellates has important implications for water quality management. The sediments of the NRE contain a vast supply of organic matter (the source for sediment nutrient loading to the water column) due to decades of anthropogenic, nutrient-driven, eutrophication (Cooper et al. 2004). For only the upper 2 cm of the sediment, the residence time for sedimentary nitrogen is estimated at 1.2 years (Luettich et al 2000). However, the zone of sediment ammoniaication extends to depths up to 40 cm (Luettich et al. 2000) suggesting that there are many years of potential sedimentary nitrogen load stored within the sediments. This source of nutrients is likely to fuel dinoflagellate blooms long after riverine load reductions are fully effective. In the long term, riverine nutrient load reductions should reduce sedimentary organic matter supply decreasing sediment nutrient loading. Patience with the current nutrient load reduction strategy is critical as it will likely take many years for the nutrient load reductions to achieve reductions in phytoplankton biomass.
Table 3.1. Select physical and chemical properties of the AVP sampling stations during the study period.

<table>
<thead>
<tr>
<th>Station</th>
<th>Property</th>
<th>Mean (STD)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR 120</td>
<td>H (m)</td>
<td>4.71 (0.20)</td>
<td>9,079</td>
</tr>
<tr>
<td></td>
<td>$Z_{pyc}$ (m)</td>
<td>2.92 (0.81)</td>
<td>10,523</td>
</tr>
<tr>
<td></td>
<td>$K_d$ (m$^{-1}$)</td>
<td>1.18 (0.27)</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>$Z_{euph}$ (m)</td>
<td>3.62 (0.73)</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Surface DIN (μM)</td>
<td>2.01 (5.81)</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Bottom DIN (μM)</td>
<td>1.38 (1.06)</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Surface DIP (μM)</td>
<td>0.64 (0.97)</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Bottom DIP (μM)</td>
<td>1.02 (1.17)</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Surface salinity (psu)</td>
<td>15.00 (4.35)</td>
<td>10,523</td>
</tr>
<tr>
<td></td>
<td>Bottom salinity (psu)</td>
<td>18.98 (3.78)</td>
<td>10,523</td>
</tr>
<tr>
<td></td>
<td>$\Delta \rho$ (kg m$^{-3}$)</td>
<td>3.19 (2.50)</td>
<td>10,523</td>
</tr>
<tr>
<td>NR 180</td>
<td>H (m)</td>
<td>7.06 (0.22)</td>
<td>18,832</td>
</tr>
<tr>
<td></td>
<td>$Z_{pyc}$ (m)</td>
<td>5.14 (1.31)</td>
<td>16,701</td>
</tr>
<tr>
<td></td>
<td>$K_d$ (m$^{-1}$)</td>
<td>1.14 (0.29)</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>$Z_{euph}$ (m)</td>
<td>4.34 (0.95)</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Surface DIN (μM)</td>
<td>0.97 (0.40)</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Bottom DIN (μM)</td>
<td>1.57 (1.27)</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Surface DIP (μM)</td>
<td>0.54 (0.65)</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Bottom DIP (μM)</td>
<td>0.73 (1.36)</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Surface salinity (psu)</td>
<td>10.33 (3.53)</td>
<td>16,701</td>
</tr>
<tr>
<td></td>
<td>Bottom salinity (psu)</td>
<td>13.62 (4.96)</td>
<td>16,701</td>
</tr>
<tr>
<td></td>
<td>$\Delta \rho$ (kg m$^{-3}$)</td>
<td>2.65 (2.48)</td>
<td>16,701</td>
</tr>
</tbody>
</table>
Table 3.2. Least squares estimates of the diel signal in $Z_{\text{max}}$ and $Z_{\text{cent}}$ from NR 120 and NR 180 under different stratification and temperature regimes.

<table>
<thead>
<tr>
<th>Record</th>
<th>Property</th>
<th>$\Delta \rho &lt;1$</th>
<th>$1&lt;\Delta \rho&lt;3$</th>
<th>$3&lt;\Delta \rho$</th>
<th>$T&lt;10$</th>
<th>$10&lt;T&lt;20$</th>
<th>$20&lt;T$</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Z_{\text{max}}$</td>
<td>Amplitude (m)</td>
<td>0.31</td>
<td>0.49</td>
<td>0.35</td>
<td>0.22</td>
<td>0.48</td>
<td>0.56</td>
<td>0.39</td>
</tr>
<tr>
<td>NR 120</td>
<td>Phase (hr)</td>
<td>0.01</td>
<td>0.17</td>
<td>0.01</td>
<td>0.79</td>
<td>0.01</td>
<td>0.59</td>
<td>0.01</td>
</tr>
<tr>
<td>R</td>
<td>0.17</td>
<td>0.28**</td>
<td>0.22**</td>
<td>0.14</td>
<td>0.27*</td>
<td>0.31**</td>
<td>0.22***</td>
<td></td>
</tr>
<tr>
<td>$Z_{\text{cent}}$</td>
<td>Amplitude (m)</td>
<td>0.01</td>
<td>0.03</td>
<td>0.04</td>
<td>0.01</td>
<td>0.00</td>
<td>0.06</td>
<td>0.03</td>
</tr>
<tr>
<td>NR 120</td>
<td>Phase (hr)</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>R</td>
<td>0.01</td>
<td>0.08</td>
<td>0.09</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.10</td>
<td>0.06</td>
</tr>
<tr>
<td>$Z_{\text{max}}$</td>
<td>Amplitude (m)</td>
<td>1.13</td>
<td>1.73</td>
<td>1.56</td>
<td>0.73</td>
<td>1.35</td>
<td>1.79</td>
<td>1.43</td>
</tr>
<tr>
<td>NR 180</td>
<td>Phase (hr)</td>
<td>0.20</td>
<td>0.00</td>
<td>-0.13</td>
<td>0.00</td>
<td>0.16</td>
<td>-0.11</td>
<td>-0.03</td>
</tr>
<tr>
<td>R</td>
<td>0.37***</td>
<td>0.55***</td>
<td>0.57***</td>
<td>0.27</td>
<td>0.43***</td>
<td>0.61***</td>
<td>0.48***</td>
<td></td>
</tr>
<tr>
<td>$Z_{\text{cent}}$</td>
<td>Amplitude (m)</td>
<td>0.08</td>
<td>0.19</td>
<td>0.21</td>
<td>0.07</td>
<td>0.15</td>
<td>0.19</td>
<td>0.15</td>
</tr>
<tr>
<td>NR 180</td>
<td>Phase (hr)</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>R</td>
<td>0.15</td>
<td>0.37*</td>
<td>0.47***</td>
<td>0.25**</td>
<td>0.28*</td>
<td>0.47***</td>
<td>0.31*</td>
<td></td>
</tr>
</tbody>
</table>

*, **, and *** indicate that R values are significant at the 0.05, 0.01, and 0.001 levels, respectively.
Table 3.3. Correlations between physical parameters and phytoplankton depth distribution indices. Pearson’s coefficients (R) for serial correlations of H and Z$_{pyc}$ versus Z$_{max}$ and Z$_{cent}$ and for Z$_{pyc}$ versus Δρ.

<table>
<thead>
<tr>
<th>Station</th>
<th>Correlates</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H and Z$_{max}$</td>
<td>0.031</td>
</tr>
<tr>
<td></td>
<td>H and Z$_{cent}$</td>
<td>0.28**</td>
</tr>
<tr>
<td>NR 120</td>
<td>Z$<em>{pyc}$ and Z$</em>{max}$</td>
<td>0.24**</td>
</tr>
<tr>
<td></td>
<td>Z$<em>{pyc}$ and Z$</em>{cent}$</td>
<td>0.36***</td>
</tr>
<tr>
<td></td>
<td>Z$_{pyc}$ and Δρ</td>
<td>-0.45***</td>
</tr>
<tr>
<td></td>
<td>H and Z$_{max}$</td>
<td>0.026</td>
</tr>
<tr>
<td></td>
<td>H and Z$_{cent}$</td>
<td>0.34***</td>
</tr>
<tr>
<td>NR 180</td>
<td>Z$<em>{pyc}$ and Z$</em>{max}$</td>
<td>0.15*</td>
</tr>
<tr>
<td></td>
<td>Z$<em>{pyc}$ and Z$</em>{cent}$</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>Z$_{pyc}$ and Δρ</td>
<td>-0.21**</td>
</tr>
</tbody>
</table>

*, **, and *** indicate that R values are significant at the 0.05, 0.01, and 0.001 levels, respectively. Values of
Figure 3.1. Time series of average water column temperature, salinity, fluorescence, and $\Delta \rho$ from the AVP deployments. $X$ axis tick marks are located on the first day of each month.
Figure 3.2. Time series of surface and near bottom DIN and PO$_4^{3-}$ concentrations during the AVP deployments. Values above 5 μM are written above or below the data marker.
DIN (µM)

PO₄⁻³ (µM)

Time 2001 2002

NR 120

Surface

Bottom

Time 2003 2004

NR 180

Surface

Bottom
Figure 3.3. Time series of $Z_{euph}$ and $Z_{pyc}$ during the AVP deployments. Average water column depth is indicated by the solid black line.
Figure 3.4. Example AVP chlorophyll and density data from 8-14 April 2002 at NR 120: a time period of dense aggregation of phytoplankton biomass at the pycnocline. Top panel: Contour plot of fluorescence profiles plotted with $Z_{\text{max}}$ (thin white line) and $Z_{\text{cent}}$ (thin black line). Water depth (H) is indicated by the thick black line at the bottom. The solar cycle is indicated above the top panel where white bars indicate time between astronomical sunrise and sunset and black bars indicate night time. Second panel: Contour plot of density plotted with $Z_{\text{pyc}}$ (white line) and H (thick black line). Contours for all panels are plotted in gray scale as indicated by the scale bar to the right of the plots. X axis tick marks are located at 00:00 of each day. Solid white areas in the contour plots represent unsampled regions. Third panel: Hourly vectoral wind speed. Whiskers point down-wind. Bottom panel: Time series of $\Delta \rho$ (solid black line) and $CV_{\text{chl}}$ (dashed black line).
Figure 3.5. Vertical profiles of *Prorocentrum minimum*, small (<20 μm diameter) centric diatoms, and PAR. Data collected at 10:30 AM on 8 April 2002 at NR 120.
Figure 3.6. Photosynthesis versus irradiance plot for *Prorocentrum minimum*. Samples collected from the railroad trestles and at the confluence of the Trent and Neuse Rivers.
% of Maximum Productivity

PAR (µmol photons m\(^{-2}\) s\(^{-1}\))

- Red: Confluence
- Blue: Trestles
Figure 3.7. Example AVP fluorescence and density data from 25 June to 2 July 2003 at NR 180: a time period displaying an obvious pattern of diel vertical migration. Figure format is the same as for figure 2.14.
Figure 3.8. Vertical profiles of *Scrippsiella trochoidea*, small (<20 μm diameter) centric diatoms, and PAR. Data collected at 10:18 AM on 30 June 2003 at NR 180.
Cell concentration (cells mL\(^{-1}\))

PAR

*S. trochoidea*

Small diatoms
Figure 3.9. Photosynthesis versus irradiance plot for *Scrippsiella trochoidea*. 
Irradiance ($\mu$mol photons m$^{-2}$ s$^{-1}$)

Photosynthesis (ng C cell$^{-1}$ h$^{-1}$)
Figure 3.10. Example AVP fluorescence and density data from 3-19 November 2003 at NR 180: a time period displaying an obvious pattern of diel vertical migration interrupted by two wind mixing events. Figure format is the same as for figure 2.14.
Figure 3.11. CV$_{chl}$ versus $\Delta \rho$ during the AVP deployments. Top panels: Scatter plots of CV$_{chl}$ versus $\Delta \rho$ for AVP deployments. Bottom panel: Average CV$_{chl}$ for each 0.2 kg m$^{-3}$ $\Delta \rho$ bin.
Mean CV$_{chl}$ of 0.2\(\Delta \rho\) Bins

NR120

R = +0.52
p < 1 \times 10^{-3}

NR180

R = +0.32
p < 1 \times 10^{-7}
Figure 3.12. Time localized wavelet power spectra of CV\textsubscript{chl}, Z\textsubscript{max}, and Z\textsubscript{cent} for the AVP data. X axis tick marks are at the first day of each month. Power is plotted in gray scale and is indicated by the scale bar to the right of the plots. The broken black bar at the top of each plot indicates time periods when data was collected.
Figure 3.13. Time averaged wavelet power spectra and Fourier power spectra of $CV_{chl}$, $Z_{\text{max}}$, and $Z_{\text{cent}}$ for the AVP data. Solid lines indicate the power spectrum. Dashed lines bracketing the power spectra are the 95% confidence interval. Dotted lines are the 95% significance levels.
Figure 3.14. Power and phase of the diel component of $Z_{\text{max}}$ and $Z_{\text{cent}}$ from the AVP data. Phase is relative to the daily solar cycle. Power and phase for $Z_{\text{max}}$ are represented by the solid line and $Z_{\text{cent}}$ is represented by the dashed line. For graphical presentation, power for $Z_{\text{cent}}$ was multiplied by 20. The broken black bar at the top of each plot indicates time periods when data was collected.
Figure 3.15. Time averaged wavelet power spectra of $Z_{pyc}$, $H$, and $\Delta\rho$ for the AVP data. Solid lines are the power spectra. Dashed lines bracketing the power spectra are the 95% confidence interval for the power spectra and dotted lines are the 95% significance levels.
Figure 3.16. Average profiles of day-time and night-time fluorescence and density gradient for the AVP data. Average depth profile of fluorescence for the total record (--), average daytime (9:00AM-3:00 PM) fluorescence profile (···), and average nighttime (9:00 P.M.-3:00 AM) fluorescence profile (---). The scale for fluorescence is the bottom x-axis. The scale for the density gradient ( — ) is the top x-axis. Only depths with at least 5,000 observations were used to generate the average profile values.
Depth (m)

0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

Fluorescence

Day Fluorescence

Night Fluorescence

NR 120

NR 180

$\Delta \rho \Delta z^{-1}$ (kg m$^{-4}$)


Chapter 4

PUTTING MARGALEF’S MANDELA TO THE TEST
Margalef’s Mandela (1978) is a classic conceptual model of phytoplankton competition and succession based upon nutrient availability and degree of mixing of the water column. This conceptual model nicely accounts for changes in composition of the phytoplankton community across a wide range of aquatic systems where mixing and nutrient availability are linked and where changes in turbulent mixing occur over seasonal time scales. We tested the applicability of the Mandela in a shallow lagoonal estuary where mixing is largely determined by wind stress and the linkage between mixing and nutrient availability is less defined than in pelagic regions of the ocean or large lakes.

Using semi-hourly decimeter scale profiles of the density structure of the lower Neuse River Estuary and phytoplankton samples collected on a weekly to biweekly basis, we used the wind induced alternations between stratified and mixed conditions in the NRE as a natural experiment to evaluate the applicability of Margalef’s (1978) Mandela in this shallow eutrophic system. In accordance with the tenets of the Mandela, diatom biomass was negatively related to the stability of the water column and under highly stratified conditions dinoflagellates dominated phytoplankton community biomass. We provide evidence for multiple interrelated mechanisms that link the wind derived energy for mixing the water column to the maintenance of the diatom community. Principal among these are variations in the vertical scale of the largest turbulent eddies, changes in the depth of the pycnocline and the resuspension of surficial sediments.

Though dinoflagellates constituted a larger fraction of total phytoplankton biomass under more stable water column conditions, under well mixed conditions dinoflagellates were
equally as likely as diatoms to dominate the phytoplankton community. Either
dinoflagellates perform equally well under stratified and mixed conditions, or the time scales
of alternations in water column stability are too rapid for competitive displacement of
dinoflagellates by diatoms in this system.
4.1. Introduction

Margalef’s (1978) conceptual model of phytoplankton succession, the “Mandela”, predicts the success of the various phytoplankton life forms based upon two primary ecosystem attributes, turbulence and nutrient availability (Fig. 4.1). In his view, other ecosystem attributes such as light fluctuations, temperature, etc. are of minor importance in comparison to these features. Phytoplankton that lack motility, typified by the diatoms, are adapted to highly turbulent conditions that are required to keep them in suspension within the photic zone (Fig. 4.1). In his view, these phytoplankton are also adapted to high nutrient concentrations as strong turbulence generally increases the transport of nutrients from deeper waters toward the surface. Alternatively, flagellated phytoplankton, typified by the dinoflagellates, are adapted to life under less turbulent conditions where their swimming allows them to eliminate sinking losses from the euphotic zone (Fig. 4.1). Additionally, in the generally low nutrient environment of low turbulence surface waters, swimming may enhance nutrient uptake by continual replacement of the water surrounding the cells and by allowing cells access to nutrient sources below the photic zone.

Over the past three decades, the Mandela has been honed and revised by numerous authors. Richardson et al. (1983) compared the photophysiology of diatoms with dinoflagellates. He found that diatoms have a more flexible photosynthetic response and lower overall light requirements for growth that were attuned to the highly variable and generally low light availability of turbulent water columns (Richardson et al. 1983). Thus, as far as light utilization is concerned, it appears that diatoms are adapted to well mixed water columns while dinoflagellates are adapted to low turbulence environments. The finding that
small scale shear associated with turbulent mixing can cause various damages to flagellate cells (Thomas and Gibson 1990; Berdalet 1992; Thomas et al. 1995) but may enhance growth of diatoms (Patel et al. 2005) also strengthens the tenet that flagellates and diatoms are adapted to low and high turbulence environments, respectively. However, it should be noted that flagellate susceptibility to damage by small scale shear is not absolute and some dinoflagellate species show unaffected or enhanced growth at naturally occurring rates of shear (Sullivan and Swift 2003). The degree to which phytoplankton adaptation to low/high nutrients and low/ high turbulence are linked is a subject of recent debate since it seems difficult to reconcile the tenets of the Mandela with the generally higher half saturation coefficients of nutrient uptake for dinoflagellates compared to diatoms (Eppley et al. 1969; Smayda 1997). Nonetheless, as turbulence and nutrient availability vary within and among systems, the Mandela provides a degree of predictability of the dominant phytoplankton life forms across broad spatial and temporal scales (Margalef 1978; Reynolds et al. 1984; Lieberman et al. 1994; Lauria et al. 1999; Huisman et al. 2004).

Along the axes of the Mandela, estuaries generally lie within the region of high nutrient availability due to riverine, groundwater, and atmospheric inputs of nutrients from land (Paerl 1997; Cloern 2001). High nutrient loads are obviously a principal reason for the high phytoplankton biomass of many estuaries (Paerl 1997; Cloern 2001). However, due to high standing stocks of phytoplankton biomass, high rates of nutrient inputs are often accompanied by high rates of uptake (Boyer et al. 1994; Carpenter and Dunham 1985; Chapter 2). As a result of the tight coupling between uptake and delivery, nutrient concentrations can at times be low enough to limit growth of the dominant phytoplankton taxa (Sunda et al. 2006; Chapter 2). So, at a given point in time or space within an estuary,
the position along Margalef’s nutrient axis will largely depend on whether nutrient concentration or flux is considered more important in determining the composition of the phytoplankton. Position of estuaries along the turbulence axis is highly variable even within a given estuarine system. The degree of vertical mixing depends on depth and the balance between buoyancy forces from freshwater input and the supply of energy for mixing from wind and tides (Dyer 1997).

The purpose of this study was to evaluate the use of Margalef’s Mandela for predicting changes in the biomass and community composition of a shallow, lagoonal estuary, the Neuse River Estuary (NRE), NC, USA. Shallow lagoonal estuaries such as the NRE are very different from other marine and limnetic systems where predictable alternations between non-flagellate and flagellated phytoplankton life forms generally follow the waxing and waning of turbulence (Fogg 1991). In the NRE, stratification is controlled primarily by salinity, rather than temperature (Chapter 2; Christian et al. 1991), and very weak tides produce negligible turbulence associated with bed shear (Luettich et al. 2000; Whipple et al. 2006). Wind is the primary source of external energy that determines the degree of vertical mixing (Whipple et al. 2006) and produces oscillations between stratified and mixed conditions on the time scale of days to weeks (Chapter 2; Whipple et al. 2006; Luettich et al. 2000). This is in marked contrast to the seasonal stratification/destratification patterns commonly observed in oceanic and limnetic systems.

In the NRE, water column depth is shallower than the critical depth (Mallin and Paerl 1992) so when the water column is fully mixed, phytoplankton can still maintain net positive photosynthetic production. However, waters below the pycnocline are normally dysphotic. Thus, phytoplankton must maintain a population within the upper mixed layer to grow.
Water column processes are tightly coupled to the sediments which can serve as both sources and sinks for nutrients (Chapter 2; Rizzo et al. 1992) and for resuspendable plankton (Shafer and Sullivan 1988; Tester et al. 1995; Cloern and Dufford 2005; Kasim and Mukai 2006). The relative importance of external riverine versus internal sediment nutrient loading is also highly variable (Paerl et al. 1997) and, when the water column is not well mixed, can produce either increasing or decreasing nutrient concentrations with depth (Chapter 2; Christian et al. 1991). Hence at times, the estuary may exhibit the peculiar “illness” described by Margalef when high nutrient concentrations and water column stability are coincident within the photic zone.

To determine the effects of turbulence and nutrients on the phytoplankton community of an estuary where conditions can change rapidly, it is imperative to have information on the conditions prior to sample collection. We used an autonomous vertical profiling system (Reynolds-Fleming et al. 2002) (AVP) to produce high resolution time series of the vertical density structure (temperature and salinity profiles) of the lower NRE. Water column stability, the density difference between bottom and surface, was then used as a proxy for the degree of turbulence in the water column. Scully et al. (2005) found that of current shear, wind speed, and water column stability, water column stability was the best predictor of vertical eddy viscosity. Phytoplankton biomass and community composition were then compared across the range of water column stabilities under which the phytoplankton community had developed to test the hypothesis that increasing water column stability would select for the flagellates and against the non-flagellates.

The nutrient conditions that occurred during the period prior to phytoplankton sample collection were poorly constrained. We only had a limited number of discrete nutrient
concentration data that were collected coincidentally with the phytoplankton samples. We also have no information on the magnitude of nutrient inputs prior to sampling. As such, greater attention is paid to the turbulence axis of the Mandela within this study. However, we do test relationships between nutrient concentrations and the phytoplankton community composition and speculate on the role of nutrient availability in determining community composition.

Structure of the phytoplankton community was investigated at the whole community level and at the level of phytoplankton groups. Largely, the groupings were made along taxonomic classes for comparison with previous research (Pinckney et al. 1998; Paerl et al. 2003; Valdes-Weaver et al. 2006; Paerl et al. 2007) that has quantified the dominant algal classes based on photopigments. Water column stability and nutrient data were compared against the biomass of each group to test the validity of the Mandela within the lower NRE. Temperature, salinity, and light attenuation data were also investigated because they are potentially confounding variables of mixing and may also provide additional information on the suite of environmental factors that shape the phytoplankton community.

A consideration of Margalef’s Mandela within a particular ecosystem requires consideration of the degree to which boundary conditions affect the conceptual model (Margalef 1978). Unlike the open-ocean or pelagic regions of lakes that are large and deep, phytoplankton populations within shallow estuaries are likely to be highly influenced by advective processes and by their relationship with the sediments. We were interested in studying the phytoplankton dynamics that occur on the time scales of the alternation between mixed and stable water columns which typically occurs on time scales from days to weeks.
As an approximation, the time scales of advective processes can be estimated by the residence time of the study location. For the entire NRE, the residence time is generally about 2 months and is greater for the lower NRE, the area of this study, due to its larger volume (Christian et al. 1991). As a result, the effect of advective processes on the phytoplankton should form a slower moving trend upon which the higher frequency signal of interest will still be detected. With typical settling rates of phytoplankton on the order of 1 m d\(^{-1}\) (Huisman and Sommeijer 2002; Reynolds 1970) and an average water column depth of only 5 m in the lower NRE (Boyer et al. 1994), the time necessary for cells to settle from the water column to the sediments is of a similar time scale to alternations in water column stability. Since the energy for mixing the water column may also lead to sediment resuspension, it would seem that a conceptual model of the effects of water column stability on phytoplankton community composition could not achieve a reasonable degree of closure without consideration of the role of the sediments as both a sink and a source for phytoplankton. As such, the likely effects of resuspension on water column phytoplankton community composition are explored by examining the composition of the benthic phytoplankton community and the frequency of resuspension events.

4.2. Methods and Materials

4.2.1. Study Sites- Data from this study were obtained from NR120 and NR180 described in Chapter 2 (Fig. 2.1).
4.2.2. Physical Data- High resolution depth profiles of temperature, salinity, fluorescence, dissolved oxygen, and turbidity were collected using the Autonomous Vertical Profiler described in detail in Chapter 2-Section 1. The average difference in density between the bottom and surface of the water column for the week prior to sample collection (\( \Delta \rho \)) was calculated from temperature and salinity profiles according to the equation of state of Pond (1983). From this point onward, \( \Delta \rho \) will be used to designate the weekly average and \( \Delta \rho \) will indicate single values of the intensity of stratification. \( \Delta \rho \) was used as an index of the intensity of stratification that was in place as the phytoplankton community developed over the week prior to sample collection. The 7 d time period over which \( \Delta \rho \) was averaged was chosen because it was believed to be long enough for phytoplankton to undergo a few divisions and for detectable expression of losses due to grazing, settling etc. on the phytoplankton community. Additionally, since the phytoplankton samples were collected at a maximum frequency of once per week and the integral time scale for autocovariation in the \( \Delta \rho \) time series (calculated according Emery and Thomson 1998 p. 268) was shorter than 7 d, each \( \Delta \rho \) value can be considered statistically independent. Due to mechanical failures of the AVP system, the \( \Delta \rho \) record was incomplete for the week prior to collection of some phytoplankton samples. We utilized phytoplankton samples for which the prior week’s \( \Delta \rho \) record was at least 90 percent complete. This criterion resulted in the use of 40 phytoplankton samples for this study.

The depth of the upper mixed layer (\( Z_{UML} \)) is an important property of the vertical water column structure that affects net population growth rates of phytoplankton (Condie and
Bormans 1997; Lucas et al. 1999; Huisman and Sommeijer 2002). Like Δρ, Z_{UML} may be influenced by current shear driven by wind stress. For each density profile, Z_{UML} was defined as the depth of the pycnocline and calculated as the depth of the maximum in the first derivative of the density profile. In instances where Δρ was less than 0.5 kg m\(^{-3}\), Z_{UML} was defined as the bottom of the water column. We justified this threshold by comparing the variance of the depth of the pycnocline to the mean Δρ of eight consecutive observations. At less than ~1 kg m\(^{-3}\), the variance of the depth of the pycnocline exponentially increases. This demonstrates that at these low levels of water column stability there is no coherent structure to the pycnocline (Fig. 4.2). So, our assignment of the 0.5 kg m\(^{-3}\) threshold is a conservative estimate of the level of stability that should indicate when the full water column can be considered the mixed layer.

The along channel wind vector (north east winds positive) was chosen to represent current shear because tidal current velocities in the lower NRE are negligible compared to wind driven currents (Luettich et al. 2000; Luettich et al. 2002) and current velocity data were unavailable. Hourly wind data were obtained from the National Data Buoy Center’s Coastal Automated Marine Network station CLKN7 located (34°37.30' N 76°31.50 W) 45 km south of NR 180 (Fig. 2.1). Assessment of the relationships between Δρ, Z_{UML}, and north east wind velocity aid in the interpretation of the results and consideration of the findings of previous researchers.

Profiles of photosynthetically active radiation (PAR) were collected at weekly-biweekly intervals using a Li-Cor 192S 4π spherical PAR sensor (Li-Cor Biosciences, Lincoln, Nebraska) and diffuse light attenuation coefficients (K\(_d\)) were calculated from least squares fits of the exponential attenuation of PAR with depth.
4.2.3. Phytoplankton and Nutrient Data-Sampling for phytoplankton community and nutrient analyses occurred biweekly as part of the Neuse River Modeling and Monitoring Program (Luettich et al. 2000) (ModMon; http://www.unc.edu/ims/neuse/modmon/index.htm) and additional samples were collected with identical methodology during weekly/biweekly maintenance trips to the AVP. Water was collected from the surface into 2-4 L polyethelene bottles. Within minutes of collection, 150 mL was poured into amber polyethelene bottles and fixed with Lugol’s solution at 1% final concentration for microscopic enumeration. The remaining sample was transported in a dark cooler at ambient temperature to the laboratory within 4 h of collection for analyses of chlorophyll a and inorganic nutrients.

Chlorophyll a measurements were made for all 40 phytoplankton samples that were microscopically enumerated during the study. Samples were gently filtered on duplicate Whatman 25 mm GFF filters (nominal pore-size 0.7 μm), and frozen at –20 °C within 6 h of collection. Filters were extracted using a tissue grinder in 90% acetone (EPA method 445.0). Chlorophyll a concentration was measured on the extracted samples using the non-acidification method of Welshmeyer (1994) on a Turner TD-700 fluorometer.

In addition to the surface phytoplankton samples, preserved phytoplankton samples were occasionally collected at 1 m depth intervals throughout the water column. At NR 180 samples were collected at 1, 2, 3, 4, and 5 m. At NR 120 samples were collected at 1, 2, 3, and 4 m. Six of these profiles of phytoplankton biomass and community composition were used to determine how well a surface sample represents average water column phytoplankton biomass and community composition. Where significant relationships between the stratification regime and group biomass were found, the error associated with use of only a
surface sample to determine water column biomass was assessed in the following manner. The surface biomass of six profiles was regressed on the mean water column biomass. The standard deviation of the residuals was then used to generate appropriately-scaled, normally distributed, random error which was added to each of the 40 surface sample observations of group biomass. Spearman’s rank correlations were then used to compare the stratification intensity versus group biomass containing the error term. One thousand repetitions of the creation and addition of the appropriately scaled noise to the surface biomass estimation provided confidence in determining the effect of this type error on relationships between stratification intensity and phytoplankton group biomass.

Coincident inorganic nutrient and light attenuation data were available for 33 of the 40 phytoplankton samples used in this study. These data included surface and bottom water concentrations of NH$_4^+$, NO$_3^-$, PO$_4^{3-}$, and dissolved silica. Methods for nutrient analyses are described in Chapter 2.

Lugol’s-preserved phytoplankton samples were stored in the dark at approximately 22 °C. Cell counts were performed using the inverted microscope technique of Utermöhl (1958) with a Leica DMIRB microscope under phase contrast at 400X magnification. A Whipple grid calibrated with a stage micrometer was used for cell measurements. The 24 h settling time was not sufficient to ensure that all of the smaller phytoplankton had settled so smaller phytoplankton are likely underrepresented in the counts. However, consistent settling time and use of the same size settling chamber (30.8 mL, 10 cm height) provide an adequate basis for comparing samples. For each sample, 100 fields were counted, providing between 114-3,438 counts of the most abundant of 12-26 cell types. Autotrophic cells with clearly visible cytoplasmic contents (for example, no empty diatom frustules or dinoflagellate thecae were
counted) were identified to the lowest possible taxonomic level. However, we were unable to identify many cell types to the genera level. As a result, many cell types were recorded by class and a drawing was made to record cell shape and size. In nearly all the samples, there were a large number of very small (~5 μm diameter) unidentifiable flagellates that were counted as other small flagellates (OSF). Larger flagellates that were not of the classes chlorophyceae, cryptophyceae, or dinophyceae were lumped into a group called other large flagellates (OLF).

Biovolume of each cell type was primarily determined using literature values from Olenina et al. (2006) or Cambell (1973). Biovolume of OSF was estimated at 65 μm³ based on the volume of a 5 μm diameter sphere according to Furnas (1982). Biovolumes for centric diatoms were based on the volume of a cylinder. Where girdle views were not observed, cell height was assumed to equal cell radius. Biovolumes of *Cylindrotheca closterium* were estimated from Olenina et al. (2006). Other pennate diatoms were estimated using measured cell dimensions and biovolumes of one of three geometric shapes: rectangular solid, oval cylinder, or parallelepiped. Where girdle views were not observed, pervalvar axis dimensions of pennate diatoms were assumed to be equal to the transapical axis dimension.

Centric diatoms are generally planktonic, while pennate diatoms are generally benthic (Vinyard 1979) but during mixing events can be resuspended contributing substantially to the planktonic diatom community of shallow estuaries (Shaffer and Sullivan 1988; Tester et al. 1995). The centric: pennate diatom ratio has been used as a paleoecological indicator of the relative importance of planktonic versus benthic diatom production (Cooper and Brush 1993; Cooper 2000). In this study, we investigated short term fluctuations in the centric: pennate
diatom ratio as an indicator of the importance of resuspension in determining diatom biomass.

To test the assumption that the centric: pennate diatom ratio is truly expressive of the contribution of diatoms due to resuspension, we collected a transect of surface sediment samples across the main axis of the lower NRE (Fig. 2.1). The six sampling stations were selected to include a range of euphotic (1 and 2 m depth, 35-10 % incident irradiance) and dysphotic depths (5 and 7.5 m < 1 % incident irradiance) that are representative of the near shore/ shoals and mid-channel benthic diatom communities, respectively. On 6 March 2009, a single sediment core was collected from each of the six stations using a WildCo (Wildlife Supply Company, Buffalo, NY) corer. The top 0.5 cm of each core was cut off and 3 mL of this material was placed into a 60 mL centrifuge vial with 35 mL of deionized water and 0.3 mL of Lugol’s solution. Samples were shaken vigorously to suspend the diatom community and 1 mL samples of the suspension were immediately collected. The 1 mL sample was subsequently diluted to levels that allowed visualization of the diatoms without obscuration from detritus and sediment grains. Counts were made of centric diatoms, pennate diatoms, and of chain forming centric diatoms in the same manner as the water column samples.

4.2.4. Data Analyses- Chlorophyll a, total phytoplankton biovolume, and total phytoplankton cell abundance were used as measures of community level phytoplankton biomass. For determining the effect of mixing regime on phytoplankton community composition, biovolume and cell abundance were summed by several different groupings of the phytoplankton: flagellated/ non-flagellated for all phytoplankton, taxonomic class for chlorophytes, cryptophytes, cyanobacteria, diatoms, and dinoflagellates, and centric/ pennate
for diatoms. For each group, biomass measures from all 40 samples were correlated with Δρ using Spearman’s rank correlation.

For the 33 phytoplankton samples for which nutrient and light attenuation data were available, a series of stepwise multiple regression models (‘stepwisefit’, Matlab version 7.0.0 R14) were used to investigate the potential for confounding factors of stratification regime and to provide additional information on factors that shape the phytoplankton community. NO$_3^-$ concentration was below the limits of detection (0.26 μM) for 27 of the 33 nutrient samples, so the sum of NO$_3^-$ and NH$_4^+$ concentrations were used in the regressions as the measure of dissolved inorganic nitrogen (DIN) availability. Though DSi was measured, it was excluded from the multiple regression models for two main reasons 1) DSi exhibited strong colinearity with salinity and 2) the lowest DSi concentration measured, 29.8 μM, is greater than an order of magnitude higher than levels expected to limit diatom uptake or growth (Dortch and Whitledge 1992). Bottom water nutrient concentrations were included in the regression model because persistent patterns of vertical migration observed during the study (Chapter 2) demonstrated that bottom water nutrient stocks are available to the flagellates. The final set of factors selected for inclusion in the step wise regression models were surface and bottom water DIN, surface and bottom water PO$_4^{3-}$, the light attenuation coefficient (K$_d$), Δρ, the average temperature of the week prior to sampling, and the salinity at the time of sampling. To satisfy the assumptions of homoscedascity of variance and normality of residuals, cell abundance and biovolume data of each group were log transformed prior to regression. Normality and homoscedascity of the residuals were tested using Lilliefors (Lilliefors 1967) and Breusch-Pagan (Breusch and Pagan 1979) tests, respectively. Instances where normality or homoscedascity were not achieved are noted. A
fake cell mL\(^{-1}\) with a volume of 100 μm\(^3\) was added to each groups cell abundance and biomass to avoid log(0) of groups that were not counted in a sample. The resulting cell abundance and biovolume of these groups that were missing from some samples were generally an order of magnitude less than the lowest actual observed group biovolume; so the addition of the fake cell had no appreciable effect on the dependent variables. For hypothesis testing, \(\alpha = 0.05\).

In a similar manner to Giffin and Corbett (2003), frequency of resuspension events was determined using average water column turbidity data collected by the AVP (Fig. 4.1). According to Luettich et al. (1990), we assumed that the horizontal component of wind generated wave orbital velocities were the dominant source of bottom stress. Using the United States Army Corps of Engineers shallow water wave hind casting models as modified by Luettich and Harleman (1990) we calculated significant wave height and period based on hourly averages of wind speed and direction from the CMAN station CLKN7 (Fig. 2.1). Wave generation was assumed to be fetch limited. Fetch was approximated by estimating the nearest distance to shore within selected divisions of the compass rose. At NR 180, the fetch for a wind direction of 0 to 90 degrees from North was set at 22 km, 90 to 180 was set to 5 km, 180 to 270 was set to 15 km, and 270 to 360 was set to 5 km. For NR 120, the fetch for a wind direction of 330 to 30 degrees was set at 4 km, 30 to 90 degrees was set to 30 km, 90 to 135 was set to 10 km, 135 to 240 degrees was set to 4 km, and 240 to 330 degrees was set to 20 km. Based on calculated wave heights and period, the maximum horizontal wave orbital velocities along the bottom were calculated using linear wave theory for intermediate depth waves. The dispersion equation was solved iteratively for wave number. Average depths, 4.71 m and 7.06 m, were used for water column depth at NR120 and NR180,
respectively. The square of the horizontal wave orbital velocities should be proportional to bottom stress (Luettich et al. 1990). So, we related this quantity to average water column turbidity to identify a threshold level of stress necessary for resuspension.

We used this threshold to understand the climatological frequency with which resuspension events are likely to occur within the lower NRE. The square of the horizontal orbital velocities at the bottom were hind casted from hourly wind data collected from 1 January 1996 through 31 December 2007. Relative bottom stress was then converted to binary form with all values above the threshold set to equal 1 and all below the threshold set to zero. The dominant modes in occurrence of the bottom stress in excess of the resuspension threshold were then investigated using time averaged wavelet power spectra with a Paul wavelet according to Torrence and Compo (1998). All analyses were performed using Matlab version 7.0 R14 (www.mathwork.com, The Mathworks, Inc. Natick, Massachusetts).

In the discussion, we compare the spatial and temporal distribution of diatom biomass within the NRE and its receiving body, the Pamlico Sound to the spatial and temporal patterns of water column stability. Data for the spatial and temporal patterns of diatoms were produced through sampling by the ModMon program and were published in Valdes-Weaver et al. (2006). We calculated the median Δρ that occurred during exactly the same time periods and geographic locations found in Valdes-Weaver et al. (2006).

4.3. Results
4.3.1. Water column stability, temperature, and salinity-The ability of the estuary to undergo rapid alterations between stratified and mixed conditions is clearly apparent in plots of $\Delta \rho$ (Fig. 4.3). Both strongly and weakly stratified conditions occurred throughout the year at both stations. However, there was a tendency toward increased duration of strongly stratified conditions during the warmer months (Fig. 4.3). This seasonality associated with stratification regime is a primary reason we included the multiple regression analyses as a follow up to simple rank correlations of biomass of each group versus $\Delta \rho$. By including temperature in the multiple regression analyses we aimed to distinguish effects of water column stability from effects of seasonal phytoplankton successional patterns in the NRE (Pinckney et al. 1998; Valdes-Weaver et al., 2006) that are believed to be due to differences in temperature optima for growth between phytoplankton taxa (Paerl and Huisman 2009).

Average water column salinities at NR120 ranged from ~5 to nearly 25 psu and from ~5 to 20 psu at NR180 over the course of the deployments (Fig. 4.3). On average, the higher salinities upstream at NR 120 than downstream at NR 180 reflect the drought conditions during the 2001-2002 deployment at NR 120.

The average depth of the upper mixed layer ($Z_{UML}$) for the week prior to sampling ranged from 2-4.5 m at NR 120 and from 5-7 m at NR 180. At both stations, increased $\Delta \rho$ was accompanied by shallower $Z_{UML}$ (Fig. 4.4). For the NR 180 data, stronger NE winds generally led to decreased water column stability. With reduced exposure to the NE wind field (Fig. 2.1), $\Delta \rho$ at NR 120 did not show a significant relationship with the NE wind component (Fig. 4.4).
4.3.2. **Nutrients and Light**—DIN generally ranged from 0.5 to 2 μM at both stations. However, there were three instances where DIN was below detection. PO$_4^{3-}$ varied seasonally at both stations from less than 0.5 μM in the cooler months to greater than 1 μM during the warmer months (Fig. 4.5). There were also three instances during the winter of 2004 at NR 180 when PO$_4^{3-}$ was below detection. Excluding data points where the ratio could not be calculated due to values below limits of detection, mean DIN:DIP ratios at both stations were ~8 and stoichiometric considerations of the Redfield ratio would suggest that nitrogen was generally the limiting nutrient for phytoplankton growth. However, during the winter DIN:DIP ratios occasionally were greater than 16 indicating the potential for phosphorous limitation. This is in agreement with numerous studies of nutrient limitation of the mesohaline region of the NRE (Paerl et al. 2004). Light attenuation was relatively constant, ranging only from 0.8 to 2.1 m$^{-1}$ and averaged ~1 m$^{-1}$ at both stations (Fig. 4.5).

4.3.3. **General description of the phytoplankton community**—Table 4.1 shows the dominant phytoplankton types counted, as well as the cell volumes used for calculated biovolume of each group. Non-chain forming small centric diatoms, less than 10 μm in diameter, and the pennate diatom *Cylindrotheca closterium* were found in nearly every sample counted. *Skeletonema costatum*, *Leptocylindrus minimum*, *Cerataulina pelagica*, and *Melosira* spp. were the principal chain forming centric diatoms. Chain forming pennate diatoms were never counted in significant numbers. The dinoflagellate community was marked by obvious seasonal successional patterns which will be discussed in a subsequent report. Briefly, *Prorocentrum minimum*, *Heterocapsa rotundata*, and *Heterocapsa triquetra* were prominent during the colder months. *Polykrikos hartmanii*, *Akashiwo sanguinea*, *Prorocentrum micans*, *Prorocentrum micans*,
Scrippsiella trochoidea, and Dinoflagellate A were common during the warmer months. Dinoflagellate A was initially identified as Gyrodinium estuariale based on the description by Campbell (1973). However, at the time of the cell counts we were unable to distinguish between Gyrodinium estuariale and Karlodinium veneficum and it is likely that Dinoflagellate A contains both species. Chlorophytes were dominated by the flagellates, Chlamydomonas sp and Pyramimonas, and the non-flagellate, Ankistrodesmus sp?. Cyanobacteria were dominated by Planktolyngbya sp? though there were occasionally significant numbers of Synechococcus type cells with diameters ~1-2 μm. The OLF group included euglenophytes, raphidophytes, chrysophytes, and the autotrophic ciliate Myrionecta rubra. Cryptophyte biomass was dominated by Teleaulax amphioxeia, though the smaller cryptophytes Chroomonas minuta and Hemiselmis virescens occurred in most of the samples.

Cell abundance was dominated by the OSF group which comprised on average ~ 45 percent of total cell abundance of each sample but due to their small size averaged ~ 5 percent of total biovolume (Table 4.2). Cyanobacteria, diatoms, dinoflagellates, and cryptophytes contributed 11 to 16 percent each to the total cell abundance and the remainder was comprised of chlorophytes and the OLF group. Dinoflagellates dominated phytoplankton biovolume (63 %) and diatoms (23 %) were the second most important group with respect to biovolume (Table 4.2). The combination of dinoflagellates and diatoms accounted for > 85 percent of mean total phytoplankton biovolume.

4.3.4. Effects of water column stability and nutrients on the phytoplankton community- At the community level, there was no significant relationship between \( \Delta \rho \) and chlorophyll \( a \) or
cell abundance. However, total phytoplankton biovolume was significantly higher in samples collected following less stratified conditions (Table 4.3).

At the class/group level, diatom cell abundance and biovolume were significantly higher under less stratified conditions (Table 4.3, Fig. 4.6). The increase in diatom biovolume under less stratified conditions drove the increase in total phytoplankton biovolume. For chlorophytes, cryptophytes, cyanobacteria, dinoflagellates, OLF, and OSF, there were no significant relationships between $\Delta \rho$ and cell abundance or biovolume (Table 4.3).

The step-wise multiple regression analyses showed significant negative relationships between $\Delta \rho$ and both diatom abundance and biovolume. This indicates that event though both diatom biovolume and cell abundance were also negatively related to temperature, the seasonal covariance between temperature and $\Delta \rho$ is unlikely to explain the observed correlation between diatom biomass and $\Delta \rho$ (Table 4.4). There were also negative temperature relationships with total phytoplankton cell abundance, cryptophyte cell abundance and biovolume, and dinoflagellate cell abundance. Cyanobacteria were the only group that showed a positive relationship with temperature. Cyanobacterial biovolume showed a negative relationship with salinity. Chlorophyll a, total cell abundance, and both diatom and dinoflagellate cell abundance were all positively correlated with light attenuation. This result was inevitable given that these phytoplankton groupings represent major components of the light absorbing and scattering materials in the water column of the estuary. Surface water DIN was positively correlated with chlorophyll a and negatively correlated with the biovolume and cell abundance of chlorophytes.
The increase in diatom biovolume under less stratified conditions was a result of both higher numbers of diatoms and an increase in average diatom size. We compared three size classes of diatoms based on their equivalent spherical diameter (ESD) calculated from their biovolume. Small sized diatoms, ESD < 10 μm, dominated total diatom abundance (Fig. 4.7) and were counted in all but one sample. Small diatoms were more abundant under less stratified conditions. Medium sized diatoms, 10 > ESD < 30, were counted in two thirds of the samples counted and were significantly more abundant under less stratified conditions. Medium sized diatoms were counted in only three samples with a Δρ greater than 4 kg m⁻³. Large diatoms, ESD > 30, were counted in only a third of the samples and were not observed in samples with Δρ exceeding 4 kg m⁻³.

The percent contribution of dinoflagellates and diatoms to total phytoplankton biovolume revealed a pattern of dominance by dinoflagellates following more intense stratification (Fig. 4.8). Under less stable water column conditions diatoms or dinoflagellates were equally as likely to dominate the phytoplankton community.

4.3.5. Profiles of diatom and dinoflagellate biomass- Diatom and dinoflagellate abundance varied by less than a factor of 10 within each profile (Fig. 4.9). The shallowest depth of a diatom cell abundance maxima was 2 m and for all six profiles, the abundance of diatoms increased from the surface to 1 m depth. Dinoflagellate abundance also increased from the surface to 1 m depth for all 6 profiles and there was a tendency toward higher dinoflagellate abundance in the middle of the water column. Variability for both diatom and dinoflagellate biovolume was generally higher than for abundance. In five of the six profiles, the biovolume of diatoms at the surface was less than at 1 m depth. In three of these cases, this
difference was nearly ten fold and reflects a general increase in size of diatoms from the surface to 1 m depth. Depth variability of dinoflagellate biovolume was generally higher than for the diatoms and there were no clear patterns to the depth distribution of dinoflagellate biovolume.

Despite the high degree of variability of diatom abundance and biovolume within a profile, the surface values reasonably predicted average water column values (Fig. 4.10). For dinoflagellates, the surface water samples predicted average water column abundance well but poorly predicted average water column biovolume. The standard deviation of the residuals for the regression of log$_{10}$ (surface diatom cell abundance) on log$_{10}$(average water column cell abundance) was 0.15. Similarly, the standard deviation of the residuals for the regression of log$_{10}$ (surface diatom cell abundance) on log$_{10}$(average water column cell abundance) was 0.16. Addition of this level of error to the diatom abundance and biovolume data did not effect the outcome of the hypotheses tested using the Spearman’s rank correlations between $\Delta \rho$ and diatom abundance or biovolume. For diatom abundance and biovolume, the largest p-values encountered from 1000 simulations with randomly generated, appropriately scaled, error were $1.2 \times 10^{-3}$ and $2.1 \times 10^{-3}$, respectively.

4.3.6. Effects of water column stability on centric and pennate diatoms—Centric diatom cell abundance and biovolume were significantly higher under less stratified conditions (Fig. 4.11). The relationships between $\Delta \rho$ and pennate cell abundance and biovolume were both weakly negative and on the cusp of statistical significance. Most of the increase in total diatom biomass under less stratified conditions was due to the increase in centric diatoms as
evidenced by the significant positive relationship between $\Delta \rho$ and the pennate diatom fraction of total diatom biovolume. Though not statistically significant, this trend was true for the pennate fraction of total diatom cell abundance as well.

4.3.7. Benthic centric and pennate diatoms—On 6 March 2009, the centric: pennate ratio of the benthic diatom community varied from less than 1 to greater than 10 across the main axis of the estuary (Fig. 4.12). At shallow euphotic sites, the centric: pennate diatom ratio varied from < 1 to ~2.5. At the deepest location, the benthic diatom community was dominated by centric diatoms and the ratio of centric: pennate diatoms was greater than 10. The median value of the centric: pennate diatom ratio from the 40 surface water samples collected during the study was 7.3.

Chain forming centric diatoms, particularly *Skeletonema costatum* and *Skeletonema potamos*, were the most abundant diatoms on the sediment surface at the two mid-channel, dysphotic sites. This was probably the result of recently deposited cells from an on going *Skeletonema* bloom. On 9 February 2009, at the location of the deepest core, surface water abundance of combined *S. costatum* and *S. potamos* was ~14,500 cells mL$^{-1}$.

4.3.8. Frequency of resuspension events—The time series of average water column turbidity from the two deployments reveal baseline values of < 5 NTU with intermittently occurring spikes up to values as great as 100 NTU (Fig. 4.3). We investigated a three month long period, 1 February 2004 through 1 May 2004, that displayed regular occurring spikes in turbidity to determine whether these spikes in turbidity were related to wind induced resuspension events. During the period at station NR 180, several large events occurred
where turbidity rapidly increased from background levels of approximately 2-5 NTU to
greater than 20 NTU (Fig. 4.13). During all the turbidity events, the water column was fully
mixed. The abrupt increase in turbidity and slower decrease observed during resuspension
events, was also noted by Giffen and Corbett (2003) and is consistent with rapid
resuspension of sediments as the critical erosion velocity is reached and then a gradual
settling of the fine grained sediments. Temporally, resuspension events occurred at
approximately 10 day intervals and there was a strong relationship between wind induced
wave orbital velocity along the bottom and water column turbidity (Fig. 4.13). These lines of
evidence lead to the conclusion that the turbidity events are in fact due to resuspension of
fine grained sediments due to wind induced wave stress along the bottom. Determination of
a threshold level of wave energy that produced resuspension was highly subjective, and in
the end the value of 0.04 m$^2$ s$^{-2}$ was chosen. This value agreed well with temporal patterns of
resuspension and wave generated bottom stress. Additionally, based on grain size analysis,
Giffin and Corbett (2003) suggested that the critical erosion velocity for the fine sediments of
the NRE should be approximately 0.20 m s$^{-1}$.

The power spectra of the occurrence of wind events producing threshold level bottom
stress were very similar for both stations (Fig. 4.14). The dominant mode of variability
corresponds with the annual cycle in wind intensity (Fig. 4.15) driven by winter storms
(Luettich 2000). However, there was a broad region of power starting at about 8 d. This is
consistent with the temporal spacing of the observed turbidity events (Fig. 4.13) and is likely
the influence of the periodicity of frontal systems (Litaker et al. 1987; Giffen and Corbett
2003).
4.4. Discussion

The phytoplankton community of the lower NRE showed a clear response to alternations in water column stability. Diatoms, the principle non-flagellate group, displayed higher abundance and were a greater fraction of the total phytoplankton biomass under more mixed conditions. This finding is fully consistent with the predictions of Margalef’s Mandela which predicts that the success of sinking phytoplankton will be critically linked to rates of mixing regardless of nutrient availability. There is evidence that in this shallow system, there are multiple interrelated mechanisms that link the wind derived energy for mixing the water column to the maintenance of the diatom community. Principal among these are variations in the vertical scale of the largest turbulent eddies, changes in the depth of the pycnocline and the resuspension of surficial sediments. There was no significant influence of water column stability on the dinoflagellate dominated flagellate community. Additionally, even though diatom biomass did increase, dinoflagellates were just as likely to dominate the phytoplankton community under well mixed conditions. So diatoms come and go within the estuary in relation to the degree of mixing but on the whole dinoflagellates dominate phytoplankton biomass.

When the ratio of the sinking velocity to the mean square of the vertical turbulent velocity fluctuations is small (Peclet number <<1), settling cells remain in suspension and the ratio of the settling velocity to upper mixed layer depth can be used to calculate the exponential loss rate from the surface layer (Condie 1999; Ferris and Lehman 2007). Under the low Peclet number situation, diatom division rates may fully or partially compensate for net losses due to settling and result in either increasing or more slowly decreasing population
levels. According to Stoke’s Law, larger cells typically have higher settling velocities which require higher degrees of turbulent mixing to maintain a homogenous vertical distribution within the upper mixed layer, ie maintain a small Peclet number situation. When the ratio of the sinking velocity to the mean square of the vertical turbulent velocity fluctuations becomes $>1$, settling cells fall from suspension and immediately disappear from the surface water (Condie 1999). Under the high Peclet number situation, division rates have no impact on population growth of the surface water.

The observed correlation between the depth of the upper mixed layer and the intensity of stratification (Fig. 4.4; Table 3.5) is probably the result of erosion of the pycnocline by wind induced turbulence decaying from the surface. This correlation has important consequences for the settling losses of the various diatom size classes. As water column stability increases the magnitude of the turbulent velocity fluctuations decreases as progressively smaller turbulent eddies are squashed at the shallowing pycnocline. Hence, increasingly lower sinking velocities will still result in a high Peclet number situation. The virtual absence of the larger diatom size fraction (>30 μm) in surface samples under the highest levels of water column stability suggests that a high Peclet number situation may be common for the larger diatoms during intensely stratified periods in the lower NRE (Fig. 4.7). Second, as the depth of the upper mixed layer decreases, even if diatoms remain in a low Peclet number situation (homogenous vertical distribution), the ratio of settling velocity to depth of the upper mixed layer increases and settling loss rates increase. This appears to be the situation for the smaller diatom fractions which were nearly always found in surface waters.

Typical sinking rates of marine diatoms are 0.5-1.5 m d$^{-1}$ (Huisman and Sommeijer 2002) and the upper mixed layer depth in the lower NRE is usually 2-5 m under stratified
conditions (Fig. 4.4). This depth also closely corresponds to the depth of the euphotic zone (depth of 1% PAR) so net growth below the pycnocline is improbable (Chapter 2). If we conservatively assume a low Peclet number situation for the diatom community of the lower NRE, the ratio of settling velocity to upper mixed layer depth provides imputed losses (Ferris and Lehman 2007) due to settling of 0.1 - 0.75 d\(^{-1}\).

Maximum intrinsic growth rates of most diatom species are higher than 0.75 d\(^{-1}\) (Smayda 1997) and should be capable of offsetting such losses. However, in natural systems phytoplankton are unlikely to achieve maximum intrinsic growth rates observed under ideal temperature, salinity, light, and nutrient conditions in cultures. Importantly, other loss factors such as grazing, infection, and cell lysis greatly reduce net population growth. In the lower NRE, average grazing losses due to the mesozooplankton (60-2,500 μm fraction) were estimated at 0.43 d\(^{-1}\) and were positively correlated with diatom abundance (Mallin and Paerl 1994). This substantial grazing loss ignores grazing by smaller ciliates and heterotrophic/mixotrophic dinoflagellates (< 60 μm) which are numerous in the lower NRE and have been shown to impart grazing losses that equal or exceed grazing by the mesozooplankton (Calbet and Landry 2004).

The importance of grazing in determining diatom biomass in the lower NRE may explain part of the observed negative relationship between temperature and diatom abundance. Several other groups of phytoplankton showed similar negative relationships with temperature. Only cyanobacteria which are known to have a higher temperature optimum for growth and generally are poorly grazed (Paerl 1988) showed a positive correlation with temperature. In the lower NRE, low temperatures have been found to correlate with lower zooplankton biomass and grazing rates (Mallin and Paerl 1994) partially releasing the
phytoplankton population from grazing losses under colder conditions. While maintaining a population under non-ideal conditions, additional losses due to sinking can shift the balance of net population growth to negative growth under poorly mixed conditions (Ferris and Lehman 2007).

While growth can compensate for sinking of the small diatoms, the large diatoms that completely disappear from the water under highly stratified conditions require another mechanism for maintaining a population within the system. Nearly 70% of the sediment surface area of the lower NRE is below the euphotic zone so sinking to the bottom should on average result in severe light limitation (Fear et al. 2004). Additionally, diatoms that initially settle within the euphotic zone are still likely to eventually be deposited below the euphotic zone as sediment focusing tends to redistribute fine grained sediments focusing them toward the deeper lower energy environments in the lower NRE (Wells and Kim 1989). We hypothesize that the deeper sediments of the lower NRE serve as an effective trap for the diatom size fraction under poorly mixed conditions. However, the turbidity data indicate that wind events commonly generate enough bed stress to resuspend the surficial sediments of even the deepest areas of the lower NRE. The meteorological drivers of the wind events are strong frontal systems (Giffen and Corbett 2003) which pass on average every 9-10 days but are more common during the winter and spring.

The periodicity of resuspension events may play an exceedingly crucial role in determining the success of the diatom community in this system. Diatoms are adapted for short periods of time in complete darkness (Furusato and Asaed 2009) but, given enough time, vegetative cells either die or form resting stages (Furusato and Asaed 2009). Generally, over the course of a few weeks in complete darkness, diatom abundance and metabolic rates
change very little (Jochem 1999) and logarhythmic growth occurs immediately upon reillumination (Jochem 1999; Furusato and Asaed 2009). Evidence from these laboratory studies and from the field (Murphy and Cowles 1997) indicates that diatoms in the lower NRE should be fully capable of surviving the short durations of darkness between resuspension events. Thus, sinking to the sediments is not the end game for diatoms in the lower NRE but rather just one half of a cyclical pelagic/benthic life history.

In this system, it is unclear whether short term variations in the ratio of centric to pennate diatom abundance in the water column are expressive of the influence of resuspension. Energy for resuspension would also mix the water column so there should be a relationship between the centric: pennate ratio and water column stability if indeed lower centric pennate ratios are indicative of resuspension. Instead, the pennate fraction of total diatom abundance showed a weak increase under more stratified conditions. In part, we believe this is because the centric: pennate ratio varies significantly with depth. At the two deepest locations, the centric: pennate ratios were 3.5 and 10.5. Similarly, Cooper (2000) found centric: pennate ratios ranging from 3.6 to 11 from deep water cores in the NRE with an average value of 7.0. These values are more similar to the ratio found in the water column (median of 7.3) than the ratios that were found in shallow euphotic areas where in situ development of the benthic diatom community is possible. Three of the four shallow water cores showed centric to pennate ratios of less than 1. So, the depth to which energy is available for resuspension should play a major factor in determining the centric to pennate ratio of the resuspended diatoms. When energy is high enough to completely mix the deeper sediments the centric to pennate diatom ratio may increase in the water column reflecting the centric: pennate ratio of this large reservoir of sedimented diatoms. Additionally, pennate diatoms, particularly
Cylindrotheca closterium, were occasionally important components of the diatom assemblage even under the most stratified conditions. These forms may have reduced settling velocities compared to some of compared to centric diatoms of equal cell volume due to increased form drag associated with their long thin shapes (Padisak et al. 2003).

The importance of water column stability for the diatom community may help to explain several prior observations of the spatial and temporal dynamics of diatom biomass in the Neuse River Estuary. As mentioned above, in the lower NRE, diatom biomass is greater during the cooler months of the winter and spring (Fig. 4.16) (this study; Valdes-Weaver et al. 2006; Mallin et al. 1991). Hobbie (1971) found a similar seasonal pattern of diatom abundance in the adjacent Pamlico River Estuary. While part of this pattern is likely due to decreased grazing losses during the colder months (Mallin and Paerl 1994), there is also a tendency toward increased duration of stratification events in the NRE during the warmer months (this study) and in the adjacent Pamlico River Estuary (Hobbie 1970). This tendency is unlikely due to the effects of temperature on the density structure of the water column because salinity dominates the density structure. Wind patterns however differ substantially on a seasonal basis. During the warmer months winds are generally lighter and the predominant south west wind direction is oriented downstream (Fig. 4.15) (Hobbie 1970). In the NRE, light winds blowing downstream tend to increase the stability of the water column rather than mix it (Luettich et al. 2000). Similar observations were made in the York River estuary by Scully et al. (2005). During the cooler months, strong wind events are more common and the predominant north east wind direction of these events (Hobbie 1970) blows upstream. Stronger winds opposing the downstream surface flow are more likely to produce a mixed water column (Luettich et al. 2000, Scully et al. 2005). Our data indicate that both
temperature and stability of the water column influence the dynamics of the diatom community. Margalef (1978) suggests that this result is because the diatom community has “fine tuned” their optimum temperature for growth to coincide with the annual peak in wind energy for mixing. An interesting test of this hypothesis would be to determine how diatom biomass corresponds to temperature in a region where the dominant wind events occurred during the warmer months.

Several authors have noted the increased relative importance of diatoms in the phytoplankton community within the well-mixed Pamlico Sound as compared to the Neuse River Estuary and Pamlico River tributary estuaries (Fig. 4.16) (Hobbie 1971; Stanley and Daniel 1985; Valdez-Weaver et al. 2006) where the water column is more stable. In these two tributary estuaries of the Pamlico Sound, diatoms are also a notably less significant component of the phytoplankton community than in other estuaries within the region such as the Newport River Estuary, Cape Fear River Estuary, and the Chesapeake Bay (Mallin et al. 1991; Stanley and Daniel 1985). These authors attributed the difference primarily to the difference in average salinities of the various estuaries. It is true that these other regional estuaries generally have higher salinities than the Neuse and Pamlico River Estuaries and that many common marine diatoms grow more rapidly at salinities closer to seawater (Radchenko and Il’yash 2006; Varum and Myklestad 1984). However, these other estuaries also differ from tributary estuaries to the Pamlico Sound in another principal aspect. They are connected directly to the open ocean and thus are mixed more regularly by tidal straining. Given our documentation of the effects of stratification regime on the diatom community and lack of an observed relationship with salinity, the lack of regular tidal mixing in the Neuse and Pamlico River Estuaries appears to be a more likely explanation of the observed pattern
in diatom dominance between the regional estuaries. In fact, Thayer (1971) recognized the importance of tidally generated turbulence for maintaining large, fast-sinking diatoms in the water column of the Newport River Estuary.

The Mandela supposes that under well mixed conditions diatoms will out compete dinoflagellates for available nutrients due to their faster growth rates (Margalef 1978). As stated in the introduction, the more recent confirmation that diatoms generally have lower light and nutrient requirements for growth and that dinoflagellates can be harmed by the small scale shear associated with mixing have supported this tenet of the Mandela. Thus, it may seem surprising that dinoflagellates were equally as likely as diatoms to dominate the water column under well mixed conditions. Notice that of these arguments for why diatoms should dominate under well mixed conditions, only damage via small scale shear directly links mixing with a negative effect on the dinoflagellate population. Competition for resources necessary for growth would indirectly affect the dinoflagellate community if light and nutrient resources are present in quantities that limit growth.

Laboratory studies have clearly demonstrated that small scale shear associated with turbulence can cause cellular damage to some dinoflagellates. *Akashiwo sanguinea* and *Gonyaulax polyedra* respond to sheer by cessation of mitosis (Berdalet 1992; Thomas et al. 1995). *Lingulodinium polyedrum* loses its longitudinal flagellum (Thomas and Gibson 1990) and in some species high shear rates can result in complete disintegration of cells (Berdalet and Estrada 1993). However, in a recent study of the effects of small scale shear on the growth of ten dinoflagellate species, three species showed reduced growth, three showed increased growth, and four showed no effect of shear (Sullivan and Swift 2003). Juhl et al. (2000) found that susceptibility to shear was influenced by other aspects of the environment.
and that scene-sensitive populations were much more likely to show negative growth effects. Some dinoflagellates thrive in strongly mixed water columns (Smyda 2001). The previously mentioned, consistently well mixed Newport River Estuary is seasonally dominated by blooms of the dinoflagellates *Heterocapsa triquetra* and *Prorocentrum minimum* (Litaker et al. 1987). Red tides also form in upwelling zones where turbulent mixing is variably intense (Smyda 2000).

During this study, dinoflagellates performing a clear pattern of DVM were thoroughly mixed by wind events greater than 10 m s\(^{-1}\) (Chapter 2, Fig. 3.20). However, resumption of the DVM pattern only hours after the winds relaxed indicates at the very least that cellular and flagellar integrity were maintained through the mixing events. It appears that the dominant dinoflagellates of the lower NRE are at least capable of tolerating the shear generated by recurrent mixing events.

Strong mixing is unlikely to lead to a light limited dinoflagellate population due to the shallowness and moderate light attenuation of the lower NRE. Under well mixed conditions, the dinoflagellate community is vertically homogenized and light limitation would presumably be at its worst. A brief conservative calculation of the light availability in a well mixed NRE water column shows that even under well mixed conditions, light levels are likely to still be near levels that saturate cellular growth. During the winter (PAR at its annual low) the daily average incident PAR to the lower NRE is ~900 µmol photons m\(^2\) s\(^{-1}\) (http://aom.giss.nasa.gov/srlocat.html). Assuming a light attenuation coefficient of 2.13 m\(^{-1}\) (the maximum observed during the study) and a water column depth of 7 m (average depth is less), the average daily photon flux density for the water column is 66 µmol photons m\(^2\) s\(^{-1}\).

This value is just above the 40-50 µmol photons m\(^2\) s\(^{-1}\) range of average daily irradiance that
saturated growth rates of 17 marine dinoflagellates (Richardson et al. 1983). Of course, when mixing is weak, dinoflagellates can use vertical migration to optimize light availability (Chapter 2; Ault 2000). So, in the lower NRE, it seems unlikely that light availability would become a selective factor favoring the competitive displacement of the dinoflagellates by diatoms.

The role of competition for nutrients in determining the relative success of any phytoplankton group within this estuary is unclear. Neither the biomass of diatoms or dinoflagellates showed any relationship with ambient nutrient concentrations. We included nutrient concentrations in the regression analyses principally because they are potential confounding variables of the degree of water column stability. However, these types of regression analyses of nutrient concentrations or light attenuation on phytoplankton biomass are conceptually flawed because the independent variables are in no way independent of phytoplankton biomass.

For the dominant diatoms, it seems that the ~ 1 μM levels of DIN that were common throughout the study are probably more than adequate to saturate growth (Sunda et al. 2007). For the dinoflagellates, moderate growth enhancement should be achieved under higher nutrient concentrations (Chapter 2). However, it may be the case that the inputs of nutrients are so high that competition between species for available nutrients is negligible. Certainly, the observed positive correlation between chlorophyll $a$ and DIN concentration would suggest that competition for available nutrients is not likely. If this is the case, then the nutrient axis of the Mandela is irrelevant within such eutrophic systems and turbulence is the only axis with any power to predict the composition of the phytoplankton community.
Perhaps if the water column remained well mixed for a long enough period of time, the diatom community, through faster growth rates and superior competitive abilities for light and nutrients, could eventually competitively displace the dinoflagellates. Reynolds et al. (1984) found alternating patterns of diatom and dinoflagellate abundance by artificially inducing mixed and stratified conditions over 3-4 week periods. However, in Reynolds’s study, there was significant carry-over of the phytoplankton community between stratified and mixed periods that dampened oscillations in dominance between diatoms and dinoflagellates. Given the relatively slow growth rates of dinoflagellates (0.1-2.7 d\(^{-1}\)) (Smayda 1997) and the rapid time scale of alternation between stratification and mixing in the NRE, carry-over of the dinoflagellate populations between stratified and mixed periods would likely obscure the effect of changing stratification regime on the dinoflagellate population. In contrast, diatoms with faster growth rates (0.2-5.9 d\(^{-1}\)) (Smayda 1997), potentially rapid losses due to sinking, and an immediate source population (benthic resuspension), may respond to changes in mixing regime on shorter time scales, leading to a greater expression of changes in mixing regime. Hence, it may also be that the Mandela is correct in its prediction of the competitive outcomes but the time scales of changes in the environment occur too rapidly to allow competition to fully run its course.
Table 4.1. Summary of the dominant phytoplankton groups and their biovolumes.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Biovolume ($\mu m^3$)</th>
<th>Reference or Method</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chlorophytes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlamydomonas sp.</td>
<td>92-1022</td>
<td>Olenina et al. (2006)</td>
</tr>
<tr>
<td>Pyramimonas sp.</td>
<td>37-723</td>
<td>Olenina et al. (2006)</td>
</tr>
<tr>
<td>Ankistrodesmus sp.</td>
<td>37</td>
<td>Olenina et al. (2006)</td>
</tr>
<tr>
<td><strong>Cryptophytes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chroomonas amphioxeia</td>
<td>230</td>
<td>Campbell (1973)</td>
</tr>
<tr>
<td>Chroomonas minuta</td>
<td>45</td>
<td>Campbell (1973)</td>
</tr>
<tr>
<td>Chroomonas sp.</td>
<td>65</td>
<td>Olenina et al. (2006)</td>
</tr>
<tr>
<td>Hemiselmis virescens</td>
<td>40</td>
<td>Campbell (1973)</td>
</tr>
<tr>
<td>Cryptomonas sp.</td>
<td>246-1387</td>
<td>Olenina et al. (2006)</td>
</tr>
<tr>
<td><strong>Cyanobacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Planktolyngbya sp.</td>
<td>75 (per filament)</td>
<td>cylinder</td>
</tr>
<tr>
<td>Synechococcus sp.</td>
<td>4.2</td>
<td>sphere</td>
</tr>
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<td><strong>Diatoms</strong></td>
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<td>single centric diatoms</td>
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<td>cylinder</td>
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<tr>
<td>pennate diatoms</td>
<td>99-96000</td>
<td>basic geometric shapes*</td>
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<td>cylinder</td>
</tr>
<tr>
<td>Skeletonema costatum</td>
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<td>Leptocylindrus minimum</td>
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<tr>
<td>Melosira sp?</td>
<td>6280</td>
<td>cylinder</td>
</tr>
<tr>
<td>Cylindrotheca closterium</td>
<td>180-396</td>
<td>Olenina et al. (2006)</td>
</tr>
<tr>
<td><strong>Dinoflagellates</strong></td>
<td></td>
<td></td>
</tr>
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<td>Katodinium rotundatum</td>
<td>190</td>
<td>Campbell (1973)</td>
</tr>
<tr>
<td>Prorocentrum minimum</td>
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<td>Campbell (1973)</td>
</tr>
<tr>
<td>Heterocapsa triqueta</td>
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<td>Campbell (1973)</td>
</tr>
<tr>
<td>Scrippsiella trochoidea</td>
<td>1356</td>
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<td>Gyrodinium estuariale</td>
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<td>Olenina et al. (2006)</td>
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<td>Olenina et al. (2006)</td>
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<td>Olenina et al. (2006)</td>
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<td>cylinder</td>
</tr>
<tr>
<td><strong>Other small flagellates</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other small flagellates</td>
<td>65</td>
<td>5 $\mu m$ diameter sphere</td>
</tr>
</tbody>
</table>
Table 4.2. Mean contribution of each phytoplankton group to total phytoplankton abundance and biovolume.

<table>
<thead>
<tr>
<th>Algal Class/ Group</th>
<th>Mean % of Total Cell Abundance (STD)</th>
<th>Mean % of Total Biovolume (STD)</th>
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</thead>
<tbody>
<tr>
<td>Diatoms</td>
<td>15.1 (16.4)</td>
<td>23.4 (28.8)</td>
</tr>
<tr>
<td>Dinoflagellates</td>
<td>11.0 (13.2)</td>
<td>62.9 (29.8)</td>
</tr>
<tr>
<td>Cryptophytes</td>
<td>13.6 (12.4)</td>
<td>4.10 (6.82)</td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>16.0 (25.7)</td>
<td>0.25 (0.64)</td>
</tr>
<tr>
<td>Chlorophytes</td>
<td>3.44 (5.66)</td>
<td>3.60 (11.3)</td>
</tr>
<tr>
<td>Other Small Flagellates</td>
<td>38.5 (27.9)</td>
<td>5.08 (6.60)</td>
</tr>
<tr>
<td>Other Large Flagellates</td>
<td>2.45 (10.4)</td>
<td>0.67 (2.19)</td>
</tr>
</tbody>
</table>
Table 4.3. Spearman’s rank correlations between $\Delta \rho$ and measures of phytoplankton biomass (abundance, biovolume, chlorophyll $a$) at the community and class/group levels. N= 40. Significant correlations appear in bold type.

<table>
<thead>
<tr>
<th>Phytoplankton Class Or Group</th>
<th>R</th>
<th>p-value</th>
<th>R</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll $a$</td>
<td>-0.0036</td>
<td>0.98</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Phytoplankton</td>
<td>-0.26</td>
<td>0.11</td>
<td><strong>-0.33</strong></td>
<td><strong>0.036</strong></td>
</tr>
<tr>
<td>Diatom</td>
<td><strong>-0.66</strong></td>
<td><strong>3.9 \times 10^{-6}</strong></td>
<td><strong>-0.72</strong></td>
<td><strong>2.0 \times 10^{-7}</strong></td>
</tr>
<tr>
<td>Dinoflagellate</td>
<td>-0.26</td>
<td>0.10</td>
<td>-0.12</td>
<td>0.46</td>
</tr>
<tr>
<td>Cryptophyte</td>
<td>-0.22</td>
<td>0.17</td>
<td>-0.29</td>
<td>0.066</td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>0.14</td>
<td>0.40</td>
<td>0.11</td>
<td>0.50</td>
</tr>
<tr>
<td>Chlorophyte</td>
<td>0.02</td>
<td>0.92</td>
<td>-0.08</td>
<td>0.61</td>
</tr>
<tr>
<td>Other Small Flagellate</td>
<td>-0.12</td>
<td>0.46</td>
<td>-0.12</td>
<td>0.47</td>
</tr>
<tr>
<td>Other Large Flagellate</td>
<td>0.23</td>
<td>0.15</td>
<td>0.22</td>
<td>0.18</td>
</tr>
</tbody>
</table>
Table 4.4. Results of step-wise multiple regression analyses for physical and chemical parameters on each phytoplankton group. Potential independent variables were light attenuation coefficient ($K_d$), surface and bottom water DIN ($DIN_{surf}$ and $DIN_{bott}$), surface and bottom water PO$_4^{3-}$ ($P_{surf}$ and $P_{bott}$), $\Delta\rho$, surface temperature (T), and surface salinity (S). Dependent variables were chlorophyll $a$, and the abundance and biovolume of phytoplankton classes or groupings. Shown are the retained independent variables, the sign of the regression coefficients, and their p-values. $N = 33$.

<table>
<thead>
<tr>
<th>Phytoplankton Group</th>
<th>Significant Independent Variables for Abundance</th>
<th>P</th>
<th>Significant Independent Variables for Biovolume</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll $a$</td>
<td>+$DIN_{surf}$</td>
<td>$2.8 \times 10^{-2}$</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>+$K_d$</td>
<td>$3.1 \times 10^{-3}$</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Total Phytoplankton</td>
<td>+$K_d$</td>
<td>$4.2 \times 10^{-3}$</td>
<td>None</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>-T</td>
<td>$3.8 \times 10^{-2}$</td>
<td>4.2 x 10$^{-3}$</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>-S</td>
<td>$1.1 \times 10^{-3}$</td>
<td>3.8 x 10$^{-2}$</td>
<td>0.012</td>
</tr>
<tr>
<td>Diatom</td>
<td>-$\Delta\rho$</td>
<td>$1.1 \times 10^{-3}$</td>
<td>-$\Delta\rho$</td>
<td>$7.3 \times 10^{-5}$</td>
</tr>
<tr>
<td></td>
<td>-$T$</td>
<td>$7.6 \times 10^{-3}$</td>
<td>-$T$</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>+$K_d$</td>
<td>0.011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dinoflagellate</td>
<td>-$T$</td>
<td>$2.9 \times 10^{-6}$</td>
<td>None</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>+$K_d$</td>
<td>$2.1 \times 10^{-3}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryptophyte</td>
<td>-$T$</td>
<td>$a3.2 \times 10^{-2}$</td>
<td>-$T$</td>
<td>$a5.6 \times 10^{-4}$</td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>+$T$</td>
<td>$9.6 \times 10^{-5}$</td>
<td>+$T$</td>
<td>$b2.2 \times 10^{-4}$</td>
</tr>
<tr>
<td></td>
<td>-$S$</td>
<td>0.018</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorophyte</td>
<td>-$DIN_{surf}$</td>
<td>$a9.0 \times 10^{-3}$</td>
<td>-$DIN_{surf}$</td>
<td>$4.6 \times 10^{-2}$</td>
</tr>
<tr>
<td>Other Small Flagellates</td>
<td>None</td>
<td>NA</td>
<td>None</td>
<td>NA</td>
</tr>
<tr>
<td>Other Large Flagellates</td>
<td>None</td>
<td>NA</td>
<td>None</td>
<td>NA</td>
</tr>
</tbody>
</table>

Superscripts above P-values indicate that hypothesis tests may be invalid due to failure to meet the assumption of $^a$normality or $^b$homogeneity of variance of the residuals.
Figure 4.1. Margalef’s Mandela.
Red Tide
Dinoflagellates

Diatoms

Turbulence

[Nutrient]

Redrawn from Margalef (1978)
Figure 4.2. Relationships between the variance of $Z_{pyc}$ and $\Delta \rho$. Top panel: Variance of $Z_{pyc}$ versus the mean $\Delta p$ from eight consecutive AVP profiles. Bottom panel: Mean variance of $Z_{pyc}$ within 0.2 kg m$^{-3}$ $\Delta p$ bins versus each 0.2 kg m$^{-3}$ increment.
Var $\left( Z_{\text{pyc}} \right)$ Over 8 Observations

Mean $\left[ \text{Var} \left( Z_{\text{pyc}} \right) \right]$ Within 0.2 $\Delta \rho$ Bins (m$^2$)

Mean $\Delta \rho$ of 8 Observations (kg m$^{-3}$)
Figure 4.3. Time series of average water column temperature, salinity, turbidity, and Δρ. X axis tick marks are located on the first day of each month. Left and right figure columns are data from the AVP deployments at NR120 and NR180, respectively.
Figure 4.4. Relationships between key parameters describing the physical structure of the water column. Top panel: Average depth of the upper mixed layer ($Z_{UML}$) versus $\Delta \rho$ at both stations. Bottom panel: Average north east wind component (NE toward SW positive) versus $\Delta \rho$ at both stations.
Figure 4.5. Time series of surface and bottom water DIN and PO$_4$$^{3-}$ and light attenuation. Data from the period of the AVP deployments. Values of nutrient concentrations above 5 μM are written above or below the data marker.
Figure 4.6. Correlation between $\Delta \rho$ and the abundance and biovolume of diatoms and dinoflagellates. $R$ and $p$ values are from a Spearman’s rank correlation between biomass and $\Delta \rho$. 
Diatoms

\[ \log_{10} \text{ (cells mL}^{-1} ) \]

Cell Abundance

\[ \log_{10} (\text{ppm}) \]

Biovolume

\[ \Delta \rho (\text{kg m}^{-3}) \]

\[ R = -0.66 \]
\[ p = 3.9 \times 10^{-6} \]

\[ R = -0.72 \]
\[ p = 2.0 \times 10^{-6} \]

Dinoflagellates

\[ \Delta \rho (\text{kg m}^{-3}) \]

\[ R = -0.26 \]
\[ p = 0.10 \]

\[ R = -0.12 \]
\[ p = 0.46 \]
Figure 4.7. Correlations between $\Delta \rho$ and the cell abundance of three size classes of diatoms. R and p values are from a Spearman’s rank correlation between biomass $\Delta \rho$. 
Figure 4.8. Correlations between $\Delta \rho$ and the cell abundance and biovolume of centric and pennate diatoms and the fraction of total diatom cell abundance and biovolume represented by pennate diatoms. $R$ and $p$ values are from a Spearman’s rank correlation between biomass and $\Delta \rho$. 
Dinoflagellates and Diatoms (% of Total Phytoplankton Biovolume)

\[ \Delta \rho (\text{kg m}^{-3}) \]

- Diatoms
- Dinoflagellates
- Diatoms + Dinoflagellates
Figure 4.9. $\Delta p$ versus the fractions of the total phytoplankton biovolume composed of diatoms and dinoflagellates.
Figure 4.10. Vertical profiles of diatom and dinoflagellate abundance and biovolume.
Average Abundance of Profile

\[ \log_{10} \text{(Cells mL}^{-1} \text{)} \]

Surface Abundance

- Diat, \( R^2 = 0.97, p = 1.1 \times 10^{-3} \)
- Dino, \( R^2 = 0.87, p = 0.025 \)

Average Biovolume of Profile

\[ \log_{10} \text{(ppm)} \]

Surface Biovolume

- Diat, \( R^2 = 0.98, p = 4.4 \times 10^{-4} \)
- Dino, \( R^2 = 0.51, p = 0.30 \)
Figure 4.11. Comparison of vertically averaged versus surface diatom and dinoflagellate abundance and biovolume.
Cell abundance \[\log_{10} \text{cells mL}^{-1} + 1] 

Centric 

Pennate 

Biovolume \[\log_{10} \text{ppm} + 10^{-4}\] 

R = -0.63 
\[p = 1.2 \times 10^{-5}\] 

R = -0.72 
\[p = 1.5 \times 10^{-7}\] 

R = +0.44 
\[p = 4.6 \times 10^{-3}\] 

R = -0.32 
\[p = 0.047\] 

R = -0.29 
\[p = 0.065\] 

R = +0.19 
\[p = 0.25\] 

R = +0.44 
\[p = 4.6 \times 10^{-3}\]
Figure 4.12. Centric and pennate diatom abundance on the sediment surface from a cross-river transect of the lower NRE. Centric diatom abundance includes both solitary and chain forming centric forms.
Abundance
$\log_{10}$ (cells cm$^{-2}$)

Pennate
Centric
Chain Centric

Centric : Pennate Ratio

Depth

Station

Depth (m)

Centric : Pennate

Depth
Figure 4.13. Resuspension events during the spring of 2004 at NR180. Time series of $\Delta \rho$ (top panel) and average water column turbidity (mid-panel) showing wind generated resuspension events. Red dots indicate time periods when the maximum wave orbital velocities along the bottom were greater than 0.2 m s$^{-1}$. The lower panel shows the square of the calculated maximum wave orbital velocities ($U_{\text{max}}^2$) along the bottom. The solid red line indicates the proposed threshold for resuspension.
Figure 4.14. Power spectra of the occurrence of super-critical wave orbital velocities. Y-axis values are arbitrary units.
Figure 4.15. Annual cycle in the NE-SW wind velocity component for the NRE. Hourly averages were smoothed using a low pass, second order Butterworth filter with a 0.0022 cpd cutoff.
Figure 4.16. Seasonal and down estuary relationship between diatom abundance and water column stability. Diatom data are from photopigment analyses and are redrawn from Valdes-Weaver et al. (2006).
4.5. LITERATURE CITED


ENVIRONMENTAL FACTORS CONTRIBUTING TO THE DEVELOPMENT AND
DEMISE OF A TOXIC DINOFLAGELLATE (KARLODINIUM VENEFICUM)
BLOOM IN A SHALLOW, EUTROPHIC, LAGOONAL ESTUARY

Nathan S. Hall, R. Wayne Litaker, Elizabeth Fensin, Jason E. Adolf, Holly A. Bowers,
Allen R. Place, and Hans W. Paerl. 2008

Estuaries and Coasts 31:402-418.

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Estuary, Volume 31, 2008, pp. 401-418, authors: Nathan S. Hall, R. Wayne Litaker,
Elizabeth Fensin, Jason E. Adolf, Holly A. Bowers, Allen R. Place, and Hans W. Paerl.
Figures 1-9.

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ABSTRACT

A dense bloom of the ichthyotoxic dinoflagellate *Karlodinium veneficum* was discovered in the Neuse River Estuary, North Carolina on 19 October 2006 and was associated with four subsequent fish kills. Microscopic, photopigment, DNA, and toxicological techniques confirmed bloom identity and toxicity. High resolution spatio-temporal data from ship-board and fixed automated sampling stations provided a unique opportunity to investigate the environmental conditions that initiated, maintained, and terminated the *K. veneficum* bloom. Bloom initiation and growth were favored by high nutrient availability and reduced dispersal during the period of declining riverine discharge following Tropical Storm Ernesto. *K. veneficum* out-competed other co-occurring dinoflagellates, perhaps due to the production of karlotoxins which are known to act as grazing deterrents and to facilitate mixotrophic feeding. Once the bloom was established, small-scale hydrodynamic processes, coupled with vertical migration, concentrated cells along a frontal convergence to high densities (> 200,000 cells mL\(^{-1}\)). By 26 October 2006, wind mixing and possible nutrient stress disrupted the bloom. Release of cell-bound toxins during the bloom collapse likely accounted for the associated fish kill events where fish were reported as exhibiting typical symptoms of karlotoxin poisoning. The dynamics of this bloom underscore the tight control of harmful algal blooms by meteorological forcing, hydrology, and sediment nutrient input in this shallow lagoonal estuary.
5.1. Introduction

Estuarine and coastal phytoplankton blooms commonly result from a sequence of events involving nutrient inputs that stimulate growth and hydrodynamic conditions that favor reduced dispersal or physical accumulation of algal cells. In certain instances, the blooms become dominated by harmful algal bloom (HAB) species that adversely affect ecosystem or human health. The success of HAB species and their ability to attain bloom concentrations generally result from one or more of the following characteristics: 1) superior ability to utilize or access available nutrients (Amano et al. 1998; Sunda et al. 2006), 2) inhibition of competing phytoplankton through release of allelopathic compounds or direct mixotrophic consumption of competitors (Smayda 1997), 3) reduced grazing losses due to direct toxicity, poor food quality or mechanical disruption of grazing (Sunda et al. 2006).

On 19 October 2006, a dense dinoflagellate bloom was discovered in the Neuse River Estuary (NRE), North Carolina, U.S.A. by the Neuse River Modeling and Monitoring Program (ModMon; www.unc.edu/ims/neuse/modmon/). Morphological, toxicological, pigment, and DNA analyses definitively identified the bloom forming dinoflagellate as *Karlodinium veneficum* (see Daugbjerg et al. 2000 for synonyms). *K. veneficum* is common to brackish waters of the U.S. Atlantic Coast and can produce dense “mahogany tides” with cell concentrations approaching $10^6$ mL$^{-1}$ (Goshorn et al. 2004). Fish kills associated with the toxins released by this organism have been reported in estuaries, brackish ponds, and aquaculture systems along the U.S. Atlantic Coast (Deeds et al. 2002; Kempton et al. 2002). In North Carolina estuaries, *K. veneficum* blooms are rare,
but background levels < 5,000 cells mL\(^{-1}\) are common, particularly during warmer months (Fensin 2004). When *K. veneficum* blooms have exceeded 30,000 cells mL\(^{-1}\) in North Carolina estuaries, they have been associated with fish kills (Fensin 2004). However, definitive attribution of these kills to *K. veneficum* was not possible because the karlotoxin concentration in the water was not measured.

Using a combination of high-resolution data from ship-board sampling and from automated water quality and meteorological monitoring stations, it was possible to investigate the sequence of environmental events and conditions that initiated, supported, and then terminated the October 2006 *K. veneficum* bloom. Of primary interest was how the hydrology of the estuary interacted with nutrient delivery and biological processes to produce the unusually high density of *K. veneficum* cells. This is of importance because a firm understanding of HAB dynamics is required to effectively manage and mitigate the globally increasing (Hallegraeff 1993) HAB problem (Cloern 2001). Secondarily, we assessed the association between the bloom and fish kills that occurred a few days after the observed *K. veneficum* biomass maximum.

### 5.2. Methods and Materials

**5.2.1. Study Site, Sample Collection, and Chemical Analyses**—Ship board data were obtained from eleven sites sampled as part of ModMon, a long-term monitoring program that collects bi-weekly water quality data (Luettich et al. 2000) and from twenty one sites checked monthly by the North Carolina Department of Environment and Natural Resources, Division of Water Quality’s (NCDENR-DWQ)
Station locations are expressed as distance downstream from the freshwater head of the estuary at Streets Ferry Bridge, New Bern, North Carolina (Fig. 5.1).

For chemical and plankton community analysis, samples were collected on 18 September, 3, 19, and 30 October 2006 by the ModMon monitoring program at all ModMon stations (Fig. 5.1). Samples were collected from the surface and from 0.5 m above the bottom using a Van Dorn sampler and dispensed in 2 L polyethylene bottles. Samples were transported in a dark cooler at ambient temperature to the laboratory within 4 hr of collection. For nutrient analyses, 100 mL of each water sample was gently filtered through a pre-combusted Whatman 25 mm GFF filter, and the filtrate was frozen at −20°C within 6 hr of collection. After nutrient filtration was completed, the filters were immediately frozen at -20°C for particulate C and N analyses. Subsequently, the frozen nutrient samples were quick thawed and NO$_2^−$ + NO$_3^−$ (reported as NO$_3^−$), NH$_4^+$, PO$_4^{−3}$ and total dissolved nitrogen (TDN) immediately determined using a Lachat Quick-chem 8000 auto-analyzer (Lachat, Milwaukee, Wisconsin) (Lachat Quik-chem methods 31-107-04-3-B, 31-107-04-1-C, 31-107-06-1-B, and 31-115-01-3-C, respectively). During the analysis of each nutrient analyte, five replicates each of a high and low concentration quality control standard were interspersed throughout each run of environmental samples to determine measurement precision. The maximum observed coefficients of variation for NO$_2^−$ + NO$_3^−$, NH$_4^+$, PO$_4^{−3}$ and TDN were 0.8, 2.3, 7.6, and 11.4 percent, respectively. Dissolved inorganic nitrogen (DIN) was subtracted from TDN to obtain the dissolved organic nitrogen (DON) fraction. For particulate C and N analyses, filters were fumed at room temperature for 12 hr with concentrated HCl to
remove excess inorganic C, and then analyzed on a Perkin Elmer Series II CHNS/O 2400 analyzer.

5.2.2. Cell Counts—Sub-samples of surface and bottom water from the ModMon stations were preserved in 1% Lugol’s solution for phytoplankton and microzooplankton counts. Surface samples from Mod Mon stations 15 - 73, collected on 18 September, 3 October, 19 October, and 30 October, as well as the bottom water sample from the bloom maximum at station 26 on 19 October 2006, were counted using 15 mL settling chambers and a Leica DMIRB inverted microscope (Wetzlar, Germany) according to Utermöhl (1958). Cells within 40-100 random Whipple grids of the settling chamber base were identified and enumerated under phase contrast at a magnification of 400X. Ninety five percent confidence intervals for each microscopic cell abundance estimate were calculated according to (Clesceri et al. 1998).

For the region of the estuary where the highest *K. veneficum* biomass developed (ModMon stations 19, 26, 28, 37, and 43, hereafter called the bloom region) the average cell concentration of each phytoplankton species was calculated from surface water concentrations using a trapezoidal approximation procedure. Briefly, the mean cell concentration between each adjacent pair of stations in the bloom region was determined and multiplied by the distance between each respective station pair. These segmental estimates were then summed and divided by the total length of the bloom region to obtain a best estimate of the average cell concentration within the bloom region.

The net rate of change (μ\text{apparent}) in the abundance of *K. veneficum* and a number of other co-occurring plankton within the bloom region were then determined for the
periods between 3-19 October and 19-30 October 2006. The equation used to calculate 
\( \mu_{\text{apparent}} \) was \([\ln (N_f N_i^{-1})] \cdot t^{-1}\), where \( N_i \) and \( N_f \) are the initial and final average cell 
concentration for each sampling interval, calculated as described above, and \( t \) is the 
number of days between sample collection.

For microzooplankton, surface samples from the stations encompassing the bloom 
region were counted on 3 October, 19 October, and 30 October. A magnification of 
200X was used to count 200 random Whipple grids. Data are reported for four broad 
groups of microzooplankton: tintinnids, oligotrich ciliates > 30 \( \mu \text{m} \) maximum dimension, 
*Oxyrrhis marina*, and other heterotrophic dinoflagellates > 10 \( \mu \text{m} \) maximum dimension. 
These were chosen because they are of the size class capable of feeding on *K. veneficum* 
(Hansen et al. 1994). *Oxyrrhis marina* was separated from other heterotrophic 
dinoflagellates because it dominated heterotrophic dinoflagellate abundance during the 
study and is known to be an important grazer of *K. veneficum* (Johnson et al. 2003). 
Average concentrations of each microzooplankton group within the bloom region were 
calculated in the same manner as for the phytoplankton.

Surface water samples for phytoplankton enumeration were collected by NCDENR-
DWQ at stations 15, 24, 30, 59 and 80 on 11 October 2006 and from the fish kill sites on 
25 October, 30 October, and 1 November 2006 (Fig. 5.1). Cells were preserved in 
Lugol’s amended with glycerin to preserve flagella (Vollenweider 1974) and counted 
according to NCDENR-DWQ protocols 
(http://h2o.enr.state.nc.us/eb/phytofolder/PhytoSOP_1_24_03.pdf) on a Leitz Diavert 
inverted microscope (Wetzlar, Germany) at 500X magnification. All cells in random 
Whipple grid fields were counted until 100 cells of a single taxon were enumerated.
5.2.3. Physical Data—On 3, 19, and 26 October 2006, vertical profiles of temperature, salinity, dissolved oxygen (DO), in vivo fluorescence, and light at each ModMon station were made at 0.5 m intervals with a YSI 6600 multiprobe sonde coupled to a LiCor LI-1925A quantum sensor that measures photosynthetically active radiation (PAR) sensor (Yellow Springs, Inc., Yellow Springs, Ohio). Euphotic zone depth (1% surface irradiance) was calculated from the diffuse light attenuation coefficient derived from PAR profiles. In vivo fluorescence profiles were post-calibrated using the regression of in vivo fluorescence versus high performance liquid chromatography (HPLC) derived chlorophyll $a$ (chl $a$) obtained from corresponding surface and bottom water samples. On 26 October, no water samples for HPLC chl $a$ were collected, so the calibration curve from 19 October was used to calibrate fluorescence profiles. Contour plots of longitudinal and vertical salinity, DO and chl $a$ (from the calibrated fluorescence profiles) were produced using the ‘contourf’ function of Matlab version 7.0.0 R14 (www.mathwork.com, The Mathworks, Inc. Natick, Massachusetts). All calculations and statistical analyses were also performed with Matlab version 7.0.0 R14.

Automated instrumentation operated by the U.S. Geological Survey (USGS) at channel markers CM9 (34º 56.917’ N, 76º 48.583’ W), CM11 (34º 59.916’ N, 76º 56.651’ W), and CM38 (35º 06.567’ N, 77º 01.967’ W) (Fig. 5.1) provided measurements of surface (1 m) and bottom (3-3.5 m) temperature, salinity, and DO every 15 min using Hydrolab Surveyor IV water quality instruments (HACH Environmental, Loveland, Colorado). The station at CM11, closest to the bloom maximum, has been operational since 1989, allowing for the comparison of conditions during this study with
historic norms (http://waterdata.usgs.gov/nc/nwis). Hourly wind and solar radiation data were obtained from the nearby U.S. Environmental Protection Agency, Clean Air Status and Trends Network (CASTNET) site 142 (34°53.088’ N, 76°37.218’ W) (Fig. 5.1) (http://www.epa.gov/castnet). Neuse River flow data were obtained from the USGS flow station at Kinston, North Carolina (http://waterdata.usgs.gov/nc/nwis), approximately 90 km upstream from the head of the NRE. Flow at this location averages two thirds of the total flow into the estuary (Luettich et al. 2000).

5.2.4. Photopigment, DNA, and Toxin Analyses—Photopigment analysis via HPLC was performed as described by Pinckney et al. (1996) except that the analyses were performed on a Shimadzu LC-20AB HPLC, coupled to a Shimadzu SPD M20A in-line photodiode array spectrophotometer (Shimadzu-Benelux, Antwerpen, Belgium).

Total DNA for genetic confirmation and quantification of *K. veneficum* was extracted from Lugol’s preserved samples using the Puregene kit from Gentra Systems (Minneapolis, Minnesota). One µL was used as template in a real-time PCR assay specific for the internal transcribed spacer locus (ITS) of the rDNA genome of *Karldinium veneficum* (Bowers, H. A. et al., unpublished data). Specificity of this assay has been confirmed against forty-five *K. veneficum* cultures (all positive) as well as twenty-six closely related dinoflagellate species (all negative, including *Karldinium armiger*, *K. conicum*, *K. austral* and two *Karenia* species; Bowers, H. A. et al., unpublished data). Each reaction contained: 0.1U Taq Pro (Denville Scientific, Metuchen, New Jersey), 1X PCR buffer, 3 mM MgCl₂, 0.2 µM forward and reverse primers, 0.3 mM each deoxynucleotide triphosphate (Invitrogen, Alameda,
California), 0.25 mg ml\(^{-1}\) bovine serum albumin (Idaho Technology, Idaho Falls, Idaho), 0.3 µM Taqman probe, molecular grade water to 10 µl and 1 µl DNA template. Samples, controls, and a serial dilution of a plasmid containing the target insert (i.e. standard curve, prepared as described in Bowers et al. 2006) were run on the ABI 7500 (Applied Biosystems, Foster City, California). The following parameters were used: an initial denaturing step for 5 min at 95\(^\circ\)C, followed by 50 cycles of 15 sec at 95\(^\circ\)C, 30 sec at 60\(^\circ\)C.

Samples for karlotoxin (KmTx 2) analysis taken at the peak of the bloom on 19 October were collected on PTFE filters which quantitatively retained particulate and dissolved toxin (Bachvaroff et al. 2007). Concentrations were measured by liquid chromatography – mass spectrometry (LC-MS) using a C8 HPLC column (LiChrosphere 125 mm x 4 mm 5 µm bead size RP-8, Waters Corporation) and an Agilent G1956A VL mass spectrometer. Karlotoxin peaks previously shown to have hemolytic activity were quantified based on calibration curves derived from pure KmTx 2 (Bachvaroff et al. 2007). Hemolytic assays of 15 s HPLC fractions from the bloom samples were performed using striped bass (Morone saxatilis) erythrocytes to confirm hemolytic activity of the KmTx 2 fraction. Saponin (10 µg) (Sigma Chemical Co.) was used as a positive hemolysin control according to Eschbach et al. (2001).

5.3. Results

5.3.1. Bloom Characteristics—On 19 October 2006 at station 26, a dense dinoflagellate bloom producing obvious water discoloration was discovered. Karlodinium veneficum
cell abundance exceeded 200,000 cells mL$^{-1}$ (Table 5.1) and numerically dominated the phytoplankton assemblage (Table 5.2). The photopigment signature from the bloom samples was consistent with that obtained from cultured *K. veneficum* and included a very high chl *a*, fucoxanthin, 19’ hexanoyloxyfucoxanthin (19’-hex), 19’ butanoyloxyfucoxanthin (19’-but), chlorophyll *c3*, diadinoxanthin, chl *c1-c2* and gyroxanthin diester (Table 5.1) (Kempton et al. 2002). In addition to *K. veneficum*, the bloom also contained high concentrations of other potentially bloom-forming dinoflagellates (Campbell 1973) that contained peridinin, as well as fucoxanthin-containing *Leptocylindrus minimum*, a small chain forming diatom, alloxanthin-containing cryptophytes, and chlorophyll *b*-containing euglenoids and chlorophytes (Tables 5.1 and 5.2). *K. veneficum* was likely the sole contributor of 19’-hex, gyroxanthin, and 19’-but, which are characteristic of this species, but found in few other dinoflagellates (Kempton et al. 2002). The 19’-hex concentration observed at the bloom maximum was 93.9 μg L$^{-1}$ (Table 5.1). The next highest value in the ModMon pigment database, which included 5,923 measurements of 19’-hex in the NRE from 4 January 1994 to 30 October 2006, was 1.93 μg L$^{-1}$.

Two putative karlotoxin peaks were detected from the bloom samples by the LC-MS analysis, one eluting at 9.1 min and one at 14.8 min (Fig. 5.2a). The mass spectra of the bloom samples contained the major ion (1367.8 amu) of purified KmTx 2 (Bachvaroff et al. 2007), but also contained a congener that is 16 amu lower (data not shown). The UV absorption spectrum of the toxin isolated from the bloom was identical to purified KmTx 2 (data not shown) with a maximum absorbance of 235 nm (Bachvaroff et al. 2007). The mass spectrum of the 9.1 min peak was consistent with sulphated congeners of the ions
present in the 14.8 min peak (data not shown). Toxin isolated from the 14.8 min peak co-eluted with the KmTx 2 standard (Fig. 5.2b), and the hemolytic activity of the toxin in this fraction was confirmed by a near 100% erythrocyte hemolysis rate as compared to the Saponin control (Fig. 5.2b).

*K. veneficum* cell abundance and toxin concentration were highly localized at station 26 (Fig. 5.3) and the linear relationship between toxin and cell abundance was highly significant ($R^2 = 0.99, P < 1 \times 10^{-7}$). Cell abundance and ITS rDNA copy number determined using qPCR also tracked each other closely throughout the study (Fig. 5.3, $R^2 = 0.9995$, Table 5.3). This highly significant correlation between the qPCR results and microscopic counts indicates both independent methods accurately quantified *K. veneficum* abundance and that there were approximately 67 detectable ITS sequences per *K. veneficum* cell in situ.

### 5.3.2 Bloom Chronology

River flow prior to Tropical Storm Ernesto (1 September 2006) was below the fifty-year median value (48 m$^3$ s$^{-1}$) but increased rapidly following the storm to more than four times the median flow (Fig. 5.4). The main body of the floodwater pulse reached the estuary ~ 10 d after the storm and freshened the estuary from surface to bottom (Fig. 5.4). As flow rates declined, the resumption of estuarine circulation was apparent as increases in bottom salinity. The progression of the salt wedge upstream as flow declined is clearly evident from the three real time data sets (Fig. 5.4). The salt wedge passed CM9 on 13 September 2006 (Fig. 5.4), followed by CM11 on 14 September (Fig. 5.4), and finally reached CM38 on 16 September (Fig. 5.4). Based on the time and distance traveled, upstream bottom water velocities were 13 cm s$^{-1}$ from
13-14 September (CM9 to CM11), 6 cm s\(^{-1}\) from 14-16 September (CM11 to CM38), and 11 cm s\(^{-1}\) for the 13-16 September upstream advance from CM9 to CM38. At the end of the high flow period on 18 September, \textit{K. veneficum} was observed only at the most downstream stations, with a maximum abundance of \(\sim 100\) cells mL\(^{-1}\) (Fig. 5.3).

Strong estuarine circulation resumed as riverine discharge declined, and in combination with weak winds, led to an unusually protracted period of stratification that lasted from 14 September to 6 October 2006 (Fig. 5.4). At the CM11 station, the minimum difference in salinity between the top and bottom over the 22 d period was 4.6 ppt. Historical records of surface and bottom salinities from 13 May 1989 to 6 October 2006 at CM 11 (6,355 observations) showed that similar 22 day periods with a continuous minimum vertical salinity difference of 4.6 occurred only 0.7 % of the time.

On 3 October 2006, surface temperature was \(\sim 23^\circ\text{C}\) (Fig. 5.5), and the estuary was strongly stratified from station 9 to the mouth of the estuary (Fig. 5.6). Surface salinity increased in a nearly linear fashion from 0 to 13 ppt from the head to mouth of the estuary (Fig. 5.6). Irradiance was high with a mid-day maximum of 750 W m\(^{-2}\) (Fig. 5.5).

Significant correlations between extracted Chl \(a\) and in vivo fluorescence were obtained which allowed post calibration of in vivo fluorescence (Table 5.3) to estimate vertical and horizontal phytoplankton biomass distributions. At stations 26, 43, and 59, three subsurface peaks in chl \(a\) greater than 20 \(\mu\text{g L}^{-1}\) were located at approximately 0.75 - 1.0 m depths (Fig. 5.6). Euphotic zone depths increased from \(\sim 2\) m at the head to \(\sim 4\) m at the mouth of the estuary (Fig. 5.6). The hypolimnion was severely hypoxic while the epilimnion was DO supersaturated from station 19 to the mouth of the estuary (Fig. 5.6). Surface and bottom water NO\(_3^-\) decreased from 50 \(\mu\text{M}\) at the head of the estuary to below
detection (0.04 μM detection level) at stations 43 and 19, respectively (Fig. 5.6). Both surface and bottom DON concentrations were relatively constant at ~ 30 μM (Fig. 5.6). Bottom water concentrations of NH₄⁺ and PO₄³⁻ were unusually high (Fig. 5.6). Peak values, 38 μM NH₄⁺ and 6.3 μM PO₄³⁻, were in the 99th percentile of 3,294 observations of bottom water NH₄⁺ and PO₄³⁻ from the NRE over the last 21 years. *K. veneficum* cells were present from stations 26 to 73 with a population maximum of ~ 4,000 cells mL⁻¹ at station 43 (Fig. 5.3).

A strongly stratified condition was maintained through 6 October (Fig. 5.5). On 7 October, a wind event (Fig 5) thoroughly mixed the water column. Normoxic hypolimnetic conditions were restored (Fig. 5.5), and a large pulse of NH₄⁺ and PO₄³⁻ was mixed throughout the water column.

On 11 October, *K. veneficum* concentrations were higher than on 3 October, and the population maximum of ~15,000 cells mL⁻¹ was located further upstream at NC DENR-DWQ station 24 (Fig. 5.3).

From 11-17 October, conditions were sunny (Fig. 5.5), winds were light (< 3 m s⁻¹) (Fig. 5.5), and the estuary was weakly stratified (Fig. 5.5). On 17 October, passage of a warm front warmed the water by ~ 3 °C (Fig. 5.5) and was accompanied by heavy cloud cover that reduced irradiance to mid-day maxima of only ~ 300 W m⁻² from 17 October through 19 October (Fig. 5.5). During this period, declining bottom DO concentrations indicated low vertical mixing (Fig. 5.5, Fig. 5.7).

From 3 - 19 October nearly all phytoplankton species within the bloom region experienced positive growth (Table 5.2). The *K. veneficum* population within the bloom region grew at 0.25 d⁻¹ (Table 5.2), an apparent growth rate greater than all other co-
occurring phytoplankton species with the exception of *Leptocylindrus minimum* (Table 5.2). Two species of 4-10 μm cryptophytes, *cf Chroomonas minuta* and *cf Hemiselmis virescens* (Campbell 1973), were abundant throughout the study. Small cryptophytes such as these are preferred prey items for *K. veneficum* (Li et al 2000). The sum of these two small cryptophytes closely tracked the *K. veneficum* population and reached a maximum abundance within the bloom region of ~ 3,000 cells mL\(^{-1}\) on 19 October 2006 (Table 5.2). Large (> 30 μm) oligotrich ciliates, tintinnids, and *Oxyrrhis marina* abundances increased as the bloom developed reaching 14, 26 and 118 cells mL\(^{-1}\), respectively, on 19 October (Table 5.2).

At the *K. veneficum* bloom maximum (station 26, 19 October), approximately 80% of water column chl \(a\) was within the upper 1 m (Fig. 5.7). Horizontally, the maximum of *K. veneficum* and chl \(a\) was located along a frontal region, marked by a steep surface salinity gradient that separated the strongly stratified region upstream from the less stratified downstream region (Fig. 5.7). The other dinoflagellates common in the bloom region showed a similar accumulation of biomass at station 26 (Fig. 5.8) while the most abundant diatoms displayed monotonic increases or decreases through the frontal region (Fig. 5.8). At station 26, the *K. veneficum* population increased from 73 cells mL\(^{-1}\) on 3 October to 219,000 cells mL\(^{-1}\) on 19 October (Fig. 5.3). This increase represents an apparent growth rate of 0.5 d\(^{-1}\) which was double the rate of *K. veneficum* when calculated over the bloom region as a whole (Table 5.2).

On 19 October, DIN was reduced to ~ 1 μM within and downstream of the bloom maximum (Fig. 5.7). The downstream distribution of surface and bottom water DON was relatively constant at approximately 25 μM except in the bloom maximum located at
the surface of station 26. Here DON was more than two fold higher than at any other station measured on either 19 October or on 3 October (Fig. 5.6, Fig. 5.7). Like DON, PO$_4$ was much higher within the bloom than surrounding stations (Fig. 5.7). Based on the particulate nitrogen measurement from the bloom (185 μM, Table 5.1) and assuming a cellular N:P molar ratio of 9 (Nielsen 1996), the excess DON and PO$_4$ within the peak of the bloom, relative to adjacent stations, constituted only 12 - 13 % of the total N and P pools found in the phytoplankton.

On the morning of 20 October 2006, strong (~ 6 m s$^{-1}$) southwest winds mixed the water column (Fig. 5.5). As the day progressed, wind direction rotated clockwise nearly 180° resulting in a ~ 5 m s$^{-1}$ northeast wind by the following morning (Fig. 5.5). Starting on 23 October, a cold front accompanied by moderate (~ 5 m s$^{-1}$) northwest winds (Fig. 5.5) cooled the estuary by ~ 5 °C over a 3 d period (Fig. 5.5).

On 26 October, maximum chl $a$ values were less than 20 μg L$^{-1}$ showing that the bloom had dissipated by this time (Fig. 5.9). Salinity between 19 and 26 October showed no evidence of a major washout event (Fig. 5.5). Irradiance was high (> 700 W m$^{-2}$) and the bulk of chlorophyll within the bloom region was distributed as a subsurface maximum at ~ 2 m extending from station 28 downstream to at least station 43 (Fig. 5.9). By 30 October 2006, maximal concentrations of $K. veneficum$ were ~ 300 cells mL$^{-1}$ (Fig. 5.3). The apparent growth rate from 19 - 30 October of the population within the bloom region was -0.52 d$^{-1}$ (Table 5.2). Other common photosynthetic dinoflagellates experienced similarly large population losses (-0.38 to -0.60 d$^{-1}$) (Table 5.2). Phytoplankton other than dinoflagellates experienced only slight declines or moderate increases over the same time period (Table 5.2). Large (> 30 μm) oligotrich ciliates and
the heterotrophic dinoflagellate *Oxyrrhis marina* abundances remained constant over this period. The other > 10 μm heterotrophic dinoflagellates besides *O. marina* nearly doubled, while tintinnid abundance decreased by half (Table 5.2).

5.3.3. *Fish Kills*—Four days after the observed bloom maximum at station 26 on 19 October, a resident living on Upper Broad Creek (Fig. 5.1) noticed fish gulping air at the surface. The next day, 24 October, the resident reported that the fish were dead and another fish kill report was made for Northwest Creek (Fig. 5.1, Table 5.4) (NCDENR Fish Kill Database 2006). *K. veneficum* concentrations of ~ 2,500 cells mL\(^{-1}\) were observed in Northwest Creek on 25 October. Two more fish kills were reported on 29 October and 1 November., but the decayed condition of the fish led to the conclusion that they occurred at the same time as the prior two fish kills. *K. veneficum* concentrations at the latter two fish kill sites were ~ 300 cells mL\(^{-1}\). A variety of common estuarine and brackish water tolerant freshwater species were killed (Table 5.4). Salinities in the fish kill waters were approximately the same as at the bloom maximum. There were no other apparent causes for fish kills such as temperature stress, hypoxia, chemical spills, or bycatch release by fishermen.

5.4. **Discussion**

5.4.1. *Summary*—The magnitude of the *Karlodinium veneficum* bloom observed in this study is unprecedented in North Carolina estuaries, exceeding previously reported maximum cell concentrations by nearly an order of magnitude (Fensin 2004). Growth of
the bloom was largely attributable to abnormally high nutrient availability, a prolonged period of water column stability, and reduced dispersal. Hydrodynamic conditions along a salinity front further concentrated the toxin containing *K. veneficum* cells to high densities (> 200,000 cells mL\(^{-1}\) and > 800 ng KmTx 2 mL\(^{-1}\)). The toxin content of these cells, if released, was more than sufficient to cause fish mortality (Kempton et al. 2002; Deeds et al. 2006).

5.4.2. *Bloom initiation*—Meteorological forcing events that alter nutrient inputs, temperatures, stratification or flow regimes in significant ways often select for bloom organisms that are not normally dominant in an ecosystem. One such example was a “surprise” dinoflagellate bloom in San Francisco Bay, which is usually dominated by diatoms. This bloom resulted from a period of high air temperatures, low wind stress, and thermal stratification that allowed dinoflagellates to grow and accumulate in an abnormally shallow upper mixed layer (Cloern et al. 2005). In the Chesapeake Bay, an unusual fall diatom bloom was initiated when wind mixing from Hurricane Isabel injected bottom water nutrients into the surface waters (Miller et al. 2006). For the *K. veneficum* bloom described in this study, the unusual meteorological event was flooding from Tropical Storm Ernesto, which was followed by a 3 wk period of uninterrupted water column stratification.

In addition to a precipitating forcing event, which species subsequently dominates the assemblage also depends on the availability of a seed population (Steidinger 1983). In the NRE, *K. veneficum* is nearly always found at background concentrations (Fensin 2004; Litaker, R. W., unpublished data). However, the cell count and qPCR data (Fig.
5.3) clearly indicate that discharge from Tropical Storm Ernesto initially flushed the background population out of the bloom region. The most likely source of cells was the small population near the mouth of the estuary, which was subsequently transported into the bloom region via subsurface transport. Support for this transport comes from the fact that following the washout from Tropical Storm Ernesto, as flow rates declined, the salt wedge moved upstream at a rate of 6-13 cm s\(^{-1}\). These values are in the range for acoustically measured bottom water velocities in the NRE (Luettich et al. 2000) and were adequate to move cells 30 km upstream from 18 September to 3 October 2006. A similar subsurface transport mechanism inoculates the upper Chesapeake Bay with bloom forming dinoflagellates annually (Tyler and Seliger 1978; Li et al. 2000).

Coincident with this subsurface transport, the water column became strongly stratified allowing the accumulation of high concentrations of sediment derived NH\(_4^+\) and PO\(_4^{3-}\) in the hypolimnion (Fig. 6). A significant portion of these regenerated nutrients were mixed into the surface waters during the wind event of 7 October 2006, supplying nutrients without the significant dilution that occurred immediately after Tropical Storm Ernesto (Fig. 5.6).

5.4.3. Growth phase of the bloom—Following the mixing event, the apparent growth rate of the \textit{K. veneficum} population within the bloom region (0.25 d\(^{-1}\), Table 5.2) was close to the maximum autotrophic intrinsic growth rate of strains isolated from southeast U.S. estuaries (0.32-0.38 d\(^{-1}\)) (Li et al. 1999; Adolf et al. 2003, 2006a). Water temperature (~20°C) and the prevailing mesohaline conditions were conducive for \textit{K. veneficum} growth (Nielsen 1996: Li et al. 2000; Fensin 2004; Goshorn et al. 2004). Prior studies have
shown that algal blooms are common in the region of the estuary where this bloom developed (Valdes-Weaver et al. 2006; Waggener 2006). One explanation is that the estuary widens in this region increasing the residence time with respect to riverine discharge (Luettich et al. 2000). Reduced advective losses and continuous nutrient supply from both riverine discharge and the underlying sediments lead to biomass accumulation as was observed during this bloom (Pinckney et al. 1997; Waggener 2006). The general favorability for phytoplankton growth in the bloom region was demonstrated in this study by increases in nearly all phytoplankton species as the *K. veneficum* population proliferated from 3 to 19 October (Table 5.2).

Under poorly mixed conditions, dinoflagellates can outcompete other phytoplankton by vertically migrating to access light and nutrient resources (Smayda 1997). Salinity data indicated that moderate stratification within the bloom region was common throughout the growth phase of the bloom, even after the mixing event of 7 October (Fig. 5.5). The observations of subsurface chl *a* maxima on 3 October and 26 October (sunny days) and surface accumulation on 19 October (overcast day) also show that vertical mixing rates within the bloom region were often low compared to phytoplankton swimming speeds and are consistent with vertical migrations to achieve light levels optimum for photosynthesis (Ault 2000).

The interaction between vertical migrations and the prevailing frontal circulation pattern most likely produced the peak in biomass discovered at station 26 on 19 October. Frontal circulation patterns, particularly two layer flow and convergent downwelling, are known to retain and even concentrate vertically migrating phytoplankton (Chang and Carpenter 1985; Janowitz and Kamykowski 2006). The importance of the interaction
between vertical migration and frontal circulation was clear from differences in the relative abundance of diatoms and dinoflagellates at the three sites encompassing the frontal region (Fig. 5.8). Non-motile diatoms did not accumulate in the front, but rather showed monotonic increases or decreases across the frontal boundary. In contrast, the vertically migrating dinoflagellates, were horizontally concentrated at the frontal boundary (Fig. 5.8).

At the time of sampling (midday), the bloom population was also skewed toward the surface (Fig. 5.7). This was probably a positive phototaxic response to alleviate light limitation caused by the low incident irradiance from 17-19 October (Fig. 5.5). Thus the surface bloom maximum on 19 October was due primarily to the hydrodynamic accumulation at the frontal region and secondarily to positive phototaxis. The dominance of hydrodynamics in concentrating the cells is supported by the fact that dinoflagellates did not accumulate to nearly the same degree at adjacent stations.

While our data show that conditions were conducive for phytoplankton growth in general, and likely dinoflagellates in particular, the question arises as to why *K. veneficum* came to dominate the phytoplankton assemblage? We speculate that one reason is that *K. veneficum*’s toxin, KmTx 2, acted as a grazing deterrent and gave *K. veneficum* an advantage over other phytoplankton. Microzooplankton, and ciliates in particular, have growth rates comparable to their phytoplankton prey and can exert high grazing pressures on algal blooms (Watras et al. 1985; Nakamura et al. 1996). Microzooplankton abundance increased as total phytoplankton biomass increased leading up to the bloom maximum. Thus, grazing potential was likely high and any advantages gained by grazing deterrence could have altered the balance of growth versus grazing in
favor of *K. veneficum*. KmTx 2 has been shown to inhibit feeding and growth of *Oxyrrhis marina* (Adolf et al. 2007). Because susceptibility to karlotoxin is dependent on cell membrane sterol composition and cell membrane sterol compositions vary widely among planktonic organisms (Adolf et al. 2006b), similar grazing deterrence is probable for other micrograzers as well.

For *K. veneficum* (Adolf et al. 2007), and toxic phytoplankton in general (Sunda et al. 2006), grazing deterrence becomes more effective as the toxic phytoplankton becomes a larger percentage of the total phytoplankton population. However, this positive feedback also implies that grazing deterrence was likely less effective at the beginning of the bloom development when *K. veneficum* was a very small portion of the phytoplankton. This suggests that some other factor or factors may also have been important for selecting *K. veneficum*.

One possibility is the enhanced growth potential of *K. veneficum* when it is feeding mixotrophically. Laboratory studies have shown that mixotrophy can enhance intrinsic growth rates of *K. veneficum* 2-3 fold compared to autotrophic growth alone (Li et al. 1999; Adolf et al. 2006a). Small cryptophytes, a preferred prey item of *K. veneficum* (Li et al. 2000), were abundant (~ 2000 cells mL\(^{-1}\)) during bloom development (Table 5.2) indicating the potential for significant mixotrophic growth enhancement. Most of the other dinoflagellates common during the bloom period are also known mixotrophs (Jeong et al. 2005). However, the karlotoxins produced by *K. veneficum* are known to immobilize prey prior to capture (Li et al. 1999; Adolf et al. 2006b), thereby increasing mixotrophic feeding efficiency relative to other dinoflagellates (Adolf et al. 2006b).
Thus, the initial importance of karlotoxin production in *K. veneficum* bloom development may be growth enhancement rather than grazing deterrence.

The chemical form or combination of available nutrients may also have selected for *K. veneficum*. Kempton et al. (2002) noted that *K. veneficum* blooms in several brackish water ponds were coincident with high NH$_4^+$ concentrations. Perhaps, the high NH$_4^+$ availability early during bloom development favored *K. veneficum*. Glibert et al. (2001) suggested that high levels of dissolved organic matter may select for harmful algal bloom species. However, in this study, the anomalously high concentrations of DON and PO$_4^{3-}$ observed at the biomass maximum on 19 October 2006 were probably not responsible for stimulating *K. veneficum*. Instead, the origin of the excess DON and PO$_4^{3-}$, which represented 12-13% of the N and P contained in the phytoplankton, was most likely the bloom itself. Accumulation of dissolved organics and PO$_4^{3-}$ due to grazing, cell lysis, or exudation has been shown to occur as dinoflagellate blooms senesce (Holmes et al. 1967). Given the high nutrient demand indicated by low residual DIN (Fig. 5.7) and enormous phytoplankton biomass at the bloom maximum (Fig. 5.7), we speculate that by 19 October 2006 the bloom was near senescence due to nutrient limitation.

5.4.4. Bloom Termination—By 26 October 2006, the dense *K. veneficum* bloom was gone (Fig 9). The negative apparent growth rates of nearly all phytoplankton species from 19-30 October show that conditions had become unsuitable for sustaining a high level of phytoplankton biomass in the bloom region (Table 5.2). Salinity data over this time period show no evidence of a major wash-out event (Fig. 5.5). Declining water temperatures over the period (Fig. 5.5) probably decreased intrinsic growth rates of the
phytoplankton (Raven and Geider 1988), but were not out of the range permissive for *K. veneficium* growth (Nielsen 1996).

The disproportionate decrease in the abundance of dinoflagellates (Table 5.2), including *K. veneficium*, suggests that environmental conditions were less favorable for dinoflagellates compared to other phytoplankton groups. Assuming that the cause of the *K. veneficium* collapse was the same as for the other dinoflagellate species, this would generally rule out infectious agents because most pathogens and parasites display a fair degree of host specificity (Park et al. 2004). It also seems unlikely that grazing would so disproportionately affect dinoflagellates, though some tintinnids are known to preferentially feed on dinoflagellates (Stoecker et al. 1981).

A more likely explanation for the catastrophic collapse of dinoflagellate biomass is that small-scale shear associated with the wind mixing event on 20-21 October 2006 (Fig. 5.5) caused physiological or structural damage to dinoflagellate cells. Dinoflagellates are generally considered the most shear-sensitive of the phytoplankton classes and display growth inhibition or mortality at shear stress levels that are orders of magnitude lower than other phytoplankton (Juhl et al. 2000). The impact of shear may have been exacerbated by its growth stage if, as the accumulation of DON and PO$_4$-3 at the bloom maximum (Fig. 5.7) suggests, the bulk of the bloom biomass was in its senescent phase (Juhl et al. 2000).

5.4.5. Association with Fish Kills—The fish kills were closely coincidental, temporally and spatially, with the declining *Karlosingium veneficium* bloom. We postulate that the strong wind mixing event on 20-21 October 2006 caused sufficient turbulence to disrupt
the *K. veneficum* bloom that was already in a senescent phase. Due to the initial strong south wind (Fig. 5.5), senescent cells and any toxin released from these cells would likely have been carried from the bloom area northward towards the affected creeks (Luettich, R.A. Jr., personal communication). Dissolved KmTx 2 retains toxic activity for up to 2 d (Deeds et al. 2002) allowing ample opportunity for lethal exposure to the fish in the affected creeks. Further, sampling by NCDENR showed *K. veneficum* was present in creek waters (Table 5.4) where fish were actively dying, though at levels lower than the 15,000 cells mL\(^{-1}\) normally required to kill fish (Deeds et al. 2002). The declining *K. veneficum* cell concentrations between the earliest fish kill reports and the fish kills reported ~ 1 wk later (Table 5.4) were consistent with the declining cell numbers observed in the main stem of the NRE (Table 5.2) indicating the likelihood that *K. veneficum* cell densities in the creeks were higher in the days before the fish kills.

Senescence and rupture of these cells in these creeks, as well as those being advected into the region from the central part of the estuary, are postulated to have released sufficient toxin to cause the fish kills. Similarly, at a fish farm in Maryland, fish death occurred only after the *K. veneficum* bloom was terminated by addition of copper sulfate (Deeds et al. 2002).

The conclusion that toxins were released from the *K. veneficum* cells is supported by the premortem observation that fish in the affected region were gulping air (Table 5.4) despite evidence that the water column was fully oxygenated (Table 5.4; Fig. 5.5). It is known that the primary ichthyocidal activity of *K. veneficum* toxin is asphyxiation resulting from damage to gill epithelial cells (Nielsen 1993; Deeds et al. 2006). Juvenile cod (*Gadus morhua*) (Nielsen 1993) and red drum (*Sciaenops ocellatus*) (Deeds et al.
2002) exposed to high densities of *K. veneficum* have been shown to gulp air at the surface and to show disoriented swimming patterns prior to death. Both behaviors are consistent with a general response to blood hypoxia in fish (Kramer 1987).

### 5.5. Conclusion

The *Karlodinium veneficum* bloom described in this manuscript underscores the tight control of harmful algal blooms by meteorological forcing, estuarine hydrology, and sediment nutrient input in this shallow lagoonal estuary. Runoff following Tropical Storm Ernesto initially resulted in increased flushing and low algal biomass as has been found for prior tropical storms (Peierls et al. 2003). As riverine discharge declined, estuarine circulation and low wind stress resulted in a prolonged period of intense vertical stratification. This stratification was associated with hypoxic bottom water and the accumulation of NH$_4^+$ and PO$_4^{3-}$ to levels rarely observed in the Neuse River Estuary. A brief wind event mixed the regenerated nutrients throughout the water column. This mixing event was followed by nearly two weeks of moderate stratification and stable flushing rates. Nutrient, salinity, light, temperature and hydrologic conditions during this period were all favorable for phytoplankton growth.

The result was a large bloom which became dominated by dinoflagellates, in part due to their ability to vertically migrate over the shallow stratified water column to acquire light and additional nutrients as needed. Vertical migration further allowed the physical concentration of dinoflagellates in the bloom region due to the prevailing frontal zone circulation pattern. We speculate that grazing deterrence and enhancing mixotrophic
nutrition due to toxin production by *K. veneficum* allowed it to out compete other potential bloom forming dinoflagellates (Adolf et al. 2006b, 2007). Similar growth advantages associated with toxin production have been postulated to account for the success of other HAB species (Sunda et al. 2006). Bloom termination was probably due to the disruption of an already senescing population by a turbulent wind mixing event. Toxin released during bloom termination was postulated to be the cause of subsequent fish kills. The conditions leading to HAB formation are typically short lived and difficult to measure at appropriate spatial and temporal scales for assessing bloom dynamics (Fogg 2002). Our ability to infer some of the factors leading to this bloom was only possible due to the combined data from ModMon, USGS, NC DENR-DWQ, and CASTNET monitoring programs. Such networks are crucial for advancement in understanding harmful algal bloom dynamics.

5.6.  

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07-172 from the University of Maryland Biotechnology Institute, Center of Marine
Biotechnology.
Table 5.1. Summary of surface and bottom *Karlodinium veneficum* abundance, particulate organic carbon, particulate nitrogen, and photopigment data from the bloom maximum at station 26 on 19 October 2006.

<table>
<thead>
<tr>
<th>Property</th>
<th>Concentration (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Karlodinium veneficum</em>, cells mL(^{-1})</td>
<td>219,481 (213,621-225,340) 626 (279-973)</td>
</tr>
<tr>
<td>Mean particulate organic carbon, μM</td>
<td>1,138 (3.1) 202 (0.5)</td>
</tr>
<tr>
<td>Mean particulate nitrogen, μM</td>
<td>185 (10.8) 21.7 (0.5)</td>
</tr>
<tr>
<td>Photopigments, μg L(^{-1})</td>
<td></td>
</tr>
<tr>
<td>chlorophyll <em>a</em></td>
<td>257.3 21.8</td>
</tr>
<tr>
<td>fucoxanthin</td>
<td>222.2 6.6</td>
</tr>
<tr>
<td>19' hexanoyloxyfucoxanthin</td>
<td>93.9 0.95</td>
</tr>
<tr>
<td>chlorophyll <em>c3</em></td>
<td>81.5(^a) 1.8(^a)</td>
</tr>
<tr>
<td>diadinoxanthin</td>
<td>59.0 1.4</td>
</tr>
<tr>
<td>19' butanoyloxyfucoxanthin</td>
<td>33.6 0.35</td>
</tr>
<tr>
<td>chlorophyll <em>c1-c2</em></td>
<td>32.0 1.1</td>
</tr>
<tr>
<td>gyroxanthin diester</td>
<td>32.1 0.32</td>
</tr>
<tr>
<td>peridinin</td>
<td>12.4 1.1</td>
</tr>
<tr>
<td>chlorophyll <em>b</em></td>
<td>9.0 0.17</td>
</tr>
<tr>
<td>Pigment</td>
<td>Amount 1</td>
</tr>
<tr>
<td>-------------------------</td>
<td>----------</td>
</tr>
<tr>
<td>alloxanthin</td>
<td>2.3</td>
</tr>
<tr>
<td>zeaxanthin</td>
<td>0</td>
</tr>
<tr>
<td>chlorophyllide a</td>
<td>0</td>
</tr>
<tr>
<td>9' cis-neoxanthin</td>
<td>0</td>
</tr>
<tr>
<td>violaxanthin</td>
<td>0</td>
</tr>
<tr>
<td>antheraxanthin</td>
<td>0</td>
</tr>
<tr>
<td>monadoxanthin</td>
<td>0</td>
</tr>
<tr>
<td>lutein</td>
<td>0</td>
</tr>
<tr>
<td>β-carotene</td>
<td>0</td>
</tr>
<tr>
<td>myxoxanthophyll</td>
<td>0</td>
</tr>
</tbody>
</table>

*a chlorophyll c3 eluted at the appropriate time but the absorbance spectrum was shifted toward the red by +/- 1 nm over the time course of the peak indicating incomplete separation from another pigment/pigments.*
Table 5.2. Average abundance and apparent growth rates of dominant phytoplankton and microzooplankton species within the bloom region (ModMon stations 19, 26, 28, 37, and 43) during the bloom growth (3-19 October) and collapse (19-30 October) phases.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Abundance (cells mL(^{-1}))</th>
<th>(\mu_{\text{apparent}}) (d(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>October</td>
<td>October</td>
</tr>
<tr>
<td>3</td>
<td>19</td>
<td>30</td>
</tr>
<tr>
<td>3-19</td>
<td>19-30</td>
<td></td>
</tr>
<tr>
<td>Dinoflagellates</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Karlodinium veneficum</em></td>
<td>801</td>
<td>43,575</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>-0.52</td>
</tr>
<tr>
<td><em>Peridinium aciculiferum</em></td>
<td>54</td>
<td>453</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.13</td>
</tr>
<tr>
<td><em>Heterocapsa rotundata</em></td>
<td>331</td>
<td>435</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>-0.38</td>
</tr>
<tr>
<td><em>Scrippsiella trochoidea</em></td>
<td>70</td>
<td>275</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.09</td>
</tr>
<tr>
<td><em>Pheopolykrikos hartmanii</em></td>
<td>17</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.11</td>
</tr>
<tr>
<td>Cryptophytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cf <em>Hemiselmis virescens</em></td>
<td>565</td>
<td>1,383</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0.06</td>
</tr>
<tr>
<td>N.A.(^a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cf <em>Chroomonas minuta</em></td>
<td>1,037</td>
<td>1,597</td>
</tr>
<tr>
<td></td>
<td>229</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-0.10</td>
</tr>
<tr>
<td><em>Cryptomonas sp?</em></td>
<td>601</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>245</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Teleaulax amphioxeia</em></td>
<td>1,050</td>
<td>2,933</td>
</tr>
<tr>
<td></td>
<td>1,025</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diatoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cylindrotheca closterium</em></td>
<td>99</td>
<td>1,365</td>
</tr>
<tr>
<td></td>
<td>197</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-0.18</td>
</tr>
<tr>
<td>15 (\mu)m length pennate</td>
<td>0</td>
<td>6,323</td>
</tr>
<tr>
<td></td>
<td>2,032</td>
<td>N.A.(^a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-0.10</td>
</tr>
<tr>
<td><em>Leptocylindrus minimum</em></td>
<td>65</td>
<td>19,985</td>
</tr>
<tr>
<td></td>
<td>25,517</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>&lt; 8 (\mu)m diameter centric</td>
<td>880</td>
<td>624</td>
</tr>
<tr>
<td></td>
<td>404</td>
<td>-0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-0.04</td>
</tr>
<tr>
<td>Species</td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------</td>
<td>-------</td>
</tr>
<tr>
<td>Skeletonema costatum</td>
<td>170</td>
<td>425</td>
</tr>
<tr>
<td>Aulacoseira sp.</td>
<td>540</td>
<td>77</td>
</tr>
<tr>
<td><strong>Chlorophytes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyramimonas sp.</td>
<td>1,028</td>
<td>1,283</td>
</tr>
<tr>
<td>Chlamydomonas sp.</td>
<td>605</td>
<td>1,403</td>
</tr>
<tr>
<td><strong>Euglenophytes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eutreptia lanowii</td>
<td>39</td>
<td>494</td>
</tr>
<tr>
<td><strong>Cyanobacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Planktolyngbya sp. b</td>
<td>302</td>
<td>5,394</td>
</tr>
<tr>
<td><strong>Other small flagellates</strong></td>
<td>4,986</td>
<td>9,687</td>
</tr>
<tr>
<td><strong>Microzooplankton</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 30 μm oligotrich ciliates</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>Tintinnid ciliates</td>
<td>3</td>
<td>26</td>
</tr>
<tr>
<td>Oxyrrhis marina</td>
<td>33</td>
<td>118</td>
</tr>
<tr>
<td>Other heterotrophic dinoflagellates &gt; 10 μm</td>
<td>25</td>
<td>33</td>
</tr>
</tbody>
</table>

N.A. indicates that $\mu_{\text{apparent}}$ could not be calculated because either the initial or final abundance was zero. b Abundance of Planktolyngbya sp. is in filaments mL$^{-1}$. 

279
Table 5.3. Regression equations and statistical results of HPLC chlorophyll \( a \) (chl \( a \)) on in vivo fluorescence and toxin concentration and DNA copy \# on *Karlodinium veneficum* cell abundance.

Chl \( a \) (\( \mu g \ L^{-1} \)) = in vivo fluorescence (units \( L^{-1} \)) \* slope (\( \mu g \ \text{unit}^{-1} \)) + intercept (\( \mu g \ L^{-1} \)).

KmTx 2 (ng mL\(^{-1}\)) = *K. veneficum* (cells mL\(^{-1}\)) \* slope (ng cell\(^{-1}\)) + intercept (ng mL\(^{-1}\)).

DNA copy \# (\( \# \ \text{mL}^{-1} \)) = *K. veneficum* (cells mL\(^{-1}\)) \* slope (\( \# \ \text{cell}^{-1} \)) + intercept (\( \# \ \text{mL}^{-1} \)).

<table>
<thead>
<tr>
<th>Regression Variables</th>
<th>N</th>
<th>Slope ( (95% \ C. \ I.) )</th>
<th>Intercept ( (95% \ C. \ I.) )</th>
<th>( R^2 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll ( a ) on in vivo fluorescence (3 October)</td>
<td>19</td>
<td>0.78 ( (\pm 0.28) )</td>
<td>-0.48 ( (\pm 3.27) )</td>
<td>0.66</td>
<td>(&lt; 0.005 )</td>
</tr>
<tr>
<td>Chlorophyll ( a ) on in vivo fluorescence (19 October)</td>
<td>22</td>
<td>0.72 ( (\pm 0.04) )</td>
<td>3.29 ( (\pm 2.60) )</td>
<td>0.99</td>
<td>(&lt; 1x10^{-20} )</td>
</tr>
<tr>
<td>Toxin KmTx 2 on <em>K. veneficum</em> abundance (19 October)</td>
<td>6</td>
<td>3.70x10(^{-3}) ( (\pm 0.15x10^{-3}) )</td>
<td>4.02 ( (\pm 13.6) )</td>
<td>0.99</td>
<td>(&lt; 1x10^{-7} )</td>
</tr>
<tr>
<td>DNA copy # on <em>K. veneficum</em> abundance (18 Sep., 3, 19, 30 Oct.)</td>
<td>36</td>
<td>67.0 ( (\pm 0.03) )</td>
<td>-8,466 ( (\pm 11,730) )</td>
<td>0.99</td>
<td>(&lt;1x10^{-4} )</td>
</tr>
</tbody>
</table>
Table 5.4. Summary of fish kill investigations following the observed *Karldininium veneficum* bloom.

<table>
<thead>
<tr>
<th>Location and date of fish kill investigation</th>
<th>Fish affected</th>
<th>Environmental conditions</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper Broad Creek, 24/10/06 16:00</td>
<td>Total ~ 502, speckled trout (30), southern flounder (15), Atlantic menhaden (15), spot (406), large mouth bass, pumpkin seed</td>
<td>Temp.=17.9 °C, Salinity=7.6 ppt, pH=7.4</td>
<td>Not assessed, Reported on 24/10/06. Fish were gulping from the surface on 23/10/06.</td>
</tr>
<tr>
<td>Northwest Creek, 25/10/06 09:42</td>
<td>Total ~ 726, spot (327), gizzard shad (80), southern flounder (58), Atlantic menhaden (36), large mouth bass, pumpkin seed</td>
<td>Temp.=14.8 °C, Salinity=7.3 ppt, pH=7.4</td>
<td>2,541 (620-4,462), Reported on 24/10/06. Fish were dying at time of investigation.</td>
</tr>
<tr>
<td>Goose Creek, 30/10/06 12:00</td>
<td>Total not assessed, southern flounder (2), speckled trout (13), sunfish (13)</td>
<td>Temp.=17.0 °C, Salinity=6.0 ppt, pH=9</td>
<td>363 (1-1,089), Reported on 29/10/06. Fish were decayed. Kill was several days old.</td>
</tr>
<tr>
<td>Fairfield Harbor Marina</td>
<td>Total ~ 4,050, bream (825), gizzard shad (276), crappie (103), large mouth bass (123), yellow perch, speckled trout, spot, striped bass, striped mullet, bay anchovy</td>
<td>Temp.=20.5 °C</td>
<td>363</td>
</tr>
</tbody>
</table>
Figure 5.1. Map of the Neuse River Estuary showing locations of sampling stations and documented fish kill events. Station identifiers are expressed as km downstream. USGS. automated instrumentation stations are labeled by the channel marker (CM) to which they are fixed. NWC= Northwest Creek. FHM= Fairfield Harbor Marina. BC= Broad Creek. GC= Goose Creek. Other acronyms are described in the Methods and Materials.
Figure 5.2. Toxin analysis from the bloom sample collected on 19 October 2006 at station 26. (A) HPLC elution profile of the methanol extract of the bloom sample monitored by absorbance at 235 nm (solid line) and total ion current (dashed line) from the mass spectrometer at ions ranging from 500 to 1500 amu. (B) Hemolytic activity of the HPLC fractions collected every 15 s from the bloom sample (bars) and the HPLC elution profile of purified KmTx 2 at 235 nm (solid line).
Figure 5.3. Downstream distribution of surface water *Karlodinium veneficum* cell abundance and DNA copy number on five sampling dates spanning the bloom period. Error bars for cell abundance data are the 95% confidence interval for the microscopic abundance measurement. KmTx 2 toxin concentration is shown for 19 October 2006.
Figure 5.4. Time series of riverine discharge and salinity. Neuse River discharge at Kinston, NC (top panel) and surface (solid line) and bottom (dotted line) salinity from three USGS automated instrumentation at channel markers CM 38, CM 11 and CM 9 (lower panels). Arrows indicate the timing of the intrusion of the salt water layer following the flushing event from Tropical Storm Ernesto.
Figure 5.5. Time series of environmental data from channel marker CM 11 for the bloom period, October 2006. Surface (solid line) and bottom (dashed line) temperature, salinity, and dissolved oxygen measured every 15 min. 4 hr block-average vectoral wind speed. Hourly block-average ground level solar irradiance.
Figure 5.6. Estuarine conditions in the downstream and vertical dimensions on 3 October 2006. Contour plots of salinity, dissolved oxygen, and chlorophyll $a$. Contour magnitudes are given by the scale bar to the left of the figure. Sampling locations are indicated by dots. Surface salinity is overlain on the salinity contour plot (solid line and triangles). The depth of 1% incident PAR is overlain on the dissolved oxygen plot (solid line and squares). Downstream distributions of surface and bottom water nutrient concentrations. Dissolved organic nitrogen (dotted line, open diamonds), $\text{NO}_3^-$ (solid line, solid triangles), $\text{NH}_4^+$ (dash-dot line, open squares), $\text{PO}_4^{3-}$ (right y-axis, dashed line, solid circles).
Figure 5.7. Estuarine conditions in the downstream and vertical dimensions on 19 October 2006. Contour plots of salinity, dissolved oxygen, and chlorophyll $a$. Contour magnitudes are given by the scale bar to the left of the figure. Note log$_2$ scale for chlorophyll $a$. Sampling locations are indicated by dots. Surface salinity is overlain on the salinity contour plot. The depth of 1% incident PAR is overlain on the dissolved oxygen plot. Downstream distributions of surface and bottom water nutrient concentrations. Dissolved organic nitrogen (black dotted line and open diamonds), NO$_3^-$ (blue solid line and solid triangles), NH$_4^+$ (red dash-dot line and open squares), PO$_4^{3-}$ (right y-axis, green dashed line and solid circles).
Figure 5.8. Surface water abundance of the numerically dominant dinoflagellates and diatoms at the three stations (19, 26, and 28) encompassing the frontal region on 19 October 2006. *K. ven.* = *Karlodinium veneficum.* *S. troc.* = *Scrippsiella trochoidea.* *P. hart.* = *Pheopolykrikos hartmanii.* *P. acic.* = *Peridinium aciculiferum.* *H. rot.* = *Heterocapsa rotundata.* *L. min.* = *Leptocylindrus minimum.* *S. cost.* = *Skeletonema costatum.* *C. clost.* = *Cylindrotheca closterium.* cent < 5 μm = non-chain forming centric diatoms < 5 μm in diameter. Error bars are the 95% confidence intervals for microscopic abundance measurements.
Figure 5.9. Downstream and vertical distribution of chlorophyll $a$ within the bloom region on 26 October 2006. Contour magnitudes are given by the scale bar to the left of the figure. Sampling locations are indicated by dots.
5.7. LITERATURE CITED

photoacclimation in Karlodinium micrum (dinophyceae) and Storeatula major

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Chapter 6

SUMMARY, CONCLUSIONS, AND MANAGEMENT IMPLICATIONS
6.1. Summary

In this shallow lagoonal estuary the vertical density structure of the estuary is an important determinant of phytoplankton community composition. As water column stability increases, mixing is not sufficient to maintain large diatoms within the surface waters and they settle out of the euphotic zone to the sediments which on an areal basis are largely dysphotic. Smaller diatoms also show a negative relationship with increasing degree of water column stratification that is likely due to enhanced settling losses associated with a shallower depth of the upper mixed layer. The sediments serve as an important reservoir of settled diatom biomass and periodic wind induced resuspension of the surficial sediments is likely an important mechanism for maintenance of the diatom community within the system. Thus, the diatom community of this shallow system is characterized by a pelagic/ benthic life history.

Under the poorly mixed conditions that lead to significant losses of the diatom community from the water column, flagellates dominate. When mixing is weak, nutrients often accumulate in the bottom waters. Since the depth of the euphotic zone is usually very close to the depth of the pycnocline, there is a strong tendency for vertically separated light and nutrient resources under stratified conditions. The dominant flagellates, dinoflagellates and cryptophytes, were observed to migrate between the well lit surface layers and nutrient rich bottom waters or aggregate along the pycnocline. Both vertical migration patterns ensure access to light and nutrients under poorly mixed conditions and should lead to enhanced intrinsic growth. However, the degree of intrinsic growth enhancement achieved
through vertical migration is unclear due to the paucity of data on the nutrient and light kinetics of many of the dominant flagellates within the system.

6.2. Conclusions

The climatological degree of water column mixing is likely the primary reason why flagellates dominate the phytoplankton biomass of the lower Neuse River Estuary. According to a simple matrix transition model Margalef (1978) postulated that water bodies with a vertical eddy diffusivity of 0.2-1 cm² s⁻¹ should be dominated by flagellates and water bodies with an eddy diffusivity of 2-100 cm² s⁻¹ should be dominated by diatoms.

We are unaware of a data set for any estuarine system that contains seasonal or annual averages of eddy diffusivity. However, for systems where tidal straining is the dominant source of water column mixing, eddy diffusivity averaged over the tidal cycle would seem to be an appropriate reference level of eddy diffusivity to compare against long term averages of community composition. Data from the diatom dominated Chesapeake and San Francisco Bays show levels of eddy diffusivity for which Margalef’s model would predict diatom dominance. In San Francisco Bay, tidally and vertically averaged eddy diffusivity is in the range 10-100 cm² s⁻¹ (Lucas et al. 1999). In the Chesapeake Bay proper, a vertically averaged eddy diffusivity of ~14 and 24 cm² s⁻¹ best predicted observed temperature and salinity distributions between neap and spring tides, respectively (Li and Zhong 2009). In the York River Estuary, a tributary estuary of the Chesapeake, vertically and tidally averaged eddy diffusivities were 4-15 cm² s⁻¹ (Scully et al. 2005).
Climatological levels of eddy diffusivity are more difficult to constrain in a system like the NRE where turbulence is principally driven by the episodic patterns of wind forcing. The observation of nearly ubiquitous phytoplankton patchiness due to vertically migrating flagellated phytoplankton may provide a valuable indication of the upper limit of the vertically averaged eddy diffusivity within the lower NRE. Maximum swimming speeds of dinoflagellates and cryptophytes are approximately 1 m h\(^{-1}\) (Sommer 1988) or 0.028 cm s\(^{-1}\).

We can describe the ability of a group of phytoplankton to maintain a patchy distribution within a turbulent water column based on the Peclet number (Condie 1999)

\[
\text{Pe} = V_{\text{swim}} w_{\text{rms}}^{-1} = V_{\text{swim}} h K_z^{-1}
\]

where Pe is the Peclet number, \(V_{\text{swim}}\) is the swimming velocity, \(w_{\text{rms}}\) is the root mean square of the turbulent vertical velocity fluctuations, \(h\) is a vertical length scale, and \(K_z\) is the vertical eddy diffusivity. In order to maintain a patch of motile cells within the water column, the Peclet number must be greater than one. Since \(V_{\text{swim}}\) is 0.028 cm s\(^{-1}\), the existence of patches dictates that \(w_{\text{rms}}\) is less than 0.028 cm s\(^{-1}\). In order to determine the eddy diffusivity, an appropriate length scale must be determined. Average dimensions of vertical patches of flagellates observed during the course of the autonomous vertical profiler deployments (Figures 2.14, 2.15, 2.17, 2.18, 2.20) suggest that a patch size of 1 m depth is typical. Thus 1 m is the maximum possible vertical scale of the largest eddies when phytoplankton patchiness exists at a scale of 1 m. In accordance with Condie (1999), this value was used as \(h\) and the Peclet number equation above provides an upper bound of 2.7 cm\(^2\) s\(^{-1}\) for eddy diffusivities.
This upper bound is only valid when flagellates are able to maintain patchiness within the water column and we observed many high energy wind events that fully mixed the water column and phytoplankton community. However, based on the relationship between the degree of stratification and levels of vertical phytoplankton patchiness (Fig. 2.21), it appears that phytoplankton patchiness was present about two thirds of the time throughout the autonomous vertical profiler records. Thus, this upper bound of vertical eddy diffusivity seems to be an appropriate reference for comparison against the eddy diffusivities of other systems.

The value, 2.7 cm s\(^{-1}\), is still higher than Margalef would have predicted for a flagellate dominated system. Since this value is an upper bound, average levels of turbulence excluding strong wind events are likely to be significantly lower. In any case, 2.7 cm s\(^{-1}\) is much lower than the eddy diffusivities determined for the diatom dominated and tidally influenced Chesapeake and San Francisco Bays and is consistent with the assertion that the inherently low levels of vertical mixing in the Neuse River Estuary are responsible for its dominance by flagellates.

6.3. Management implications

6.3.1. Improved eutrophication models—Mechanistic models of nutrient loading on the water quality of estuaries are increasingly being used to guide management strategies aimed at curbing the deleterious effects of eutrophication. In recognition that the effects of eutrophication within coastal and estuarine systems are significantly influenced by both the levels of stimulated phytoplankton production and by changes in the composition of the
phytoplankton community, recent eutrophication models have sought to improve the predictive capability for key environmental parameters such as chlorophyll $a$ and dissolved oxygen by including several different functional groupings of phytoplankton (Cerco and Cole 1993; Bowen and Heironymus 2000; Hieronymus and Bowen 2006). For the Neuse River Estuary, the Neuse Estuary Eutrophication Model (NEEM) was developed to predict how various nutrient load reduction scenarios for the Neuse River would affect water quality within the estuary.

The NEEM model breaks the phytoplankton community into three functional groups with different settling speeds and nutrient/ light kinetics for growth (Bowen and Heironymus 2000; Hieronymus and Bowen 2006). These groups consist of: 1) cryptophytes and chlorophytes 2) cyanobacteria and 3) dinoflagellates and diatoms (Bowen and Heironymus 2000; Hieronymus and Bowen 2006). NEEM explained only 36 percent of the observed variability in chlorophyll $a$ over a two year period (Hieronymus and Bowen 2006). In contrast, using similar phytoplankton groupings, a eutrophication model of the diatom dominated Chesapeake Bay explained 65 percent of the variability in chlorophyll $a$ (Cerco and Cole 1993). In the Neuse River Estuary, where much of the variability in chlorophyll $a$ is due to flagellates, grouping of dinoflagellates and diatoms seems likely to produce the comparatively poor model performance.

By grouping dinoflagellates and diatoms together, the model implicitly ignores fundamental differences in their ecology such as maximum growth rates, nutrient and light utilization kinetics, and principally the fact that diatoms sink and dinoflagellates swim. Our data suggest that sinking losses are an important term in the growth equation of the diatom community and provide no justification for making sinking losses an appreciable term...
affecting net dinoflagellate growth. If separated from the diatoms, simple representations of
vertical migration patterns of the dominant flagellates within the system could be included in
the eutrophication model to more realistically represent the growth advantages associated
these behaviors. The effects of separating these groups and including vertical migration
patterns for the flagellates are likely to broadly change observed model behavior and may
alleviate some discrepancies between NEEM model predictions and the observed
phytoplankton biomass in the estuary.

Vertical migrations within the ambient velocity gradients of estuaries are known to
significantly reduce advective losses (Seliger et al. 1970; Anderson and Stolzenbach 1985)
and would likely reduce modeled advective losses of the flagellate community. There are
several indications that the current NEEM model overestimates advective losses. During the
construction of the model it was recognized that the longitudinal chlorophyll \( a \) maximum
maintained a higher degree of patchiness than could be explained with longitudinal
dispersion coefficients that correctly parameterized the longitudinal salinity distribution
(Bowen and Hieronymus 2000). Secondly, the current NEEM model significantly
overestimates the time that it takes the phytoplankton community to reestablish itself after
major flushing events such as tropical storms and hurricanes (Hieronymus and Bowen 2006).
Both of these observations, suggest that, in reality, phytoplankton biomass within the estuary
tends to experience advective losses less than those calculated based on the model generated
flows.

In addition to more accurately portraying advective losses, a representation of vertical
migration behavior of the flagellates would dictate that nutrient stocks within the bottom
water would be directly available to the flagellate community without requisite mixing into
the surface waters. This more direct linkage between sediment nutrient loading and phytoplankton uptake would more accurately represent the potentially important feedback between flagellate biomass and bottom water NH$_4^+$ concentrations. In doing so, the ninety percent error in modeled versus predicted NH$_4^+$ produced by the current NEEM model may be reduced.

The NEEM model also consistently underestimates observed chlorophyll $a$ concentrations of the most intense algal blooms. These blooms are usually due to flagellates (Pinckney et al. 1998) and we suggest that this model outcome is likely to occur regardless of how well the model is parameterized. In a flagellate dominated system like the NRE, ephemeral small scale physical processes are likely to interact with the vertical migration velocities of the flagellates to result in very high localized concentrations of flagellates. The frontal zone that allowed the accumulation of *Karlodinium veneficum* to extremely high concentrations in Chapter 4 provides an excellent example. These processes occur at the sub-grid scale of eutrophication models and generate model error that must be accepted as inevitable within a flagellate dominated system.

6.3.2. Phytoplankton as indicators—Estuarine phytoplankton communities are currently being used as indicators of ecological change due to anthropogenic nutrient pollution and climatically induced hydrologic variations (Lacouture et al. 2006; Pærl et al. 2003, 2007). However, our ability to interpret changes in the phytoplankton community suffers from a lack of a mechanistic understanding of the complex interactions between anthropogenic and natural factors that shape the phytoplankton community (Cloern et al. 2001). Water quality managers using phytoplankton community structure as indicators of water quality and
ecosystem health should recognize that the degree of mixing can be an important natural
determinant of phytoplankton community structure within lagoonal estuaries.

The changes in phytoplankton community structure observed in this study occurred on
time scales of days to weeks. Typically water quality assessment protocols average over
these short term fluctuations to reveal long term annual or decadal trends that are presumably
of anthropogenic origin (National Research Council 2000). However, the global climate
system is changing rapidly and global circulation models predict regional alterations in
riverine discharge (Giorgi et al. 1994) and wind speeds (Suursaar and Kullas 2000) that are
the primary determinants of stratification and mixing in lagoonal estuaries. Thus, the
potential exists for longer term shifts in phytoplankton community structure via changes in
mixing regimes. Improved understanding of the role of mixing in determining phytoplankton
community structure will aid managers in discerning forcing features of the phytoplankton
community structure that can and cannot be feasibly managed.


